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Dietary contribution of fermented grain pellets to the growth of juvenile *Litopenaeus vannamei* raised in an intensive biofloc-based rearing system

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Abstract
This study investigated the dietary contribution of fermented grain pellets (FGP) to the growth of juvenile *Litopenaeus vannamei* raised in a biofloc-based system. Grain pellets made of agricultural plant by-products were fermented with distilled water containing dehydrated live probiotic yeast, *Saccharomyces cerevisiae*. Shrimp were fed a feed with 396.8 g kg^{-1} crude protein (CP) under the following proportions: 100:0 (percentage of feed and FGP to the total daily ration, respectively), 75:25, 50:50, 25:75, and 0:100. Juvenile shrimp of 1.15 ± 0.12 g were stocked under 133 animals m^{-2} and reared for 77 days in thirty-five 1-m^3 outdoor tanks. Final shrimp survival reached 89.3 ± 5.7% and was unaffected by dietary treatment. Shrimp body weight decreased significantly from 12.68 ± 1.48 (100:0) and 11.71 ± 0.67 g (75:25) to a low of 5.23 ± 0.40 g (0:100). No differences were found in weekly shrimp growth between shrimp fed under 100:0 (1.06 ± 0.14 g) and 75:25 (0.97 ± 0.06 g). Feed replacement at 25% caused no loss in yield (1,290 ± 87 g m^{-2}) compared to no feed replacement at all (1,365 ± 148 g m^{-2}). The 75:25 proportion of feed to FGP was able to partially spare feed inputs leading to 0.08 USD kg^{-1} savings in feeding costs. Results indicated that a feed replacement of 25% compensated by an equivalent amount of FGP as part of the daily ration led to no detriment in shrimp growth performance in a biofloc-based system.

Keywords Fermentation · Grain pellets · Biofloc · Feed costs

Introduction

Industrially compounded feeds represent the single highest variable cost in shrimp farming (Engle et al. 2017; Castro et al. 2021; Weldon et al. 2021). Efforts to reduce the direct costs with feed usage in shrimp culture have basically evolved on promoting pond natural

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productivity and establishing more rational feed management practices. Such strategies have included the increase in feeding frequency (Arnold et al. 2016; Nunes et al. 2019a; Reis et al. 2021), calibration of ration size (Arnold et al. 2016; Nunes et al. 2006, 2019a; Weldon et al. 2021), and implementation of feed automation and feeding schedules (Reis et al. 2021).

Under extensive and semi-intensive culture systems, natural productivity act as a supplemental source of dietary nutrients which significantly contributes to shrimp growth (Burford et al. 2003, 2004b, 2020; Huang et al. 2020; Nunes and Parsons 2000a). This reduces the reliance on formulated feeds making production more economically-efficient despite lower yields and profits (Nguyen et al. 2019). As opposed, under high-density culture, nutrient-dense feeds are the primary source of shrimp growth (Burford et al. 2004a; Castro et al. 2021; Weldon et al. 2021). In these systems, the application of carbohydrates in water as carbon sources to control the carbon to nitrogen (C:N) ratio and promote heterotrophic bacterial growth is a recommended practice (Roy et al. 2010; Correia et al. 2014; Xu et al. 2016; Abakari et al. 2021). It has been found that an increased C:N ratio mitigates the accumulation of toxic nitrogen compounds (Wasielesky et al. 2006; Xu et al. 2016, 2021; Huang et al. 2022) allowing a significant reduction in water exchange rates. The commonly used organic carbon sources are agricultural by-products derived from corn, rice, soybean, sugarcane, tapioca, and wheat (Burford et al. 2003; Wang et al. 2016; Leite et al. 2020; Abakari et al. 2021; Huang et al. 2022). These by-products serve as a prebiotic for the growth of beneficial bacteria forming what is known as synbiotic. Application of synbiotic supplements to culture water has been shown to improve shrimp gut microbiota flora (Ramírez et al. 2013; Zubaidah et al. 2015), their immunity and disease resistance (Zubaidah et al. 2015; Huynh et al. 2018; Situmorang et al. 2021), acting as a carbon source (Leite et al. 2020; Pimentel et al. 2022) for the bacterial community in shrimp rearing systems (Hussain et al. 2021).

While continuous inputs of grain pellets have been reported to promote the development of heterotrophic bacteria in high-intensity, zero-water exchange shrimp ponds (Burford et al. 2003), there is scarce information of their dietary contribution, if any, to shrimp growth in biofloc-based culture systems. Our previous work has shown that culture water treated with a mix of rice by-products improved the final body weight (BW), feed conversion ratio (FCR), and the yield of juvenile *Litopenaeus vannamei* farmed under limited water exchange at 127 animals m^{-2} in a tank rearing system (Leite et al. 2020). We hypothesized that improvement in shrimp performance occurred due to a direct ingestion of rice particles colonized by heterotrophic bacteria and *Bacillus* spp. In the present work, we investigated if grain pellets made of agricultural plant by-products and subjected to fermentation could act as a supplemental source of dietary nutrients to juvenile whiteleg shrimp, *L. vannamei*, fed under a progressive feed replacement regime in a biofloc-based culture system.

Materials and methods

Preparation of feed and grain pellets

A practical feed was formulated to meet the recommended nutrient levels for the farming of penaeid shrimp (NRC 2011; Table 1). The feed was designed to achieve a CP and lipid content of 396.8 and 81.1 g kg^{-1} (as-is basis), respectively. Soybean meal (SBM), salmon meal, and soy protein concentrate were used as the main protein sources, with dietary inclusions of 380.0, 120.0, and 50.0 g kg^{-1} (as-is), respectively. The feed was supplemented with L-lysine, DL-methionine, and L-threonine in order to meet the minimum levels of these amino acids

(AAs) required to maximize shrimp growth under green-water culture conditions (Façanha et al. 2016, 2018; Nunes et al. 2019b). Total lysine, methionine (Met plus cysteine, Cys), and threonine reached 21.49, 7.58 (13.69), and 17.87 g kg^{-1} of the diet (dry matter basis, DM), respectively (Table 2). The total formula cost of the practical feed was estimated at 0.72 USD kg^{-1} based on local market prices of ingredients and feed additives.

Grain pellets were made of agricultural plant by-products which included broken rice, SBM, wheat bran, and rice bran (Table 1). These raw materials were chosen taking into account their local availability and lowest possible economical cost. Grain pellets were formulated to meet a CP, total fiber and a C:N ratio of 185.5, 32.8 g kg^{-1}, and 19:1, respectively. Liquid sugar-cane molasses was included to raise organic carbon. Soybean oil was added for pellet lubrification allowing feed extrusion. A synthetic binder was used to increase pellet water stability after immersion in seawater. The total formula costs of the grain pellets were estimated at 0.43 USD kg^{-1}, not including the costs with fermentation.

Both the feed and grain pellets were prepared in the lab following the methodology described by Nunes et al. (2011). In short, all dried ingredients were ground to 300 µm, weighed, and mixed with the liquid raw materials and feed additives in a planetary mixer until a feed dough was formed. The feed dough was then manually broken in small particles by forcing it though a rigid net. The particles were introduced into a laboratory extruder adjusted to operate at 95 °C. After extrusion, pellets of 2.4 mm in diameter by 4.8 mm in length were steamed for 10 min. and then dried in a convection oven until moisture was reduced to less than 120 g kg^{-1}. Dried pellets were stored under 16 °C until use.

Fermentation of grain pellets

After preparation, grain pellets were subjected to fermentation following a modified method described by Yabaya et al. (2009) and Sharawy et al. (2016). Each 100 g of grain pellets was top-coated with 2,500 mL of distilled water containing 2.50 g of a dehydrated live probiotic yeast, *Saccharomyces cerevisiae* SC 47 (Procreatin®, strain NCYC 996, Phileo Lesaffre Animal Care, São Paulo, Brazil) with a density of 1.5×10^{10} cells g^{-1}. The mixture was placed in a 10 l glass flask covered with an aluminum foil and incubated for 24-h under 40 **°C** in a convection oven. The 24-h incubation was chosen since preliminary assessments had indicated it provided the highest increase in the total CP content of grain pellets compared to 12 and 48 h (Kjeltech auto-analyzer, Analyzer unit 6500; FOSS, Hoganas, Sweden). After drying, moisture levels of grain pellets ranged between 180 and 200 g kg^{-1}. During the study, fermentation was carried out on a daily basis following the amounts needed of fermented grain pellets (FGP) for the next day's application.

Physical analyses of feed and grain pellets

Both the feed and FGP were analyzed for water stability following a modified method described by Obaldo et al. (2002) and Nunes et al. (2019a). Initially, a sample with 25 g of feed and 25 g of FGP were weighed and separated in plastic bags in triplicate. Each sample was transferred to 250 mL Erlenmeyer flasks with 100 mL seawater (35 g l^{-1} salinity). Flasks were placed on a temperature-controlled horizontal shaker (Incubator Lac-INR-1000®, Láctea, São Paulo, Brazil) adjusted to operate at 100 ± 15 RPM s^{-1} at 27.5 °C for 30 min. Samples were then transferred to a Tyler #20 mesh sieve (equivalent to 0.85 mm) and washed with distilled water. The retained samples were allowed to drain

Table 1 Ingredient and proximate composition (g kg^{-1}, as-is basis) and formula cost (USD kg^{-1}) of the practical feed and grain pellets, before and after fermentation

Ingredients	Dietary inclusion (g kg^{-1})		
	Feed	Grain pellets	
		Non-fermented	Fermented
Broken rice[1]	-	420.3	420.3
Soybean meal[2]	380.0	224.7	224.7
Wheat flour[3]	250.0	-	-
Wheat bran[3]	-	150.0	150.0
Salmon meal[4]	120.0	-	-
Rice bran[1]	-	100.0	100.0
Sugar-cane molasses	-	60.0	60.0
Soy protein concentrate[5]	50.0	-	-
Cassava starch	30.0	-	-
Salmon oil[4]	30.0	-	-
Calcium carbonate	21.9	20.0	20.0
Soy lecithin	21.8	-	-
Wheat gluten[6]	21.3	-	-
Soybean oil[7]	-	20.0	20.0
Sodium monophosphate[8]	13.9	-	-
Magnesium sulfate	12.4	-	-
Salt	8.2	-	-
Potassium chloride	7.4	-	-
Vitamin and mineral premix[9]	10.0	-	-
Kaolin	9.3	-	-
Synthetic binder[10]	5.0	5.0	5.0
L-lysine[11]	4.1	-	-
L-threonine[12]	2.0	-	-
DL-methionine[13]	1.9	-	-
Vitamin C[14]	0.8	-	-
Formula cost (USD kg^{-1})[15]	0.72	0.43	-
Proximate composition (g kg^{-1}, dry matter basis)			
Dry matter	884.0	886.7	813.3
Crude protein	396.8	209.2	252.7
Ether extract	81.1	68.9	85.1
Crude fiber	27.9	37.0	45.4
Ash	121.4	58.9	75.7
NFE[16]	329.5	555.1	440.1
Gross energy (MJ kg^{-1})[17]	19.03	18.80	18.79

[1]Sucesso Agroindústria Ltda. (Eusébio, Brazil)

[2]Bunge Alimentos S.A. (Luiz Eduardo Magalhães, Brazil)

[3]J. Macedo (Fortaleza, Brazil)

[4]Pesquera Pacific Star S.A. (Puerto Montt, Chile)

[5]X-Soy 600®, CJ Selecta S.A. (Araguari, Brazil)

[6]Amytex 100. Tereos Syral S.A.S. (Marckolsheim, France)

[7]ADM do Brasil Ltda. (Campo Grande, Brazil)

[8]60 g kg^{-1} calcium, 207 g kg^{-1} of phosphorous, 141.2 g kg^{-1} of avail-

Table 1 (continued)

able phosphorus

[9]Rovimix® Camarao Intensivo. DSM Produtos Nutricionais Brasil Ltda. (São Paulo, Brazil). Guarantee levels per kg of product: vitamin A, 1,250,000 IU; vit. D3, 350,000 IU; vit. E, 25,000 IU; vit. K3, 500 mg; vit. B1, 5,000 mg; vit. B2, 4,000 mg; vit. B6; 10 mg; nicotinic acid, 15,000 mg; pantothenic acid, 10,000 mg; biotin, 150 mg; folic acid, 1,250 mg; vit. C, 25,000 mg; choline, 50,000 mg; inositol, 20,000 mg; Fe 2,000 mg; Cu, 3,500 mg; chelated Cu, 1,500 mg; Zn, 10,500 mg; chelated Zn, 4,500 mg; Mn, 4,000 mg; Se, 15 mg; chelated Se, 15 mg; I, 150 mg; Co, 30 mg; Cr, 80 mg; filler, 1,000 g

[10]Nutri-Bind Aqua Veg Dry, Nutri-Ad International NV (Dendermonde, Belgium)

[11]Biolys®, Evonik Nutrition & Care GmbH (Hanau, Germany). L-Lysine, 546 g kg^{-1}

[12]ThreAMINO®, Evonik Nutrition & Care GmbH (Hanau, Germany). L-Threonine, 985 g kg^{-1}

[13]Evonik Nutrition & Care GmbH (Hanau, Germany). DL-Methionine, 990 g kg^{-1}

[14]Rovimix® Stay C® 35. Minimum of 350 g kg^{-1} of phosphorylated vitamin C activity. DSM Nutritional Products AG (Schweiz, Switzerland)

[15]Based on local ingredient prices. Cost of fermentation for grain pellets not included

[16]Nitrogen free extract. Calculated by subtraction [dry matter—(crude protein + ether extract + crude fiber + ash)]

[17]Gross energy given on a dry matter (DM) basis. Calculated as GE = (4,143 + (560 × EE [DM]) + (150 × crude protein [DM]) – (440 × crude ash [DM])) × 0.0041868. Source:Ewan (1989)

excess water and dried at 130 °C for 24 h. Dried samples were weighed and the water stability (%) determined by the formula: [final weight (g) of the dried sample/initial weight (g) of the sample] × 100. Final weight of sample was multiplied by its moisture content to convert it into dry weight. Moisture was determined in a forced-air convection oven by drying five replicate samples of 3 g each at 105 °C for 72 h. Pellet hardness was measured with a Kahl pellet hardness tester, manually operated (Amandus Kahl GmbH & Co, Hamburg, Germany). Crushing readings (expressed in kg) were carried out with 30 individual pellets per feed type. Pellet length and hardness were measured using an electronic caliper.

Shrimp and biofloc rearing system

Shrimp were reared in 35 circular independent outdoor tanks of 1 m^3 with a bottom area of 1.02 m^3. Tanks were round, blue in color, and made from polypropylene. Rearing tanks were equipped with continuous air-diffusion aeration provided by one 7.5-hp blower connected to PVC lines. Air was delivered to the bottom of each tank by a 0.5-m aeration tubing (Aero-Tube™, Tekni-Plex Aeration, Austin, Texas, USA). Aeration was supplied to nearly saturate water with dissolved oxygen (DO) during the complete rearing cycle. Water exchange (10% of total tank volume) took place only twice during the experimental period. In this case, freshwater was added to control the increase in water salinity due to evaporation. Water preparation consisted of filling 90% of total tank volume with sand-filtered seawater pumped from an estuary and the remaining with biofloc water from a shrimp nursery tank. Heterotrophic bacteria development was promoted through supplementation

Table 2 Amino acid profile (g kg^{-1}, dry matter basis, DM) of the feed and grain pellets, before and after fermentation

Amino acids	Composition (g kg^{-1}, DM)		
	Feed	Grain pellets	
		Non-fermented	Fermented
Essential amino acids (EAA)			
Arginine	167.87	15.11	17.71
Histidine	8.94	5.07	6.02
Isoleucine	15.38	8.35	10.08
Leucine	27.6	16.35	19.92
Lysine	21.49	9.25	12.17
Methionine	7.58	3.50	4.30
Methionine + cysteine*	13.69	7.33	9.34
Phenylalanine	17.42	10.26	12.30
Threonine	17.87	8.35	9.59
Tyrosine	11.99	7.44	9.10
Valine	15.84	9.36	11.31
Sum EAA	167.87	93.04	112.50
Non-essential amino acids (NEAA)			
Alanine	18.10	10.04	11.56
Aspartic acid	33.82	16.24	13.65
Cysteine	6.11	3.83	5.04
Glycine	19.80	9.36	11.31
Glutamic acid	66.86	32.37	33.20
Proline	23.19	10.60	12.79
Serine	17.08	9.47	11.43
Taurine	11.99	-	-
Sum NEAA	196.95	91.91	98.98
Sum EAA + NEAA	364.82	184.96	211.48

*Sulfur amino acids

of 10 g m^{-3} day^{-1} (as-is basis) of ground shrimp feed (minimum of 350 g kg^{-1} CP) with 36 g m^{-3} day^{-1} of liquid sugar-cane molasses over five consecutive days. Three additional days with strong aeration were allowed prior to shrimp stocking.

Juvenile *L. vannamei* of 1.15 ± 0.12 g body weight (BW, mean ± standard deviation; $n = 4{,}760$) were stocked under 133 animals m^{-2}. After stocking, shrimp were acclimated for 6 days with a crumbled commercial feed reported to contain a minimum of 400 g kg^{-1} CP (Camanutri 40 CR2, Neovia Nutrição e Saúde Animal Ltda., São Lourenço da Mata, Brazil). All rearing procedures were performed in compliance with relevant laws and institutional guidelines, including those related to animal welfare. Shrimp were reared for a total of 77 days.

Feeding

To investigate the dietary contribution of FGP to shrimp growth, the inputs of feed were progressively reduced and the missing amounts replaced by FGP. Therefore, feed and FGP were delivered individually or mixed together on a daily basis under the following proportions: 100:0 (percentage of feed and FGP to the total daily ration, respectively), 75:25, 50:50, 25:75, and 0:100. Seven rearing tanks were designated for each proportion. The daily rations

were calculated based on the equation $MM = 0.0931BW^{0.6200}$, where MM is the maximum amount of feed that can be consumed daily by an individual with a specific BW (Nunes and Parsons 2000b). Previous work had shown that the MM can be reduced by 28.8% without any detrimental effect to shrimp growth performance (Nunes et al. 2006). Therefore, the daily meals (feed and FGP alone or combined) were reduced by 30% across all treatments to control FCR. Meals were adjusted daily, assuming a fixed daily drop in shrimp survival and BW gain of 100 mg shrimp day^{-1}. Starting in the 15th day of rearing, 10 shrimp $tank^{-1}$ were sampled weekly to determine their BW gain. Until the next sampling, meals were adjusted assuming an average daily weight gain achieved in the previous week for each specific culture tank, maintaining a fixed 0.07% daily drop in shrimp survival. Shrimp were fed 10 times during daylight using an automatic feeder (Nunes et al. 2019a). No feed remains were collected.

Water quality

Water salinity, temperature, pH, and DO were measured daily in each tank between 2:00 pm and 3:00 pm. Observed mean (±standard deviation, sd) values were 31 ± 3.0 g l^{-1} (n = 1,995), 29.2 ± 1.2 °C (n = 1,995), 7.81 ± 0.16 (n = 1,995), and 6.41 ± 0.48 mg l^{-1} (n = 1,995), respectively. Water alkalinity was measured biweekly with a visible spectrophotometer (DR 2800 Spectrophotometer, Hach Company, Loveland, USA). Mean alkalinity reached 148 ± 23.7 mg $CaCO_3$ l^{-1} (n = 27). Water alkalinity and pH were controlled by adding 30 g m^{-3} of diluted sodium bicarbonate whenever levels dropped below 140 mg $CaCO_3$ l^{-1} and 7.0, respectively (Castro et al. 2021).

Total ammonia nitrogen (mg TAN l^{-1}), nitrite (mg NO_2^- l^{-1}), and nitrate (mg NO_3^- l^{-1}) were also determined with a visible spectrophotometer. Readings were carried one day after shrimp stocking and a day before shrimp harvest in three samples per dietary treatment, each comprised from a pool of five tanks. Similarly, on days 1, 25, and 75 of culture, chlorophyll-*a* (Clr-*a*, µg l^{-1}) was determined through spectrophotometric analysis (APHA Sect. 10200H) following APHA (2012). The procedures to analyze total suspended solids (TSS, mg l^{-1}; APHA Sect. 2540D), fixed suspended solids (FSS, mg l^{-1}; APHA Sect. 2540E), and volatile suspended solids (VSS, mg l^{-1}; APHA Sect. 2540E) were outlined by APHA (2012). Settleable solids (SS, ml l^{-1}) were determined in each rearing tank every 2 days using 1-l Imhoff cones (Castro et al. 2021).

Shrimp growth performance and feeding costs

At harvest, all live shrimp were counted and weighed individually using a 0.01-g precision scale. Final shrimp survival (S, %) was calculated as:

$$S = \left(\frac{POPf}{POPi}\right) \times 100$$

where

POPi = number of stocked shrimp and POPf = number of shrimp at harvest.

The weekly weight gain (WWG, g $week^{-1}$) was determined by the formula:

$$WWG = \left(\frac{BWf - BWi}{t}\right) \times 7$$

where

BWi = wet shrimp body weight (BW, g) at stocking, *BWf* = final shrimp BW at harvest, and t = number of days in culture.

The gain in shrimp yield (YIE, g of shrimp biomass gained m^{-2}) was determined as:

$$YIE = \frac{\text{BIOf} - \text{BIOi}}{\text{tank bottom area } (m^2)}$$

where

BIOi = initial shrimp biomass per tank (g), *BIOf* = final shrimp biomass (g), and tank bottom area = 1.02 m^2.

The FCR was calculated as the sum of the total inputs of feed (g, DM basis) and FGP (g, DM basis) delivered during the experimental rearing period. The apparent feed intake (AFI, g of feed delivered divided by the number of stocked shrimp) was calculated by dividing the total amount of feed (g, DM basis) by the number of stocked shrimp. The cost of feeding per kg of shrimp produced (USD kg^{-1}) was determined by multiplying the respective formula costs (USD kg^{-1}) for the total amount of feed (kg) and FGP (kg) used during culture. The result was then divided by the gained shrimp biomass (kg) attained in each rearing tank.

Relative AFI of feed and FGP

In order to assess if shrimp consumed FGP, a 5-day evaluation of AFI was carried out. After harvest, shrimp from each dietary treatment were randomly selected, transferred to 15 tanks of 1.5 m^3 (bottom area of 1.61 m^2), and stocked under 70 animals m^{-2} (112 shrimp $tank^{-1}$). Shrimp were fed in excess, at 0800 am and 0100 pm, exclusively in a feeding tray (one unit per tank) measuring 50.3 cm^2. The amount of feed and FGP delivered in each tray followed the proportions previously defined (100:0, 75:25, 50:50, 25:75, and 0:100%). The relative AFI [(%, number of pellets recovered/number of pellets delivered) × 100] was determined by counting the total number of feed pellets and/or FGP at time of feeding and one hour after feed delivery. In this case, only the pellets recovered from each feeding tray was counted. The distinction between the feed (dark brown) and FGP (light brown) was done visually by their color.

Chemical composition

The chemical composition was determined for the feed, grain pellets (non-fermented and fermented), harvested shrimp (whole animal including the carapace, 15 animals per dietary treatment), and natural food (five samples per dietary treatment). For the purpose of this work, the algal material, feed remains and detritus attached to the interior walls above the water surface of rearing tanks is collectively called natural food (Façanha et al. 2016). After water from the tanks were drained, this organic material was scrapped from each tank and washed with distilled water to remove excess salt for proximate composition.

All samples (feed, grain pellets, shrimp, and natural food) were first stored in plastic containers after collection, kept frozen at − 80 °C in an ultra-freezer for 24 h before freeze-drying. Analyses followed standard methods (AOAC 2005). DM was determined in a convection oven for 24 h at 105 °C; CP was analyzed by the Kjeldahl method of nitrogen estimation (AOAC 2005); ash content was determined by burning samples in a muffle at 600 °C for 2 h (AOAC 942.05); and crude fiber by enzymatic–gravimetric determination (AOAC 992.16). The AA composition was determined for the feed and grain pellets (non-fermented and fermented) using high performance liquid chromatography (Hagen et al. 1993; White et al. 1986).

Digestive enzyme activity

Total protease, lipase, and amylase activity was determined in the shrimp hepatopancreas (total of 100 hepatopancreas = 4 shrimp tank^{-1} × 5 treatments × 5 replicate tanks) collected at harvest. After capture, shrimp were sacrificed; the hepatopancreas were dissected and immediately homogenized with ultrapure water (dilution 1:4) at 10,000 g for 25 min under 4 °C. The supernatant was removed, and the resulting aliquots stored under − 80 °C until analysis. Measurement of enzyme activity was performed in triplicate with a spectrophotometer reader (Thermo Scientific™ GENESYS 10S UV–Vis, Waltham, USA).

Total protease activity was determined by azocasein-hydrolysis following a modified method described by Diógenes et al. (2018). A reaction mixture containing azocasein at 1% (w/v), buffer (50 mM Tris–HCl, pH 7.6), and supernatant from the homogenates was incubated for 1 h at 37 °C. The reaction was terminated by adding trichloroacetic acid (TCA) solution (20%; w/v) to the enzyme–substrate mixture. Subsequently, the reaction mixture was centrifuged at 10,000 g at 25 °C for 10 min and the supernatant was mixed with 2 N NaOH to interrupt the reaction and the absorbance measured at 420 nm against blanks. A saline solution at 9% was used as a control blank. Lipase activity was determined using a commercial kit (ref. 304, Gold Analisa Diagnóstica Ltda., Belo Horizonte, Brazil) determined by the resulting reaction of thioalcohol with 5,5-dithio-bis-2-nitrobenzoic acid followed at 412 nm. α-amylase activity was determined with a commercial kit (ref. 311, Gold Analisa Diagnóstica Ltda., Belo Horizonte, Brazil). Following the hydrolysis of soluble starch by amylase, a solution of potassium iodate (16.7 mmol l^{-1}), potassium iodide (271 mmol l^{-1}), and hydrochloric acid (112 mmol l^{-1}) was added and quantified at 660 nm. One unit (U) of enzyme activity was defined as µmol of product generated per minute under the measurement conditions described above and expressed per specific activity). Protein concentration was determined using Bradford's method (Bradford 1976), with bovine serum albumin solution as standard.

Statistical analyses

Statistical analyzes were performed with the Statistical Package for Social Sciences, package 23 (IBM® SPSS® Statistics, Chicago, Illinois, USA). The effect of the different proportions of feed and FGP on water quality, shrimp growth performance, and their digestive enzyme activity were analyzed using One-way ANOVA. When significant differences were detected, two-by-two comparisons were performed using the Tukey's HSD test. The Student's *t*-test was applied to evaluate the differences in the physical characteristics of the feed versus FGP. This test was also used to determine the differences in the relative AFI when shrimp were fed the feed or FGP alone and a combination of both at different percentages. The significant level of 5% was set in all statistical analyses.

Results

Effect of fermentation on grain pellets

The proximate composition and AA profile varied between the non-fermented and fermented grain pellets (Tables 1 and 2). The fermented pellets showed a higher CP and AA content compared to the non-fermented ones. Fermentation increased the CP and

ether extract content of the grain pellets from 209.2 to 252.7 g kg^{-1} and from 68.9 to 85.1 g kg^{-1}, respectively. Similarly, fermentation resulted in an increase of 21.10 ± 4.45% in the sum of all essential AA (EAA) and 18.56 ± 9.47% of the non-essential AA (NEAA). In comparison, the feed showed higher levels of all EAA and NEAA compared to FGP, including the presence of taurine, which was absent in the later.

The feed and FGP also showed different physical characteristics (Table 3). While moisture content of the FGP was statistically higher than the feed (185.3 ± 3.8 versus 114.0 ± 9.5 g kg^{-1}, respectively), the former displayed a lower physical stability in water (83.55 ± 1.49 versus 87.53 ± 0.42%, respectively, Table 3; $P > 0.05$). Hardness was also lower for the FGP compared to the feed (2.1 ± 1.0 versus 3.5 ± 0.8 kg). There was no difference in pellet diameter of the feed and FGP (mean of 2.47 ± 0.17 mm), but the FGP displayed a longer pellet length than the feed (5.22 ± 1.01 versus 4.37 ± 0.37 mm, respectively).

Water quality

The concentration of TAN, NO_2^-, NO_3^-, Clr-*a*, TSS, FSS, and VSS did not differ statistically between dietary treatments (Table 4, $P > 0.05$). Among the nitrogenous compounds, only NO_3^- increased significantly between the day of shrimp stocking (day 1, 1.60 ± 0.45 mg l^{-1}) and 2 days before harvest (day 75, 3.10 ± 1.59 mg l^{-1}). An increment was also found in the concentration of Clr-*a*, from 0.07 ± 0.02 on day 1 to 0.22 ± 0.07 µg l^{-1} on day 25. No difference was observed in this parameter between day 25 and shrimp harvest ($P > 0.05$). TSS and FSS only differed when concentrations observed on days 1 and 25 were compared with day 75. In this case, TSS levels increased from 416 ± 72 and 449 ± 91 to 577 ± 84 mg l^{-1}, respectively. FSS increased from 347 ± 72 and 349 ± 74 to 432 ± mg l^{-1}, respectively. There was a progressive and significant increase in VSS along the culture period, from 69 ± 23 mg l^{-1} on day 1 to 145 ± 39 mg l^{-1} before harvest. The concentration of SS increased progressively, from an average of 1.1 ml l^{-1} in the first week after shrimp stocking to more than 5.7 ml l^{-1} in the last week of culture (Fig. 1). The 75:25 proportion (feed to FGP) produced more SS in culture water in comparison to all other proportions ($P < 0.05$).

Shrimp growth performance and feeding cost

Final shrimp survival reached an average of 89.3 ± 5.7% and was unaffected by higher levels of FGP in the daily rations (Table 5, $P > 0.05$). However, final shrimp BW, weekly growth, gained yield, and FCR were all significantly affected by the reduction of feed inputs and increase of FGP, starting at the 50:50 proportion. Shrimp final BW decreased linearly with higher inputs of FGP, from a high of 12.68 ± 1.48 (100:0) and 11.71 ± 0.67 g (75:25) to a low of 5.23 ± 0.40 g

Table 3 Physical characteristics of the feed and fermented grain pellets (FGP). The mean values (± standard deviation) represent 30 measurements for each parameter, except for the moisture content ($n = 5$) and water stability ($n = 10$). Different letters in the same column are statistically different ($P < 0.05$) according to Student's *t*-test

Diets	Moisture (g kg^{-1})	Water stability (%)	Hardness (kg)	Pellet diameter (mm)	Pellet length (mm)
Feed	114.0 ± 9.5a	87.53 ± 0.42a	3.5 ± 0.8a	2.48 ± 0.18a	4.37 ± 0.37a
FGP	185.3 ± 3.8b	83.55 ± 1.49b	2.1 ± 1.0b	2.46 ± 0.16a	5.22 ± 1.01b
Mean	-	-	-	2.47 ± 0.17	-

(0:100). Weekly growth was also depressed in a similar pattern. A higher percentage of FGP relative to the feed resulted in slower growth rates ($P < 0.05$). However, no statistical differences were found in weekly shrimp growth between animals fed under 100:0 (1.06 ± 0.14 g) and 75:25 (0.97 ± 0.06 g) feed and FGP proportion. Gained yield was also significantly affected by feed replacement, despite higher inputs of FGP. Feed replacement at 25% (75:25) caused no loss in yield (1,290 ± 87 g m^{-2}) compared to no feed replacement at all (100:0, 1,365 ± 148 g m^{-2}). However, yields decreased progressively starting at 50:50 ($P < 0.05$).

The AFI (g of feed ingested per stocked shrimp) also reduced in response to lower inputs of the feed. The highest AFI was found when shrimp were kept on feed alone (100:0, 15.14 ± 0.69 g) compared to the treatment with only FGP (0:100, 0.66 ± < 0.001 g). The FCR increased significantly with the inputs of both feed and FGP. The lower the proportion of feed to FGP, the higher was the FCR. Under the 100:0 treatment, FCR reached 1.32 ± 0.11 compared to 3.78 ± 0.61 when shrimp were fed 0:100 ($P < 0.05$). There was no statistical difference between the FCR achieved between treatments 100:0 and 100:75.

The highest cost of feeding was achieved when only FGP was delivered (0:100) at 1.29 ± 0.21 USD kg^{-1}. The feeding costs of all other treatments were not statistically different ($P > 0.05$) and varied from 0.87 ± 0.04 (75:25) to 1.04 ± 0.11 USD kg^{-1}.

AFI of feed versus FGP

Assessment of the relative AFI indicated that shrimp consumed FGP in the presence or absence of feed (Fig. 2). However, AFI responded to the proportion of feed to FGP. As feed was restricted and higher amounts of FGP delivered (75:25), there was a statistically higher AFI for the later in comparison to the feed ($P < 0.05$). The opposite was observed when shrimp were fed more feed in comparison to FGP (25:75). Under the 50:50 proportion, there was a statistically higher relative AFI for FGP.

Digestive enzyme activity and proximate composition of shrimp and natural food

Shrimp hepatopancreas showed increased levels of protease, lipase, and amylase when animals were fed the 0:100 feed to FGP proportion (Fig. 3, $P < 0.05$). No other statistical

Table 4 Water quality parameters during the intensive culture of juvenile *L. vannamei* fed on different proportions of a practical feed and fermented grain pellets. Different letters on the same line indicate a statistically significant difference according to the Tukey's HSD test at the significance level of $\alpha = 0.05$

Water quality parameter[1]	Days of Rearing			*P* ANOVA	Mean ± SD
	1	25	75		
TAN (mg l^{-1})	0.03 ± 0.02a	-	0.14 ± 0.07a	0.027	0.09 ± 0.08
NO_2^- (mg l^{-1})	9.00 ± 2.22a	-	4.00 ± 1.61a	0.424	7.00 ± 2.93
NO_3^- (mg l^{-1})	1.60 ± 0.45a	-	3.10 ± 1.59b	< 0.0001	-
Clr-*a* (µg l^{-1})	0.07 ± 0.02a	0.22 ± 0.07b	0.21 ± 0.05b	< 0.0001	-
TSS (mg l^{-1})	416 ± 72a	449 ± 91a	577 ± 84b	< 0.0001	-
FSS (mg l^{-1})	347 ± 72a	349 ± 74a	432 ± 82b	< 0.0001	-
VSS (mg l^{-1})	69 ± 23a	104 ± 52b	145 ± 39c	< 0.0001	-

[1] *TAN*, total ammonia nitrogen; NO_2^-, nitrite; NO_3^-, nitrate; *Clr-a*, chlorophyll *a*; *TSS*, total suspended solids; *FSS*, fixed suspended solids; *VSS*, volatile suspended solids

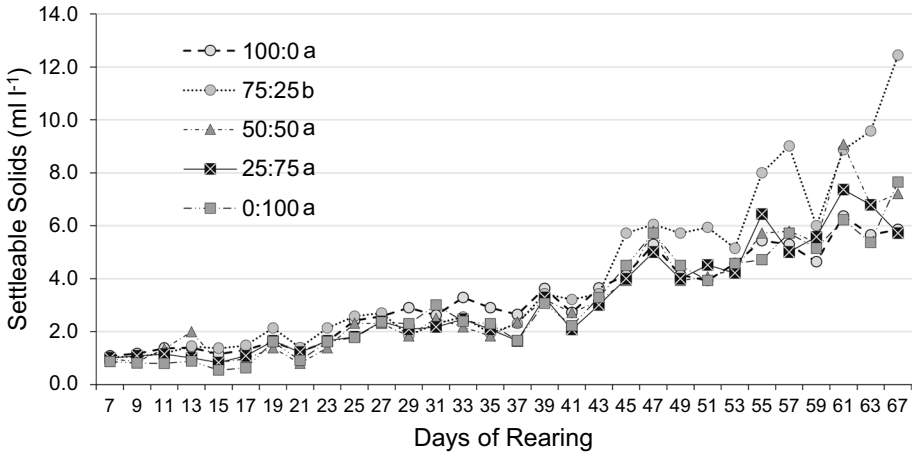


Fig. 1 Dynamics of total settleable solids (TSS, ml l^{-1}) in culture water during the rearing of *L. vannamei* in a biofloc-based system. Shrimp were fed a practical feed (100:0) or fermented grain pellets (0:100) alone and a combination of both at different percentages (50:50, 75:25, and 25:75). Different letters indicate statistically significant difference according to the Tukey's HSD test at $\alpha = 0.05$. Each data point represents the mean of five rearing tanks

difference was observed among other proportions. However, there was an increment in amylase activity when shrimp were fed 50:50 and 75:25 which were comparable to 0:100 proportion ($P > 0.05$).

The CP and ether extract content of shrimp whole body at harvest reduced from 706.9 and 39.2 g kg^{-1} to 680.6 and 35.0 g kg^{-1} under the 100:0 and 0:100 proportions, respectively (Table 6). However, such a trend was not observed for the natural food which showed minimum and maximum values for CP of 218.9 and 227.9 g kg^{-1} and a mean of 1.0 g kg^{-1} for ether extract respectively. Crude fiber and ash levels were less consistent in shrimp and natural food and did not seem to respond to the different feed to FGP proportions. For crude fiber, minimum and maximum levels reached 27.8 and 40.0 g kg^{-1} in natural food and 38.8 and 52.5 g kg^{-1} in shrimp. High ash levels were observed in natural food with an average of 469.5 ± 24.1 g kg^{-1}. Ash content increased in the shrimp's whole body from an average of 113.6 ± 0.70 g kg^{-1} in the 100:0 through 25:75 proportions to 128.5 g kg^{-1} when FGP was delivered alone (100:0).

Discussion

Results from the present study indicated that a feed reduction of 25% compensated by an equivalent amount of FGP as part of the daily ration led to no detriment in shrimp growth performance in a biofloc-based culture system. The 75:25 proportion of feed to FGP was able to partially spare feed inputs leading to 0.08 USD kg^{-1} savings in feeding costs (from a mean of 0.95 to to 0.87 USD kg^{-1} under the 75:25 proportion, feed manufacturing costs were not included). It seems this combination of feed and FGP provided enough dietary nutrients to maximize shrimp growth performance. On a dry-matter basis, a 25% feed

Table 5 Growth performance of juvenile shrimp *L. vannamei* and cost of feeding with a practical feed and/or fermented grain pellets. Different letters on the same line indicate statistically significant difference according to the Tukey's HSD test at the significance level of $\alpha = 0.05$

Performance parameters	Percentage of feed to fermented grain pellet (%)					*P* ANOVA	Mean ± SD
	100:0	75:25	50:50	25:75	0:100		
Initial body weight (g)	1.16 ± 0.12a	1.15 ± 0.12a	1.15 ± 0.12a	1.15 ± 0.12a	1.15 ± 0.12a	0.360	1.15 ± 0.12
Final body weight (g)	12.68 ± 1.48a	11.71 ± 0.67a	9.93 ± 0.62b	7.37 ± 0.50c	5.23 ± 0.40d	< 0.0001	-
Final survival (%)	90.2 ± 5.7a	92.5 ± 4.5a	90.7 ± 3.8a	89.3 ± 6.0a	84.9 ± 6.1a	0.174	89.3 ± 5.7
Growth (g week^{-1})	1.06 ± 0.14a	0.97 ± 0.06a	0.81 ± 0.06b	0.57 ± 0.05c	0.38 ± 0.04d	< 0.0001	-
Gained yield (g m^{-2})	1,365 ± 148a	1,290 ± 87a	1,050 ± 110b	723 ± 68c	440 ± 74d	< 0.0001	-
Feed delivered (g tank^{-1})	1,824 ± 84a	1,352 ± 47b	879 ± 16c	447 ± 9d	80 ± < 0.1e	< 0.0001	-
FGP delivered (g tank^{-1})	-a	390 ± 14b	734 ± 15c	1,012 ± 25d	1,185 ± 42e	< 0.0001	-
AFI (g shrimp^{-1})[1]	15.14 ± 0.69a	11.22 ± 0.39b	7.29 ± 1.13c	3.67 ± 0.09d	0.66 ± 0.00e	< 0.0001	-
FCR[2]	1.32 ± 0.11a	1.63 ± 0.07ab	1.90 ± 0.20b	2.56 ± 0.28c	3.78 ± 0.61d	< 0.0001	-
Feeding costs (USD kg^{-1})	0.95 ± 0.08 a	0.87 ± 0.04 a	0.89 ± 0.09 a	1.04 ± 0.11 a	1.29 ± 0.21 b	< 0.0001	-

[1] *AFI*, apparent feed intake

[2] *FCR*, feed conversion ratio accounting for the total inputs of feed and fermented grain pellets

[3] Cost of feeding shrimp with feed and/or FGP per kg of shrimp produced. Feed manufacturing and other costs not included

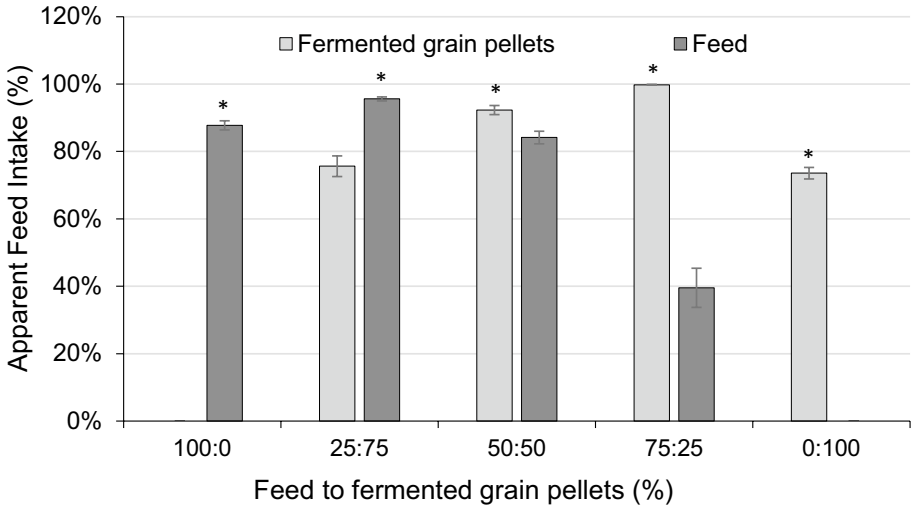


Fig. 2 Apparent shrimp feed intake (%)fed under different proportions of feed to fermented grain pellets in feeding trays. Asterisks indicate statistically significant differences according to the Student's *t*-test ($P < 0.05$). Each column represents the mean (± standard error) of 50 observations of feed intake (5 tanks × 5 days of observations × 2 feedings per day)

replacement for FGP reduced the daily dietary protein inputs from 396.8 to 297.6 g kg^{-1} (on a DM basis). The addition of equivalent amounts of FGP raised the daily inputs to 360.8 g kg^{-1} CP. This should have been sufficient to meet shrimp daily requirements for CP, and essential AAs (EAAs). However, a feed replacement starting at 50% with equivalent increments of FGP caused a slower shrimp growth, reduced BW, increased FCR, and lower yield at harvest. This clearly indicates that under high-density conditions, half or more of the daily meal in the form of FGP is not adequate and will not supply adequate amounts of nutrients to sustain a maximum shrimp growth performance in a biofloc system. The most significant loss was observed in gained yield which reduced from 26 to 171% when feed was restricted beyond 25%.

The effects of moderate and severe feed deprivation on shrimp growth performance have been investigated previously. Nunes et al. (2006) reported that restricting the period to which shrimp is exposed to feed on a daily basis (from 6 to 5, 4, 3, and 2 h day^{-1}) negatively impacts feed intake, leading to a reduced final BW, and yield. Additionally, Nunes et al. (2006) reported that when fed to satiation, feed rations could be reduced by 50% without detrimental effects to shrimp performance. However, in this case, authors stocked shrimp of 9.1 ± 1.44 g at 36 animals m^{-2} and raised them in clear water for only 28 days under feed restriction. Although these conditions differ from the present study, it is possible that the optimal proportion of feed to FGP in biofloc systems vary in response to shrimp stocking density, shrimp BW, and days of exposure to feed replacement. Arnold et al. (2016) also found growth benefits of a 20% restricted ration for juvenile *Penaeus monodon*. They fed shrimp for 6 weeks with a high CP-diet with and without the addition of a microbial biomass-based ingredient included at 100 g kg^{-1} (as-fed basis).

The application of grain pellets in culture water has been reported by Burford et al. (2003) in high-intensity, zero water exchange shrimp ponds to promote bacterial growth. Their grain pellets contained a similar ingredient composition (SBM, wheat grain, and corn), CP content (219 g kg^{-1}, as-is basis), and water stability (84% versus 83.55% for FGP) as the ones used the current study. However, unlike the FGP, pellets used by Burford et al. (2003) were not fermented. Application also gradually decreased in relation to feed inputs as the culture period progressed and no feed replacement was carried out. Such conditions may not have favored a significant dietary contribution of grain pellets to shrimp growth, but their direct consumption by shrimp may not be discarded. We have found that shrimp ingested FGP regardless of the presence or the proportion of feed in feeding trays. Penaeid shrimp are known to be opportunistic feeders which is consistent with our findings.

In the present study, feed intake appeared to have been driven more by food availability in detriment of palatability and/or physical characteristics of pellets. The higher the proportion of FGP in feeding trays in relation to feed, the higher AFI of the former and vice-versa. Fermentation undoubtedly enhanced the nutrient value of grain pellets, potentially improving their digestibility and reducing the antinutritional factors (ANFs). Fermentation is considered an inexpensive method which has been applied by the feed industry to improve the quality of plant ingredients and allow higher replacement levels of fish meal in shrimp feed formulas (Shiu et al. 2015; Chiu et al. 2016; Sharawy et al. 2016; Sun et al. 2016; Jannathulla et al. 2018). Previous work has shown that a 48-h solid-state fermentation (SFF) with yeast (*S. cerevisiae*) can increase the CP, lipid, and total AA content of SBM by 13.6, 11.6, and 16.3%, respectively (Sharawy et al. 2016). In their work, the total AA levels alone were increased from 385.4 to 448.1 g kg^{-1}. Authors also reported a significant reduction in the concentration of ANFs, such as phytic acid and trypsin inhibitors. Similarly, Sun et al. (2016) applied SSF to cottonseed meal (CSM) and reported that phytic acid and free gossypol were reduced from 7.8 to 0.9 g kg^{-1} and from 879.2 to 356.3 mg kg^{-1} after fermentation, respectively. In the present work, since the nutrient value of FGP in terms of CP, lipids, and total EAA was relevant (252.7, 85.1, and 112.5 g kg^{-1}, respectively), some level of dietary contribution to shrimp growth was inevitable.

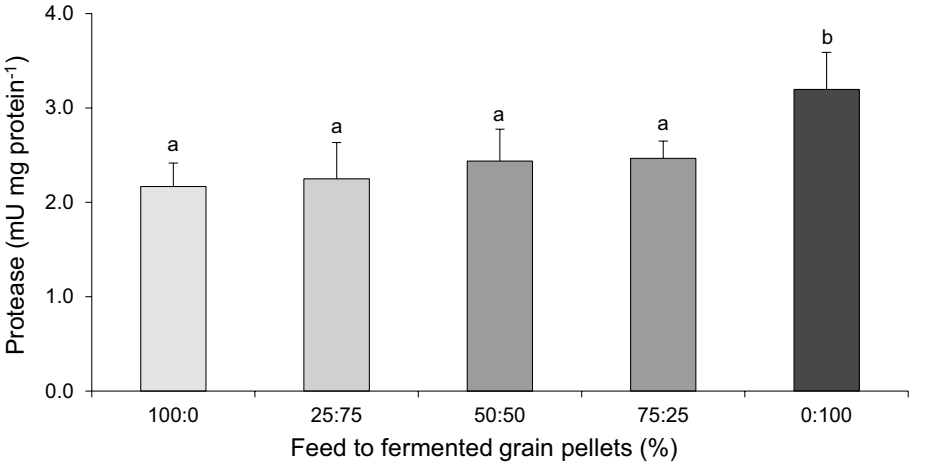
Under the biofloc system, the daily application of FGP at 25% of the total ration did not significantly reduce feeding costs, but provided 0.08 USD kg^{-1} savings compared to the 100:0 treatment. Although further work is needed to precisely determine the economic advantages of FGP application, such feed sparing effect alone may justify the adoption of FGP in biofloc-based systems. However, the daily provision of FGP had no significant effect on nitrogenous compounds. Although tanks operated under minimum water exchange, concentration of TAN, NO_2^-, and NO_3^- was low and did not appear to be affected by the addition FGP or by the proportions of feed to FGP. We have recorded a progressive accumulation of organic matter during culture as verified by the increasing levels of TSS and SS. In the current work, FGP was not applied with the sole intention of controlling the C:N ratio, but also to serve as a potential food source to juvenile whiteleg shrimp. It has been found that the addition of a grain pellets in combination with molasses is able to promote the growth of heterotrophic bacteria, but limits nitrification (Burford et al. 2003). In the present study, despite the development of phytoplankton in the outdoor tanks shown by the increased levels of Chl-*a* during culture, their levels were very low (0.07–0.21 µg l^{-1}) compared to semi-intensive shrimp ponds (levels between 5.1–31.3 µg l^{-1}; Guerrero-Galván et al. 1998). This suggests the system was

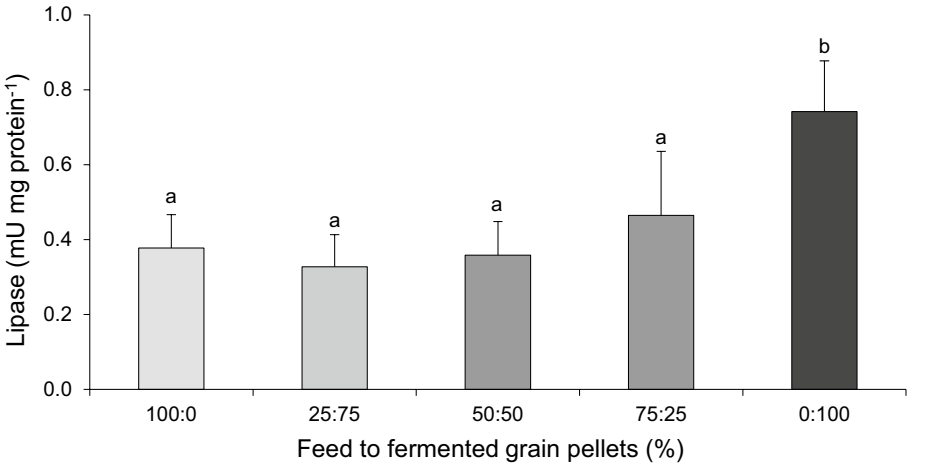
Fig. 3 Digestive enzyme activity of protease, lipase, and amylase in the hepatopancreas of *L. vannamei* fed a practical feed (100:0) or fermented grain pellets (0:100) alone and a combination of both at different proportions (50:50, 75:25, and 25:75). Different letters indicate a statistically significant differences according to the Tukey's HSD test at $\alpha = 0.05$

biofloc-dominated with maximum SS recorded at 12 ml l^{-1} (maximum recommended levels of 10–12 ml l^{-1}, Samocha et al. 2017).

The combination of 75:25 feed to FGP positively contributed to a higher concentration of SS in water compared to other proportions. This factor may have favored an increased accumulation of natural food attached to the interior walls of rearing tanks which could have also acted as a nutrient source to farmed shrimp, particularly under restricted feed rations. During the study, shrimp were often found near the water surface grazing on natural food around tank walls. However, the different feed to FGP proportions had no effect on the proximate composition of natural food. We observed a trend towards higher levels of CP and lower levels of ash in shrimp whole body at harvest with more restricted feed ratios. However, in general, proximate composition of both natural food and shrimp whole body was not substantially altered with the different proportions of feed to FGP. Similar results were found by Façanha et al. (2016) when investigating the effect of graded levels of dietary Met (4.8, 6.2, 7.2, 8.1, or 9.4 g kg^{-1} of the diet, DM basis) and shrimp stocking density (50, 75, and 100 shrimp m^{-2}) over the growth performance of juvenile *L. vannamei*. Authors reported that under a green-water culture system, natural food composition (CP, Met, Met + Cys, and total EAAs) collected from the inner walls of outdoor tanks did not seem to respond to the dietary Met content and shrimp stocking density. They found a CP content of natural food ranging from 215 and 247 g kg^{-1} (DM basis) consistent with our findings (218.9 and 227.9 g kg^{-1}, DM basis).

There was a significant increase in the activity of protease, lipase, and amylase in shrimp hepatopancreas when animals were fed only under the provision of FGP (0:100). The higher availability of FGP containing live *S. cerevisiae* may have favored the intake of food enriched with beneficial bacteria with the ability to increase digestive enzyme activity (Sumon et al. 2018; Rachmawati et al. 2019; Wang et al. 2020). Sumon et al. (2018) also found a significant enhancement in digestive protease and amylase activity when the giant Malaysian prawn, *Macrobrachium rosenbergii*, were fed for 60 days with the probiotic bacteria *Clostridium butyricum*. Authors associated this finding with the synbiotic behavior of probiotics in the animal's digestive system. Rachmawati et al. (2019) reported a significant improvement in protein digestibility when fingerlings of the Java barb (*Barbonymus gonionotus*) were fed a diet enriched with 3 g kg^{-1} of *S. cerevisiae*. According to authors, this was likely driven by a higher digestive enzyme activity. Wang et al. (2020) reported that a mixed probiotic bacteria induced the digestive activity of protease, lipase, amylase, and cellulase in the gut of *P. monodon*. In the current study, higher levels of carbohydrate in the 0:100 proportion compared to the 100:0 and 75:25 could also have led to an increased amylase activity. Molina-Poveda and Morales (2004) also reported a similar finding when juvenile *L. vannamei* were fed diets with higher replacement levels of marine protein (shrimp head meal, fish meal, squid meal) for a mixture of wheat gluten meal and barley-based fermented grains (BFG). They found that amylase activity in shrimp hepatopancreas increased seven to nine times under 33 to 100% substitution levels compared to shrimp fed a control diet without any replacement of marine protein. Authors associated a higher amylase activity with an increased content of soluble carbohydrate in BFG.


Protease (mU mg protein-1)
4.0
3.0
2.0
1.0
0.0
a
a
a
a
b
100:0
25:75
50:50
75:25
0:100
Feed to fermented grain pellets (%)


Lipase (mU mg protein-1)
1.0
0.8
0.6
0.4
0.2
0.0
a
a
a
a
b
100:0
25:75
50:50
75:25
0:100
Feed to fermented grain pellets (%)

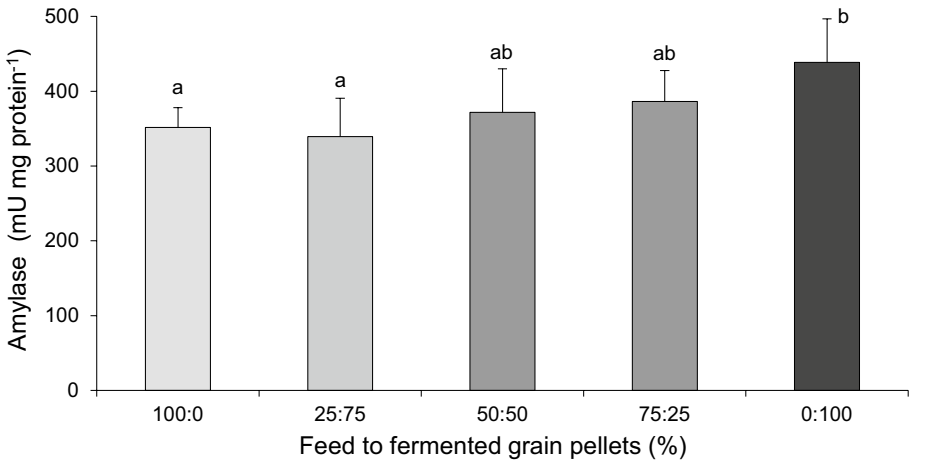

Amylase (mU mg protein-1)
500
400
300
200
100
0
a
a
ab
ab
b
100:0
25:75
50:50
75:25
0:100
Feed to fermented grain pellets (%)

Table 6 Proximate composition (g kg^{-1}, dry matter basis) of natural food (NF) and whole shrimp (shell-on, head-on) after 77 days of rearing. Shrimp were fed under different proportions of feed and fermented grain pellets. Each value for NF and shrimp represents a total of five and 15 samples, respectively

Composition (g kg^{-1}, dry matter)	Sample	Proportion of feed to fermented grain pellet (%)				
		100:0	75:25	50:50	25:75	0:100
Crude protein	NF	218.9	222.0	221.4	219.4	227.9
	Shrimp	706.9	705.8	682.3	686.2	680.6
Ether extract	NF	1.0	1.0	1.0	1.0	1.0
	Shrimp	39.2	51.2	48.8	37.5	35.0
Crude fiber	NF	36.7	33.1	40.0	27.8	30.3
	Shrimp	47.9	38.8	52.5	52.0	47.9
Ash	NF	505.6	481.8	455.2	458.8	446.2
	Shrimp	114.4	113.7	113.4	112.8	128.5

Conclusions

This study has demonstrated that fermentation significantly enhances the nutrient value of grain pellets, increasing CP and total AA content. A continuous provision of FGP as part of the daily ration in a biofloc-based rearing system provides a supplemental dietary contribution to shrimp growth, either through its direct ingestion and (or) increased availability of natural food. We have found that a feed replacement of up to 25% could be adopted as long as equivalent amounts of FGP are added as part of the daily ration for juvenile *L. vannamei*. Under such condition, shrimp were able to achieve a similar survival, final BW, growth, and yield, compared to animals raised under no feed replacement. Thus, the use of FGP into the biofloc culture of the whiteleg shrimp can partially spare the use of complete feeds, act as a source of carbohydrates to control the C:N ratio in water, and (or) as a synbiotic to deliver probiotics and other functional feed additives to farmed shrimp. The success of such replacement may change according to different culture conditions, and the abundance and nutrient profile of natural productivity.

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Author contribution Jordana Sampaio Leite: conceptualization, data curation, formal analysis, roles/writing of original draft. Alexandre Firmino Diógenes: chemical analyses of enzymes, writing—review and editing. Alberto Jorge Pinto Nunes: conceptualization, data curation, formal analysis, funding acquisition, roles/writing—original draft investigation, methodology, project administration, project administration, supervision.

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Data availability The data that support the findings of this study are available from the corresponding author upon reasonable request.

Declarations

Ethics approval All rearing procedures were performed in compliance with relevant local laws and institutional guidelines, including those related to animal welfare. All applicable international, national, and/or institutional guidelines for the care and use of animals were followed by the authors.

Consent to participate Not applicable.

Conflict of interest Phileo Lesaffre Animal Care donated the live yeast used in this study. This did not alter, influence, or affect the development of the study, including study design, sampling, analysis of results, interpretation of data, or decision for publication. All other authors declare no competing interests.

References

Abakari G, Luo G, Kombat EO, Alhassan EH (2021) Supplemental carbon sources applied in biofloc technology aquaculture systems: types, effects and future research. Rev Aquac 13:1193–1222. https://doi.org/10.1111/raq.12520

AOAC (2005) Official Method of Analysis, 18th edn. Association of Officiating Analytical Chemists, Washington DC

APHA (2012) Standard methods for the examination of water and wastewater, 22nd edn. American Public Health Association, Washington DC

Arnold S, Smullen R, Briggs M et al (2016) The combined effect of feed frequency and ration size of diets with and without microbial biomass on the growth and feed conversion of juvenile Penaeus monodon. Aquac Nutr 22:1340–1347. https://doi.org/10.1111/anu.12338

Bradford M (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein using the principle of protein dye-binding. Anal Biochem 72:248–254. https://doi.org/10.1006/abio.1976.9999

Burford MA, Thompson PJ, McIntosh RP et al (2003) Nutrient and microbial dynamics in high-intensity, zero-exchange shrimp ponds in Belize. Aquaculture 219:393–411. https://doi.org/10.1016/S0044-8486(02)00575-6

Burford MA, Preston NP, Minh TH et al (2004a) Dominant sources of dietary carbon and nitrogen for shrimp reared in extensive rice-shrimp ponds. Aquac Res 35:194–203. https://doi.org/10.1111/j.1365-2109.2004.01012.x

Burford MA, Thompson PJ, McIntosh RP et al (2004b) The contribution of flocculated material to shrimp (Litopenaeus vannamei) nutrition in a high-intensity, zero-exchange system. Aquaculture 232:525–537. https://doi.org/10.1016/S0044-8486(03)00541-6

Burford MA, Hiep LH, Van Sang N et al (2020) Does natural feed supply the nutritional needs of shrimp in extensive rice-shrimp ponds? – A stable isotope tracer approach. Aquaculture 529:735717. https://doi.org/10.1016/j.aquaculture.2020.735717

Castro LF, Pinto RCC, Nunes AJP (2021) Nutrient value and contribution of microbial floc to the growth performance of juvenile shrimp, Litopenaeus vannamei, fed fatty acid and amino acid-restrained diets under a zero-water exchange intensive system. Aquaculture 531:735789. https://doi.org/10.1016/j.aquaculture.2020.735789

Chiu S-T, Wong S-L, Shiu Y-L et al (2016) Using a fermented mixture of soybean meal and earthworm meal to replace fish meal in the diet of white shrimp, Penaeus vannamei (Boone). Aquac Res 47:3489–3500. https://doi.org/10.1111/are.12799

Correia ES, Wilkenfeld JS, Morris TC et al (2014) Intensive nursery production of the Pacific white shrimp Litopenaeus vannamei using two commercial feeds with high and low protein content in a biofloc-dominated system. Aquac Eng 59:48–54. https://doi.org/10.1016/j.aquaeng.2014.02.002

Diógenes AF, Castro C, Miranda AC et al (2018) Dietary replacement of fishmeal by corn distillers dried grains with solubles (DDGS) in diets for turbot (Scophthalmus maximus, Linneaus, 1758) juveniles. Aquaculture 492:113–122

Engle CR, McNevin A, Racine P et al (2017) Economics of sustainable intensification of aquaculture: evidence from shrimp farms in Vietnam and Thailand. J World Aquac Soc 48:227–239. https://doi.org/10.1111/jwas.12423

Ewan RC (1989) Predicting the energy utilization of diets and feed ingredients by pigs. In: van der Honing Y, WH Close (eds) Energy metabolism. European Association of Animal Production Bulletin No. 43, Pudoc, Wageningen, p 271–274

Façanha FN, Oliveira-Neto AR, Figueiredo-Silva C, Nunes AJP (2016) Effect of shrimp stocking density and graded levels of dietary methionine over the growth performance of Litopenaeus vannamei reared in a green-water system. Aquaculture 463.https://doi.org/10.1016/j.aquaculture.2016.05.024

Façanha FN, Sabry-Neto H, Figueiredo-Silva C et al (2018) Minimum water exchange spares the requirement for dietary methionine for juvenile Litopenaeus vannamei reared under intensive outdoor conditions. Aquac Res 49.https://doi.org/10.1111/are.13624

Guerrero-Galván SR, Páez-Osuna F, Ruiz-Fernández AC, Espinoza-Angulo R (1998) Seasonal variation in the water quality and chlorophyll a of semi-intensive shrimp ponds in a subtropical environment. Hydrobiologia 391:33–45. https://doi.org/10.1023/A:1003590625379

Huang Q, Olenin S, Li L et al (2020) Meiobenthos as food for farmed shrimps in the earthen ponds: Implications for sustainable feeding. Aquaculture 521.https://doi.org/10.1016/j.aquaculture.2020.735094

Hussain AS, Mohammad DA, Sallam WS et al (2021) Effects of culturing the Pacific white shrimp Penaeus vannamei in "biofloc" vs "synbiotic" systems on the growth and immune system. Aquaculture 542:736905. https://doi.org/10.1016/j.aquaculture.2021.736905

Hagen SR, Augustin J, Grings E, Tassinari P (1993) Precolumn phenylisothiocyanate derivatization and liquid chromatography of free amino acids in biological samples. Food Chem 46:319–323. https://doi.org/10.1016/0308-8146(93)90127-2

Huang HH, Liao HM, Lei YJ, Yang PH (2022) Effects of different carbon sources on growth performance of Litopenaeus vannamei and water quality in the biofloc system in low salinity. Aquaculture 546:737239. https://doi.org/10.1016/j.aquaculture.2021.737239

Huynh TG, Cheng AC, Chi CC et al (2018) A synbiotic improves the immunity of white shrimp, Litopenaeus vannamei: Metabolomic analysis reveal compelling evidence. Fish Shellfish Immunol 79:284–293. https://doi.org/10.1016/j.fsi.2018.05.031

Jannathulla R, Dayal JS, Vasanthakumar D et al (2018) Effect of fungal fermentation on apparent digestibility coefficient for dry matter, crude protein and amino acids of various plant protein sources in Penaeus vannamei. Aquac Nutr 24:1318–1329. https://doi.org/10.1111/anu.12669

Leite JS, Melo CSB, Nunes AJP (2020) Utilization of rice byproducts as carbon sources in high-density culture of the Pacific white shrimp, Litopenaeus vannamei. Rev Bras Zootec 49. https://doi.org/10.37496/rbz4920190039

Molina-Poveda C, Morales ME (2004) Use of a mixture of barley-based fermented grains and wheat gluten as an alternative protein source in practical diets for *Litopenaeus vannamei* (Boone). Aquac Res 35:1158–1165. https://doi.org/10.1111/j.1365-2109.2004.01140.x

Nguyen TAT, Nguyen KAT, Jolly C (2019) Is super-intensification the solution to shrimp production and export sustainability? Sustainability 11:1–22. https://doi.org/10.3390/su11195277

NRC (2011) Nutrient requirements of fish and shrimp. National Research Council, The National Academies Press, Washington, D.C. https://doi.org/10.17226/13039

Nunes AJP, Parsons GJ (2000a) Effects of the Southern brown shrimp, Penaeus subtilis, predation and artificial feeding on the population dynamics of benthic polychaetes in tropical pond enclosures. Aquaculture 183:125–147. https://doi.org/10.1016/S0044-8486(99)00278-1

Nunes AJP, Parsons GJ (2000b) Size-related feeding and gastric evacuation measurements for the Southern brown shrimp Penaeus subtilis. Aquaculture 187:133–151. https://doi.org/10.1016/S0044-8486(99)00386-5

Nunes AJP, Sabry-Neto H, da Silva FHP et al (2019a) Multiple feedings enhance the growth performance and feed efficiency of juvenile Litopenaeus vannamei when fed a low-fish meal amino acid-supplemented diet. Aquac Int 27.https://doi.org/10.1007/s10499-018-0330-7

Nunes AJP, Sabry-Neto H, Masagounder K (2019b) Crude protein in low-fish meal diets for juvenile Litopenaeus vannamei can be reduced through a well-balanced supplementation of essential amino acids. J World Aquac Soc 50:1093–1107. https://doi.org/10.1111/jwas.12605

Nunes AJP, Sá MVC, Carvalho EA, Neto HS (2006) Growth performance of the white shrimp Litopenaeus vannamei reared under time- and rate-restriction feeding regimes in a controlled culture system. Aquaculture 253:646–652. https://doi.org/10.1016/j.aquaculture.2005.09.023

Nunes AJP, Sá MVC, Sabry-Neto H (2011) Growth performance of the white shrimp, Litopenaeus vannamei, fed on practical diets with increasing levels of the Antarctic krill meal, Euphausia superba, reared in clear-versus green-water culture tanks. Aquacult Nutr 17:511–520. https://doi.org/10.1111/j.1365-2095.2010.00791.x

Obaldo LG, Divakaran S, Tacon AG (2002) Method for determining the physical stability of shrimp feeds in water. Aquac Res 33:369–377. https://doi.org/10.1046/j.1365-2109.2002.00681.x

Pimentel OALF, de Oliveira VQ, Oliveira CR, do R, et al (2022) Assessment of different ionic adjustment strategies in low-salinity water on the growth of Litopenaeus vannamei and microbial community stoichiometry in a synbiotic nursery system. Aquac Res 53:50–62. https://doi.org/10.1111/are.15552

Rachmawati D, Hutabarat J, Samidjan I et al (2019) The effects of saccharomyces cerevisiae-enriched diet on feed usage efficiency, growth performance and survival rate in java barb (barbonymus gonionotus) fingerlings. AACL Bioflux 12:1841–1849

Ramírez NB, Seiffert WQ, Vieira F, do N, et al (2013) Dieta suplementada com prebiótico, probiótico e simbiótico no cultivo de camarões marinhos. Pesqui Agropecu Bras 48:913–919. https://doi.org/10.1590/S0100-204X2013000800015

Reis J, Weldon A, Ito P et al (2021) Automated feeding systems for shrimp: effects of feeding schedules and passive feedback feeding systems. Aquaculture 541.https://doi.org/10.1016/j.aquaculture.2021.736800

Roy D, Hassan K, Boopathy R (2010) Effect of carbon to nitrogen (C:N) ratio on nitrogen removal from shrimp production waste water using sequencing batch reactor. J Ind Microbiol Biotechnol 37:1105–1110. https://doi.org/10.1007/s10295-010-0869-4

Samocha TM, Patnaik S, Speed M et al (2007) Use of molasses as carbon source in limited discharge nursery and grow-out systems for Litopenaeus vannamei. Aquac Eng 36:184–191. https://doi.org/10.1016/j.aquaeng.2006.10.004

Sharawy Z, Goda AMAS, Hassaan MS (2016) Partial or total replacement of fish meal by solid state fermented soybean meal with Saccharomyces cerevisiae in diets for Indian prawn shrimp, Fenneropenaeus indicus, Postlarvae. Anim Feed Sci Technol 212:90–99. https://doi.org/10.1016/j.anifeedsci.2015.12.009

Shiu Y-L, Wong S-L, Guei W-C et al (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. Aquac Res 46:382–394. https://doi.org/10.1111/are.12186

Sumon MS, Ahmmed F, Khushi SS et al (2018) Growth performance, digestive enzyme activity and immune response of Macrobrachium rosenbergii fed with probiotic Clostridium butyricum incorporated diets. J King Saud Univ Sci 30:21–28. https://doi.org/10.1016/j.jksus.2016.11.003

Situmorang ML, Nurwidayanti P, Suantika G, Caruso D (2021) Synbiotic containing Kappaphycus alvarezii, Spirulina sp., and Halomonas alkaliphila improves survival, growth, and vibriosis resistance in whiteleg shrimp (Litopenaeus vannamei) post-larval culture. Aquat Living Resour 34:1–8. https://doi.org/10.1051/alr/2021009

Sun H, Tang J, Yao X et al (2016) Effects of replacement of fish meal with fermented cottonseed meal on growth performance, body composition and haemolymph indexes of Pacific white shrimp, Litopenaeus vannamei Boone, 1931. Aquac Res 47:2623–2632. https://doi.org/10.1111/are.12711

Wang C, Pan L, Zhang K et al (2016) Effects of different carbon sources addition on nutrition composition and extracellular enzymes activity of bioflocs, and digestive enzymes activity and growth performance of Litopenaeus vannamei in zero-exchange culture tanks. Aquac Res 47:3307–3318. https://doi.org/10.1111/are.12784

Wang Y, Al Farraj DA, Vijayaraghavan P et al (2020) Host associated mixed probiotic bacteria induced digestive enzymes in the gut of tiger shrimp Penaeus monodon. Saudi J Biol Sci 27:2479–2484. https://doi.org/10.1016/j.sjbs.2020.07.010

Wasielesky W, Atwood H, Stokes A, Browdy CL (2006) Effect of natural production in a zero exchange suspended microbial floc based super-intensive culture system for white shrimp Litopenaeus vannamei. Aquaculture 258:396–403. https://doi.org/10.1016/j.aquaculture.2006.04.030

Weldon A, Davis DA, Rhodes M et al (2021) Feed management of Litopenaeus vannamei in a high density biofloc system. Aquaculture 544:737074. https://doi.org/10.1016/j.aquaculture.2021.737074

White JA, Hart RJ, Fry JC (1986) An evaluation of the Waters Pico-Tag system for the amino-acid analysis of food materials. J Automat Chem 8:170–177. https://doi.org/10.1155/S1463924686000330

Xu WJ, Morris TC, Samocha TM (2016) Effects of C/N ratio on biofloc development, water quality, and performance of Litopenaeus vannamei juveniles in a biofloc-based, high-density, zero-exchange, outdoor tank system. Aquaculture 453:169–175. https://doi.org/10.1016/j.aquaculture.2015.11.021

Xu W, Xu Y, Su H et al (2021) Production performance, inorganic nitrogen control and bacterial community characteristics in a controlled biofloc-based system for indoor and outdoor super-intensive culture of Litopenaeus vannamei. Aquaculture 531.https://doi.org/10.1016/j.aquaculture.2020.735749

Yabaya A, Akinyanju JA, Jatau ED (2009) Yeast enrichment of soybean cake. World J Dairy Food Sci 4:141–144

Zubaidah A, Yuhana M, Widanarni (2015) Encapsulated synbiotic dietary supplementation at different dosages to prevent Vibriosis in white shrimp, Litopenaeus vannamei. HAYATI J Biosci 22:163–168. https://doi.org/10.1016/j.hjb.2015.10.007