Latin American Journal of Aquatic Research, 54(4): 625-634, 2026
DOI: 10.3856/vol54-issue4-fulltext-3645

Research Article

Dietary supplementation of calcium butyrate modulates hepatopancreatic trypsin activity in Pacific white shrimp *Penaeus vannamei*

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ABSTRACT. The use of feed additives and innovative shrimp diets aims at improving feed quality, leading to animal health and performance gains. Organic acids like calcium butyrate are used as a dietary supplement for marine shrimp. They are effective in promoting shrimp health and growth, and benefit the digestibility and availability of different nutrients. This study evaluated the growth performance and hepatopancreatic digestive enzyme activity of juvenile *Penaeus vannamei* fed a diet supplemented with commercial calcium butyrate (Butifour®CCB) for 27 days. For this purpose, 80 *P. vannamei* juveniles were randomly assigned to two experimental groups, with eight replicates each: control (0.0%) and calcium butyrate treatment (0.2% Butifour®CCB). Additionally, water quality analyses were performed to ensure a suitable environment for the juveniles. Shrimp survival remained above 95% for all treatments, and no significant differences were observed in growth performance, weight gain, specific growth rate, or protein efficiency rate between groups ($P > 0.05$). Similarly, the dietary use of calcium butyrate did not grossly alter the hepatopancreas composition (inc. protein and triglycerides) or the hepatopancreatic activity of amylase, lipase, and chymotrypsin digestive enzymes. Notably, the activity of hepatopancreatic trypsin showed a 30% increase ($P = 0.0281$) in shrimp fed the calcium butyrate diet (0.08 ± 0.01 nmol min^{-1} mg $protein^{-1}$) compared to shrimp fed the control diet (0.05 ± 0.01 nmol min^{-1} mg $protein^{-1}$). Therefore, the inclusion of 0.2% calcium butyrate in *P. vannamei* juvenile diet promotes trypsin activity in the hepatopancreas and may benefit protein digestion and nutrient availability.

Keywords: animal nutrition; organic acids; decapod crustacean; digestive enzyme; midgut gland; aquaculture

Associate Editor: Carlos Alvarez

INTRODUCTION

Among aquaculture sectors, shrimp culture has stood out in several countries worldwide due to high global demand, technological advances in farming systems, and its strong economic returns (Emerenciano et al. 2022). In 2018, Pacific white shrimp (*Penaeus vannamei*) was the most produced worldwide, with 4.9 million tons (FAO 2020). *P. vannamei* has a high growth potential and the ability to advantageously convert controlled diets into weight gain, promoting a good economic return from the activity (Barros-Nogueira et al. 2021). The search for increased productivity is needed to maintain the shrimp industry's attractiveness. Innovative diets and feed supplements aim for high-quality products that meet the species' nutritional requirements. The most studied and used feed additives include probiotics, prebiotics, symbiotics, immune-stimulants, organic acids, nucleotides, and medicinal herbs (Dawood et al. 2018).

Organic acids are non-nutritional feed supplements and an important tool for aquaculture. They are effective feed preservatives and increase the shelf life of aquafeed; as lipophilic acidifiers, organic acids inhibit the growth of microorganisms like mould/fungi and pathogenic bacteria (Lim et al. 2015). Their use as a feed supplement is associated with improved zootechnical development, intestinal health through epithelial proliferation, and pathogen control in production systems (Gomez-Gil et al. 2000, Defoirdt et al. 2011, Vijayaram et al. 2022). Dietary organic acids improve nutrient utilization and mineral availability; the acidic pH in the diet and intestine benefits pepsin activation, thus enhancing nitrogen retention and the bioavailability and absorption of minerals (Ng & Koh 2017, Zhang et al. 2022). They also modulate intestinal microbiota by promoting bacterial richness and diversity and stimulating immune-related genes (He et al. 2017, Pourmozaffar et al. 2017).

Butyric acid is an organic compound belonging to the class of carboxylic acids and consists of a chain of four carbon atoms. Like other short-chain fatty acids, it is produced during the fermentation of dietary fibers by the anaerobic microbiota associated with the epithelium of the digestive tract. It is the main respiratory fuel of intestinal bacteria (Rimoldi et al. 2016). However, when used in animal feed, most butyric acid is derived from chemical synthesis, although a trend toward bioproduction has been observed (Jiang et al. 2018). Structurally, butyric acid has a hydrogen ion of the hydroxyl group (-OH) that is weakly bound and easily substituted; when in solution, this hydrogen ion is lost, and the organic salt binds to molecules such as sodium and calcium, becoming a free salt form of butyric acid, or sodium butyrate or calcium butyrate, respectively (Ahsan et al. 2016, Ng & Koh 2017).

Butyric acid is associated with different benefits for aquaculture. Microencapsulated sodium butyrate dietary supplementation improves common carp's (*Cyprinus carpio*) immunological and intestinal health (Gatlin et al. 2014). Dietary sodium butyrate increased the availability of several amino acids and nucleotide derivatives, positively affecting the growth of sea bream (*Sparus aurata*) (Robles et al. 2013). Coated sodium butyrate, as a feed additive, presents an antimicrobial effect (*Aeromonas hydrophila*, *Streptococcus agalactiae*) and increases fish performance (high yield, biomass gain) and health (increased red blood cells and monocytes; Jesus et al. 2019). In marine shrimp culture, butyrate's effectiveness is mainly characterized by increasing resistance and combating bacterial diseases such as vibriosis (Silva et al. 2016). Butyrate also acts as a feed attractant, leading to increased feed consumption and consequently benefiting the growth and survival of *P. vannamei* (da Silva et al. 2013).

Considering the beneficial characteristics of butyrate, we hypothesized that calcium butyrate has the potential to be used as a feed additive for *P. vannamei* at low inclusion rates. Therefore, this study aimed to evaluate the growth performance and hepatopancreas enzymatic activities of *P. vannamei* juveniles fed a diet supplemented with 0.2% calcium butyrate.

MATERIALS AND METHODS

Experimental design

The feeding trial was conducted in the Shrimp Culture Laboratory, UFPR, Brazil. The experimental design was randomly divided into two experimental groups, with eight replicates (n = 8) distributed over 16 experimental units. Each experimental unit, with a useful volume of 40 L, was arranged in a recirculation aquaculture system with a biological filter and constant aeration. The photoperiod was a 12:12 h light:dark cycle using artificial light. Five *P. vannamei* juveniles were randomly stocked in each experimental unit, with a total of 80 ind. The animals were acclimated to the experimental environment for 24 h before starting the feeding trial.

The water used in the system was obtained from the public supply; it was filtered and de-chlorinated, then artificially salinized using Red Sea® salt (23 g L^{-1}; Red Sea, Herzliya, Israel). The water quality was evaluated daily and remained within the adequate values for *P.*

vannamei farming: temperature (29.2 ± 0.8°C), dissolved oxygen (4.9 ± 0.3 mg L^{-1}), pH (7.8 ± 01), ammonia (0.12 ± 0.08 mg L^{-1}), nitrite (0.08 ± 0.07 mg L^{-1}), alkalinity (142 ± 38 mg L^{-1} $CaCO_3$) and hardness (1,580 ± 362 mg L^{-1} $CaCO_3$).

Experimental diets

Two experimental groups were used: control (0.0%; diet without any additive) and calcium butyrate treatment (0.2%; control diet with 0.2% Butifour®CCB added before extrusion). Butifour®CCB (Impextraco® Araucária, Brazil) is a commercial calcium butyrate dietary supplement composed of 85% calcium butyrate (69% butyrate - 16% calcium) and 15% inert material (coating); according to the manufacturer, the coating material prevents butyrate leaching. For feed preparation, a commercial shrimp feed mixture (GuabiTech Active®, 40% crude protein, Guabi©, Campinas, Brazil) was homogenized and sieved for each experimental group. The mixture was pre-wetted with hot water (55ºC), extruded into 1 mm diameter pellets, dried in a forced-air oven (45ºC, 24 h), and stored at 4ºC.

All experimental diets were analyzed for their chemical composition (AOAC 2005) in the Laboratory of Animal Nutrition and Feeding (LANA, by its Portuguese acronym), UFPR, Brazil (Table 1). The proximate composition of feed samples was determined in triplicate. Moisture content and dry matter were determined by drying the samples to a constant weight at 105°C for 24 h (method 930.15).

Crude protein content was determined via the Kjeldahl method (method 984.13) using a conversion factor of 6.25. Ether extract was determined gravimetrically using a Soxhlet extraction apparatus with petroleum ether (method 920.39). Mineral matter was measured gravimetrically by incinerating the samples at 550°C for 16 h (method 942.05). Neutral detergent fiber and acid detergent fiber were analyzed according to the Van Soest methods (method 2002.04 and method 973.18, respectively). Gross energy was measured by complete combustion of the samples using a bomb calorimeter.

The amount of feed offered was calculated based on the expected shrimp growth of one gram per week, using an expected feed conversion ratio (FCR) of 1.5:1 (feed grains: shrimp live weight increment). Feed consumption of each unit was monitored daily and adjusted in case of mortality and/or decrease or increase in food consumption. The experimental diets were offered five times daily (0:00, 5:00, 9:30, 13:30, and 17:00 h), thus providing greater food availability throughout the day. Feces and uneaten pellets were siphoned every morning. The feeding trial duration was 27 days.

Table 1. Centesimal chemical composition of dry matter of experimental diets for *Penaeus vannamei* supplemented with 0.2% calcium butyrate.

Chemical composition	0.0%	0.2%
Moisture content (%)	0.00	0.00
Dry matter (%)	100.00	100.00
Crude protein (%)	44.33	43.19
Ether extract (%)	11.06	11.31
Mineral matter (%)	12.52	12.60
Fiber in neutral detergent (%)	29.84	30.13
Fiber in acid detergent (%)	4.56	4.18
Gross energy (kcal g^{-1})	4.890	4.890

Growth parameters

At the end of the feeding trial, all animals were weighed and measured individually, in batches corresponding to each experimental unit. Survival was calculated at the end of the feeding trial. Feed intake was estimated based on the total amount of feed offered and according to the feeding protocol; uneaten feed pellets were not quantified separately. The growth parameters were calculated as follows:

$$\text{Survival (\%)} = 100 \times (\text{final number of shrimp} - \text{initial number of shrimp})$$

$$\text{Weight gain (g)} = \text{final weight} - \text{initial weight}$$

$$\text{Daily weight gain (g)} = \frac{\text{weight gain}}{\text{days of feeding trial}}$$

$$\text{Weekly weight gain (g)} = \frac{\text{weight gain}}{\text{weeks of feeding trial}}$$

$$\text{Length (cm)} = \text{distance between the tip of the rostrum and the tip of the telson}$$

$$\text{Specific growth rate (g d}^{-1}\text{)} = 100 \times \frac{\left(\begin{matrix}\text{Ln final weight} - \\ \text{Ln initial weight}\end{matrix}\right)}{\text{days of feeding trial}}$$

$$\text{Feed conversion ratio (FCR)} = \frac{\text{weight of total feed provided}}{\text{weight gain}}$$

$$\text{Condition factor (K)} = 100 \times \frac{\text{final body weight}}{\text{final body lenght}^3}$$

$$\text{Protein efficiency rate (PER)} = \frac{\text{weight gain}}{\text{protein intake}}$$

Chemical analyses

At the end of the 27-day feeding trial, 16 shrimp were randomly sampled from each experimental group and euthanized by thermal shock (iced water, 5 min). The hepatopancreas, also known as the midgut gland, was sampled and immediately stored at -80ºC for subsequent analyses. Samples were analyzed individually for

biochemical and enzymatic determinations, and the mean value of the two shrimp from each tank was used for statistical analyses (n = 8). Samples were then sent to the Biochemistry and Genetics Laboratory, Federal University of Fronteira Sul, Brazil. The hepatopancreas samples were homogenized in cold 0.8% saline solution using an electric homogenizer (T10 basic Ultra-Turrax®, IKA, Staufen, Germany), centrifuged (4°C, 10 min, 12,000 *g*; Sigma 3-16KL, Sigma, Osterode am Harz, Germany), and the supernatant was used for the enzymatic analyses. Protein content was determined using the Bradford method (Bradford 1976). Triglyceride content was measured using a commercial colorimetric kit (Triglycerides Monoreagent, Bioclin, Belo Horizonte, Brazil) adapted for microplates. Amylase and lipase activities were determined using commercial colorimetry kits (Amylase Kinetic and Lipase Automation, Bioclin, Belo Horizonte, Brazil), in 96-well flat-bottom microplates (de Seixas-Filho 2003). Chymotrypsin and trypsin activities were determined using the Hummel method (Hummel 1959), adjusted for microplates (Pelison da Fonseca et al. 2025).

Statistical analysis

For all analyses, the tank was considered the experimental unit, and mean values from each tank were used for statistical comparisons (n = 8). Data are expressed as mean ± standard deviation (SD). Homoscedasticity and normality between experimental groups were assessed using Levene's and Shapiro-Wilk's tests, respectively. Statistical analyses of the zootechnical performance and enzymatic activity data were then performed using an independent-samples *t*-test at a 95% confidence level, using Minitab® 17 (USA) software.

RESULTS

Growth performance

At the end of the feeding trial, all experimental groups (0.0 and 0.2%) presented a high survival rate (>95%) and satisfactory growth performance for commercial shrimp farming, with similar performance observed among all the analyzed parameters (Table 2). Although feeding behavior was not quantitatively assessed during the present study, no obvious differences in feed acceptance or activity were observed among experimental groups.

Biochemical measures of the hepatopancreas

Total protein levels in the hepatopancreas tissues were similar between control and calcium-butyrate-reared animals (Fig. 1; Table S1). There was an approximately 33% increase in triglyceride levels in the calcium-butyrate-reared animals, but was determined non-significant (P = 0.1256). Similarly, enzyme profiles (lipase, amylase) were also consistent; however, trypsin levels were significantly enhanced (>30% increase; P = 0.0281).

DISCUSSION

The use of organic acids in aquafeed is associated with multiple benefits to animal growth and health, including growth enhancement (Romano et al. 2015), their role as feed attractant (da Silva et al. 2013), promotion of digestive enzyme activity and improved nutrients digestibility (Romano et al. 2015), antimicrobial activity (da Silva et al. 2013), and an immunostimulant effect (Chowdhury et al. 2021). These functional properties are mainly attributed to the ability of organic acids to modulate intestinal microbiota, improve gut health, and influence digestive physiology. In the present study, we investigated the effect of dietary calcium butyrate on the survival and growth performance of juvenile *P. vannamei*. Shrimp survival was above 95% regardless of the experimental group, suggesting that the inclusion of 0.2% calcium butyrate in the shrimp diet did not affect shrimp survival. Likewise, the dietary use of 0.2% calcium butyrate did not influence shrimp growth parameters, with similar performance among shrimp fed a calcium butyrate-supplemented diet and those fed a control diet. Thus, our results suggest that 0.2% calcium butyrate did not affect shrimp survival or growth performance.

Conversely, improved survival and growth performance have been reported for fish (Luz et al. 2019, Dawood et al. 2020) and shrimp (da Silva et al. 2016, Duan et al. 2017, Ding et al. 2022) fed diets supplemented with different forms of butyrate, such as sodium butyrate, calcium butyrate, tributyrin, and hydroxybutyrate. These beneficial effects are often associated with butyrate's role as an energy source for intestinal epithelial cells, as well as its capacity to regulate microbial communities and intestinal barrier function (Rimoldi et al. 2016, Lin et al. 2023). Organic acids and their salts have high solubility, and, consequently, leaching losses may occur (Defoirdt et al. 2011). Thus, it is hypothesized that a higher inclusion

Table 2. Zootechnical performance of *Penaeus vannamei* juveniles reared on diets supplemented with calcium butyrate organic acid. SGR: specific growth rate. FCR: feed conversion rate. Absence of different superscript letters on the same row indicates no significant differences. Data presented as mean ± standard deviation, n = 8. Independent *t*-test, *P*-value < 0.05. N/A: not applicable.

	0.0%	0.2%	*t*-test	
			t	*P*
Initial weight (g)	1.84 ± 0.15	1.84 ± 0.15	N/A	N/A
Final weight (g)	6.08 ± 1.12	6.30 ± 0.81	0.87	0.389
Weight gain (g)	4.24 ± 1.12	4.46 ± 0.83	0.86	0.393
Daily weight gain (g)	0.15 ± 0.04	0.16 ± 0.03	0.86	0.393
Weekly weight gain (g)	1.10 ± 0.29	1.15 ± 0.21	0.86	0.393
Initial length (cm)	6.80 ± 0.32	6.80 ± 0.32	N/A	N/A
Final length (cm)	9.40 ± 0.58	9.57 ± 0.86	1.02	0.309
Initial biomass (cm)	6.90 ± 0.15	6.90 ± 0.15	N/A	N/A
Final biomass	29.05 ± 3.77	29.86 ± 3.42	0.45	0.660
Survival (%)	95.00 ± 9.26	95.00 ± 9.26	0.00	1.000
SGR (g d^{-1})	4.37 ± 0.79	4.54 ± 0.55	0.97	0.337
FCR	1.03 ± 0.11	1.06 ± 0.06	1.16	0.248
Condition factor (K)	0.71 ± 0.08	0.72 ± 0.10	0.63	0.530
Protein efficiency rate (PER)	1.50 ± 0.39	1.50 ± 0.28	0.00	1.000

level of calcium butyrate in shrimp feed (>0.2%) may be necessary to observe a potentially positive impact on shrimp survival and growth performance. In such a case, a cost analysis is recommended to determine the feasibility of higher dosages.

The activity of digestive enzymes in the hepatopancreas of *P. vannamei* and its composition were also investigated in the present study. Our results showed a higher trypsin activity index in the hepatopancreas of shrimp fed a 0.2% calcium butyrate-supplemented diet compared to shrimp fed the control diet. This finding suggests that dietary butyrate may modulate digestive physiology by stimulating proteolytic enzyme production or secretion. Notably, one may observe a slight baseline drop (approximately 1.14%) in the crude protein content of the treatment group compared to the control group, which suggests that both diets remained nutritionally comparable and the 30% increase in trypsin activity was triggered by the organic salt itself, rather than a higher baseline protein intake.

Trypsin is one of the most abundant proteolytic enzymes in the digestive system of crustaceans and contributes, together with chymotrypsin, to at least 60% of protein digestion in penaeids (Fernández-Gimenez et al. 2002). This digestive endoprotease is produced in the shrimp hepatopancreas, which, in turn, releases its content into the short midgut (Kibenge & Strange 2021). In addition to digestion, trypsin also contributes to the activation of zymogens (enzyme precursors), food intake, and nutrient assimilation (Sainz et al. 2004). Its activity in *P. vannamei* is influenced by the feeding regime and the source, quality, and quantity of protein in the diet, the molting stage, genetic factors, and stress (Sainz-Hernández & Cordova-Murueta 2009).

Considering this, one theorizes that the increase in trypsin activity observed in the hepatopancreas of shrimp fed the butyrate diet could enhance protein hydrolysis and nutrient digestibility, particularly nitrogen assimilation. Nevertheless, the present results did not show a significant increase in protein efficiency ratio in shrimp fed the butyrate diet, nor in other growth parameters. Notably, shrimp growth is more intense during the last four to six weeks of the growth cycle, close to the shrimp's final harvesting weight (i.e. approximately 25 g) (Cheng & Hardy 2004). Given the 27-day feeding trial, the observed increase in trypsin activity may indicate an early physiological and enzymatic response rather than a long-term performance outcome. Although sufficient to detect immediate changes in digestive enzyme activity, the trial duration may not have been long enough to translate physiological adjustments into measurable growth performance.

Furthermore, these responses may reflect transient adaptations that require longer observation time to confirm their persistence and biological relevance.

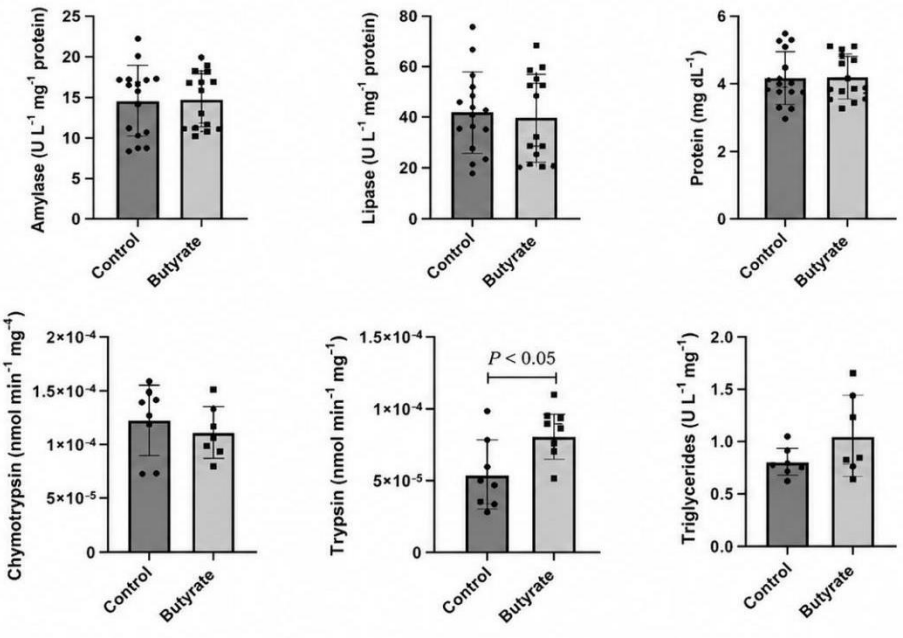

Figure 1. Hepatopancreas composition and digestive enzyme activity of *Penaeus vannamei* juveniles reared on diets supplemented with calcium butyrate organic acid. Data presented as mean ± standard deviation, n = 8. Independent *t*-test, $P < 0.05$.

Therefore, the observed increased trypsin activity should be interpreted as an immediate physiological effect. Further long-term studies are suggested to determine whether this effect can translate into improved growth performance and nutrient utilization.

Correa et al. (2018), when studying the dietary use of 2% sodium butyrate in shrimp nursery (postlarvae 30; 0.03 g), also did not observe a significant effect on *P. vannamei* growth performance; this dosage, however, increased the count of lactic acid bacteria in the shrimp intestine, suggesting a possible modulation of the gut microbiota without immediate effects on growth. Thus, the juvenile shrimp used in the present study may not have reached sufficient weight gain to display a significant difference in protein-use efficiency or growth performance. Other studies corroborate the present results regarding the increased activity of digestive enzymes in the intestine or hepatopancreas of fish and shrimp fed with butyrate, as well as the potential for increased nutrient digestibility due to the dietary supplementation with butyrate.

In *P. vannamei*, the dietary use of 2% sodium butyrate and 2% polyhydroxy butyrate positively modulates the final weight as well as the feed efficiency and protein efficiency ratio and increases the amount of trypsin in the shrimp intestine and hepatopancreas, resulting in higher nutrient digestibility, including polysaccharides and lipids (da Silva et al. 2016, Silva et al. 2016). Protected butyrate also alleviates the negative impact associated with diets containing reduced fishmeal percentage levels on *P. vannamei* performance and immunity (Yarahmadi et al. 2022). In the giant tiger prawn *Penaeus monodon*, the dietary inclusion of 2% butyrate or a 3% mixture of organic acids (butyrate, succinate, fumarate) decreases FCR. It increases prawn survival, biomass gain, and the efficiency of macronutrient retention, including crude protein (Rombenso et al. 2020). Likewise, the use of sodium butyrate in fish diets significantly increases the hepatic trypsin activity of yellow catfish (*Pelteobagrus fulvidraco*; 0.1% inclusion) (Zhao et al. 2021); the intestinal trypsin, lipase, and amylase activities of lemon carp (*Ctenopharyngodon idella*; 0.01-0.03% inclusion) (Tian et al. 2017); and the intestinal trypsin and lipase activities of cross carp (*Carassius auratus*; 0.2-0.4% inclusion) (Fang et al. 2021). Organic salts, such as sodium and calcium butyrate, act as facilitators of protein digestion by reducing the pH and thus facilitating protein denaturation (Lückstädt 2008). The acidic environment facilitates access of proteins to proteolytic enzymes, leading to increased enzymatic activity in the digestive system and greater digestibility, absorption, and utilization of nutrients (Zhou et al. 2019). In this regard, future studies shall quantitatively

assess whether dietary organic salts influence feed acceptance and feeding activity in shrimp.

Likewise, calcium butyrate was evaluated in the present study at a single dietary inclusion level (0.2%) as an initial assessment of its effects on growth performance and digestive enzymes in *P. vannamei* juveniles. While this approach allowed the identification of physiological responses at a practical supplementation level, future studies investigating higher inclusion levels are recommended to establish dose-response effects, outline optimal dietary inclusion levels, and assess possible adverse effects at higher dosages. Additionally, further studies are suggested to use adult shrimp closer to the harvesting weight.

In summary, this study suggests that the use of 0.2% calcium butyrate as a dietary supplement for juvenile *P. vannamei* maintains shrimp growth performance and survival. This dosage also increases trypsin enzyme activity in the shrimp hepatopancreas, with a potential improvement in protein digestion capacity and nutrient availability.

Credit author contribution

C. de Souza Valente: data handling, results interpretation, intellectual content and writing - review & editing; A.P. de Oliveira: conceptualisation, data curation, formal analysis, funding acquisition, investigation, methodology, project administration, validation, visualisation, writing - original draft and writing - review & editing; R. Ortiz Kracizy & C.C. Bra Brazão: data curation, formal analysis, investigation, validation, visualisation and writing - review & editing; C.J. Coates: data handling, results interpretation, intellectual content and writing - review & editing; A. Koppenol: conceptualisation, data curation, formal analysis, funding acquisition, methodology, resources, supervision and visualisation; L.H. Cazarolli: data handling, results interpretation, intellectual content and writing - review and editing; E.L. Cupertino Ballester: conceptualisation, data curation, formal analysis, funding acquisition, methodology, resources, supervision, visualisation, writing - original draft and writing - review & editing.

Conflict of interest

The authors declare that they have no competing financial interests or personal relationships that could have influenced the work reported in this paper.

Ethics

The current Brazilian animal protective legislation (Law No 11.794, 2008, Brazil) does not require the approval of studies with decapod crustaceans by an Animal Welfare Committee. Nevertheless, this study was performed in a research facility laboratory from a Brazilian federal university and followed the recommended commercial and academic standards for shrimp farming, including adequate water quality and proper feed quality and ratio. Torpor with thermal shock was induced before euthanasia. This research does not contain any study with cephalopods, vertebrates, or humans.

Funding

This study was funded by the Funding Authority for Studies and Projects of the Ministry of Science and Technology (FINEP), Coordination for the Improvement of Higher Education Personnel (CAPES), and the National Council for the Development of Science and Technology (CNPq), Brazil. Ana Paula de Oliveira was a recipient of a CAPES scholarship (Process No 88887.501264/2020-00). Rafael Ortiz Kracizy (Process No 140656/2018-9) and Claudia Caramelo Brazão (Process No 140655/2018-2) were recipients of CNPq scholarships. Eduardo Luis Cupertino Ballester was the recipient of a Research Productivity Grant (PQ Process No 311456/2020-0).

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Received: April 27, 2026; Accepted: June 29, 2026

SUPPLEMENTARY MATERIAL

Table S1. Hepatopancreas composition and digestive enzyme activity of *Penaeus vannamei* juveniles reared on diets supplemented with calcium butyrate organic acid. Data presented as mean ± standard deviation, n = 8. Independent *t*-test, $P < 0.05$. *Indicates a significant difference between treatments.

Parameter	0.0%	0.2%	*P*-value
Amylase (U L^{-1} mg^{-1} protein)	14.47 ± 4.13	14.69 ± 3.34	0.8790
Lipase (U L^{-1} mg^{-1} protein)	41.78 ± 15.38	39.50 ± 16.66	0.7045
Protein (mg dL^{-1})	4.14 ± 0.76	4.16 ± 0.64	0.9563
Chymotrypsin (nmol min^{-1} mg^{-1})	0.12 ± 0.03	0.11 ± 0.02	0.4676
Trypsin (nmol min^{-1} mg^{-1})	0.05 ± 0.02	0.08 ± 0.01	0.0281*
Triglycerides (U L^{-1} mg^{-1})	0.77 ± 0.13	1.02 ± 0.35	0.1256