Postprint: Optimal Dietary Calcium and Phosphorus Supplementation Levels for Juvenile Litopenaeus vannamei

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Abstract

This experiment aimed to investigate the effects of different dietary calcium and phosphorus supplementation levels on growth performance, body composition, tissue calcium and phosphorus levels, and serum indices of juvenile Litopenaeus vannamei. A two-factor experimental design was adopted, with calcium supplementation levels of 0, 0.50%, and 1.00%, and phosphorus supplementation levels of 0, 0.40%, 0.80%, 1.20%, and 1.60% to formulate 15 experimental diets with varying calcium and phosphorus levels. One thousand eight hundred shrimp with an initial weight of (0.38±0.01) g were randomly divided into 15 groups, with each group fed one experimental diet, each group having 3 replicates and each replicate containing 40 shrimp. The culture lasted for 8 weeks. The results showed: 1) Dietary calcium level and the interaction between calcium and phosphorus levels extremely significantly affected the weight gain rate (WGR), specific growth rate (SGR), protein efficiency ratio (PER), and feed conversion ratio (FCR) of shrimp ($P<0.01$); dietary phosphorus level extremely significantly affected the WGR, SGR, PER, FCR, and survival rate (SR) of shrimp ($P<0.01$). 2) Dietary calcium level extremely significantly affected the contents of crude protein (CP), crude lipid (CL), and crude ash (Ash) in shrimp muscle ($P<0.01$), and significantly affected the CL and Ash contents in whole shrimp ($P<0.05$); dietary phosphorus level extremely significantly affected the CP and CL contents in whole shrimp, and CP, CL, and Ash contents in muscle ($P<0.01$); the interaction between dietary calcium and phosphorus levels extremely significantly affected the CL content in whole shrimp, and CP, CL, and Ash contents in muscle ($P<0.01$). 3) Dietary calcium and phosphorus levels and their interaction extremely significantly affected the calcium and phosphorus levels in whole shrimp, muscle, and shrimp shell ($P<0.01$). 4) Dietary calcium level extremely significantly affected the activities of serum alkaline phosphatase (ALP) and phenoloxidase (PO), and calcium ion (Ca2+) content ($P<0.01$); dietary phosphorus level extremely significantly affected the activities of serum ALP and PO, and the contents of cholesterol (CHOL), triglyceride (TG), and inorganic phosphorus (IP) ($P<0.01$), and significantly affected the serum Ca2+ content ($P<0.05$); the interaction between dietary calcium and phosphorus levels extremely significantly affected serum ALP activity, and Ca2+ and IP contents ($P<0.01$), and significantly affected serum TG content ($P<0.05$). Under the conditions of this experiment, with SGR as the evaluation criterion and through quadratic regression curve model analysis, the optimal supplementation levels of calcium and phosphorus in diets for juvenile Litopenaeus vannamei were determined to be 1.00% and 1.26%, respectively.

Full Text

Optimum Calcium and Phosphorus Supplemental Levels in Diets for Juvenile Litopenaeus vannamei

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Abstract

This experiment investigated the effects of different dietary supplemental levels of calcium (Ca) and phosphorus (P) on growth performance, body composition, tissue mineralization, and serum biochemical indices of juvenile Litopenaeus vannamei, and determined the optimal supplemental levels of these minerals. A two-factor experimental design was employed with three Ca supplemental levels (0%, 0.50%, and 1.00%) and five P supplemental levels (0%, 0.40%, 0.80%, 1.20%, and 1.60%), resulting in 15 experimental diets. A total of 1,800 juvenile shrimp with an initial weight of (0.38±0.01) g were randomly allocated into 15 groups, with each group fed one experimental diet. Each treatment consisted of three replicates, with 40 shrimp per replicate. The feeding trial lasted for 8 weeks.

The results showed: (1) Dietary Ca level and the interaction between Ca and P levels significantly affected weight gain rate (WGR), specific growth rate (SGR), protein efficiency ratio (PER), and feed conversion ratio (FCR) ($P<0.01$). Dietary P level significantly influenced WGR, SGR, PER, FCR, and survival rate (SR) ($P<0.01$). (2) Dietary Ca level significantly affected crude protein (CP), crude lipid (CL), and ash contents in muscle ($P<0.01$), and significantly affected CL and ash contents in whole body ($P<0.05$). Dietary P level significantly affected CP and CL contents in whole body, and CP, CL, and ash contents in muscle ($P<0.01$). The interaction between dietary Ca and P levels significantly affected CL content in whole body and CP, CL, and ash contents in muscle ($P<0.01$). (3) Dietary Ca and P levels and their interaction significantly affected Ca and P concentrations in whole body, muscle, and exoskeleton ($P<0.01$). (4) Dietary Ca level significantly affected serum alkaline phosphatase (ALP) and phenoloxidase (PO) activities, and Ca^{2} concentration ($P<0.01$). Dietary P level significantly affected serum ALP and PO activities, and cholesterol (CHOL), triglyceride (TG), and inorganic phosphorus (IP) concentrations ($P<0.01$), and significantly affected serum Ca^{2} concentration ($P<0.05$). The interaction between dietary Ca and P levels significantly affected serum ALP activity and Ca^{2} and IP concentrations ($P<0.01$), and significantly affected serum TG concentration ($P<0.05$). Under the experimental conditions, based on SGR and analyzed through quadratic regression curve modeling, the optimal supplemental levels of Ca and P in diets for juvenile L. vannamei were determined to be 1.00% and 1.26%, respectively.

Keywords: Litopenaeus vannamei; calcium; phosphorus; growth performance; body composition; tissue calcium and phosphorus deposition; serum indexes

Introduction

Calcium is a crucial structural component in crustaceans, contributing not only to exoskeleton formation but also to muscle contraction, blood coagulation, nerve transmission, osmoregulation, prophenoloxidase system activation, and maintenance of cell membrane integrity and permeability [1-2]. Phosphorus is an essential constituent of nucleic acids and cell membranes, directly influencing cellular energy metabolism and playing a vital role in animal growth and skeletal mineralization. Phosphorus deficiency significantly inhibits shrimp growth [3].

Litopenaeus vannamei, commonly known as Pacific white shrimp, represents one of the three major cultured shrimp species worldwide. While substantial research has been conducted on dietary requirements for protein, amino acids, lipids, biotin, and vitamins in juvenile L. vannamei, studies on mineral requirements have primarily focused on calcium, magnesium, and chromium [5], with limited investigation into Ca and P requirements. Deshimaru et al. [6] reported that Marsupenaeus japonicus required no dietary Ca supplementation, with 2% dietary P sufficient for optimal growth. In contrast, Kanazawa et al. [7] found that 1% dietary P was adequate. Fenneropenaeus chinensis exhibited optimal growth when fed diets containing 0.91% P and 2.2% Ca [8]. For Penaeus monodon, maximum growth was achieved with 1.0-1.5% P at 1.25% dietary Ca level [9]. Penaeus indicus showed optimal growth performance at 1.25% Ca and 1.32% P [10]. Davis et al. [11] demonstrated that in seawater, L. vannamei fed basal diets with 0.35% P maintained normal growth and survival without Ca supplementation; however, with 1% and 2% Ca supplementation, 0.5-1.0% and 1.0-2.0% P, respectively, were required to sustain normal growth. Cheng et al. [12] observed growth inhibition in L. vannamei at 2% Ca supplementation in low-salinity water, suggesting that Ca levels should be lower than P levels. However, no studies have simultaneously evaluated optimal dietary Ca and P supplemental levels for juvenile L. vannamei, and results vary among studies due to differences in salinity conditions and developmental stages.

Excessive dietary Ca and P levels increase mineral excretion into the aquatic environment, causing water pollution. Some researchers recommend treating the dietary Ca/P ratio as an independent mineral index [13], emphasizing minimal supplementation while meeting shrimp requirements. Due to high fish meal prices, plant protein sources have increasingly replaced fish meal in commercial feeds. Fish meal typically contains 1.5-2.5% P, whereas 40-90% of P in plant proteins exists as phytate-bound phosphorus, which is poorly utilized by aquatic animals, resulting in reduced Ca and P levels in commercial shrimp feeds [14]. This study investigated the effects of different Ca and P supplemental levels on growth performance, body composition, tissue mineralization, and serum indices of juvenile L. vannamei to determine optimal supplemental levels, providing fundamental data for efficient feed formulation.

Materials and Methods

1.1 Experimental Diets and Design The basal diet was formulated using fish meal, shrimp shell meal, soybean meal, peanut meal, and corn gluten meal as protein sources; fish oil, soybean oil, and soybean lecithin as lipid sources; and wheat flour as carbohydrate source. The composition and nutrient levels are presented in Table 1 .

Using calcium lactate (Sinopharm Chemical Reagent Co., Ltd.) as the Ca source and sodium dihydrogen phosphate (Sinopharm Chemical Reagent Co., Ltd.) as the P source, 15 experimental diets were prepared with three Ca supplemental levels (0%, 0.50%, and 1.00%) and five P supplemental levels (0%, 0.40%, 0.80%, 1.20%, and 1.60%). Feed ingredients were ground to pass through an 80-mesh sieve and weighed according to formulation. The premix was prepared using the progressive enlargement method. After initial mixing for 15 minutes, fish oil, soybean oil, and soybean lecithin were added, mixed thoroughly, and passed through an 80-mesh sieve. Distilled water (30%, V/m) was added before mixing and pelleting into 1.0 mm and 1.5 mm diameter pellets. The pellets were cooked at 60°C for 30 minutes, air-dried, labeled, and stored at -20°C until use.

1.2 Experimental Shrimp and Culture Management The feeding trial was conducted at the indoor marine culture system of the Marine Biology Research Base of Guangdong Ocean University, Donghai Island, Zhanjiang. Juvenile L. vannamei were purchased from Donghai Island Zhonglian Shrimp Hatchery and acclimated in outdoor cement tanks to the required size. Prior to the experiment, shrimp were fed the basal diet for one week for dietary adaptation.

According to the experimental design, 15 groups were established, each with three replicates. Each replicate consisted of a 0.30 m^3 fiberglass tank stocked with 40 shrimp. After 24-hour feed deprivation, uniformly sized, healthy, and active shrimp [initial weight (0.38±0.01) g] were randomly distributed among the tanks. The culture period lasted 8 weeks. Shrimp were hand-fed to satiation four times daily (07:00, 11:00, 17:00, and 21:00). Uneaten feed was checked one hour after feeding, and feeding rates were adjusted based on consumption and weather conditions. Continuous aeration was maintained. During the first two weeks, water exchange occurred every two days; subsequently, daily water exchange was implemented. Water temperature was maintained at 28-31°C, salinity at 21-24, pH at 7.8-8.2, dissolved oxygen 5 mg/L, ammonia nitrogen <0.2 mg/L, and nitrite <0.05 mg/L.

1.3 Sample Collection and Analysis

1.3.1 Sample Collection At the end of the trial, after 24-hour feed deprivation, shrimp were weighed and counted for growth parameter calculation. Fifteen shrimp per tank were randomly selected, and hemolymph was individually collected from the ventral sinus of the fifth pereiopod using 1 mL syringes. Hemolymph samples were pooled in Eppendorf tubes, stored at 4°C overnight, and centrifuged at 3,000 r/min for 10 minutes to collect serum, which was stored at -80°C for subsequent analysis. Additionally, ten shrimp per tank were sampled, surface moisture removed, and stored in sealed bags. The remaining shrimp were dissected to separate exoskeleton and muscle, which were stored at -20°C for proximate composition analysis.

1.3.2 Proximate Composition Analysis Moisture content in diets, whole body, and muscle samples was determined by oven drying at 105°C to constant weight. Crude protein (CP) content was measured using the Kjeldahl method (KjeltecTM 8400, Sweden). Crude lipid (CL) content was determined by Soxhlet extraction using petroleum ether as solvent. Ash content was measured by incineration in a muffle furnace at 550°C.

1.3.3 Calcium and Phosphorus Analysis Dried and ground samples were placed in stoppered test tubes with 5 mL Merck nitric acid and digested in a 70°C water bath for 2 hours, followed by acid evaporation on a digestion block for 5 hours. One milliliter of hydrogen peroxide was added, and samples were evaporated to 1 mL before dilution. Calcium and phosphorus concentrations were determined using inductively coupled plasma optical emission spectrometry (ICP).

1.3.4 Serum Index Analysis Serum alkaline phosphatase (ALP) activity was measured using assay kits from Nanjing Jiancheng Bioengineering Institute. Phenoloxidase (PO) activity was determined according to the method of Huang et al. [15]. Cholesterol (CHOL), triglyceride (TG), calcium ion (Ca^{2}), and inorganic phosphorus (IP) concentrations were analyzed using an automatic biochemical analyzer (Hitachi 7020HITACHI, Japan).

1.3.5 Growth Parameter Calculation Weight gain rate (WGR, %) = [(final mean weight - initial mean weight) / initial mean weight] × 100
Specific growth rate (SGR, %/d) = [(ln final mean weight - ln initial mean weight) / feeding days] × 100
Protein efficiency ratio (PER) = (final body weight - initial body weight) / (feed intake × dietary CP content)
Feed conversion ratio (FCR) = dry feed intake / (final body weight - initial body weight)
Survival rate (SR, %) = (number of shrimp at experiment end / number of shrimp at experiment start) × 100

1.4 Statistical Analysis Data were analyzed using two-way ANOVA with SPSS 17.0. When significant differences were detected, Duncan' s multiple range test was applied to compare means among groups. Results are expressed as "mean ± standard deviation." $P<0.05$ indicated significant difference, and $P<0.01$ indicated highly significant difference.

Results

2.1 Effects of Dietary Calcium and Phosphorus Levels on Growth Performance Growth performance results are presented in Table 2 . Dietary Ca level significantly affected WGR, SGR, PER, and FCR ($P<0.01$), but not SR ($P>0.05$). Dietary P level significantly affected WGR, SGR, PER, and FCR ($P<0.01$). WGR and SGR increased with increasing dietary P level, with no significant differences among 0.80-1.60% P levels ($P>0.05$). PER increased initially then decreased with increasing P level, reaching maximum at 0.80% P, which was significantly higher than other levels ($P<0.05$). FCR decreased initially then increased, with minimum value at 0.80% P. All P supplementation levels except 1.20% significantly improved SR ($P<0.05$). The interaction between dietary Ca and P levels significantly affected WGR, SGR, PER, and FCR ($P<0.01$), but not SR ($P>0.05$).

Based on SGR and analyzed through quadratic regression and broken-line models, the optimal dietary P levels were determined to be 1.17% without Ca supplementation, 1.00% with 0.50% Ca supplementation, and 1.26% with 1.00% Ca supplementation. Shrimp achieved maximum SGR at 1.00% Ca and 1.26% P supplementation (Figure 1 [Figure 1: see original paper]).

2.2 Effects of Dietary Calcium and Phosphorus Levels on Whole Body and Muscle Composition Whole body and muscle composition results are presented in Table 3 . Dietary Ca level significantly affected muscle CP, CL, and ash contents ($P<0.01$), and significantly affected whole body CL and ash contents ($P<0.05$), but not whole body CP content ($P>0.05$). Dietary P level significantly affected whole body CP and CL contents, and muscle CP, CL, and ash contents ($P<0.01$), but not whole body ash content ($P>0.05$). The interaction between dietary Ca and P levels significantly affected whole body CL content and muscle CP, CL, and ash contents ($P<0.01$), but not whole body CP and ash contents ($P>0.05$).

Different Ca supplementation levels significantly increased whole body ash and muscle CP contents ($P<0.05$), while significantly decreasing whole body CL and muscle CL and ash contents ($P<0.05$). Whole body and muscle CP contents increased with increasing P level and then stabilized. Dietary P supplementation at 1.60% significantly decreased whole body CL content ($P<0.05$). Different P levels significantly decreased muscle CL content ($P<0.05$), and 0.80-1.60% P supplementation significantly increased muscle ash content ($P<0.05$).

2.3 Effects of Dietary Calcium and Phosphorus Levels on Tissue Calcium and Phosphorus Concentrations Tissue mineral concentrations are presented in Table 4 . Dietary Ca and P levels and their interaction significantly affected Ca and P concentrations in whole body, muscle, and exoskeleton ($P<0.01$). Different Ca supplementation levels significantly increased whole body Ca and P concentrations and exoskeleton Ca concentration ($P<0.05$), while significantly decreasing muscle Ca concentration ($P<0.05$). Muscle P concentration decreased initially then increased with increasing dietary Ca level, reaching maximum at 1.00% Ca with significant differences among levels ($P<0.05$). Whole body Ca and P concentrations and exoskeleton Ca concentration increased initially then decreased with increasing dietary P level. Muscle P concentration increased continuously, while exoskeleton P concentration increased and then plateaued with increasing P level.

2.4 Effects of Dietary Calcium and Phosphorus Levels on Serum Indices Serum indices are presented in Table 5 . Dietary Ca level significantly affected serum ALP and PO activities and Ca^{2} concentration ($P<0.01$), but not CHOL, TG, and IP concentrations ($P>0.05$). Dietary P level significantly affected serum ALP and PO activities and CHOL, TG, and IP concentrations ($P<0.01$), and significantly affected serum Ca^{2} concentration ($P<0.05$). The interaction between dietary Ca and P levels significantly affected serum ALP activity and Ca^{2} and IP concentrations ($P<0.01$), and significantly affected serum TG concentration ($P<0.05$), but not PO activity or CHOL concentration ($P>0.05$).

Different Ca supplementation levels significantly increased serum ALP and PO activities while decreasing Ca^{2} concentration ($P<0.05$). Different P supplementation levels significantly decreased serum ALP activity and CHOL and TG concentrations ($P<0.05$), though ALP activity showed no significant differences among 0.80-1.60% P levels ($P>0.05$), and TG concentrations showed no significant differences between 0.40% and 0.80% or between 1.20% and 1.60% levels ($P>0.05$). Serum PO activity increased initially then decreased with increasing P level, reaching maximum at 0.40% P. Different P levels significantly increased serum Ca^{2} and IP concentrations ($P<0.05$), with no significant differences among 0.40-1.60% P levels ($P>0.05$).

Discussion

3.1 Effects on Growth Performance Davis et al. [11] reported that L. vannamei could maintain normal growth and survival with 0.35% P in basal diets without Ca supplementation; however, with 1% Ca supplementation, maximum WGR and SR were achieved at 2% P. Cheng et al. [12] found that in low-salinity water (salinity 2), L. vannamei required 0.77% available P (0.93% total P) in Ca-free diets for optimal growth, but needed 1.22% available P (2% total P) when 1% Ca was supplemented. Our results showed that dietary Ca and P levels and their interaction significantly affected WGR, SGR, PER, and FCR. At the same Ca level, shrimp fed P-free diets showed significantly lower WGR, SGR, and PER compared to other groups, and the basal diet group without Ca and P supplementation showed significantly lower SR. These findings align with Davis et al. [11] and Cheng et al. [12]. Shrimp can absorb minerals from water, but ambient P levels are generally insufficient to meet growth requirements, necessitating dietary P supplementation. In this study, WGR, SGR, and PER increased with increasing dietary P level, consistent with reports in L. vannamei [16], P. monodon [9], Eriocheir sinensis [17], Larimichthys crocea [18], and Rachycentron canadum [19]. However, Peñaflorida [20] reported that P. monodon achieved maximum WGR and SR at 0.5% P without Ca supplementation, with WGR and SR decreasing substantially at higher P levels. Ambasankar et al. [9] observed maximum WGR and SR in P. monodon at 1.5% P with 1.25% Ca, but minimum SR at 2.5% P, indicating growth inhibition by excessive P. Similar results were reported in Melanogrammus aeglefinus [21] and Sparus macrocephalus [22]. The absence of growth inhibition by excessive P in our study may be attributed to the rapid growth and frequent molting of juvenile shrimp requiring higher P levels. Niu et al. [23] also reported higher P requirements during early developmental stages of L. vannamei.

Shrimp require substantial Ca and P for growth and molting. Generally, shrimp can absorb adequate Ca from culture water through gills and intestinal epithelium, making dietary Ca supplementation unnecessary [24]. However, dietary Ca level affects P absorption and utilization; excessive Ca can combine with phosphate to form calcium phosphate, reducing P digestibility [25]. Davis et al. [26] reported that dietary Ca should not exceed 3% in shrimp feeds, a recommendation supported by Cheng et al. [12]. Our dietary Ca levels ranged from 1.62% to 2.70%, consistent with these recommendations.

3.2 Effects on Whole Body and Muscle Composition Our results demonstrated that increasing dietary P level improved CP contents in whole body and muscle of L. vannamei. Wang et al. [2] reported that muscle CP content in Cherax quadricarinatus increased with dietary P level (0.72-1.74%). Similar trends were observed in Lateolabrax japonicus [27]. These findings align with reports in M. aeglefinus [21], Ictalurus punctatus [28], and S. macrocephalus [29]. Conversely, body lipid content decreased significantly with increasing dietary P level in L. japonicus [27] and C. quadricarinatus [2]. Our observation that dietary P reduced whole body and muscle CL contents is consistent with these studies and with findings in Procambarus clarkii [30]. Roy et al. [21] suggested that P deficiency inhibits fatty acid -oxidation, reducing lipid utilization and increasing protein catabolism for energy, resulting in lipid accumulation and decreased protein content. Adequate P enhances fatty acid -oxidation, improving lipid utilization for energy, increasing glycogen synthesis, and promoting protein deposition, thereby increasing protein and decreasing lipid contents. You et al. [31] reported improved lipid digestibility with increasing dietary P in grass carp, supporting the role of P in promoting lipid oxidation and metabolism. Dietary P supplementation increased muscle ash content in our study, consistent with reports in L. vannamei [13, 12], E. sinensis [32], C. quadricarinatus [2], P. clarkii [30], and F. chinensis [33]. Luo [13] suggested that increased P and mineral interactions enhance mineral deposition in tissues.

3.3 Effects on Tissue Calcium and Phosphorus Concentrations Tissue Ca and P concentrations are commonly used as reference indicators for determining optimal dietary mineral levels in fish and shrimp [25,34]. Our study showed that dietary Ca and P supplementation increased Ca and P concentrations in whole body and exoskeleton of L. vannamei. Similar results were reported in P. clarkii [30] and E. sinensis [32], where whole-body P increased with dietary P level. Huang et al. [35] found that dietary calcium dihydrogen phosphate significantly affected whole-body and exoskeleton Ca and P in L. vannamei. Studies in C. quadricarinatus [2], F. chinensis [33], and L. vannamei [13,16] demonstrated positive relationships between dietary mineral levels and tissue mineralization. However, Peñaflorida [21] reported no correlation between whole-body P and dietary P in P. indicus, and Davis et al. [41] found no direct relationship between dietary Ca/P levels and tissue mineral content in L. vannamei. Similar observations were reported in M. japonicus [42] and Homarus americanus [43]. These discrepancies may be related to the molting cycle, as Ca and P concentrations in exoskeleton and muscle fluctuate before and after ecdysis [43].

3.4 Effects on Serum Indices Alkaline phosphatase is a phosphomonoesterase that catalyzes phosphate hydrolysis and group transfer reactions, providing inorganic phosphate for ADP phosphorylation to ATP [44]. Studies in C. quadricarinatus [2] and L. vannamei [12] reported decreased hepatic ALP activity with increasing dietary P level. Our results showing decreased serum ALP activity with increasing P supplementation are consistent with these findings, likely because increased IP provides adequate phosphate groups for biochemical reactions, reducing the demand for ALP-catalyzed phosphate production. However, L. japonicus showed increased plasma ALP activity with dietary P level (0.31-1.17%) [27], and S. macrocephalus exhibited lowest plasma ALP at 1.12% total P with increasing activity at higher levels [29]. These differences may be attributed to species differences, dietary composition, and the fact that shrimp are invertebrates with innate immune systems [45].

The prophenoloxidase activating system is a cascade system present in hemolymph granulocytes of crustaceans. Phenoloxidase can be activated by bacterial lipopolysaccharides (LPS), Ca^{2} , and trypsin to produce active PO [46]. Fan et al. [47] reported that Mg^{2} and Ca^{2} enhanced PO activity in F. chinensis. Our results showing increased serum PO activity with dietary Ca supplementation are consistent with this finding. The initial increase followed by decrease in PO activity with increasing P level may be explained by the formation of insoluble phosphate salts with iron and magnesium, reducing absorption and metabolism of these ions [48] and consequently affecting PO activity, as Mg^{2} is known to enhance PO activity.

Serum CHOL and TG concentrations decreased with increasing dietary P level in R. canadum [19] and L. crocea [49]. Our results showing reduced serum CHOL and TG with P supplementation are consistent with reports in S. macrocephalus [22], L. japonicus [27], Myxocyprinus asiaticus [50], and Protonibea diacanthus [51]. Huang [51] suggested this relates to lipid metabolism, where P deficiency increases lipogenesis and adequate P reduces lipogenesis, decreasing blood CHOL and TG levels. Our findings support this mechanism.

Serum IP concentration increased initially then plateaued with increasing dietary P level, consistent with reports in R. canadum [52], M. aeglefinus [21], Epinephelus malabaricus [53], and L. japonicus [27].

Based on the interaction between dietary Ca and P levels, maximum SGR was achieved at 1.00% Ca and 1.26% P. Single-factor studies have reported P requirements of 1.16-1.37% for F. chinensis postlarvae [54], consistent with our results. However, other studies reported different requirements: 0.25% P for L. vannamei in 30% seawater salinity [13] and 1.84% P for P. clarkii [(5.02±0.51) g] [30]. These variations may be attributed to species differences, P sources, culture systems, and developmental stages. Ca studies generally indicate no need for dietary Ca supplementation. Channel catfish and common carp cultured in water containing 14 and 20 mg/L Ca showed no Ca deficiency signs when fed diets with only 0.03% and 0.05% Ca, respectively [55-56]. Our two-factor analysis yielded results similar to some single-factor studies but differed from others.

Nutrients interact directly or indirectly through synergistic, antagonistic, or regulatory relationships. Therefore, two-factor or multi-factor experimental designs considering nutrient interactions and environmental factors should provide more practical results for aquaculture. However, multi-factor experiments require substantial effort and accurate data collection, warranting further investigation to determine whether such results can serve as foundational data for nutritional parameter databases in L. vannamei.

Conclusions

1. Based on SGR and analyzed through quadratic regression and broken-line models, the optimal dietary P supplemental levels were 1.17%, 1.00%, and 1.26% corresponding to Ca supplementation levels of 0%, 0.50%, and 1.00%, respectively.
2. Juvenile L. vannamei achieved maximum SGR at dietary Ca and P supplemental levels of 1.00% and 1.26%, respectively.

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