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Research Article

Fishmeal replacement and its effects on nutrition and the physiology of *Penaeus vannamei* shrimp juveniles

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ABSTRACT. The replacement of fishmeal with plant protein sources has been studied in *Penaeus vannamei.* However, it has only been studied from the point of view of protein replacement, forgetting the integral role that fishmeal plays in the nutrition of shrimp. This study aimed to determine if a supplement of dietary phosphorus can sustain the fishmeal replacement by a blend of plant protein feedstuffs, with betaine and tauren as attractants and still supporting homeostasis, growth, digestive enzymes, and lipid-soluble antioxidant systems at normal levels in juveniles of *P. vannamei*. A standard feed (25% fishmeal) for juvenile *P. vannamei* was partially replaced with a mix of plant ingredients (soybean and canola paste, soy protein concentrate, and wheat meal). These feedstuffs were supplemented with amino acids (MET, LYS, TAU) plus microencapsulated exogenous phytase, betaine, and dicalphosphate. *P. vannamei* juveniles (2.56 g) were maintained in 100 L fiber tanks for 70 days. Biomass was significantly affected by the treatments ($P < 0.05$), being better as the fishmeal was replaced in the diet and comparable to commercial feed. No significant changes were observed in blood parameters. Muscle superoxide dismutase activity was significantly affected by the diets ($P < 0.05$). There were adaptive changes in digestive enzymes, and homeostasis remained stable. Fishmeal replacement may depend on supplementation with taurine and soluble phosphorus, not only for performance but flesh quality, and here, the shear strength of the shrimp muscle remained within the acceptable value (69-81 mJ).

Keywords: *Penaeus vannamei*; phytase; fishmeal replacement; plant protein; physiology

INTRODUCTION

Fishmeal (FM) has been a major component in feed for juveniles of *Penaeus vannamei* but is scarce and expensive (Azaza et al. 2023). Finding alternative protein sources is complex (Tacon 2008, Hardy 2010). Moreover, an "unknown growth factor" has been suspected of profoundly influencing the physiology of many farmed animals, including crustaceans; in the case of shrimp, there is a requirement for a high mineral content of up to 20% (Lemos et al. 2021, Truong et al. 2022).

FM has an integral blend of additives, including taurine. Deshimaru & Yone (1978) proposed that taurine was one of the best palatable molecules when it was included in diet shrimp. Shi-wei et al. (2016) measured the taurine content of FM and different plant protein ingredients, and the highest concentration was obtained in FM (0.67%), soybean meal (0.17%), soybean protein concentrate (0.21%); wheat meal (0.03%).

In considering plant proteins to replace FM in grow-out feed (Colvin & Brand 1977, Cruz-Suarez et al. 2001, Suárez et al. 2009), the focus has been not only

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on taurine but also on minerals from phosphates and trace minerals (Koss 1979, Troung et al. 2022), as well as salmon, phagostimulating substances as betaine plus vitamins (Hemre 2014).

Huynh et al. (2018) have described betaine as a chemoattractant. Also, a combination of taurine and betaine was attractive in a 10-6 M concentration for *Peneaus monodon* (Comman et al. 1996).

Regarding mineral requirements, shrimp need phosphorus concerning molting (Civera & Guillaume 1989, Davis et al. 1993, Vijayan & Diwan 1996), but little is known about the influence of its form (soluble or not) upon digestibility (Civera & Guillaume 1989), and hence on its effects for growth and molting frequency (Lemos et al. 2021, Truong et al. 2022). An almost doubling of the mineral content of feed for juveniles of *Marsupenaeus japonicus* can enhance their growth and molting frequency compared with their performance on formulated feeds with a 10-12% mineral content (Aquacop 1989). However, there needs to be more study of this or the form of phosphates included in experimental diets. During the 1970s, there were disturbing occurrences of disorders such as molt death syndrome or black death disease at the laboratory level (Gallagher et al. 1978, Baticados et al. 1986); these may also have been occurring in the wild, but detection would have been less easy, and natural productivity (worms, copepods, mysids) may have averted a lack of dietary phosphorus.

The present study aimed to determine the extent to which the supply of dietary phosphorus to *P. vannamei* could be capable of sustaining the FM replacement by a blend of plant protein feedstuffs in feed in which betaine and taurine were added and still supporting homeostasis, maintaining survival, growth, digestive proteolytic enzymatic activity (Casillas-Hernández et al. 2006), and sodium dismutase activity at normal levels. Also, it included two postmortem measurements: shear texture and caloric content of the shrimp.

MATERIALS AND METHODS

Experimental dispositive and water quality

Penaeus vannamei juveniles (2.56 ± 0.31 g) were obtained from Aquaculture Farm 2000, Campeche (Mexico). Under controlled conditions, juveniles were maintained for 70 days (10 days acclimatization and 60 days experimental period) in a recirculating system. The quality water parameters were salinity: 36 measured with a refractometer; temperature: 28 ± 1°C (controlled in the recirculation system with a cooler brand Acuabone); dissolved oxygen: (6.5 ± 0.5 mg L^{-1}), monitored using a Hach oximeter (model hqd40) twice daily (07:00 and 19:00 h), with a photoperiod of 12:12 (day:night). Seawater was passed on a sand filter, 5 µ cartridge filter, UV, and inside a recirculation system equipped with a 20 µm cartridge filter and a skimmer. The 36 rectangular fiberglass tanks of 100 L capacity received 20 ind $tank^{-1}$ (stocking density of 66 ind m^{-2} (Venkateswarlu 2019, Araneda et al. 2020, Suwoyo & Hendrajat 2021).

Experimental design and diets composition

The completely randomized design was used with six treatments with six replicates per treatment: T_1, T_2, T_3, T_4, and T_5, with an animal:vegetal proteic ratio of 50:40, 40:49, 30:53, 20:63, and 10:86, respectively, plus a commercial diet: T_6. Diets were prepared at the Nutrition Laboratory of UMDI Sisal, Sciences Faculty. The raw materials were ground, sifted, homogenized at 250 µm, and mixed, and then the oils mix was mixed with a binder to form a paste, passed through a meat mincer, and the pellets were dried at 60°C for 12 h. Microencapsulated phytase (Ronozyme^{TM} CT P5000) was added (5000 U kg^{-1} feed) to the dry ingredients in diets T_1 to T_5 (the Ronozyme was derived from *Peniophora lycii*, produced by fermentation with *Aspergillus oryzae* (range of activity pH 4-7) (Table 1). Diets were analyzed in triplicate following AOAC (1995) procedure for moisture (60°C, 24 h), crude protein (micro Kjeldahl), ether extract (Soxhlet), crude fiber (successive acid-base hydrolysis), and ash (muffle at 550°C, 24 h). Shrimp were fed thrice daily at 7:00, 13:00, and 19:00 h with a ration calculated on 3% biomass d^{-1} (Zainuddin et al. 2019, Suwoyo & Hendrajat 2021) while monitoring and adjusting for any feed excess.

Zootechnical parameters

Weight gain was calculated from the difference between final mean wet weight and the initial mean wet weight for each treatment.

Survival rate was calculated as (final / initial) × 100, and weight gain as specific growth rate = 100 (ln final-ln initial mean weight) / days. Also recorded for each treatment was biomass, using the final weight and the number of survivors in each tank.

Texture (shear strength, mJ) of body muscle (2^{nd} abdominal segment) was measured in 12 shrimp from each treatment at intermolt stage C (Bourgeois & Cuzon 1975); a Brookfield press with a 1 mm knife blade in the form of a guillotine, and at a speed of 25 mm min^{-1}, allowed penetration of 3 mm.

Table 1. Diet formulation of experimental diets. Results expressed in g kg^{-1}. [1]Soy proteic concentrate: Profine, Central Soya Inc. (70%CP), [2]cod liver oil Cedrosa, [3]Rovimix®Roche, [4]DSM Ronozyme 5000 U kg^{-1}. **Commercial feed (Api camarón, 35% of crude protein, Malta Cleyton).

AP:VP ratio	T_1	T_2	T_3	T_4	T_5	T_6**
	50:60	40:49	30:53	20:63	10:86	
Menhaden FM	250	200	150	100	50	
Canola meal	80	110	110	140	220	
Soybean meal	120	160	170	190	250	
[1]SPC	10	30	50	80	80	
Wheat meal	430	349.2	339.2	294.2	149.2	
L-Lys	9	9	9	9	9	
DL-Met	7	7	7	7	7	
Taurine	-	10	10	10	20	
Fish oil[2]	20	30	50	40	60	
Soy lecithin	20	20	20	20	20	
Cholesterol	1	1	1	1	1	
Stay-C	0.3	0.3	0.3	0.3	0.3	
Vitamin mix	5	5	5	5	5	
Mineral mix[3]	5	11	16	18	24	
$CaHPO_4$	22	32	32	50	64	
Betaine	-	5	10	15	20	
CMC	20	20	20	20	20	
Phytase ronozyme[4]	0.5	0.5	0.5	0.5	0.5	
Total	1000	1000	1000	1000	1000	

Biochemical parameters

Trypsin and chymotrypsin (60% of the protease activity of the hepatopancreas) were assayed at intermolt stages C or D_0; protease activity at those stages coincided with an 18% increase in food intake in *P. vannamei*, so in the present study the enzymatic activity in shrimp hepatopancreas extracts was measured at those stages (12 extracts/treatment) and tissue was kept in Ependorff™ microtubes in liquid nitrogen (-70°C). Each extract was placed in a microtube with 500 µL distilled water, homogenized on ice for 30 s at 4000 *g*, and then centrifuged for 20 min at 14,000 g and 4°C. The supernatant (raw extract) was decanted to measure soluble protein concentration and trypsin and chymotrypsin activity. Samples remained at a low temperature to avoid enzyme denaturation and raw extracts were diluted immediately for analysis.

Soluble protein concentration was measured with a Sigma micro protein determination kit (Bio-Rad 500-0006) (Bradford 1976). Trypsin (benzoyl-arginine-p-nitroanilide (Sigma B7632) in Tris buffer 0.1 M, pH 8 (Geiger & Fritz 1988). The rate of hydrolysis coincided with an increase in absorbance (Spectronic model 21D) at 405 nm during 2 min; to read a difference in absorbance between the 1st and 2nd minutes required at extinction coefficient $\varepsilon 405 = 1.02l\ mol^{-1}\ cm^{-1}$ (Geiger 1988). Chymotrypsin activity was measured with succinyl-alanine-2-proline-phenyl-p-nitroanilide as substrate (Geiger 1988) expressed in units (U) per g dry weight of tissue or milli-units (mU) per mg soluble protein in the extract (1 unit represents the conversion of 1 µM of substrate per minute under test conditions).

Superoxide dismutase (SOD) activity was measured in shrimp muscle at intermolt stages (C-D_0) from 10 individuals per treatment. 100 mg frozen shrimp muscle was placed in the homogenizer containing 0.5 mL phosphate buffer solution (50 mM, pH 7.8) centrifuged at 5724 *g*, 5 min at 4°C, and the supernatant heated 5 min at 65°C. The supernatant was centrifuged and stored at -20°C (samples were kept on ice throughout the extraction process). Enzyme-substrate reaction and tetrazolium dye xanthine oxidase (SOD assay kit Sigma, 19160) led to an absorbance measured in a Spectronic 21D at 450 nm after 20 min reaction time at 37°C. Inhibition percentage was normalized in mg of protein units of SOD, and a one-way ANOVA processed results (SAS Program 2006) at $P < 0.05$ (Zar 1999).

Blood parameters

For total hemocyte count and measurement of osmotic pressure in hemolymph from 20 individuals per

treatment at intermolt stage C or D_0, 20 µL were collected by puncture from the first abdominal segment with a 1 mL syringe in an isotonic solution SIC-EDTA (NaCl 450 mM, KCl 10 mM, HEPES 10 mM and EDTA 20 mM, pH 7.3) at 8°C, and equal proportions of anticoagulant andhemolymph (Rosas et al. 2002) to obtain plasma. Twenty five microliters were centrifuged for 10 min at 800 *g* and 4ºC, placed in a hemocytometer, automatic cell counter TC10 (Bio-Rad, Hercules, CA, USA), and 20 µL for osmotic pressure, in a micro-osmometer (3 MO-Plus Advanced Instruments, USA).

Statistical analysis

For the survival and final wet weight, parameters of energetic balance, enzymatic activity, hemocyte count, and osmotic pressure, one-way ANOVA was used to determine the significant differences between the treatments after corroborating normality and homogeneity of variances. Due to the presence of a control treatment (T_6) when significant differences were detected, the Dunnett range test was used. A confidence level of 0.05 was used (Zar 1999). The Statistical program version 10 was employed. All statistical data analyses and graphic visualizations were performed in R software 4.2.2 (R Core Team 2022). Significance was assigned at $P < 0.05$. A regression model was used to estimate the relationship among response variables: survival and calorimetry measurement of the organism with ratio animal:vegetal protein of experimental diet. Commercial diet was excluded from regression analysis because the animal:vegetal protein ratio was unknown.

RESULTS

The proximate composition of experimental diets and commercial feed (Table 2), did give a uniform response in terms of stability in seawater for at least 1 h after feeding ($P > 0.05$). Therefore, the hardness or pellet compression grade did not differ significantly between the commercial feed and T_1, T_2, T_4, and T_5. T_3 had a range of values like the one of feed.

Zootechnical parameters

Survival percentages differed significantly among treatments ($P < 0.05$), and the highest values were obtained in T_4 and T_6 (Table 3). As shown in Figure 1, it was observed that survival has a reduction associated with the proportion of animal:vegetal protein in the diet ($F[1, 28]$, $P \leq 0.01$). Biomass was significantly affected by diets. The best results for biomass were achieved with T_6 and T_4 ($P < 0.05$, Table 3).

Digestive enzymatic activity

No significant effects were observed between dietary treatments for the digestive enzymatic activity of both endoproteinases (trypsin and chymotrypsin) (Table 4).

Muscle parameters

Concerning the muscle parameters, there were significant differences between treatments in terms of SOD activity, being the lowest values observed for T_3 and T_4. In contrast, the other treatments were not different from the control. The texture of muscle tissue showed no significant difference ($P > 0.05$, Table 5). The gross energy content of shrimp fed in function proportion animal: vegetal protein in diet there a significant increment was observed ($F[1,18] = 14.25$, $P \leq 0.01$, Fig. 2).

Blood parameters

For the blood parameters measured, there were no significant differences between treatments in total hemocyte count or osmotic pressure (mOsm kg^{-1}), did not reflect any change in relation with dietary treatments on one side, and no effect of confinement at such stocking density on the other (Table 6).

DISCUSSION

The results of this study showed that FM replacement by a blend of plant feedstuffs and the addition of the mix of additives as amino acids, attractants, and appetizers (betaine and taurine), phytase and phosphorous was very successful for the survival and final biomass of the juveniles of *Peneaus vannamei*. Since usually the traditional diet for raising *P. vannamei* juveniles contained 25% FM (diet $T_{1)}$ in the present study could be considered as a control even though several papers have indicated a total replacement (Suárez et al. 2009, Glencross et al. 2014). However, in the present study, the five experimental diets were formulated with the first limiting factor of protein mix supported by threonine. Since plant ingredients such as soybean meal contain an adequate amino acid profile (Shigeno 1975, Lawrence et al. 1986) and wheat protein is a highly digestible, partial replacement of FM was feasible. On the other hand, the present physiological data provide useful information on mineral requirements. Although calcium can be retrieved from seawater (Li & Cheng 2012), phosphorus cannot, and any feed formulation must ensure a balance between those two essential minerals. Whatever the partial replacement of FM, the diet must contain an adequate source of minerals, considering

Table 2. Proximate composition of experimental diets (results expressed in g kg^{-1}) and analytical data in %; leaching (mean ± standard deviation, SD). Values on the hardness of diets expressed in mJ (mean ± SD). The same letters indicate no significant difference between treatments ($P < 0.05$). Analytitical data in %; leaching (mean ± SD). The same letters indicate no significant difference between treatments ($P < 0.05$). *g 100 g^{-1}. THR indicate threonine as a limitant aminoacid, that means lower content related to the shrimp content. EE: ether extract, NFE: nitrogen free extract, DE: digestible energy, P. sol. intake: dissolved phosphorus intake.

	T_1	T_2	T_3	T_4	T_5	T_6**
Moisture*	5.6	6.4	4.9	6.3	5.8	
Protein*	33.8	35.2	34.6	35.7	36.1	36
Limiting factor	THR	THR	THR	THR	THR	
EE*	6.9	6.9	7.8	6.1	7.8	8
Fiber*	3.1	2.9	2.5	3.3	4.7	4
NFE	40	40	40	39	34	37
Ash*	8.6	9.0	8.6	10.3	11.18	9
DE kJ g^{-1} calculated	16	16	17	17	16	17
P_{total}*	1.5	1.6	1.5	1.7	1.8	-
$P_{available}$*	0.9	0.8	0.7	0.6	0.5	-
Phytate (by #)	0.6	0.8	0.8	1.1	1.3	-
Ca/P	1.3	1.3	1.2	1.2	1.3	
P. sol. intake (mg d^{-1})	30	15	15	19	20	
DM recovered%	87.3 ± 10.	88.9 ± 12.3	89.2 ± 9.1	88.1 ± 10.2	84.9 ± 8.6	92 ± 6

Table 3. Zootechnical parameters by treatment. SGR: specific growth rate, FCR: feed conversion ratio. Same letters indicate no significant difference between treatments ($P < 0.05$). Initial weight 2.56 ± 0.31 g. Initial weight 2.56 ± 0.31 g. *Different letters in superscript in the same row indicate significant differences between treatments ($P < 0.05$).

Zootechnical parameters	T_1	T_2	T_3	T_4	T_5	T_6
Final wet weight (g)	$7.47 \pm 1.9^{a*}$	7.18 ± 1.5^{a}	7.31 ± 1.6^{a}	7.33 ± 1.5^{a}	6.97 ± 1.6^{a}	7.31 ± 1.3^{a}
Biomass (g)	89.6 ± 10.2^{c}	89.7 ± 9.9^{c}	99.9 ± 9.4^{b}	119.7 ± 8.4^{ab}	106 ± 13^{ab}	126.6 ± 17.5^{a}
Survival (%)	70.8 ± 4.9^{c}	77.5 ± 8.8^{bc}	85.0 ± 4.5^{ab}	92.5 ± 6.9^{a}	82.5 ± 9.4^{abc}	91.7 ± 6.8^{a}
Weight gain (g $week^{-1}$)	0.48	0.46	0.47	0.47	0.44	0.47
SGR (% d^{-1})	1.78 ± 0.1^{a}	1.71 ± 0.1^{a}	1.75 ± 0.1^{a}	1.75 ± 0.07^{a}	1.67 ± 0.1^{a}	1.73 ± 0.1^{a}
FCR	7.8 ± 1.3^{a}	8.3 ± 1.6^{a}	4.7 ± 0.6^{ab}	3.2 ± 0.2^{bc}	4.2 ± 0.8^{ac}	2.25 ± 0.4^{c}
Protein efficiency ratio	0.4 ± 0.06^{c}	0.3 ± 0.07^{c}	0.6 ± 0.07^{bc}	0.8 ± 0.07^{ab}	0.7 ± 0.09^{bc}	1.12 ± 0.2^{a}

that not all added phosphates have the same digestibility and plant protein sources can cause concern due presence of antinutritional factors.

Survival, weight gain, and hemocyte count all remained stable, perhaps partly because leaching was controlled to avoid any artifact, such as a loss of phosphorus supplied in soluble form, which is more digestible than regular dicalphos (Cuzon & Aquacop 1982). Phosphorus was incorporated in two main forms: soluble, such as monopotassium phosphate (Deshimaru 1981), or low solubility dicalphos, and ensured sufficient weight gain (0.4 g $week^{-1}$) and survival.

Molt stages (Bourgeois & Cuzon 1975, Vijayan & Diwan 1996) have rarely been considered, and reference to a thick skeleton (Drach & Jacques 1976) did not coincide necessarily with the denomination for a soft-shell species (Baticados et al. 1986). In microcosm tests (Gaxiola & Cuzon 2015), the number of exuvia per tank per month with the Japanese feed Nippaï was higher than with the standard FM diet (150 *vs.* 60 molts respectively for four weeks in triplicate). Part of the reason derived from some formulations for *P. japonicus* contained up to 19% minerals in the 1970s. The molt results in a waste of minerals even though part of the calcium is retrieved prior to exuviation (Li & Cheng 2012). At present, the feed industry proposed a ready-made pre-mixed formula that tends to decrease the attention on critical nutrients such as trace elements. For example, in *Farfantepenaeus californiensis*, a severe mineral imbalance has led to a "molt death syndrome" and a strong cannibalism trend at molt (Gallagher et al. 1978).

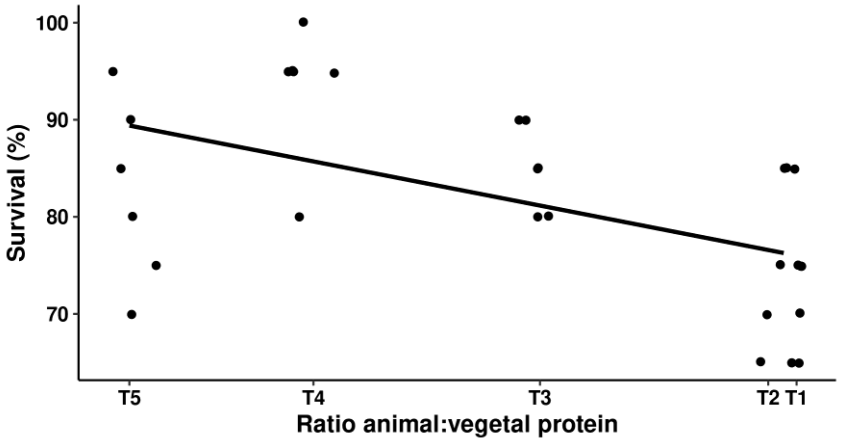

Figure 1. Survival of juvenile *Penaeus vannamei* in the function of ratio animal:vegetal protein in the diet. The solid line represents the prediction of the regression equation calculated: $y = 91.52\text{-}18.29x$. The points indicated the survival of each tank in the experiment.

Table 4. Digestive enzymes (U per g hepatopancreas tissue). Same letters indicate no significant difference between treatments ($P < 0.05$). Initial weight 2.56 ± 0.31g. The same letters in the same row indicate no significant difference between treatments ($P < 0.05$).

Digestive enzymatic activity	T_1	T_2	T_3	T_4	T_5	T_6
Soluble protein	15.4 ± 1.1[a]	14.2 ± 4.4[a]	15.0 ± 3.2[a]	15.49 ± 7.3[a]	13.1 ± 9.3[a]	11.6 ± 4.3[a]
Trypsin	5.3 ± 2.0[a]	6.2 ± 2.0[a]	6.3 ± 1.0[a]	5.3 ± 1.6[a]	12.6 ± 4.8[a]	7.4 ± 0.9[a]
Chymotrypsin	16.8 ± 3.3[a]	8.4 ± 2.8[a]	23.8 ± 7.4[a]	14.8 ± 4.8[a]	15.7 ± 3.9[a]	10.0 ± 3.8[a]

Table 5. Muscle texture and sodium dismutase activity of *P. vannamei* juveniles fed diets with fishmeal replacement. Different letters in superscript in the same row indicate significant differences between treatments ($P < 0.05$).

Muscle	T_1	T_2	T_3	T_4	T_5	T_6
Super oxide dismutase	10.5 ± 2.0[a]	10.4 ± 1.3[a]	9.5 ± 0.6[b]	9.1 ± 1.7[b]	10.1 ± 2.4[ab]	10.4 ± 1.2[a]
Shear strength (mJ)	79.4 ± 12.1[a]	73.5 ± 13.1[a]	81.3 ± 13.1[a]	78.8 ± 21.9[a]	69.7 ± 10.1[a]	74.5 ± 12.0[a]

P concentration in solution (H_3PO_4, H_2PO_4, HPO_4^{--}, PO_4^{-}) is pH dependent, and some phosphate sources (Horne & Goldman 1994), such as dicalphos, dissociate in acid pH; since the *P. vannamei* digestive tract is slightly alkaline (pH > 7), and the apparent digestibility coefficient as low as 20% will limit phosphorus release (Davis & Arnold 2000).

Optimal digestion occurred during enzymatic peak hours (Focken et al. 1998, Casillas-Hernández 2006), and fractioning meals would favor feed intake and digestion. Moreover, some digestive enzymes could have hydrolyzed phytate, as was seen with alkaline phosphatase in *P. japonicus* (Civera & Guillaume 1989), and improved weight gain. However, phytase facilitated minerals uptake from plant proteins such as soybean, canola, and wheat (Bulbul et al. 2015).

The potential lack of available phosphorus with a decrease in the FM component of the feed could be compensated by phytate-supplemented plant ingredients.

Also, energy partitioning helped verify that retained energy was similar for all treatments, including a commercial feed, to corroborate weight gain results. All parameters were at the same level except feed composition, but energy partition (Bureau et al. 2000) would not change so much, especially for maintenance (Jiménez-Yan et al. 2006); major variation, if any, would have been expected for HiE (Teshima et al. 1977). Within the range of dietary phosphorus tested here, it was not expected to see a difference in relation to the build-up of energy molecules that would have had an incidence on the energy budget.

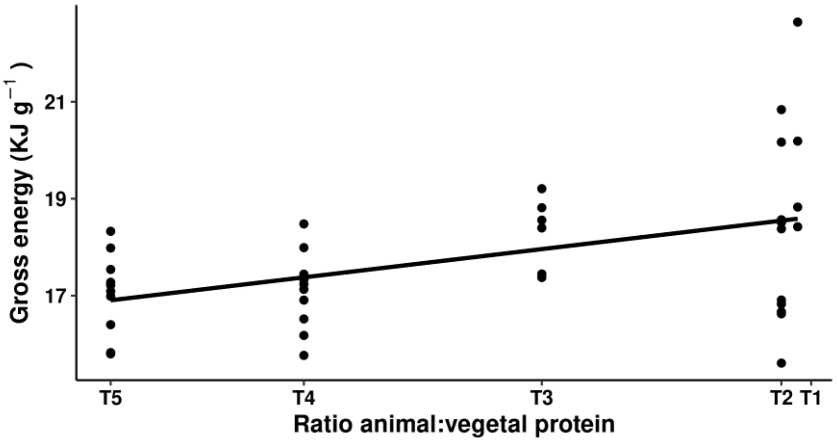

Figure 2. Gross energy of shrimp in function ratio animal:vegetal protein in the diet. The solid line represents the prediction of the regression equation calculated: $y = 16.63+2.34x$. The points indicated the gross energy of the shrimp in each tank in the experiment.

Table 6. Blood parameters of the juveniles fed diets with fishmeal replacement.

Blood parameters	T_1	T_2	T_3	T_4	T_5	T_6
Total haemocyt count $\times 10^4$	230 ± 70^a	240 ± 50^a	260 ± 30^a	240 ± 20^a	$290 \pm .60^a$	230 ± 60^a
Osmotic pressure (mOsm kg^{-1})	890 ± 31.4^a	922.5 ± 55.9^a	907 ± 33.4^a	900.5 ± 23.4^a	896.8 ± 35.6^a	918.3 ± 22.4^a

Hemolymph phosphatemia would be a key indicator, but little information (104 mg L^{-1}) was available (Shimizu et al. 2001). Such situations have led to mortality peaks, especially when molting. Davis et al. (1993) found calcification to decline in shrimp lacking trace minerals and particularly Se. Finally, with respect to phosphate measured on cultured shrimp gave values well below (35 mg L^{-1}) and connected with mortalities at molt. Moreover, at present, the such parameter is not so easy to address because of rapid clotting, and variability can be high in the absence of molt stage determination and could miss in further studies. A range of variation from 35 to 100 mg L^{-1} was observed in the floating cage in the lagoon where P-PO_4^- remained at 0.05 ppm except during the rainy season, after 100 days culture, a peak of mortality (Goguenheim 2014; *comm. pers.*) noticed in premolt stage and phytoplankton blooms can modify the culture conditions (Martin & Bianchi 1980).

Metabolism: the combination of taurine and microbial phytase at 1000 U kg^{-1} inclusion could stabilize the nutritional input in case of drastic FM reduction in current formulations. *P. vannamei* juveniles stood in a microcosm. A progressive decrease in FM content was compensated to a certain extent by the presence of taurine (Yue et al. 2013).

Physiology and muscle texture (shear strength) indicated diet position without altering performances. This status looking for maximum weight gain at molt is achieved when environmental conditions and feed composition will meet physiological requirements. Such status is certainly difficult to sustain under intensive culture, whatever peneid species.

Immune response, stress: parameters taken into account allowed us to see that juveniles were not in stress situation considering the variations of an enzyme of a lipid-soluble system ($P > 0.05$) that contribute to eliminating free radicals issued from lipid tissue (Cardona et al. 2016); it could have happened in a slight effect with commercial feed, but an immune response cannot be questioned here in spite of a decline in survival due to diet T_1.

FM limitation in shrimp feed brings protein and mineral nutrition aspects. Most of the experimental diets for grow-out do not contain more than 12-13% ash; on top of that, plant-based ingredients bring phytate. Trapped phosphorus is no longer available for shrimp, although one study displayed an action of alkaline phosphatase. Then, trials in oligotrophic lagoon waters showed a lack of soluble forms (PO_4^-) for juveniles raised in floating cages, for example, with relatively low ash content of 12-13% compared to 20%, half of which NaH_2PO_4 (Deshimaru 1981).

The present study, therefore, provides *P. vannamei* with an answer as to mineral filler to provide juvenile shrimp on the one hand and boost the ability to hydrolyze phytate by the action of exogenous phytase, while taking account of Ca or Na phosphate intake, preferably in soluble forms. In case it will be given dical ($ADC_{phosphorus}$, 16%), an increase in % incorporation is needed.

In large-scale production, a risk of phosphorus released by shrimp excretion or leaching from pellets. Such inputs can be controlled by an algal development or control via "floc," which will reduce total reactive phosphorus load. In this context, one can think of improving the performances of farmed shrimp, whatever species (*M. japonicus*, *P. vannamei*), while controlling mineral intake for a few months.

So, on a low dietary FM content (high in vegetable protein), options between soluble forms or less soluble phosphates with the use of phytases and attractants and appetizers would reduce inorganic load. There are ways for a better adequation between diet, growth, and environment.

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