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ORIGINAL ARTICLE

Aquaculture

Evaluation of a single-cell protein as a dietary fish meal substitute for whiteleg shrimp *Litopenaeus vannamei*

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Abstract
A 9-week feeding trial was conducted to evaluate the optimum dietary level of PROTIDE (PRO), a single-cell protein obtained from the bacteria *Corynebacterium ammoniagenes*, as a substitute for fish meal in the diet of whiteleg shrimp *Litopenaeus vannamei*. Five diets were formulated that replaced fish meal at 0% (PRO_0), 10% (PRO_2), 20% (PRO_4), 30% (PRO_6) and 40% (PRO_8). Fifty shrimp averaging 0.15 ± 0.02 g (mean ± SD) body weight were randomly distributed between 20 experimental tanks and fed one of the five experimental diets. At the end of the experiment, final weight, weight gain, specific growth rate and feed conversion ratio of shrimp fed PRO_0 and PRO_2 diets were significantly improved compared to those fed PRO_6 and PRO_8 diets ($P < 0.05$). The proximate composition of muscle and the whole body indicated an increase in crude protein content with an increase in dietary PRO level. These results suggest that, for whiteleg shrimp, the optimum dietary level of PRO when used as a replacement for fish meal should be greater than 10% (PRO_2) but less than 20% (PRO_4) without any additional dietary amino acid supplementation.

Keywords Growth performance · Crude protein content · Optimal diet · Feed production · Penaeid

Introduction

The whiteleg shrimp *Litopenaeus vannamei* is the most important cultivated shrimp species in the world and has the highest value of all traded crustacean products. The global production of whiteleg shrimp increased from 154,515 tons in 2000 to 4,155,827 tons in 2016 with more than an 18 billion US dollars (USD) increase in its aquacultural value during that time (FAO 2018). This species has several merits that make it more suitable for aquaculture than other penaeid shrimps, such as a relatively low dietary protein requirement, high density tolerance, and adaptability to wide ranges of salinity and temperature (Argue et al. 2002; Lightner et al. 2009; Moss et al. 2001, 2007, 2010).

The significant growth in shrimp aquaculture has led to a corresponding expansion of feed production for shrimp. Commercial shrimp feed formulations commonly include between 25 and 50% fish meal (FM), representing the primary and most expensive protein ingredient of these products (Gonzalez-Rodriguez and Abdo de la Parra 2004). In the mid-1990s, FM cost ca. 500 USD per million tons, but as of May 2018 the average cost had risen to 1,510 USD per million tons (World Bank Commodity 2018). The economic success of the aquaculture industry depends in part on a reduction in the use of FM in fish feed (Choi et al. 2004). Consequently, fish nutritionists have conducted several studies to evaluate the efficiency of various conventional and unconventional protein sources when used as substitutes for FM in fish feed. Some of these studies evaluated the substitution of FM by single-cell protein (SCP) in fish and shrimp diets (Beck et al. 1979; Davies and Wareham 1988; Lara-Flores et al. 2003; Glencross et al. 2014; Vizcaíno et al. 2014) and found that SCPs have significant potential for use in aquafeed formulations.

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SCPs are produced from single-cell microorganisms (e.g. bacteria, yeast, fungi, and algae) that utilize different carbon sources for growth (Ravinda 2000). SCPs are commonly sourced from the wastes produced by other agricultural industries including cereal husks, crop peel, cocoa processing wastes, sugar cane (bagasse), citrus, mango and coconut waste, etc. Some of the wastes comprise lignocelluloses, recalcitrant compounds that can resist enzymatic and microbial treatments (Karimi and Taherzadeh 2016). These wastes are of public and environmental concern due to the large quantities in which they are produced and the difficulty of their disposal. Converting these wastes into SCPs serves the dual purposes of mitigating degradation of the environment from waste accumulation and providing a good protein source for fish diets (Palmegiano et al. 2005). Bacteria present a promising source of SCP due to their rapid growth in various substrates, high protein value, and cellular vitamins and photopigments (Kantachote et al. 2005; Kornochalert et al. 2014; Chumpol et al. 2018). PROTIDE (PRO) is a new SCP concentrate obtained from the bacteria *Corynebacterium ammoniagenes* by a specific fermentation process (patented by CJ Cheiljedang, Republic of Korea). The nutrient profile of PRO closely resembles that of Peruvian and menhaden FM with a crude protein content of 64.7% on a dry matter basis. Moreover, the production of PRO does not rely on finite marine resources, which is a significant hurdle to sustainable aquaculture.

Given its good nutritional properties, PRO is of particular research interest for use as a FM substitute. However, it has yet to be evaluated for its potential in whiteleg shrimp diets. Therefore, the present study was conducted to evaluate the effects of a dietary SCP (PRO) as a substitute for FM, and its effects on the growth, whole-body proximate composition and amino acid content of whiteleg shrimp *L. vannamei*.

Materials and methods

Experimental design and diet preparation

Commercially available PRO was provided by CJ Cheiljedang (http://www.cjbio.net/html/product/application.asp). The Gram-positive aerobic bacteria *C. ammoniagenes* undergoes several fermentation processes with inosine-5′-monophosphate until the final product, PRO, is achieved (Fig. 1). The proximate composition and formulation of the diets are presented in Table 1. Five isonitrogenous (45% crude protein) and isocaloric (16.4 kJ gross energy/g diet) experimental diets were formulated to contain 0 (PRO_0), 2 (PRO_2), 4 (PRO_4), 6 (PRO_6) and 8 % (PRO_8) PRO to replace 0, 10, 20, 30 and 40% FM, respectively. These amounts were based on the results of a previous study (Chumpol et al. 2018). The proximate composition analysis and the amino acid profile of dietary PRO and FM are summarized in Tables 2 and 3, respectively. FM and dehulled soybean meal were included as the major protein sources, fish and soybean oil were the main lipid sources, while carbohydrates derived from wheat meal and corn starch were used to adjust the energy content of the diet.

Diet preparation and storage followed Bai and Kim (1997). First, dry ingredients were mixed and then soybean oil, fish oil, and water (30% of diet) were added to the mixture to form a paste. A laboratory mincing machine (Shinsung, Seoul) was used to obtain uniform pellets of 0.6-mm and 1.5-mm diameter. The feed pellets were dried and stored at − 20 °C until use.

Experimental shrimp and feeding trial

Post-larval whiteleg shrimp were transported from the Taean hatchery of the National Fisheries Research and Development Institute to the Feeds and Foods Nutrition Research Centre, Pukyong National University, Busan, Republic of Korea for the feeding trial. All the shrimp were fed a commercial shrimp diet (DongA One, Seoul) for 1 week for acclimation to the experimental conditions. Fifty shrimp with an initial average weight of 0.15 ± 0.02 g (mean ± SD) were randomly distributed between 20 tanks. Each tank was then randomly assigned to one of the four replicated treatments for five experimental diets. Shrimp were fed four times per day (at 0900, 1200, 1500 and 1800 hours) for 9 weeks at a fixed rate of 7% of body weight per day. Total shrimp weight in each experimental tank was determined every 3 weeks and the feeding rate was adjusted accordingly. This feeding trial was conducted using a semi-recirculating system, supplied with filtered seawater at the rate of 0.8 l min^{-1} from a reservoir tank (8 tons). Aeration was provided to maintain dissolved oxygen at 6.0 ± 0.5 mg l^{-1}. Water temperature, salinity and pH during the experiment were maintained at 27 ± 0.5 °C, 32 ± 1 practical salinity units and 7.5 ± 0.3, respectively. Each rearing tank was siphoned every day in the morning, and dead shrimp removed, weighed, and recorded daily.

Sample collection and analyses

When the feeding trial was completed, all the shrimp from the experimental tanks were weighed and counted in order to calculate weight gain (WG), specific growth rate (SGR), feed conversion ratio (FCR), protein efficiency ratio (PER) and survival.

After the final weighing, six shrimp were randomly collected from each tank and frozen at − 20 °C for whole-body and muscle proximate analysis. Muscle samples were collected by removing the exoskeleton, head, telson and other internal organs. Proximate composition analyses of

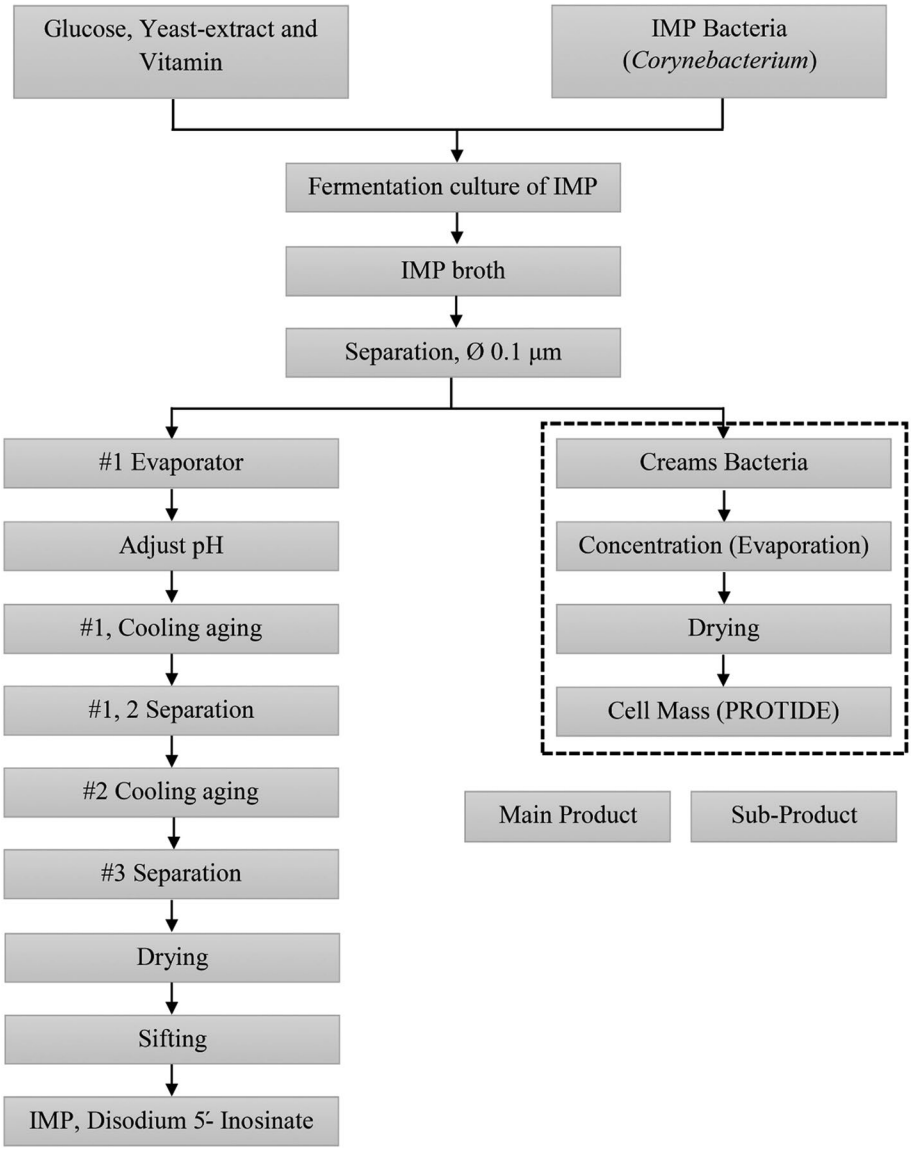

Fig. 1 PROTIDE manufacturing procedure with inosine-5′-monophosphate (provided by CJ Cheiljedang, Seoul, Republic of Korea)

experimental diets and shrimp body were performed using the standard methods of the Association of Official Analytical Chemists (AOAC) (2005). Samples of diets and shrimp were dried at 105 °C to a constant weight to determine their moisture content. Ash content was determined by incineration at 550 °C, crude protein by the Kjeldahl method (nitrogen × 6.25) after acid digestion and crude lipid by Soxhlet extraction using the Soxhlet system 1046 (Tacator, Hoganas, Sweden). Amino acid analyses for PRO, fish meal and shrimp muscle were performed according to Macchi et al. (2000). Freeze-dried samples (0.02 g) were hydrolysed with 15 ml of 6 N HCl at 110 °C for 24 h. Hydrolysed samples were evaporated, recovered in sodium citrate buffer (0.2 N, pH 2.2) and filtered (0.2 μm). Following ninhydrin reaction, sample absorbance was measured at 570 nm and 440 nm using a S433 amino acid analyser (Sykam, Eresing, Germany). Samples were hydrolysed using performic acid for methionine and cystine analysis.

Sample collection and analyses

Assumptions for statistical tests such as normality and homogeneity of variances were checked. Data were analysed according to one-way ANOVA to test the effects of the dietary treatments. When significant differences were observed in the treatments, a least significant difference test for multiple comparisons was performed. Treatment effects were considered significant at the $P < 0.05$ level. All statistical analyses were carried out by SAS version 9.0 software (SAS Institute, Cary, NC).

Table 1 Formulation and proximate compositions of the experimental diets (% dry matter; *DM*)

Ingredients	Diets				
	PRO_0	PRO_2	PRO_4	PRO_6	PRO_8
Fish meal[a]	20.0	18.0	16.0	14.0	12.0
PROTIDE (PRO)[b]	0	2.0	4.0	6.0	8.0
Dehulled soybean meal[a]	39.8	39.9	39.9	39.9	40.0
Squid liver powder[a]	5.0	5.0	5.0	5.0	5.0
Wheat gluten meal[a]	9.0	9.0	9.0	9.0	9.0
Wheat flour[a]	14.9	14.8	14.9	14.9	14.8
Corn starch[a]	4.5	4.5	4.5	4.5	4.5
Lecithin[a]	1.0	1.0	1.0	1.0	1.0
Soybean oil[a]	1.5	1.5	1.4	1.4	1.4
Fish oil[c]	2.0	2.0	2.0	2.0	2.0
Vitamin mix[d]	1.0	1.0	1.0	1.0	1.0
Mineral mix[e]	1.0	1.0	1.0	1.0	1.0
Calcium phosphate[f]	0.3	0.3	0.3	0.3	0.3
Proximate composition (% DM)					
Moisture	7.27	7.23	7.05	7.25	7.03
Crude protein	44.8	45.0	45.5	45.3	45.2
Crude lipid	7.6	7.3	6.9	6.9	7.2
Crude ash	9.0	8.8	8.5	8.2	8.0

[a]Peruvian fish meal, menhaden: crude protein 64.7% (DM basis) (Suhyup Feeds, Uiryeong, Republic of Korea)

[b]CJ Feed, Seoul, Republic of Korea

[c]Jeil Feed, Hamman, Republic of Korea

[d]Vitamin mix (mg/kg in diet): ascorbic acid, 300; DL-calcium pantothenate, 150; choline bitate, 3000 l inositol, 150; menadione, 6; niacin, 150; pyridoxine hydrochloride, 15; riboflavin, 30; thiamine mononitrate, 15; DL-α-tocopherol acetate, 20 1; retinyl acetate, 6; biotin, 1.5; folic acid, 5.4; cobalamin, 0.06

[e]Mineral mix (mg/kg in diet): NaCl, 437.4; $MgSO_4 \cdot 7H_2O$, 1379.8; $ZnSO_4 \cdot 7H_2O$, 226.4; iron citrate, 299; $MnSO_4$, 0.016; $FeSO_4$, 0.0378; $CuSO_4$, 0.00033; calcium iodate, 0.0006; MgO, 0.00135; $NaSeO_3$, 0.00025

[f]$CaHPO_4$ (Sigma-Aldrich, Yongin, Republic of Korea)

Table 2 Proximate composition of fish meal and PRO used in the experimental diets (% DM)

Ingredients	Moisture	Protein	Lipid	Ash
PRO[a]	3.21	63.6	8.77	10.0
Fish meal[b]	3.01	64.7	8.41	24.2
Pooled SEM[c]	0.21	2.01	0.57	1.03

Results reported on a DM basis. For abbreviations, see Table 1

[a]CJ Feed

[b]Peruvian fish meal, menhaden: crude protein 64.7% (DM basis) (Suhyup Feed)

[c]$SD/\sqrt{n}$

Table 3 Amino acid composition of fish meal and PRO

Amino acids	PRO	Fish meal	Pooled SEM
Asp	8.01 a	7.32 b	0.20
Thr	2.66	2.69	0.03
Ser	2.59 a	2.20 b	0.11
Glu	8.89 a	7.84 b	0.30
Pro	2.09 b	2.83 a	0.22
Gly	3.40 b	4.36 a	0.28
Ala	5.63 a	3.75 b	0.54
Val	3.87 a	3.00 b	0.26
Ile	2.85 a	2.45 b	0.12
Leu	4.88 a	4.15 b	0.22
Tyr	1.04 b	1.59 a	0.16
Phe	2.75 a	2.37 b	0.11
His	2.30 a	1.86 b	0.13
Lys	2.03 b	4.75 a	0.79
Arg	2.63 b	3.46 a	0.24
Cys	0.12 b	0.32 a	0.06
Met	0.50 b	0.78 a	0.08

Results of one-way ANOVA. Results reported on a DM basis; averages of duplicates. For abbreviations, see Tables 1 and 2

In each row, means with *different lowercase letters* are significantly different ($P < 0.05$)

Results

Growth performance

The growth performance and survival of whiteleg shrimp fed the five experimental diets for 9 weeks are shown in Table 4. At the end of the feeding trial, the weights of shrimp fed the PRO_2 diet were significantly higher than those fed the PRO_6 and PRO_8 diets ($P < 0.05$). However, there were no significant differences in the final weight among shrimp fed the PRO_0, PRO_2 and PRO_4 diets, among shrimp fed the PRO_0, PRO_4 and PRO_6 diets, or among those fed the PRO_4, PRO_6 and PRO_8 diets ($P > 0.05$). WG and SGR of shrimp fed the PRO_0 and PRO_2 diets were significantly higher than those fed PRO_6 and PRO_8 diets. There were no significant differences in WG and SGR among shrimp fed the PRO_0, PRO_2 and PRO_4 diets, or among those fed the PRO_4, PRO_6 and PRO_8 diets. The FCR was significantly higher for shrimp fed the PRO_4, PRO_6 and PRO_8 diets compared to those fed the PRO_0 and PRO_2 diets, but the differences in FCR were not significant between shrimp fed the PRO_0 and PRO_2 diets. PER was significantly higher for shrimp fed the PRO_0 and PRO_2 diets than for those fed the PRO_4, PRO_6, and PRO_8 diets. However, there were no significant differences in PER among shrimp fed the PRO_4, PRO_6 and PRO_8 diets. The survival of shrimp fed the PRO_6 and PRO_8 diets was significantly higher than that of shrimp fed the PRO_0 and PRO_2

Table 4 Growth performances of whiteleg shrimp fed five different experimental diets for 9 weeks (% of DM basis)

Parameters	Diets					Pooled SEM
	PRO_0	PRO_2	PRO_4	PRO_6	PRO_8	
FW[a]	1.15 ab	1.16 a	1.11 abc	1.05 bc	1.03 c	0.02
WG[b]	638 a	647 a	580 ab	551 b	547 b	14.83
FCR[c]	1.59 b	1.56 b	1.80 a	1.84 a	1.92 a	0.05
PER[d]	1.35 a	1.35 a	1.15 b	1.13 b	1.09 b	0.03
SGR[e]	3.63 a	3.65 a	3.49 ab	3.41 b	3.39 b	0.04
Survival (%)[f]	72.0 b	73.0 b	77.0 ab	82.0 a	84.0 a	1.50

Values are means from quadruplicate groups of shrimp. *In each row*, means with *different lowercase letters* are significantly different ($P < 0.05$)

FW Final weight, *WG* weight gain, *FCR* feed conversion ratio, *PER* protein efficiency ratio, *SGR* specific growth rate; for other abbreviations, see Tables 1 and 2

[a]FW = FW/final number of shrimp

[b]WG = (final weight − initial weight) × 100/initial weight

[c]FCR = (dry feed intake/wet weight gain)

[d]PER = weight gain/dietary protein intake

[e]SGR = (ln final weight − ln initial weight)/days × 100

[f]Survival (%) = (initial no. shrimp − final number no. shrimp) × 100/initial no. shrimp

diets. However, there were no significant differences in survival among shrimp fed the PRO_0, PRO_2 and PRO_4 diets or among those fed the PRO_4, PRO_6 and PRO_8 diets.

Whole-body and muscle proximate composition

Table 5 shows the proximate composition of whiteleg shrimp fed the five experimental diets for 9 weeks. Whole-body crude protein contents of shrimp fed PRO_4, PRO_6 and PRO_8 diets were significantly higher than those of shrimp fed the control diet. However, there were no significant differences among shrimp fed PRO_4, PRO_6 and PRO_8 diets, among shrimp fed PRO_2, PRO_4 and PRO_8 diets, or among those fed PRO_0 and PRO_2 diets. Furthermore, the muscle protein contents of PRO_0 and PRO_2 groups were significantly lower than those of all other groups.

Whole-body crude lipid contents of shrimp fed the PRO_8 diet were significantly higher than those of shrimp in all the other dietary treatments. The control group showed the lowest whole-body lipid content, although this was not significantly different from the content in the PRO_4 group. However, the muscle lipid contents of shrimp fed the PRO_0 and PRO_2 diets were significantly higher than those of shrimp fed the PRO_2, PRO_6 and PRO_8 diets.

The whole-body crude ash contents of shrimp fed the PRO_6 and PRO_8 diets were significantly higher than those

Table 5 Whole-body and muscle proximate composition of whiteleg shrimp fed five different experimental diets for 9 weeks (% of DM basis)

Parameters	Diets					Pooled SEM
	PRO_0	PRO_2	PRO_4	PRO_6	PRO_8	
Crude protein						
Whole	73.76 c	73.94 bc	74.72 ab	75.22 a	74.42 ab	0.19
Muscle	85.36 b	85.70 b	86.95 a	87.26 a	87.94 a	0.34
Crude lipid						
Whole	2.86 b	2.64 c	2.70 bc	2.69 c	3.54 a	0.11
Muscle	2.20 a	1.57 b	0.99 e	1.08 d	1.30 c	0.15
Ash						
Whole	13.83 ab	13.51 c	13.66 bc	14.00 a	14.08 a	0.07
Muscle	6.92 ab	6.96 a	6.95 a	6.81 b	6.81 b	0.03
Moisture						
Whole	78.07	77.03	77.40	78.08	77.37	0.19
Muscle	76.69	76.49	76.75	76.96	76.70	0.07

Values are means from quadruplicate groups of shrimp. *In each row*, means with a *different lowercase letter* are significantly different ($P < 0.05$). For abbreviations, see Tables 1 and 2

fed the PRO_2 and PRO_4 diets. However, there were no significant differences among shrimp fed the PRO_0, PRO_6 and PRO_8 diets, among shrimp fed the PRO_0 and PRO_4 diets, or among those fed the PRO_2 and PRO_4 diets. Furthermore, the muscle ash contents of shrimp fed the PRO_2 and PRO_4 diets were significantly higher than those fed the PRO_6 and PRO_8 diets. Whole-body and muscle moisture contents did not show any significant differences among shrimp fed any of the experimental diets.

Muscle amino acid composition

The muscle amino acid compositions of whiteleg shrimp are presented in Table 6. Although significant differences were found for some of the amino acids, no clear trends were observed. Serine, glycine and alanine levels of shrimp fed the PRO_8 diet were significantly higher than levels of those fed the PRO_0, PRO_2, PRO_4 and PRO_6 diets. The levels of valine, isoleucine, leucine, phenylalanine and lysine of shrimp fed the PRO_0, PRO_2, PRO_4 and PRO_6 diets were significantly higher than those of shrimp fed the PRO_8 diet. Also, the glutamine and proline levels of shrimp fed the PRO_4 and PRO_6 diets were significantly higher than those of shrimp fed the PRO_0, PRO_2 and PRO_8 diets. The asparagine level of shrimp fed the PRO_4 diet was significantly higher than that of shrimp fed the PRO_8 diet. Furthermore, the cysteine level of shrimp fed the PRO_6 diet was significantly higher than that of shrimp fed the PRO_0 diet. However, there were no significant differences in threonine, tyrosine and methionine levels among shrimp fed the different experimental diets.

Discussion

In the present study, the formulation and proximate composition of the diets are comparable to those of commercial diets used for whiteleg shrimp. Also, the proximate and amino acid compositions of dietary PRO closely resemble those of dietary fish meal (Tables 2, 3), as indicated by the results obtained for growth and survival with no visibly deleterious effects on shrimp health. During the experiment, shrimp readily consumed all the feed, indicating that the experimental diets were palatable to them.

Research on the potential use of SCP in fish diets started in the late 1970s (Beck et al. 1979; Bergstrom 1979; Dabrowski et al. 1980; Davies and Wareham 1988); because of the encouraging results obtained, research is still ongoing in this field. In Mozambique tilapia *Oreochromis mossambicus* and sea bass *Dicentrarchus labrax* up to 40% and 50% FM was replaced with an industrial SCP and brewer's yeast, respectively (Davies and Wareham 1988; Oliva-Teles and Goncalves 2001). Gamboa-Delgado et al. (2016) used torula yeast *Candida utilis* as a protein source in the diet of whiteleg shrimp and succeeded in replacing 60% of the FM with this. Replacement of up to 45% FM with yeast extract in the diet of *L. vannamei* had no adverse effects on the digestibility of the feed or the growth of the shrimp

Table 6 Muscle amino acid composition of whiteleg shrimp fed five different experimental diets for 9 weeks (% of DM basis)

Amino acids	Diets					Pooled SEM
	PRO_0	PRO_2	PRO_4	PRO_6	PRO_8	
Asp	12.06 ab	12.47 ab	12.71 a	12.55 ab	11.88 b	0.12
Thr	2.88 a	2.90 a	2.96 a	2.89 a	2.82 a	0.02
Ser	2.73 b	2.65 b	2.69 b	2.61 b	3.00 a	0.05
Glu	12.28 ab	12.40 ab	12.76 a	12.62 a	12.03 b	0.10
Pro	5.73 ab	5.88 ab	6.11 a	5.97 a	5.36 b	0.10
Gly	6.25 b	6.45 b	6.65 b	6.82 b	7.74 a	0.18
Ala	3.03 b	3.13 b	3.2 b	3.22 b	4.75 a	0.22
Val	3.63 a	3.68 a	3.77 a	3.74 a	3.36 b	0.05
Ile	3.55 a	3.53 a	3.63 a	3.64 a	3.23 b	0.05
Leu	6.16 a	6.18 a	6.27 a	6.28 a	5.78 b	0.07
Tyr	1.53 a	2.66 a	2.72 a	2.73 a	2.51 a	0.24
Phe	3.49 a	3.49 a	3.55 a	3.54 a	3.22 b	0.04
His	2.11 ab	2.19 a	2.16 ab	2.08 b	1.93 c	0.03
Lys	7.11 a	7.06 a	7.21 a	7.18 a	6.65 b	0.08
Arg	6.23 ab	6.28 ab	6.41 ab	6.48 a	6.08 b	0.06
Cys	0.40 b	0.43 ab	0.43 ab	0.48 a	0.42 ab	0.01
Met	0.86 a	0.89 a	0.84 a	0.93 a	0.92 a	0.02

Values are means from quadruplicate groups of shrimp. *In each row*, means with *different lowercase letters* are significantly different ($P < 0.05$). For abbreviations, see Tables 1 and 2

(Zhao et al. 2015). Although few studies have investigated bacteria as a SCP in the diet of shrimp, it has been well proven that other types of SPC can have a positive effect on their immune system and growth (Biswas et al. 2012; Deng et al. 2013). Viola and Zohar (1984) found that a bacterial SCP, Pruteen, which was used to replace 50% of the dietary FM protein for hybrid tilapia *Oreochromis niloticus* × *Oreochromis aureus*, was beneficial to its immune system and growth. Other studies have used a SCP to replace a smaller percentage of FM in feed. In a recent study, Rosales et al. (2017) reported that SCP (dried yeast) can be used to replace up to 50% of the crude protein (40%) provided by half FM and half soybean meal in the diet of red drum *Sciaenops ocellatus*; in effect, SCP replaced approximately 10% of the FM in this experiment. Our experiment showed that up to 20% of the fish meal in a practical diet for whiteleg shrimp could be effectively replaced by PRO, a SCP derived from the bacteria *Corynebacterium ammoniagenes*, without any significant reduction in WG. In accordance with our results, Lunger et al. (2006) concluded that 25% of feed FM could be replaced with SCP without growth reduction in cobia *Rachycentron canadum*. However, 15% of yeast-based SCPs in the diet of Red-Stirling tilapia *Oreochromis niloticus* (Ribeiro et al. 2014) and gray mullet *Mugil cephalus* (Luzzana et al. 2005) resulted in growth reduction. The adverse effects on growth in our and several other experiments that used SCPs as FM substitutes could be related to differences in target species, sources of SCP, feed formulations, and physicochemical conditions of the experiments. A more accurate comparison could be made by knowing the cost of each diet before and after FM substitution.

In the present study, an increasing trend of survival was observed with increasing PRO in the diet. To the best of our knowledge, only a few studies have observed significant relationships between bacterial SCPs and fish/shrimp survival. Christensen et al. (2003) found that application of whole-cell bacterial SPC had a statistically significant impact on a specific systemic immune response in mice. Chumpol et al. (2018) also showed that administration of bacterial SPC in the diet of *L. vannamei* increases its innate immunity, as evidenced by elevated superoxide dismutase and phenoloxidase activities. Therefore, in the present study, the observed trend of increased survival may have been due to the amplification of non-specific immune responses by PRO. According to the results of our study, up to 20% of FM can be substituted by PRO without a significant drop in growth factors such as WG, SGR and FW. However, substitution of more than 10% FM caused a reduction in PER and increased the FCR. Similar effects have been observed in other FM replacements studies (e.g. El-Sayed 1998) when replacing FM with shrimp meal, blood meal, meat and bone meal and poultry by-product meal, as the PER and FCR were significantly slowed down. PER is calculated from fish body mass gain and intake of protein, and defines the quality of protein in feed. In contrast, FCR defines the efficiency of converting feed into fish body mass (NRC 2011). Both of these values are closely related to the quality, digestibility and utilisation of feed. It is likely that there are multiple factors that influence these parameters in diets with a higher SCP content. For example, it was previously suggested that the cell walls of SCP are the main cause of low nitrogen digestibility in feed (Yamada and Sgarbieri 2005). Tlusty et al. (2017) replaced 55% of FM with a specific bacterial SCP from *Methylobacterium extorquens* in the diet of Atlantic salmon *Salmo salar* and reported lower digestibility of the SCP-containing diet.

The whole-body and muscle proximate composition of shrimp in this study did not seem to show a specific trend in response to different levels of dietary PRO, although the whole-body lipid content of shrimp fed 8.0% PRO was higher than that of shrimp fed all the other diets. Similar results were reported for red drum (Rosalesa et al. 2017), hybrid striped bass (Gause and Trushenski 2011) and rainbow trout (Hauptman et al. 2014) when FM was replaced with SCPs. This could have been related to the retention of lipids, and lipid storage, in the body of shrimp due to an imbalance in the protein composition of the PRO. In the present study, the muscle amino acid composition of whiteleg shrimp corresponded to the amino acid composition of PRO and FM based on dietary inclusion levels. In contrast, significantly lower levels of essential amino acids such as arginine, histidine, isoleucine, lysine, leucine, phenylalanine and valine were retained in the muscle of whiteleg shrimp fed higher amounts of PRO. This could indicate that the amino acid requirements of shrimp were not met by the higher level of PRO in the diet, as evidenced by shrimp growth reduction. Methionine and cysteine contents in the whole body of finfish and shellfish have been reported to be adversely affected in various fish meal replacement studies (Xie et al. 2016). Therefore, additional dietary supplementation of these amino acids in fish meal-replacement feed has been recommended as the justified approach to maintain their profile in shrimp. However, in the present investigation, none of the experimental diets were formulated with additional supplementation of methionine or cysteine. In order to achieve a higher percentage substitution of FM, future research should focus on the efficacy of methods that can be used to increase the digestibility of SCP (cell wall disruption) and provide essential amino acids in the diet at higher replacement levels. Most of the works reviewed here have evaluated FM replacements from a biological or nutritional point of view, whereas economic analyses of these protein sources have scarcely been undertaken. Therefore, it is highly recommended that future experiments should also focus on the economic consequences of FM replacement by SPCs.

In conclusion, the results of the present experiment indicate that the optimum level of PRO, as a replacement for fish meal, should be greater than 10% but less than 20% for whiteleg shrimp culture, without any additional dietary amino acid supplementation. PRO, derived from the bacteria *C. ammoniagenes*, can be recommended as a SCP for shrimp feed, providing farmers with an under-utilized and cost-effective protein source.

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