Aquaculture Reports 33 (2023) 101830

Contents lists available at ScienceDirect

Aquaculture Reports

journal homepage: www.elsevier.com/locate/aqrep

Effect of different types of bacterial single cell protein on feed intake, digestibility, growth and body composition of Pacific white shrimp (*Penaeus vannamei*)

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ARTICLE INFO

Keywords:
Novel ingredients
Bacterial meal
Fishmeal replacement/alternative
Methanotrophs
Protein source

ABSTRACT

This study assessed the potential of four bacterial (*Methylococcus capsulatus*) single cell protein (SCP) products as alternative protein sources for Pacific white shrimp (*Penaeus vannamei*) diets. A growth trial and a digestibility trial were undertaken, during which the bacterial SCP products were compared with a high-quality fishmeal and a soy protein concentrate, regarding their impact on ingredient digestibility, growth, feed intake and whole-body composition of juvenile *P. vannamei*. Seven diets were formulated; one reference diet (REF) and six test diets. The test diets consisted of 85% of the REF diet and 15% of a test ingredient. Ingredients tested were four bacterial SCP products (SCP1–4), which differed in processing conditions, fishmeal (FM) and soy protein concentrate (SoyProt). Growth and feed utilization were similar for *P. vannamei* fed either the FM diet or one of the bacterial SCP diets, whilst lowest growth and feed utilization were observed for shrimp fed the SoyProt diet. Final whole-body protein content did not differ between shrimp fed the FM diet or one of the four bacterial SCP diets. However, shrimp fed the SCP diets had a significantly higher final phosphorus body content and a higher phosphorus retention than shrimp fed the FM or SoyProt diets. This indicates a higher phosphorus availability in the bacterial SCP products compared to FM and SoyProt. Protein digestibility of the SCP products was similar to FM, whilst amino acid (AA) digestibility was comparable to FM for three of the four SCP products (SCP1, SCP2 and SCP4). The SCP3 product showed the lowest digestibility for most AA, indicating a possible influence of processing conditions on AA availability of bacterial SCPs. Overall, this study highlights that bacterial SCP originating from *M. capsulatus* is a viable alternative protein source for Pacific white shrimp diets, but processing conditions should be taken into account.

1. Introduction

Shrimp aquaculture has grown rapidly over the last decade, with Pacific white shrimp (*Penaeus vannamei*) being the most cultivated shrimp species globally (FAO, 2022). Due to the nutritional quality, high digestibility and palatability, fishmeal is still a major protein source used in shrimp diets, of which the dietary inclusion ranges between 5% and 40%, depending on the country (Tacon and Metian, 2008; Tacon et al., 2022). However, use of fishmeal is considered unsustainable among others due to limited supply, resource-use conflicts and environmental concerns. To keep up with the growing shrimp industry and the likewise increasing demand for high-quality shrimp feeds, there is thus an interest in sustainable, novel protein sources in shrimp diets (e.g., Amaya et al., 2007, Panini et al., 2017, Shao et al., 2019, Chen et al., 2021).

Several ingredients originating from either animals, plants or microorganisms have been tested as protein sources in shrimp diets (Sánchez-Muros et al., 2020; Nunes et al., 2022). Among protein sources of plant origin, soy products have been used widely, but the use of soy products also raises sustainability concerns (Malcorps et al., 2019; Song et al., 2021; Nunes et al., 2022) and feed-food competition (Mottet et al., 2017). In addition, most plant ingredients contain anti-nutritional factors (Tacon and Basurco, 1997; Krogdahl et al., 2022) and other adventitious toxins (Gonçalves et al., 2018), reducing the nutritional value of feeds.

Single cell proteins (SCP), which can be of algal, fungal or bacterial origin, have been suggested as alternative protein sources for aquafeeds

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https://doi.org/10.1016/j.aqrep.2023.101830
Received 8 June 2023; Received in revised form 31 October 2023; Accepted 1 November 2023
Available online 8 November 2023

(Glencross et al., 2020; Jones et al., 2020). Of the three SCP sources, bacterial SCPs have in general the highest protein levels (50–80% on a dry basis) (Glencross et al., 2020) and many studies have highlighted their potential for use in shrimp diets (Hamidoghli et al., 2019; Chen et al., 2021; Jintasataporn et al., 2021; Chen et al., 2022; Felix et al., 2023).

Methylococcus capsulatus (Bath) is a naturally occurring gammaproteobacteria which is known to be highly efficient for the production of bacterial protein, using methane for carbon and energy and ammonia as a nitrogen source (Bothe et al., 2002; Øverland et al., 2010). Promising results have been reported for bacterial protein meal based on primarily *M. capsulatus* as the alternative protein source partly replacing fishmeal in diets of many finfish, such as Atlantic salmon (*Salmo salar*) (Aas et al., 2006), Atlantic halibut (*Hippoglossus hippoglossus*) (Aas et al., 2007), Japanese yellowtail (*Seriola quinqueradiata*) (Biswas et al., 2020), rainbow trout (*Oncorhynchus mykiss*) (Rajesh et al., 2022), American eel (*Anguilla rostrata*) (Lu et al., 2023), spotted seabass (*Lateolabrax maculatus*) (Yu et al., 2023) and turbot (*Scophthalmus maximus*) (Zheng et al., 2023). For *P. vannamei,* Chen et al. (2021) reported that up to 45% replacement of fishmeal with *M. capsulatus* bacterial meal did not affect growth, whilst Jintasataporn et al. (2021) reported that even 100% replacement of fishmeal (15% included in their control diet) was possible as growth, survival and feed conversion ratio (FCR) were not affected. In addition, these studies also indicate that *M. capsulatus* derived bacterial meal can improve disease resistance of *P. vannamei* (Chen et al., 2021; Jintasataporn et al., 2021).

The success of a novel ingredient depends among others on its impact on palatability, feed intake and nutrient bioavailability (i.e., digestibility, incorporation in body tissue and growth) (Glencross et al., 2007; Glencross, 2020). Studies on the digestibility of *M. capsulatus* bacterial products for shrimp are scarce, but Felix et al. (2023) reported an apparent protein digestibility of more than 90% in *P. vannamei.* Functionality of SCP products do not only depend on characteristics of the microbial biomass itself, but the production process can have an influence as well. For instance, Tibbetts et al. (2017) and Teuling et al. (2019) showed that cell wall disruption of microalgae (*Chlorella vulgaris* or *Nannochloropsis gaditana*) can increase digestibility in Atlantic salmon and Nile tilapia. Agboola et al. (2021) demonstrated that besides the strain of yeast, downstream processing during yeast production could affect the potential to counteract enteritis in Atlantic salmon fry. For bacterial meal it has been reported that downstream processing by autolysis can improve digestibility in mink, whilst this was not the case for rainbow trout (Øverland et al., 2006). In addition, Biswas et al. (2020) did not find any specific effect of post-production processing of bacterial SCP in the Japanese yellowtail. Earlier studies focusing on *M. capsulatus* bacterial meal for *P. vannamei* diets evaluated for example different inclusion levels, but each study used a SCP product which was produced with a single processing method (Chen et al., 2021; Jintasataporn et al., 2021; Felix et al., 2023). It is unknown to what extent differences in processing methods could influence the functionality of bacterial SCP products for shrimp diets. Considering the above, the main objective of the current study was to assess the potential of four bacterial (*M. capsulatus*) SCP products, which differ in their production process, as alternative proteins for shrimp diets. The bacterial SCP products were compared with a high-quality fishmeal (LT-70) and a soy protein concentrate regarding the impact on ingredient digestibility, feed intake, growth and body composition of juvenile Pacific white shrimp (*P. vannamei*).

2. Materials and methods

A growth trial followed by a digestibility trial was conducted at the Aquaculture Research Facility (ARF) of the Wageningen University (Wageningen, The Netherlands). The same group of animals, diets and rearing conditions were used in both trials.

2.1. Diets

The four bacterial SCP products evaluated in this study were produced by String Bio Pvt. Ltd. (Bengaluru, India; https://www.stringbio.com). For all four products, the production process comprises the steps of fermentation, cell separation and drying, which were carried out in a centralized facility with large scale fermentation for the conversion of gaseous substrates into value-added products. The products were made from the gammaproteobacteria *Methylococcus capsulatus*, which were grown with methane as a carbon source, either derived from biogas or natural gas. All four SCP products were produced by the continuous aerobic fermentation process using String Bio's patented proprietary fermentation process (String Integrated Methane Platform; SIMP™ technology) as described in Subbian et al. (2021) (https://patents.google.com/patent/EP3455341A4/en).

Certain aspects of upstream and downstream processes varied, resulting in the four SCP products, referred to as SCP1, SCP2, SCP3 and SCP4 (Fig. 1). For SCP1, after the cell separation step, harvested biomass was subjected to a high temperature (135 °C) during the drying procedure (drum-drying). Although the drying procedure was the same as for SCP1, an additional step of hydrolysis was included after cell separation for both SCP2 and SCP3. This was done to enhance the peptide levels in these products. For SCP2, this additional step consisted of heating of the cell mass at 70 °C for 30 min before the drying step, whilst in the case of SCP3 an enzymatic hydrolysis step was included before the drying step. For SCP4, the same upstream and downstream processes were followed as for SCP1, with minor modifications in the media components. As osmotic stress can accommodate changes in the cytoplasmic water activity by accumulating osmoprotectants in bacteria (Cayley et al., 1992), the modifications in the media components were assumed to increase the levels of osmoprotectant in SCP4, as a result of osmotic stress.

To measure the digestibility of the four SCP products and compare them to that of fishmeal (fishmeal LT-70; FM) and soy protein concentrate (SoyProt), seven diets were formulated: one reference diet (REF) and six test diets (Table 1). The test diets consisted of 85% of the REF diet and 15% of the test ingredient. An 15% inclusion level was chosen to prevent potential negative effects of higher inclusion levels on either palatability, digestibility or both. The REF diet did not contain any fishmeal, which is currently not the case in commercial shrimp diets. However, there is a continuous trend in the reduction of fishmeal in aquafeeds (Naylor et al., 2021). In order to have relevant diets for the future, the REF diet was formulated to have no fishmeal. Additionally, in order to make a better comparison of the digestibility of the four SCP product test ingredients with the digestibility of the fishmeal test ingredient, it was also decided to not add any fishmeal to the REF diet. It should be noted that although the REF diet was high in plant-based

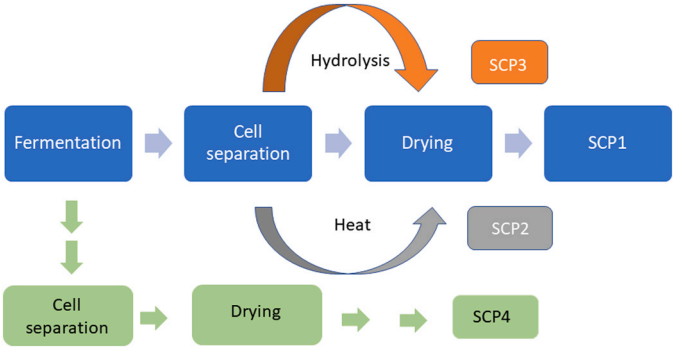

Fig. 1. Schematic overview of the differences in upstream and downstream processes resulting in the four different bacterial single cell protein products (i.e. SCP1, SCP2, SCP3 and SCP4) that were evaluated as potential feed ingredients for *Penaeus vannamei*.

Table 1
Ingredient composition of the reference diet and test diets.

Ingredient (%)	Reference diet	Test diet X
Test ingredient X[1]	**0.00**	**15.00**
Basal mixture	**100.00**	**85.00**
Whole wheat	43.15	36.68
Wheat bran	21.18	18.00
Wheat gluten	5.88	5.00
Soy protein concentrate	17.65	15.00
Krill meal	2.35	2.00
Soya lecithin	1.76	1.50
Salmon oil	2.35	2.00
Cholesterol	0.24	0.20
L-Lysine	0.47	0.40
DL-Methionine	0.47	0.40
L-Threonine	0.24	0.20
L-Tryptophan	0.12	0.10
$CaCO_3$	1.18	1.00
Monocalciumphosphate	1.76	1.50
Mineral and vitamin premix[2]	1.18	1.00
Yttrium oxide	0.02	0.02

[1] Tested ingredients are LT70 fishmeal, soy protein concentrate and four bacterial single cell protein products.
[2]Premix composition. Vitamins (IU or mg/g premix): vitamin B1, 3; vitamin B2, 3; vitamin B3, 7; vitamin B5, 7; vitamin B6, 6; biotin, 0.05; B-12, 0.015; folic acid, 0.6; vitamin C, 12.5; vitamin E, 30 IU; A-vitamin A palmitate, 300 IU; D-Rovimix D3-500, 300 IU; K_3 K-menadione sodium bisulphite (51%), 4; Inositol, 30; Choline, 100. Minerals (mg/g premix): Fe (as ferric sulphate), 5; Zn (as zinc sulphate), 7.5; Co (as cobalt sulphate), 0.005; Cu (as copper sulphate), 4; Se (as sodium selenite), 0.03; Mn (as manganese sulphate), 2; Mg (as magnesium sulphate), 30; I (as potassium iodate), 0.2.

ingredients, it did contain ~5% marine derived ingredients (salmon oil and krill meal; Table 1). Crystalline amino acids were supplemented to make the basal diet balanced regarding the amino acid profile (on g/kg crude protein basis; NRC, 2011). Yttrium oxide (Y_2O_3) was included in the basal diet (at 0.02%) as inert marker for digestibility calculations. The analyzed nutrient composition of the ingredients and experimental diets are summarized in Table 2 and Table 3, respectively. Diets (2 mm pellets) were produced by Research Diet Services (Wijk bij Duurstede, The Netherlands), by steam pelleting. Diets were stored at 4 °C throughout the duration of the experiment.

2.2. Shrimp, rearing conditions and housing facilities

Pacific white shrimp (*Penaeus vannamei*) were obtained from a commercial shrimp farm (CreveTec, Ternat, Belgium). Prior to the start of the experiment, shrimps were housed in 120-L tanks and fed a commercial shrimp diet (Crevetec Starter Feeds for Crustacean postlarvae and small shrimps; moisture 10% max; crude protein 54% min; lipids 12% min; HUFA 1.6% min; cholesterol 0.5% min; crude fiber 1% max; ash 10% max; phosphorous 1.2% min; http://www.crevetec.be/starterfeeds.htm). The same shrimps were used in both the growth and digestion trials (in succession). For both trials, the shrimps were fed the same experimental diet. A total of 28 tanks and 14 tanks were used for the growth trial and digestibility trial, respectively. The tanks were rectangular, glass tanks of 120-L (90 cm * 45 cm * 45 cm; l * w * h). At the start of the growth trial, each tank was stocked with 30 shrimps, with an average start weight of 2.70 ± 0.16 g. For the digestibility trial, tanks were stocked with 20 shrimps per tank (average start weight of 13.5 ± 1.5 g). All tanks were connected to the same recirculating system, consisting of a sump, settling tank and trickling filter, ensuring the same water quality for the inflow of each tank. Water flow through each tank was set at 2.5 L min^{-1} with a hand-held liquid rotameter. Tanks were closed by a lid and provided with an air stone. The photoperiod was set at 12 h light: 12 h dark.

Water quality parameters were monitored regularly to ensure that they remained within the pre-set ranges optimal for *P. vannamei*. Water temperature ranged between 27.1 and 28.7 °C (Testo 110; pre-set range 28.0 ± 0.5 °C), salinity ranged between 19.3‰ and 23.0‰ (WTW-multi 3430; pre-set range 20.0 ± 1.0‰) and pH ranged between 7.2 and 8.3 (WTW-pH 325; pre-set range 7–8). Dissolved oxygen levels of the common outflow ranged between 6.2 and 9.0 mg/L (WTW-Oxi 340i; pre-set > 4 mg/L). Merck tests were used to measure concentrations of total ammonia nitrogen (TAN, Merck Aquamerck Colometric Ammonium test), nitrite (NO_2, Merck Aquamerck Colometric Nitrite test) and nitrate (NO_3, Merck MQuant Nitrate test strips). TAN, nitrite and nitrate of the outflow remained below 0.5 mg/L, 0.1 mg/L and 100 mg/L respectively.

Table 2
Analyzed nutrient and amino acid (AA) composition of the test ingredients.

	Ingredients[1]					
	FM	SoyProt	SCP1	SCP2	SCP3	SCP4
Nutrient[2]						
Dry matter (DM, g/kg)	922	903	954	957	946	951
Crude protein (N * 6.25)	747	686	731	756	746	745
Crude fat	84	16	72	69	67	65
Gross energy (kJ/g DM)	20.7	20.3	23.2	23.4	23.6	23.1
Carbohydrates[3]	–	228	106	104	102	105
Ash	168	71	92	71	86	85
Phosphorus	24.1	8.0	21.7	17.6	19.8	20.0
Essential AA[2]						
Arginine	44	46	48	49	51	51
Histidine	21	23	22	22	23	23
Isoleucine	35	33	36	38	38	37
Leucine	53	50	56	57	59	58
Lysine	58	48	49	50	54	52
Methionine	23	12	20	21	22	21
Phenylalanine	32	36	36	37	37	37
Threonine	30	26	32	34	33	33
Tryptophan	6	7	11	11	11	9
Tyrosine	24	62	31	30	30	29
Valine	35	33	43	45	46	45
Non-essential AA[2]						
Alanine	49	28	49	52	52	52
Aspartic acid	71	78	62	67	65	67
Glutamic acid	100	122	73	78	81	80
Glycine	43	26	38	39	40	39
Proline	32	29	21	34	31	30
Serine	28	30	23	23	24	24
Sum of AA[2]	684	689	651	689	697	688

[1] FM, LT70 fishmeal; SoyProt, soy protein concentrate; SCP1-4, single cell protein product 1-4.
[2] Values are in g/kg dry matter (DM), unless stated otherwise.
[3] Carbohydrates, calculated as 1000 – (crude protein + crude fat + ash). Since carbohydrates are calculated indirectly, they may contain fractions that should not be classified as carbohydrates, such as phenolic compounds.

2.3. Experimental procedure

2.3.1. Growth trial

The growth trial lasted 10 weeks (70 days) and consisted of two 5-week growth periods (Period I; 36 days and Period II; 34 days), during which each diet was tested using 4 replicates. At the start of Period I, each tank was randomly stocked with 30 shrimps. In addition, an extra 30 shrimps were randomly selected, killed (ice-water) and stored (−20 °C) for initial whole-body composition analyses. At the start of Period II, the number of shrimps per tank was reduced to 25 shrimps. This was done to equalize the biomass post mortality of the first 5-week growth period and to lower the biomass per tank for the second growth period. However, in one of the tanks (SCP2 diet), 26 shrimps were stocked. This was noted the day after stocking, and not corrected (Supplementary Table 1).

At the start and end of both growth periods, shrimps were batch-weighed and counted, to determine growth and survival. The day before each weighing, shrimps were not fed in order to empty their gastro-intestinal tract and the temperature was gradually lowered to

Table 3
Analyzed nutrient and amino acid (AA) content of the experimental diets.

	Diets[1]						
	REF	FM	SoyProt	SCP1	SCP2	SCP3	SCP4
Nutrient[2]							
Dry matter (DM, g/kg)	894	900	907	910	909	912	912
Crude protein (N * 6.25)	290	361	350	359	365	362	362
Crude fat	59	64	53	59	65	63	63
Gross energy (kJ/g DM)	19.5	19.7	19.5	20.1	20.1	20.2	20.2
Carbohydrates[3]	588	497	533	515	507	510	508
Ash	63	77	64	67	63	66	66
Phosphorus	10.5	13.0	10.1	12.4	11.2	12.1	12.3
Essential AA[2]							
Arginine	17	20	22	23	22	22	21
Histidine	9	10	12	12	11	11	11
Isoleucine	13	16	17	17	16	17	16
Leucine	20	24	26	26	24	25	25
Lysine	18	24	24	23	22	23	23
Methionine	7	10	8	9	8	9	9
Phenylalanine	17	19	20	20	19	20	19
Threonine	12	14	15	16	15	15	15
Tryptophan	4	4	4	5	5	5	4
Tyrosine	9	11	12	12	11	12	11
Valine	13	16	17	19	17	18	17
Non-essential AA[2]							
Alanine	12	16	14	18	17	17	16
Aspartic acid	25	30	33	32	30	30	30
Glutamic acid	68	68	80	72	67	69	68
Glycine	11	15	13	15	14	14	13
Proline	18	19	18	17	19	18	15
Serine	12	13	15	14	13	13	13
Sum of AA[2]	286	328	349	350	331	339	326

[1] REF, reference diet; FM, LT70 fishmeal; SoyProt, soy protein concentrate; SCP1-4, single cell protein product 1-4.
[2] Values are in g/kg dry matter (DM), unless stated otherwise.
[3] Carbohydrates, calculated as 1000 – (crude protein + crude fat + ash). Since carbohydrates are calculated indirectly, they may contain fractions that should not be classified as carbohydrates, such as phenolic compounds.

23 °C to reduce stress during weighing. After weighing, the temperature during the first days within each period was gradually increased by 1 °C per day until 28 °C was reached. At the end of Period II, 10 shrimps per tank were randomly selected, killed (ice-water) and stored (−20 °C), for final whole-body composition analyses. The remaining shrimps were used in the digestibility trial.

During both growth periods, shrimps were fed to apparent satiation. Each tank was continuously fed over a period of 16 h, using a belt feeder, between 16.00 h and 8.00 h, which gradually dropped the feed into the tank. Every morning at 9.00 h for each tank the presence of uneaten pellets was recorded. On the first day of Period I, each tank was given 1 g of feed, whilst on the first day of Period II each tank was given 5 g of feed. If no feed refusal was recorded (at 9.00 h), the feeding level of that tank was increased by 1 g/d. In the case of feed refusal, the feeding level of that tank was reduced by 0.5 g/d. If during the days thereafter there was no feed refusal, the feeding level was increased by 0.2 g/d; if three days in a row, no feed refusal was recorded for a tank, the feeding level was increased by 0.5 g/d. Using this procedure, feed spillage was minimized in order to have a proper estimate of feed intake.

2.3.2. Digestibility trial

The remaining shrimps of the growth trial were used in the digestibility trial. The digestibility trial lasted 10 weeks, which included 1 week of acclimatization and 9 weeks of feces collection, during which each diet was tested using 2 replicates. At the start, shrimps were redistributed over the tanks, in such a way that each tank was stocked with 20 shrimps, which had the same diet during the growth trial.

Shrimps were fed a fixed amount of feed (10 g $tank^{-1}day^{-1}$), aimed to be close to satiation. During the acclimatization period, for each tank, the daily amount of feed given was increased from 6 g to 10 g, in steps of 1 g/d. Feed was provided to the tank over a period of 16 h using a belt feeder, between 16.00 h and 8.00 h, which gradually dropped the feed into the tank.

The faeces was collected every other day (i.e. 3 times a week) per tank. The night before faeces collection, tanks were fed 7 g feed, using a belt feeder. The remaining 3 g of feed were given in the morning, by hand, over a period of 1 h. After the 1 h feeding, tanks were cleaned by siphoning out leftover pellets, old faeces and exoskeletons. One hour after cleaning, faeces were collected by siphoning the faeces out of the tank. After collection, faeces were rinsed twice with deionized water, and stored in the freezer (−20 °C).

2.4. Analyses

Prior to analyses, shrimps were freeze dried and ground using a mixer mill (Retsch Germany, model ZM2000). Chemical composition of shrimps, diets and ingredients were determined according to ISO-standard analysis for dry matter (DM; 103 °C for 6 h; ISO 6496, 1983), crude ash (550 °C for 4 h; ISO 5984, 1978), crude fat (Soxhlet method using petroleum ether for crude fat extraction; ISO 6492), energy (bomb calorimeter; IKA® werke, C7000; IKA analysentechnik, Weitershem, Germany; ISO 9831, 1998) and crude protein (Kjeldahl-method; ISO 5983, 1997; as N x 6.25). Yttrium (Y) and phosphorus (P) were analyzed after H_2SO_4/H_2O_2/Se destruction, using inductively coupled plasma-mass spectrometry (ICP-OES) according to the standard NEN 15510 (2007).

Fecal samples were pre-dried at 70 °C (96 h), pooled per tank, and ground using a bullet mill (Retsch Germany, model MM2000, 11.55 mm bullets). For the digestibility calculations, P, Y and total nitrogen content in fecal samples were determined after H_2SO_4/H_2O_2/Se destruction using inductively coupled plasma-mass spectrometry (ICP-OES) according to the standard NEN 15510 (2007) for P and Y and segmented flow analysis (SFA-Nt) for the total nitrogen. Gross energy was determined for feed, ingredients and feces by combustion in a calorimeter

(bomb calorimeter; IKA® werke, C7000; IKA analysentechnik, Weitershem, Germany; ISO 9831, 1998). Amino acids were analyzed by String Bio Pvt. Ltd. (Bengaluru, India) in the feed, ingredients and fecal samples. The amino acid profile of the samples of diets and whole shrimp were separated and quantified using High-Performance Liquid Chromatography (Model: 1260 Infinity II with a quaternary pump, Agilent Technologies) equipped with Advance Bio AAA column (4.6 × 100 mm, 2.7 μm, Agilent Technologies). 100 mg of sample was hydrolyzed with 10 ml of 6 N HCl at 110 °C for 24 h in a hot air oven and filtered using 0.22 μm PES filters and diluted with 0.1 N HCl before analysis by HPLC. Norvaline (2 mg) was used as an internal standard to calculate the recovery. Analysis of tryptophan involved prior hydrolysis with 5 ml of 4 M NaOH at 110 °C for 16 h in a hot air oven, neutralized and filtered with a 0.22 μm PES filter. Samples were pre-column derivatized using ortho-phthalaldehyde (OPA) and fluorenylmethoxy chloroformate (FMOC). Mobile phase for HPLC consisted of a mixture of A: 10 mM dibasic sodium phosphate and 10 mM sodium borate, pH 8.2 and B: Methanol: Acetonitrile: Water, 45:45:10 (v:v:v) with 1.0 ml/min flow rate. Tryptophan analysis was done using Poroshell 120 EC C18 column (4.6 × 250 mm, 4 μm, Agilent Technologies). The mobile phase for HPLC consisted of a mixture of 0.91 A (25 mM Sodium acetate, pH 7) and 0.09 B (Acetonitrile) with a 0.9 ml/min flow rate. The samples were monitored at 338 nm or 262 nm. Peak areas obtained from standards and samples were used to quantify the amino acids and expressed as relative content in percentage.

2.5. Calculations

Shrimp growth performance parameters were first calculated for period I and period II separately, after which the means were taken for the performance over the whole growth trial. Survival per tank (in %) was calculated as (Nf / Ni) x 100, where Nf is the final number of shrimps and Ni the initial number of shrimps per growth period. From the start and final biomass and shrimp numbers, individual body weights (initial body weight [Wi] and final body weight [Wf]) were derived. The growth (daily gain; in g/shrimp/d) was calculated per tank as the difference between Wi and Wf, divided by the duration of each growth period in days (d). Specific growth rate (SGR; in % body weight/d) was calculated as (ln (Wf) – ln (Wi)) x 100 / d. The daily feed intake (FI; in g/shrimp/d) was calculated as FI_{tot} / (n x d), where n is the number of shrimp per tank corrected for mortality and FI_{tot} the total amount of feed given to the tank. The feed conversion ratio (FCR) was calculated as average daily FI /daily gain.

Yttrium was used as inert marker to calculate the apparent digestibility coefficient (ADC; in %) of crude protein, gross energy, phosphorus, and AA of the diets, according to the following formula: ADC_{diet} (%) = (1 – ((Y_{diet}/Y_{feces}) x (N_{feces}/N_{diet}))) x 100, where Y is the concentration of Yttrium in the diet and feces and N is the concentration of nutrients, or energy, in the diet and feces. The ADC of crude protein, gross energy, and AA of the test ingredients were calculated according to the following formula (Bureau and Hua, 2006): $ADC_{ingredient}$ (%) = $ADC_{test\ diet}$ + ($ADC_{test\ diet}$ – $ADC_{reference\ diet}$) x (0.85 x $N_{reference\ diet}$ / 0.15 x $N_{test\ ingredient}$), where $ADC_{test\ diet}$ and $ADC_{reference\ diet}$ are the ADC (%) of the nutrients or energy in the test diet and reference diet, respectively. $N_{reference\ diet}$ and $N_{test\ ingredient}$ are the concentration of nutrients or energy in the reference diet and test ingredient, respectively. Phosphorus retention (in mg P day^{-1}) was calculated as the difference between final and initial whole body P content (mg $shrimp^{-1}$) divided by the duration of the growth trial (70 days).

2.6. Statistical analyses

Statistical analyses were performed using the statistical software program SAS (Statistical Analysis System) version 9.4. Tanks were considered as the experimental units. The residuals were assumed to be normal distributed. The performance data were analyzed for the effect of diet within each growth period by one-way ANOVA (PROC GLM of SAS). The combined performance data of period I and period II were also analyzed for the effect of diet, period and their interaction by repeated measures ANOVA (PROC GLM of SAS). The effect of diet was tested against the between tank variation within diets, whilst the effect of period and the interaction effect were tested against the within tank variation between periods. During period I of the growth trial, 10 shrimps escaped via the outlet of one of the tanks (REF diet), as the screen preventing shrimp escaping was disconnected overnight. Data of this tank were therefore omitted from the statistical analysis of the performance data (Supplementary Table 1). The final whole-body composition, P retention and nutrient, energy and AA digestibility (ingredients and diets) were analyzed for the effect of diet by one-way ANOVA (PROC GLM of SAS). When the effect of diet was significant ($p < 0.05$) a pairwise comparison of the means was done using Tukey's multiple range test.

3. Results

3.1. Performance

Performance data per growth period (period I and II) can be found in Supplementary Table 1, whilst mean shrimp performance over the 10 wk growth trial is shown in Table 4. The mean initial weight was similar for the dietary treatments ($p > 0.05$). Overall, survival was high (>90%) during the growth trial, with no differences between the dietary treatments ($p > 0.05$). All other performance parameters were affected by dietary treatments ($p < 0.001$). Feed intake was the lowest for the REF and SoyProt diet (respectively 0.246 and 0.245 g/shrimp/day) and highest for the SCP1 and SCP3 diet (respectively 0.274 and 0.273 g/shrimp/day). However, there was no difference ($p > 0.05$) in feed intake between FM, SoyProt and the SCP groups. The lowest specific growth rate (SGR) was observed in shrimp fed the REF and SoyProt diets (respectively 1.87% and 2.11%/day), whilst the highest growth was observed for shrimp fed the SCP1, SCP3 and SCP4 diets (respectively 2.37%, 2.30% and 2.36%/day). Shrimp fed the SCP diets did not differ in growth and had a comparable growth as shrimp fed the FM diet. Feed efficiency, indicated by FCR, showed a similar pattern as the growth results; shrimp fed the SCP1, SCP3 and SCP4 diets had the lowest FCRs (respectively 1.64, 1.68 and 1.58), which were lower than the REF and SoyProt diets (respectively 2.22 and 1.93). No differences in FCR were found between shrimp fed the FM diet and shrimp fed the SCP diets.

3.2. Body composition and P retention

The initial and final body composition of the shrimps are shown in Table 5. No differences were found in final crude protein and fat content of the shrimp. At the end of the growth trial, dry matter, energy and P content of the shrimp were affected by diet ($p < 0.01$). Energy content was lower in shrimp fed the SCP diets compared to shrimp fed the SoyProt, although the differences were minor (20.8–21.0 vs. 21.5 kJ/g DM, respectively). The P content of shrimp fed the SCP diets was higher compared to the other dietary treatments ($p < 0.001$). Highest P content was found for shrimp fed the SCP1 diet, which was greater than compared to shrimp fed the SCP2 and SCP3 diet, but similar to shrimp fed the SCP4 diet (Table 5). A comparable pattern was observed for the P retention by the shrimp (Fig. 2); a higher P retention was observed for shrimp fed the SCP diets compared to shrimp fed one of the other diets ($p < 0.0001$). Highest P retention was observed for shrimp fed the SCP1 and SCP4 diets, whereby shrimp fed the SCP4 diet had a significantly higher P retention then shrimp fed the SCP2 diet (Fig. 2; $p < 0.05$).

3.3. Digestibility

All data on the ADC of the nutrients, AA and energy of the different diets are reported in Supplementary Table 2, whilst data on the ADC of

Table 4
Performance of Pacific white shrimp fed the experimental diets during a 10-wk growth trial. Values are mean and standard error of the mean (SEM).

	Diets[1]								
	REF	FM	SoyProt	SCP1	SCP2	SCP3	SCP4	SEM	Diet[2]
Initial weight (g)	2.75	2.70	2.55	2.68	2.76	2.75	2.69	0.084	ns
Final weight (g)	10.69^{a}	13.57^{b}	11.64^{a}	14.31^{b}	13.57^{b}	14.20^{b}	14.48^{b}	0.269	* **
Survival (%)	96	96	95	98	97	94	93	1.3	ns
Feed intake (g/shrimp/d)	0.246^{a}	0.259^{a,b}	0.245^{a,b}	0.274^{b}	0.262^{a,b}	0.273^{b}	0.264^{a,b}	0.0047	* **
Daily gain (g/shrimp/d)	0.110^{a}	0.153^{c}	0.127^{b}	0.166^{c}	0.153^{c}	0.162^{c}	0.167^{c}	0.0034	* **
SGR (%/d)	1.87^{a}	2.26^{b,c}	2.11^{b}	2.37^{c}	2.24^{b,c}	2.30^{c}	2.36^{c}	0.040	* **
FCR	2.22^{a}	1.71^{b,c}	1.93^{b}	1.64^{c}	1.71^{b,c}	1.68^{c}	1.58^{c}	0.051	* **

[1] REF, reference diet; FM, LT70 fishmeal; SoyProt, soy protein concentrate; SCP1-4, single cell protein product 1–4.
[2] ns, not significant P > 0.1; # P < 0.10; * P < 0.05; * * P < 0.01; * ** P < 0.001. [abc] Values in the same row lacking common superscripts are different (P < 0.05) according to Tukeys' multiple comparison test.

Table 5
Initial and final whole-body composition of Pacific white shrimp fed the experimental diets during a 10-wk growth trial. Values are mean and standard error of the mean (SEM).

		Final body composition[1]								
		Diets[2]								P-value[3]
	Initial body composition[1]	REF	FM	SoyProt	SCP1	SCP2	SCP3	SCP4	SEM	Diet
Dry matter (g/kg)	219	233^{a}	237^{a,b}	238^{a,b,c}	244^{a,b,c}	245^{b,c}	243^{a,b,c}	250^{c}	2.6	* *
Crude protein	785	782	784	782	791	786	791	792	4.7	ns
Fat	42	49	46	44	44	46	45	44	2.0	ns
Energy (kJ/g DM)	20.2	21.2^{a,b}	21.2^{a,b}	21.5^{b}	21.0^{a}	21.0^{a}	20.8^{a}	20.9^{a}	0.09	* **
Ash	131	111	106	104	112	116	112	112	3.0	ns
Phosphorus	12.9	9.1^{a,b}	9.5^{b}	9.1^{a}	12.3^{d}	11.7^{c}	11.8^{c}	12.0^{c,d}	0.09	* **

[1]Values are in g/kg dry matter (DM), unless stated otherwise.
[2] REF, reference diet; FM, LT70 fishmeal; SoyProt, soy protein concentrate; SCP1–4, single cell protein product 1–4.
[3] Effect of diet was tested excluding the initial body composition. ns, not significant P > 0.1; # P < 0.10; * P < 0.05; * * P < 0.01; * ** P < 0.001. [abc] Values in the same row lacking common superscripts are different (P < 0.05) according to Tukeys' multiple comparison test.

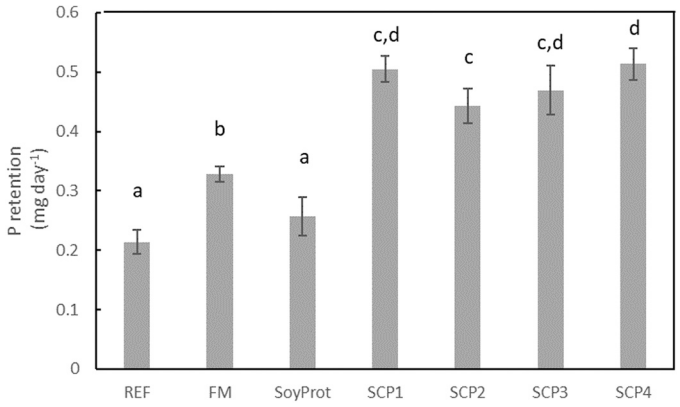

Fig. 2. Phosphorus retention (mg day^{-1}) of Pacific white shrimp fed the experimental diets during a 10-wk growth trial. REF, reference diet; FM, LT70 fishmeal; SoyProt, soy protein concentrate; SCP1–4, single cell protein product 1–4. Bars represent mean values (n = 4 tanks treatment^{-1}) and error bars represent standard deviations. Treatments lacking a common letter differ significantly (Tukey HSD; P < 0.05).

the nutrients, AA and energy of the ingredients are reported in Table 6.

Crude protein and energy digestibility did not differ between the tested ingredients.

Based on the ADC of the sum of all AA, amino acid availability was different between the ingredients (p < 0.05), with the highest value found for SoyProt followed by SCP1, which were both significantly different from SCP3. The latter had the lowest value for amino acid availability. No difference in ADC of the sum of all AAs was found between FM and the other ingredients (Table 6). Looking at the ADC values of individual AAs, all AAs, except for lysine and proline, differed in digestibility between the ingredients (p < 0.05). SCP3 had the lowest ADC values, which was for most AAs significantly lower compared to those of SoyProt (with the exception of tyrosine and phenylalanine) and SCP1 (with the exception of glutamic acid, glycine, tryptophan, phenylalanine, isoleucine and leucine). In general, the highest ADC values for the AAs were found for SoyProt, with the exception of tyrosine which was the highest for SCP1 and phenylalanine, isoleucine and leucine which were all the highest for FM. Overall, SCP1, SCP2, and SCP4 did not differ from FM in AA digestibility (p > 0.05), whilst SCP3 had a significantly lower digestibility compared to that of FM for alanine, valine, methionine, phenylalanine, isoleucine and leucine. Of the four SCP ingredients, highest digestibility per AA was found for SCP1 (Table 6).

4. Discussion

Total replacement of FM (15% dietary inclusion level) with one of the four bacterial SCP products did not compromise shrimp performance in terms of survival, growth and feed utilization. There was similar growth performance, feed utilization and nutrient digestibility for Pacific white shrimp fed either a diet with a high-quality FM (15% dietary inclusion) or diets in which FM was fully replaced by bacterial SCP originating from *M. capsulatus*. Protein digestibility of the SCP products was similar to that of FM. Whole-body protein content did not differ between shrimp fed the FM diet or one of the four bacterial SCP diets. These results highlight the potential of this bacterial SCP as a novel protein source for shrimp diets. The SCP3 product showed the lowest values for the availability of most AA, indicating that processing conditions can affect the quality of bacterial SCP products.

Data obtained here are in line with those from previous studies, which also demonstrated that survival, growth and FCR of Pacific white shrimp did not change when FM was (partly) replaced by *M. capsulatus* products in the diets (Chen et al., 2021; Jintasataporn et al., 2021; Felix

Table 6
Apparent digestibility coefficient (ADC) of protein, energy and amino acids in ingredients fed to Pacific white shrimp during a 9-wk digestibility trial. Values are mean and standard error of the mean (SEM).

	Ingredients[1]							
ADC (%)	FM	SoyProt	SCP1	SCP2	SCP3	SCP4	SEM	P-value[2]
Nutrient								
Crude protein (N * 6.25)	81.5	91.1	79.6	79.4	74.8	76.9	2.8	#
Energy	65.7	78.4	76.1	70.4	66.9	71.7	7.5	ns
Essential AA								
Arginine	90.5^{a,b}	98.2^{a}	96.1^{a}	90.0^{a,b}	84.3^{b}	89.7^{a,b}	2.0	*
Histidine	88.7^{a,b,c}	100.1^{a}	95.2^{a,b}	86.2^{b,c}	83.1^{c}	87.5^{b,c}	2.1	*
Isoleucine	94.0^{a}	93.6^{a}	88.1^{a,b}	83.0^{a,b}	76.6^{b}	88.0^{a,b}	2.2	* *
Leucine	94.9^{a}	92.1^{a}	87.9^{a,b}	82.7^{a,b}	76.1^{b}	87.0^{a,b}	2.7	*
Lysine	95.5	98.1	97.9	94.3	88.2	96.1	2.2	ns
Methionine	93.9^{b,c}	104.9^{d}	100.0^{c,d}	95.1^{c}	87.4^{a}	88.2^{a,b}	1.1	* **
Phenylalanine	95.1^{a}	82.4^{a,b}	80.3^{a,b}	70.2^{a,b}	65.0^{b}	81.8^{a,b}	4.5	*
Threonine	91.6^{a,b}	98.7^{a}	93.3^{a}	81.3^{a,b}	74.1^{b}	84.0^{a,b}	3.2	*
Tryptophan	83.8^{a,b}	89.9^{a}	75.9^{a,b}	79.2^{a,b}	70.1^{b}	76.8^{a,b}	3.2	*
Tyrosine	78.7^{a,b}	89.8^{a,b}	92.1^{a}	82.5^{a,b}	75.9^{b}	82.6^{a,b}	2.6	*
Valine	91.8^{a,b}	99.0^{a}	92.5^{a,b}	85.5^{b,c}	78.2^{c}	85.8^{b,c}	2.0	* *
Non-essential AA								
Alanine	91.8^{a,b}	96.5^{a}	89.6^{a,b}	82.8^{b,c}	76.4^{c}	80.8^{b,c}	2.3	* *
Aspartic acid	89.3^{a,b}	99.1^{b}	97.0^{b}	89.8^{a,b}	82.2^{a}	87.2^{a,b}	2.6	*
Glutamic acid	94.1^{a,b}	100.1^{a}	97.2^{a,b}	88.4^{a,b}	79.5^{b}	87.0^{a,b}	3.4	*
Glycine	90.0^{a,b}	96.0^{a}	91.7^{a,b}	82.7^{a,b}	73.6^{b}	77.3^{a,b}	3.9	*
Proline	91.5	107.1	111.2	102.2	87.8	92.9	4.8	#
Serine	87.9^{a,b,c}	98.7^{a}	91.2^{a,b}	79.4^{a,b,c}	67.4^{c}	76.4^{b,c}	3.8	*
Sum of AA	91.9^{a,b}	97.1^{b}	93.2^{b}	86.2^{a,b}	78.8^{a}	86.4^{a,b}	2.4	*

[1] REF, reference diet; FM, LT70 fishmeal; SoyProt, soy protein concentrate; SCP1–4, single cell protein product 1–4.
[2] ns, not significant $P > 0.1$; # $P < 0.10$; * $P < 0.05$; * * $P < 0.01$; * ** $P < 0.001$. [abc] Values in the same row lacking common superscripts are different ($P < 0.05$) according to Tukeys' multiple comparison test.

et al., 2023). This suggests that for the Pacific white shrimp, the nutritional value of *M. capsulatus* products is equivalent to that of FM. It should be noted that diets used in the current study were in the first place formulated to determine ingredient digestibility, and interpretations on growth performances should therefore be done with care. Nevertheless, as the test diets were fairly similar in nutrient composition, growth comparisons are still informative. Looking at the nutrient content of the ingredients, the SCP products tested in this study had a similar protein content and essential AA profile as that of the high-quality FM used. The higher growth and lower FCR for shrimp fed the SCP diets compared to shrimp fed the SoyProt diet, suggests that the SCP products had a higher biological value compared to that of SoyProt. Protein, fat and methionine levels were lower in SoyProt compared to the SCP products. As both FM and soy products are common protein sources in shrimp feeds (Sánchez-Muros et al., 2020), these results highlight the potential of *M. capsulatus* SCPs as an alternative protein ingredient for use in the diets of shrimp.

To evaluate the potential of novel ingredients for use in feeds for fish or shrimp, besides growth, survival and feed utilization, other aspects should also be assessed, like palatability of the ingredient (Glencross, 2020). Palatability issues have been mentioned as one of the challenges of using bacterial SCPs in aquafeeds (Jones et al., 2020), however, data from the literature is not conclusive. Whilst some studies have reported a reduced feed intake with increased dietary bacterial SCP in for example Atlantic halibut (Aas et al., 2007), Rainbow trout (Zamani et al., 2020) and Yellowtail Kingfish (Pilmer et al., 2022), other studies report no effect on feed intake when bacterial SCP was included in diets of Atlantic salmon (Berge et al., 2005; Aas et al., 2006; Salze and Tibbetts, 2021), rainbow trout (Ruiz et al., 2023), barramundi (*Lates calcarifer*; Woolley et al., 2023) or gilthead sea bream (*Sparus aurata*; Marchi et al., 2023). Based on the measured feed intake in the current study, it can be stated that for the Pacific white shrimp, the palatability of the four SCP products was equal to that of FM. This is in line with previous studies, which also showed similar feed intake for Pacific white shrimp fed either a FM diet or diets in which (part of the) FM was replaced by *M. capsulatus* products (Jintasataporn et al., 2021; Chen et al., 2022; Felix et al., 2023).

Information regarding nutrient digestibility is also of importance for ingredient evaluation and values on ingredient digestibility in the target species are required to formulate species-specific balanced diets. Ingredient digestibility's are commonly determined using test diets in which a known part of a reference diet is substituted by the test ingredient. In the current study, an inclusion level of 15% of the test ingredient was chosen. This inclusion level may have had an influence on the accuracy of the digestibility results (Glencross et al., 2023), but this resembles practical diets in which it is common to use a 15% inclusion level, or even lower, for ingredients. In addition, as palatability issues have been mentioned as one of the main challenges of using bacterial SCPs in aquafeeds (Jones et al., 2020), the 15% inclusion level in the current study was also chosen to avoid potential negative effects on palatability. The four SCP products tested in the current study had similar protein digestibility compared to FM and three out of the four SCP products (SCP1, SCP2 and SCP4) had also similar AA availability as FM, both for the sum of AAs as well as for the individual AAs. In combination with the earlier mentioned similar protein content and essential AA profile, this indicates that protein quality of SCP1, SCP2 and SCP4 was comparable to the FM used in this study. Studies on the digestibility of bacterial SCP on ingredient level are scarce and most studies report digestibility of the whole diet including the bacterial SCP. For fish, protein digestibility of diets including bacterial SCP range between 79% and 89%, depending among others on bacterial species and inclusion levels (reviewed in Glencross et al., 2020) and diet protein ADCs measured in the current study with Pacific white shrimp fall within this range. Looking at the protein digestibility on ingredient level, results of the four SCP products tested in our study are in line with previous reported protein digestibility values for *M. capsulatus* fed to Atlantic salmon (71–84%; Skrede et al., 1998, Storebakken et al., 2004, Glencross et al., 2023), whilst Salze and Tibbetts (2021) reported for Atlantic salmon a slightly higher protein digestibility of 86% for *M. extorquens*. A higher digestibility was reported by Felix et al. (2023), as they found for Pacific white shrimp a protein apparent digestibility coefficient of 91%, using a similar product as in the current study.

We also found that the protein digestibility values of the SCP products were lower compared to the digestibility of the sum of amino acids

(75–80% vs. 79–93% respectively), which was also shown to be the case in the study of Glencross et al. (2023). This could be due to the presence of non-protein nitrogen sources, such as nucleic acids, known to be present in bacterial SCP products. The distinct difference in crude protein content (measured as N x 6.25) and sum of AAs within each SCP product supports the presence of non-protein nitrogen sources. As stated by Glencross et al. (2023) protein digestibility of bacterial SCP should therefore be calculated using the sum of AAs instead of total nitrogen.

Notable is that for all AAs measured, the lowest digestibility values were found for the SCP3 product. This indicates that downstream processing conditions can affect the quality of bacterial SCP products. Earlier studies also showed the effect of processing conditions on the quality of SCP products, but highlighted improved quality due to processing. Both Teuling et al. (2019) and Agboola et al. (2019) demonstrated for example that disrupting the cell wall of the microalgae *Nannochloropsis gaditana*, using either physical (pasteurization, freezing, freeze drying) or mechanical (bead milling) treatments, increased the protein and fat digestibility for Nile tilapia (*Oreochromis niloticus*) and African catfish (*Clarias gariepinus*) respectively, with the highest increase found when cells were disrupted by bead milling. For brewer's yeast (*Saccharomyces cerevisiae*) mechanical disruption of the cells can improve the quality as was reflected by an increased protein digestibility in rainbow trout (Rumsey et al., 1991) and Atlantic salmon (Hansen et al., 2021). Autolysis has also been shown as an effective method to improve yeast quality for protein and AA digestibility in Atlantic salmon, although this was dependent on the yeast species used (Agboola et al., 2022). In a study by Øverland et al. (2006) comparable protein and AA digestibility was found for rainbow trout fed either bacterial protein meal or autolyzed bacterial protein meal, suggesting that autolysis did not affect the quality of the bacterial SCP. Biswas et al. (2020), who used a similar SCP but treated differently (grinding, hydrolysis), did also not find any difference in the response of Japanese Yellowtail. A potential explanation for the lower AA digestibility of SCP3 might be attributed to the formation of Maillard reaction products, which can result in lower digestibility of amino acids (Plakas et al., 1985; Deng et al., 2005). Several processing conditions of either diets or ingredients can induce Maillard reactions (Salazar-Villanea et al., 2017; Teuling et al., 2019) and this might have been the case during the production of SCP3. Nevertheless, Maillard reaction products were not measured, and further studies are warranted to elucidate if the Maillard reaction indeed played a role. Overall, these studies and the result of the current study highlight the importance of not only knowing which species the SCP product consists of, but also under which conditions the products have been produced.

All four SCP diets resulted in a higher final phosphorus whole-body composition and an accompanied higher phosphorus retention compared to the FM and SoyProt diet. As phosphorus content of the SCP products was lower compared to that of FM (17.6–21.7 g/kg vs. 24.1 g/kg DM, respectively), this shows a higher phosphorus availability in the SCP products. Previous studies have also reported an increased phosphorus digestibility and retention for salmonids (Aas et al., 2006; Rajesh et al., 2022) and Pacific white shrimp (Felix et al., 2023) fed diets in which FM was partly replaced by bacterial SCP. It was suggested that this can be ascribed to the form of phosphorus present in the ingredients; in bacterial SCP, phosphorus is mainly in the form of nucleic acids and phospholipids (Øverland et al., 2010), which are better digested than calcium-hydroxyapatite complexes, the major form of phosphorus in FM (Hua and Bureau, 2006; Rajesh et al., 2022). Another reason could be the presence of enzymes, like phytase, in bacterial protein meal which could facilitate phosphorus digestibility (Cao et al., 2007).

Earlier studies have highlighted that the addition of bacterial SCP to shrimp diets can have beneficial effects on the health of the animal, as higher survival was observed for shrimp fed bacterial SCP diets in challenge tests with *Vibrio* spp. (Chen et al., 2021; Jintasataporn et al., 2021). Health benefits of bacterial SCP have also been reported for fish species, like gilthead sea bream (Marchi et al., 2023), rainbow trout (Ruiz et al., 2023) and spotted seabass (*Lateolabrax maculatus*; Yu et al., 2023, Zhang et al., 2023). Further studies are warranted to elucidate if the SCP products used in the current study also have similar beneficial effects on health and welfare, but were beyond the scope of this study. From a life cycle analysis point of view, feed and feedstuffs constitute the largest contributors of most environmental impacts of aquaculture operations (Aubin et al., 2009; Boissy et al., 2011), demonstrating the need for responsible ingredients. From a sustainability perspective, methanotrophic bacteria are highlighted as a sustainable protein source, as they can be produced harnessing natural gas, biogas or wastewater and hence less dependent on finite resources (Øverland et al., 2010; Zha et al., 2021; Salehi and Chaiprapat, 2022; Jain et al., 2023). In addition, such protein sources also avoid direct feed-food competition, as it is with terrestrial agricultural products (Nyyssölä et al., 2022).

To conclude, based on similar growth, nutrient digestibility and utilization as FM, bacterial SCP originating from *M. capsulatus* is a promising novel protein source for Pacific white shrimp diets. This, in combination with the higher phosphorus availability, shows the potential of bacterial SCP to replace fishmeal in shrimp diets. The lower AA availability of one of the SCPs tested, indicates also that processing conditions can affect the quality of bacterial SCP products, which warrants attention at all stages of production of such SCPs.

CRediT authorship contribution statement

Marit AJ Nederlof: Conceptualization, Methodology, Formal analysis, Investigation, Data curation, Writing – original draft. **Sachi J Kaushik:** Conceptualization, Methodology, Writing – review & editing, Funding acquisition. **Johan W Schrama:** Conceptualization, Methodology, Formal analysis, Data curation, Writing – review & editing, Project administration, Funding acquisition.

Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Marit Nederlof reports financial support was provided by String Bio Pvt. Ltd. (Bengaluru, India).

Data availability

Data will be made available on request.

Acknowledgement

The authors would like to thank Menno ter Veld and the staff of ARF-CARUS for their technical support in preparation and execution of the experiments. We thank Fariz Harwinda and Remon van Ginkel for their help during the execution of the experiments. We would also like to thank Tino Leffering, Samara Hutting and Ronald Booms for their support during the chemical analyses. At last, we would like to acknowledge the three anonymous reviewers for their comments and suggested changes to an earlier version of this paper. This research was financed by String Bio Pvt. Ltd. (Bengaluru, India), who also carried out the amino acid analyses.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.aqrep.2023.101830.

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