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Substituting dietary fishmeal with silkworm pupae meal in diets of Pacific white shrimp (*Penaeus vannamei*): Effects on growth performance, nutrient utilisation, whole-body composition and digestive enzyme activities

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Abstract

The present study evaluated the dietary substitution effect of silkworm pupae meal (SWP) for fish meal (FM) on growth performance, feed utilisation, whole-body proximate composition, amino acid profile and digestive enzyme activities of Pacific white shrimp (*Penaeus vannamei*). The shrimp were fed with six isonitrogenous (36% crude protein), isolipidic (8% crude lipid) and isocaloric (16 MJ kg^{-1}) experimental diets, containing 0, 20, 40, 60, 80 and 100% SWP, respectively, to replace dietary FM. A total of 360 shrimps (average initial weight of 3.86±0.20 g) were distributed into 18 tanks (20 shrimp per tank). The experimental diets were fed to triplicate groups of *P. vannamei* three times a day until satiation for 45 days. Significantly ($p<0.05$) higher growth performance and feed utilisation were found in shrimp fed diet with 60% incorporation level of SWP meal. No significant differences ($p>0.05$) were observed in the survival rate, whole-body proximate composition and amino acid profile of shrimp fed SWP meal-incorporated diets. Additionally, higher specific activities of digestive enzymes such as amylase, trypsin and lipase were observed in shrimps fed diet with 60% incorporation level of SWP meal. The present study revealed that dietary fish meal protein up to 60% can be substituted with SWP without compromising growth performance, feed utilisation, whole-body proximate composition, amino acid profile and digestive enzyme activities of Pacific white shrimp fed on SWP meal based diets.

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Introduction

Pacific white shrimp (*Penaeus vannamei*) is one of the largest cultured shrimp species, and it contributed about 51.7% of the total crustacean aquaculture production in 2020 (FAO, 2022). Due to the availability of viral pathogen-free brood stock, high larval survival, fast growth, disease resistance, tolerance to high stocking density, adaptability to a wide range of salinity and temperature as well as low dietary protein requirement for development and physiological process, *P. vannamei* is the most widely cultured shrimp species in the world (Liao and Chien, 2011).

Protein is the most expensive nutrient component in diets of *P. vannamei* and fish meal is used as a major ingredient source of protein in commercial shrimp diets (Tacon, 1993). The lower availability and increased use of fish meal in chicken and piglet feed have decreased the dependence on the fish meal in aquatic diets and increased the cost of fish meal (El-Sayed, 1999). Therefore, many studies have attempted partially or completely replacing fish meal with non-conventional, less expensive and high-protein ingredients in shrimp and fish diets (Samocha *et al.,* 2004; Xu *et al.,* 2018; Rahimnejad *et al.,* 2019; Sathishkumar *et al.,* 2021; Hodar, 2022).

Since the 1960's, silkworm pupae (*Bombyx mori*) meal has been used as a fish feed ingredient in Indo-Pacific regions (Hora and Pillay, 1962; KH, 1966). The spent silkworm pupae is a major by-product obtained from silk producing factories, and large quantities are discarded in open environments or used as fertilisers (Begum *et al.,* 1994; Gangadhar *et al.,* 2017). However, silkworm pupae meal is rich in protein (50-70%), unsaturated fatty acids (61.50-77.29% of total fatty acids) and cheaper than fish meal (Begum *et al.,* 1994; Rahimnejad *et al.,* 2019). Therefore, it is used as an alternate protein ingredient in livestock feeds, especially for monogastric animals such as poultry, pigs and fish (Trivedy *et al.*, 2008). Due to their nutritional quality, local availability and lower cost compared to fish meal, several studies have been undertaken in the past to investigate the effect of silkworm pupae as dietary fish meal replacers in various aquatic species, such as *Macrobrachium dayanum* (Seema *et al.,* 2011), Carps (*Catla catla, Labeo rohita, Labeo fimbriatus* and *Cyprinus carpio*) (Begum *et al.,* 1994; Rangacharyulu *et al.,* 2003; Gangadhar *et al.,* 2017), Abalone (*Haliotis discus hannai Ino*) (Cho, 2010), GIFT tilapia (Feng *et al.,* 2021; Sathishkumar *et al.,* 2021), *Penaeus vannamei* (Rahimnejad *et al.,* 2019; Hodar, 2022), *Clarias gariepinus* (Olaniyi and Babasanmi, 2013) and mirror carp (*Cyprinus carpio* var. *specularis*) (Xu *et al.,* 2018). However, only limited studies have been carried out in *P. vannamei* using silkworm pupae meal as a fish meal replacer and therefore, the present study was undertaken to evaluate the effect of substituting FM with SWP in the diet on growth performance, nutrient utilisation, proximate composition and digestive enzyme activity of Pacific white shrimp, *P. vannamei.*

Materials and methods

Experimental design and diet preparation

Six experimental diets were formulated to replace the inclusion level of fish meal (FM) while increasing the content of silkworm pupae meal (SWP). The FM was replaced at 0% (control), 20, 40, 60, 80 and 100% level by SWP (Table 1). All the experimental diets were formulated and prepared to be isonitrogenous (36% of crude protein), isolipidic (8% of crude lipid) and isocaloric (16 MJ kg^{-1}) to meet the nutrient requirement of *P. vannamei.* All the dry feed ingredients were finely ground using a hammer mill, passed through a 180-micron mesh and

Table 1. The ingredient and proximate composition of experimental diets (g kg^{-1} of diet) with 0% (Control), 20, 40, 60, 80 and 100% substitution of silkworm pupae meal

	Experimental diets					
Ingredients (g kg^{-1})	Control	SWP 20	SWP 40	SWP 60	SWP 80	SWP 100
Fish meal[a]	230	184	138	92	46	0
Silkworm pupae meal[b]	0	60.4	120.8	181.3	241.7	302.1
Soybean meal[a]	300	300	300	300	300	300
Wheat flour[a]	240	240	240	240	240	240
Corn flour[a]	95	86.6	76.2	65.7	57.3	46.9
Shrimp meal[a]	40	40	40	40	40	40
Fish oil[a]	20	20	20	20	20	20
Palm oil[a]	30	24	20	16	10	6
Soy lecithin[c]	10	10	10	10	10	10
Dicalcium phosphate[d]	10	10	10	10	10	10
DL-Methionine[e]	2	2	2	2	2	2
L-Lysine[f]	2	2	2	2	2	2
Vitamin mix[g]	5	5	5	5	5	5
Mineral mix[h]	5	5	5	5	5	5
Vitamin C[d]	1	1	1	1	1	1
Pellet binder[i]	10	10	10	10	10	10
Nutrient composition (g kg^{-1})						
Moisture	95.1	93.7	89.5	95.7	90.1	92.6
Crude protein	363.9	361.2	359.7	362.4	360.8	359.8
Crude lipid	81.7	80.5	81.1	81.8	80.5	81.2
Crude fibre	44.2	47.8	46.8	42.7	46.9	53.2
Total ash	132.5	124.7	103.8	137.4	125.8	113.5
Gross energy (MJ kg^{-1})	16.27	16.35	16.85	16.19	16.41	16.49

[a]Mahindra feeds Pvt. Ltd., Namakkal, Tamil Nadu, India
[b]Silvermine Pvt. Ltd., Udumalaipet, Tamil Nadu, India
[c]Otto Chemie Pvt. Ltd., Mumbai, India
[d]Jain Industrial Chemicals, Chennai, India
[e]Evonik AG (DL-methionine: MetAMINO® – 99%)
[f]Ajinomoto Heartland, Inc., Chicago (L-Lysine HCL-98.5%)
[g]Anicare Pvt. Ltd., Chennai, Tamil Nadu, India. Composition of vitamin premix (Quantity per kg): Vit. A-10,000,000 IU, Vit. B1-5000 mg, Vit. B2-5000 mg, Vit. B3-6000 mg, Vit. B5-6000 mg, Vit. B6-6000 mg, Vit. C-60,000 mg, Vit. D3-2,000,000 IU, Vit. E-10,000 IU, Vit. H-200 mg
[h]Anicare Pvt. Ltd., Chennai, Tamil Nadu, India. Composition of mineral premix (Quantity per kg): Magnesium-2800 mg, Iodine-7.4 mg, Iron-7400 mg, Copper-1200 mg, Manganese-11,600 mg, Zinc-9800 mg, Chlorides cobalt-4 mg, Potassium-100 mg, Selenium-4 mg, Calcium carbonate-27.25%, Phosphorous-7.45 mg, Sulphur-0.7 mg, Sodium-6 mg, Calpan-200 mg, Aluminium-1500 mg and Choline chloride-10,000 mg
[i]PEGABIND®, Bentoli Agrinutrition India Pvt. Ltd., Chennai, India

then mixed thoroughly. Then, the oil sources (fish oil, soy lecithin), all the additives and the appropriate amount of water were added to the ground ingredients and were mixed thoroughly for 15 min using an electric blender (Gaocheng - GC-MT1200, Mogli Labs, India Pvt. Ltd.) for homogenisation. After proper mixing, the soft dough was cooked at 80^{0}C for 15 min. Then, the cooled dough was pelletised in a tabletop pelletiser having a 2 mm die. The pellets were dried in a hot air oven at 60^{0}C for 12 h to reach approximately 10% moisture. Finally, the dried pellets were placed in air tight plastic container and stored at -20^{0}C until used.

Experimental shrimp and feeding trial

Pacific white shrimp (*P. vannamei*) post-larvae (PL-11) were procured from Star Aqua hatchery, Koovathur, Tamil Nadu, India and transported to the Indoor Aquaculture Unit, Directorate of Incubation and Vocational Training in Aquaculture (DIVA), TNJFU, Chennai, Tamil Nadu, India. Initially, the seeds were stocked into a cement tank of 16,000 l capacity and fed a commercial diet (36% crude protein) for four weeks to acclimatise them to experimental conditions. After nursery rearing, a total of 360 healthy shrimp seeds (average weight 3.86±0.20 g) were randomly distributed into 18 indoor plastic circular tanks (80 l capacity) at the rate of 20 shrimps per tank. Each experimental diet was fed to the shrimp in triplicates. The shrimps were fed with experimental diets three times daily at 08.00, 13.00 and 18.00 hrs. The feed offered was readjusted based on the animal's average weight, survival and feed intake. After one hour of feeding, the unconsumed feed pellets were removed frequently by the siphon method and were dried at 60^{0}C for 12 h in a hot air oven to calculate feed intake on a daily basis. During the feeding trial, 20% of the water was exchanged every day. The water quality parameters, including salinity, temperature, dissolved oxygen, pH, total ammonia N, nitrite-N and nitrate N, were measured during the feeding trial and it was recorded as 24.23±0.78 ppt, 28.70±0.60^{0}C, 6.43±0.87 mg l^{-1}, 8.31±0.54, 0.21±0.05 mg l^{-1}, 3.10±0.59 mg l^{-1} and 0.12±0.01 mg l^{-1}, respectively. All the water quality parameters were estimated following standard protocols of APHA (2012).

Growth and feed utilisation analysis

At the end of the experiment, all the shrimps in each treatment were counted, and their total weight was recorded for analysis of the survival, growth performance and feed utilisation. The growth performance and feed utilisation were estimated using the following formulae:

Weight gain (WG) (g) = Final body weight (g) - Initial body weight (g)

Survival rate (SR) (%) = (No. of shrimps survived/No. of shrimps stocked) × 100

Average daily growth (ADG) (g) = (Mean final weight (g) - Mean initial weight (g))/Days of culture

Feed conversion ratio (FCR) = Total feed consumed (g)/Wet weight gain of shrimp (g)

Specific growth rate (SGR) (%/day) = [(Ln (final weight)) - (Ln (initial weight)/No. of days)] × 100

Protein efficiency ratio (PER) = Wet weight gain of shrimp (g)/Protein intake (g)

Feed intake (% BW/day) = (Dry feed intake (g)/Final shrimp weight (g)/Days fed) × 100

Proximate and amino acid composition

At the end of the experiment, 10 shrimps from each replicate of the experiment were ice killed and taken for analysis of the whole-body proximate composition and amino acid profile. The proximate composition of experimental diets and whole-body carcass samples were estimated following standard protocols of AOAC (2010). The moisture content was determined using a hot air oven at 105°C for 5 h, the crude protein content was determined using the Kjeldhal method (Kelplus-Distyl Em Ba, Pelican equipments, Chennai, Tamil Nadu, India) and the ether extract was estimated using the soxhlet method (Socsplus - SCS 04 AS DLSTS, Pelican equipments, Chennai, Tamil Nadu, India). The samples were digested using 1.25% sulphuric acid (45 min) followed by 1.25% sodium hydroxide (45 min) using fibercap method (FIBRAPLUS FES 04, Pelican equipments, Chennai, Tamil Nadu, India) for determining the crude fibre levels. The total ash content was estimated using a muffle furnace at 550°C for 6 h. The gross energy content of experimental diets and whole-body carcass samples were analysed using Bomb calorimeter (IKA-C 6000, IKA® India Private Limited, Bengaluru, India).

The amino acid profile of test diets and whole-body carcass samples were determined by ultra-pressure liquid chromatography (UPLC; Model-Waters ACQUITY-UPLC, Waters), following the method of Ishida *et al.* (1981) (Table 2).

Digestive enzyme activities

At the end of the feeding trial, the shrimp intestine samples were collected from each replicate treatment. The samples were rinsed with ice-cold distilled water and homogenised (1:5, w: v) in phosphate buffer (pH: 7.5).The homogenates were centrifuged at 4000 ×g for 5 min at 4°C and then the supernatant was collected and stored at -20°C until analysis of digestive enzyme activity.

The total protein content of the samples was determined by following the method of Bradford (1976) using bovine serum as a substrate. The α - amylase activity was estimated by the 3,5-dinitrosalicylic acid (DNS) method (Worthington, 1991). The starch substrate (1% w/v) was diluted in a buffer at pH 7.0, 0.1 M NA_2HPO_4 and 0.1 M NAH_2PO_4. The substrate (0.1 ml) was incubated with enzyme extract (0.1 ml) for 30 min at 37°C. Then, 2 ml of DNS solution was added and the mixture was boiled for 5 min. After boiling, 10 ml of distilled water was added to the mixture and the absorbance of the solution was recorded at 540 nm. Blanks were similarly prepared but without the addition of enzyme extracts. Maltose (0.3-5 µm ml^{-1}) was used to prepare the standard curve. The α - amylase activity was expreseed as µmol of maltose produced per min per mg protein at the specified condition. Trypsin activity was determined by following method of Erlanger *et al*. (1961) using N-α-benzoyl-dl-arginine-p-nitroaniline (BAPNA) as substrate. One unit of trypsin enzyme activity was expressed as 1 µmol of p-nitroaniline released per min. The method of Cherry and Crandel (1932) was used to determine the lipase activity by measuring the fatty acid release caused by enzyme hydrolysis of olive oil.

Statistical analysis

The data were statistically analysed using one-way ANOVA, followed by Duncan's multiple range test using statistical software SPSS 20.0 for Windows to determine the significant difference ($p<0.05$) among the treatments. All the data values were expressed as mean ± standard deviation (SD) of all three replicatess per treatment (n=3).

Table 2. Amino acid profile of experimental diets (g kg^{-1} of diet) with 0% (Control), 20, 40, 60, 80 and 100% substitution of silkworm pupae meal

Amino acids (g kg^{-1} of diet)	Control	Experimental diets				
		SWP 20	SWP 40	SWP 60	SWP 80	SWP 100
Essential amino acids						
Arginine	21.50	21.79	21.48	21.59	21.10	21.89
Histidine	8.67	8.12	8.45	8.65	8.39	8.57
Isoleucine	14.65	15.1	14.87	14.68	14.23	14.78
Leucine	25.63	25.89	26.14	25.87	25.54	25.37
Lysine	20.34	19.97	20.41	20.77	20.16	19.85
Methionine	9.01	8.94	9.16	9.27	9.02	9.15
Phenylalanine	16.95	16.3	16.58	16.28	16.74	16.5
Threonine	13.25	13.54	12.98	12.87	13.38	13.27
Tryptophan	3.8	3.85	3.79	3.9	3.91	3.93
Valine	17.16	17.64	16.95	17.25	17.37	17.29
Total EAA	150.96	151.14	150.81	151.13	149.84	150.6
Non-essential amino acids						
Alanine	19.58	18.52	19.27	19.65	19.32	19.56
Aspartic acid	31.98	32.5	32.14	32.57	32.7	32.14
Glutamic acid	55.2	51.9	52.4	52.6	52.87	52.19
Glycine	18.4	18.3	18.7	18.2	18.5	18.9
Proline	18.6	18.12	18.07	19.1	18.9	18.2
Serine	15.21	15.74	15.98	15.46	15.32	15.49
Total NEAA	158.97	155.08	156.56	157.58	157.61	156.48
Total amino acids	309.93	306.22	307.37	308.71	307.45	307.08

The amino acid composition values are expressed as means of three replicates per treatment (n=3).

Results

Growth performance and survival rate of *P. vannamei*

Growth parameters such as initial weight (IW), final weight (FW), weight gain (WG), average daily growth (ADG), survival rate (SR) and specific growth rate (SGR) of *P. vannamei* fed diets with different levels of silkworm pupae meal are shown in Table. 3. Significant differences ($p<0.05$) were observed in IW, FW, WG, ADG and SGR of *P. vannamei* fed diets with different levels of silkworm pupae meal. Among the treatment diets, significantly higher growth performance was found in shrimps fed the SWP 60 diet, than in other diets including the control diet. No significant difference ($p>0.05$) was observed in the survival rate of *P. vannamei* fed different levels of silkworm pupae meal.

Feed utilisation by *P. vannamei*

Feed utilisation parameters such as feed conversion ratio (FCR), protein efficiency ratio (PER) and feed intake (FI) of *P. vannamei* fed diets with different inclusion levels of SWP meal are displayed in Table.4. Significant difference ($p<0.05$) were observed in feed utilisation parameters of *P. vannamei* fed diets with different inclusion levels of silkworm pupae meal. Significantly ($p<0.05$) higher FCR, PER and FI were observed in *P. vannamei* fed SWP 60 diet than in other diets including the control diet.

Whole body proximate and amino acid composition of *P. vannamei*

The whole-body proximate composition such as crude protein, ether extract and total ash and amino acid composition of *P. vannamei* fed diets with different levels of silkworm pupae meal are shown in Table 5 and 6, respectively. .No significant difference ($p>0.05$) was observed in whole-body proximate composition and amino acid profile of *P. vannamei* fed SWP incorporated diets.

Digestive enzyme activity of *P. vannamei*

The digestive enzyme activity, including amylase, trypsin and lipase activity of *P. vannamei* fed diets with different inclusion levels of

Table 3. Growth performance of *P. vannamei,* fed diet with 0% (Control), 20, 40, 60, 80 and 100% substitution of silkworm pupae meal

Parameters	Control	Experimental diets					
		SWP 20	SWP 40	SWP 60	SWP 80	SWP 100	P value
IW (g)	3.9±0.08	3.86±0.07	3.91±0.21	3.75±0.13	3.86±0.05	3.86±0.19	0.742
FW (g)	$7.4±0.18^{bc}$	$7.5±0.27^{bc}$	$7.86±0.27^{ab}$	$8.36±0.23^{a}$	$7.12±0.34^{c}$	$6.62±0.33^{d}$	<0.001
WG (g)	$3.50±0.12^{c}$	$3.63±0.26^{bc}$	$3.95±0.09^{b}$	$4.61±0.15^{a}$	$3.25±0.31^{c}$	$2.75±0.34^{d}$	<0.001
ADG (g)	$0.08±0.003^{c}$	$0.08±0.006^{bc}$	$0.09±0.002^{b}$	$0.10±0.003^{a}$	$0.07±0.007^{c}$	$0.06±0.007^{d}$	<0.001
SR (%)	93.33±2.88	91.66±2.88	93.33±2.88	90.00±5.00	95.00±5.00	93.33±2.88	0.674
SGR (% / day)	$1.42±0.03^{bc}$	$1.47±0.08^{bc}$	$1.55±0.04^{b}$	$1.78±0.04^{a}$	$1.35±0.08^{c}$	$1.19±0.14^{d}$	<0.001

Growth performance values are expressed as mean±SD of three replicates per treatment (n=3) and values with different superscript letters are significantly different ($p<0.05$) among treatments.
IW: Initial weight; FW: Final weight; WG: Weight gain; ADG: Average daily growth; SR: Survival rate; SGR: Specific growth rate.

Table 4. Feed utilisation of *P. vannamei* fed a diet with 0% (Control), 20, 40, 60, 80 and 100% substitution of silkworm pupae meal

Parameters	Control	Experimental diets					
		SWP 20	SWP 40	SWP 60	SWP 80	SWP 100	p value
FCR	1.97±0.02^{bc}	2.04±0.17^{ab}	2.05±0.09^{ab}	1.81±0.01^{c}	2.14±0.08^{ab}	2.23±0.14^{a}	0.006
PER	1.41±0.02^{b}	1.36±0.12^{bc}	1.35±0.06^{bc}	1.53±0.01^{a}	1.30±0.05^{bc}	1.25±0.08^{c}	0.004
FI (% BW day^{-1})	2.07±0.01	2.20±0.11	2.29±0.15	2.21±0.03	2.16±0.02	2.05±0.13	0.069

Feed utilisation values are expressed as mean±SD of three replicates per treatment (n=3), and values with different superscript letters are significantly different (p<0.05) among treatments.
FCR: Feed conversion ratio; FER: Feed efficiency ratio; PER: Protein efficiency ratio; FI: Feed intake

Table 5. Whole-body proximate composition (g 100 g^{-1} of dry weight) of *P. vannamei,* fed diets with 0% (Control), 20, 40, 60, 80 and 100% substitution of silkworm pupae meal

Parameters	Initial	Control	Experimental diets					
			SWP 20	SWP 40	SWP 60	SWP 80	SWP 100	P value
Crude protein (%)	65.68	68.63±0.31	68.73±0.19	67.95±0.66	68.06±0.69	68.37±0.38	68.30±0.68	0.450
Crude lipid (%)	3.68	4.49±0.28	4.25±0.42	3.92±0.35	4.51±0.20	4.33±0.43	4.34±0.39	0.424
Total ash (%)	15.36	15.17±0.90	14.68±0.15	14.65±0.62	14.60±0.36	14.77±0.62	15.02±0.66	0.846

Whole-body proximate values are expressed as mean ± SD of three replicates per treatment (n=3).

Table 6. Whole-body amino acid profile (g kg^{-1} of dry matter basis) of *P. vannamei,* fed diets with 0% (Control), 20, 40, 60, 80 and 100% substitution of silkworm pupae meal

Amino acids (g kg^{-1} dry weight basis)	Initial	Control	Experimental diets					
			SWP 20	SWP 40	SWP 60	SWP 80	SWP 100	p value
Essential amino acids								
Arginine	29.6	32.93±3.1	33.86±2.6	33.43±1.5	33.6±3.1	33.2±3.9	33.83±1.5	0.998
Histidine	4.9	11.4±2.1	12.96±1.0	13.2±2.6	11.23±1.2	11.76±2.1	13.63±0.5	0.449
Isoleucine	16.9	21.03±1.3	22.50±1.3	21.90±1.3	22.50±1.2	23.23±0.6	22.46±2.6	0.641
Leucine	38	43.23±0.7	42.93±1.1	42.90±1.3	43.06±0.9	42.36±1.4	41.73±0.7	0.564
Lysine	33.6	32.63±3.1	35.90±1.1	35.30±2.1	33.63±2.1	35.36±2.2	33.36±1.3	0.388
Methionine	21.6	25.33±0.9	25.23±2.1	23.50±2.1	24.90±2.3	25.03±1.7	24.63±2.2	0.878
Phenylalanine	15.1	21.06±2.7	24.93±1.0	24.36±1.5	23.70±2.0	23.43±2.8	24.16±2.2	0.379
Threonine	18.5	22.63±1.5	24.00±2.7	24.90±1.7	24.73±1.5	22.00±1.3	24.50±1.5	0.313
Valine	26.8	32.50±0.6	33.63±1.5	34.16±1.7	32.16±2.2	33.80±0.8	35.43±0.6	0.531
Total EAA	205	242.74±1.8	231.01±1.6	253.65±1.6	249.51±1.8	250.17±1.9	253.73±1.4	
Non-essential amino acids								
Alanine	61.6	62.33±3.1	65.20±1.3	63.20±2.4	62.90±2.4	64.23±2.7	65.06±0.7	0.570
Aspartic acid	53.9	55.43±1.4	52.00±1.9	52.90±1.2	55.16±2.3	52.13±2.0	54.30±1.6	0.141
Cysteine	3.6	6.56±0.5	5.3±1.7	5.23±2.0	4.26±2.6	4.43±0.8	4.43±1.0	0.533
Glutamic acid	80.5	83.33±1.7	82.93±1.0	83.93±0.7	83.66±1.1	85.06±0.5	84.06±1.5	0.419
Glycine	86.7	90.80±1.4	92.20±2.4	92.50±1.7	93.00±1.1	94.66±1.5	93.00±3.5	0.434
Proline	26.8	30.80±1.4	33.90±0.7	30.86±2.2	33.06±1.6	33.66±1.0	34.36±2.5	0.633
Serine	24.5	30.86±2.3	30.46±0.6	32.20±0.8	32.63±0.2	32.10±3.1	34.13±1.5	0.356
Total NEAA	337.6	360.11±1.7	361.99±1.4	360.82±1.6	364.67±1.6	366.27±1.6	369.34±1.8	
Total amino acids	542.6	602.85±1.7	593±1.5	614.47±1.7	614.18±1.7	616.44±1.8	623.07±1.6	

Whole-body amino acid values are expressed as mean ± SD of three replicates per treatment (n=3).

silkworm pupae meal are presented in Fig. 1. Significant differences (p<0.05) were observed in digestive enzyme activities (including amylase, trypsin and lipase activities) of *P. vannamei* fed different graded levels of silkworm pupae meal. Significantly (p<0.05) higher amylase, trypsin and lipase activities were observed in *P. vannamei* fed SWP 60 diet than in other diets, including the control diet.

Discussion

Due to the nutritional significance of insect meals and the unsustainable production of fish meal, more research has been conducted to evaluate the utility of insect meals as fish meal replacers in shrimp diets (Wang *et al.,* 2021; Chen *et al.,* 2021; He *et al.,* 2022; Hodar, 2022; Yildirim-Aksoy *et al.,* 2022). Among these insect meals, only limited research has been conducted to evaluate the effects of replacement of fish meal with silkworm pupae meal on growth performance, feed utilisation, carcass composition, serum antioxidant capacity and biochemical parameters of Pacific white shrimp (*P. vannamei*) (Rahimnejad *et al.,* 2019; Hodar, 2022). The results of the present study showed that 60% of SWP can replace fish meal protein in diets of *P. vannamei* without any negative impacts on growth performance, feed utilisation, whole-body composition and specific activities of digestive enzymes, which might

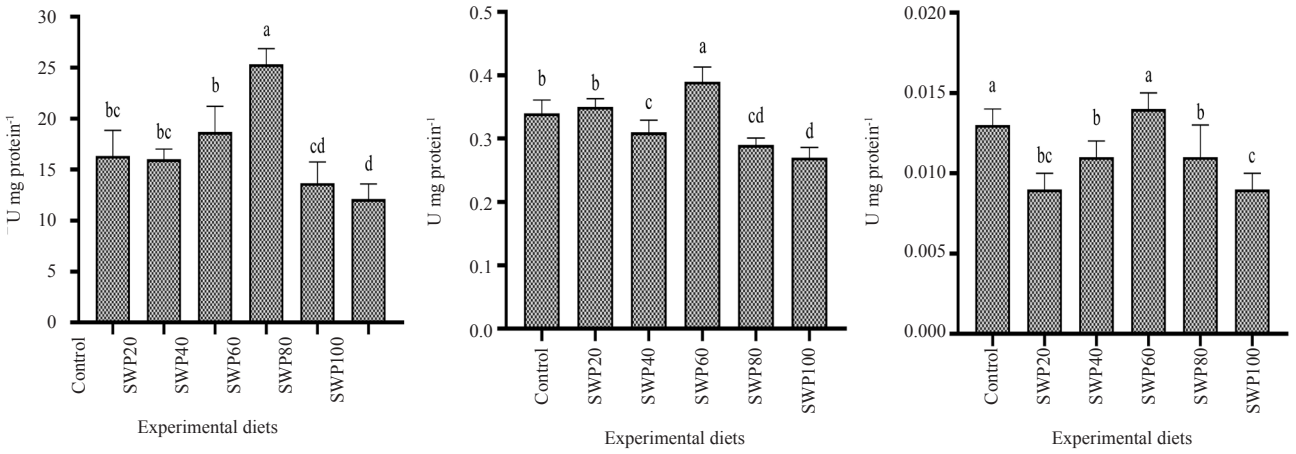

Fig. 1. Digestive enzyme activities (U mg^{-1} protein) of (a). Amylase, (b). Trypsin and (c). Lipase in *P. vannamei,* fed diets with 0% (Control), 20, 40, 60, 80 and 100% substitution of silkworm pupae meal. Data (mean±SD) with different letters are significantly different ($p<0.05$) among treatments.

be due to the presence of high crude protein content and adequate levels of essential amino acids in SWP, that meets the requirements of *P. vannamei.* Similarly, 75% of non-defatted silkworm pupae meal and combination of 50% of silkworm pupae meal and soybean meal can replace the fish meal in the diets of *P. vannamei* (Rahimnejad *et al.,* 2019; Hodar, 2022). The findings of our present study showed that a 100% inclusion level of SWP could reduce the growth performance and feed utilisation in *P. vannamei* diets. Similar to our research, it was found that SWP and BSFL meal cannot completely replace the fish meal protein in the diets of *P. vannamei* (Cummins *et al.,* 2017; Wang *et al.,* 2021; He *et al.,* 2022; Hodar, 2022). This could be attributed to imbalanced amino acid and fatty acid profiles and the rancidity of SWP meal. Additionally, no significant difference ($p>0.05$) was observed in the survival rate of shrimps fed with varying levels of SWP meal diets. The present finding agreed with the previous results of Rahimnejad *et al.* (2019), Chen *et al.* (2021); Richardson *et al.* (2021); Wang *et al.* (2021) and Hodar (2022) in *P. vannamei* diets.

The whole-body proximate composition of *P. vannamei* was not significantly influenced by feeding with different levels of SWP in the present study which was also confirmed by results from previous studies in *P. vannamei* fed with silkworm pupae meal (Rahimnejad *et al.,* 2019; Hodar, 2022) and black soldier fly larvae (BSFL) meal (Yildirim-Aksoy *et al.,* 2022). *P. vannamei* fed with different levels of mealworm meals does not influence the whole-body composition such as moisture, crude protein and ash levels. However, high lipid content in mealworm diets has increased the lipid deposition of shrimp muscles (Panini *et al.,* 2017). Wang *et al.* (2021) reported that defatted BSFL meal did not significantly affect the whole-body composition except the crude protein content of *P. vannamei.* Similarly, our results showed that *P. vannamei* fed with different inclusion levels of SWP did not significantly influence the whole-body amino acid composition and has been confirmed with previous findings in *P. vannamei* fed with defatted silkworm pupae meal. However, the significant reduction of whole-body methionine levels in 100% SWP inclusion diets could be attributed to lower methionine content in their diets (Rahimnejad *et al.,* 2019).

Digestive enzyme activity, utilisation plays a significant role in digestion and nutrient utilisation capacity of experimental diets (Dabrowski and Glogowski, 1977). Previous findings have confirmed that digestive enzyme activities were positively or negatively correlated with the dietary inclusion level of various feed ingredients (Gangadhar *et al.,* 2017; Feng *et al.,* 2021; Hosseini Shekarbi *et al.,* 2021). In our present study, digestive enzymes were significantly influenced by different inclusion levels of dietary SWP. Higher specific activity of digestive enzymes including amylase, trypsin and lipase was observed in 60% inclusion level of SWP incorporated diet. He *et al.* (2021) reported that the protease activity of *P. vannamei* was influenced by BSFL meal and higher protease activity was achieved in a 25% BSFL meal-incorporated diet. However, BSFL meal does not affect the specific activities of amylase and lipase in the diets of *P. vannamei.*

The findings of the present study revealed that dietary silkworm pupae meal could replace up to 80% of fish meal in diets of *P. vannamei* without affecting the growth performance, feed utilisation, whole-body proximate and amino acid profile and digestive enzyme activities. However, the test diet incorporated with 60% SWP meal achieved higher growth performance, feed utilisation, whole-body proximate composition and amino acid profile, and digestive enzyme activity of *P. vannamei.*

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References

AOAC 2010. *Official methods of analysis,* 18th edn. Association of the Official Analytical Chemists, Washington DC, USA.

APHA 2012. *Standard methods for the examination of water and waste water,* 22nd edn. American Public Health Association., New York, USA.

Begum, N. N., Chakraborty, S. C., Zaher, M., Abdul, M. M. and Gupta, M. V. 1994. Replacement of fishmeal by low-cost animal protein as a quality fish feed ingredient for Indian major carp, *Labeo rohita,* fingerlings. *Journal of the Science of Food and Agriculture,* 64(2): 191-197. https://doi.org/10.1002/jsfa.2740640207.

Chen, Y., Chi, S., Zhang, S., Dong, X., Yang, Q., Liu, H. and Xie, S. 2021. Evaluation of the dietary black soldier fly larvae meal (*Hermetia illucens*) on growth performance, intestinal health and disease

resistance to *Vibrio parahaemolyticus* of the Pacific white shrimp (*Litopenaeus vannamei*). *Front. Mar. Sci.,* 1072. https://doi.org/10.3389/fmars.2021.706463.

Cherry, I. S. and Crandall Jr. L. A. 1932. The specificity of pancreatic lipase: Its appearance in the blood after pancreatic injury. *American Journal of Physiology-Legacy Content,* 100(2): 266-273. https://doi.org/10.1152/ajplegacy.1932.100.2.266 .

Cho, S. H. 2010. Effect of fishmeal substitution with various animal and/or plant protein sources in the diet of the abalone *Haliotis discus hannai* Ino. *Aquac. Res.,* 41(10): e587-e593. https://doi.org/10.1111/j.1365-2109.2010.02561.x.

Cummins Jr., V. C., Rawles, S. D., Thompson, K. R., Velasquez, A., Kobayashi, Y., Hager, J. and Webster, C. D. 2017. Evaluation of black soldier fly (*Hermetia illucens*) larvae meal as partial or total replacement of marine fish meal in practical diets for Pacific white shrimp (*Litopenaeus vannamei*). *Aquaculture,* 473: 337-344. https://doi.org/10.1016/j.aquaculture.2017.02.022.

Dabrowski, K. and Glogowski, J. 1977. Studies on the role of exogenous proteolytic enzymes in digestion processes in fish. *Hydrobiologia,* 54(2): 129-134. https://doi.org/10.1007/BF00034986.

El-Sayed, A. -F. M. 1999. Alternative dietary protein sources for farmed tilapia, *Oreochromis* spp. *Aquaculture,* 179(1-4): 149-168. https://doi.org/10.1016/S0044-8486(99)00159-3.

Erlanger, B. F., Kokowsky, N. and Cohen, W. 1961. The preparation and properties of two new chromogenic substrates of trypsin. *Archives of Biochemistry and Biophysics,* 95(2): 271-278. https://doi.org/10.1016/0003-9861(61)90145-X.

FAO 2020. *The state of world fisheries and aquaculture 2018-Meeting the sustainable development goals.* Food and Agriculture Organisation of the United Nations, Rome, Italy.

Feng, Q. F., Wang, S. W., Huang, X. Z., Li, H., Liu, C. J., Lu, H., and Luo, L. 2021. Effects of oxidized silkworm (*Bombyx mori* L.) pupae on growth performance and intestine, liver and muscle histology and function of Gif Tilapia (*Oreochromis niloticus*). *Aquac. Res.,* 52(9): 4127-4137. https://doi.org/10.1111/are.15251.

Gangadhar, B., Umalatha, H., Ganesh, H., Saurabh, S. and Sridhar, N. 2017. Digestibility of dry matter and nutrients from three ingredients by the carps, *Labeo fimbriatus* (Bloch, 1795) and *Cyprinus carpio* Linnaeus, 1758 with a note on digestive enzyme activity. *Indian J. Fish.,* 64(3): 75-84. https://DOI: 10.21077/ijf.2017.64.3.69091-11.

He, Y., Liu, X., Zhang, N., Wang, S., Wang, A., Zuo, R. and Jiang, Y. 2022. Replacement of commercial feed with fresh black soldier fly (*Hermetia illucens*) larvae in Pacific white shrimp (*Litopenaeus vannamei*). *Aquac. Nutr.,* https://doi.org/10.1155/2022/9130400.

Hodar, A. S. R. 2022. Effect of fishmeal replacement with soybean meal and silkworm (*Bombyx mori*) pupae meal on growth performance, feed utilization and carcass composition in diet of juvenile white leg shrimp (*Litopenaeus vannamei*). *J. Exp. Zool. India,* 25: 1459-1467. https://connectjournals.com/03895.2022.25.1459.

Hora, S. L. 1962. Handbook of fish culture in the Indo-Pacific region. *FAO Fish Biol. Tech. Pap*., 14. Food and Agriculture Organisation of the United Nations, Rome, Italy, 203 p.

Hosseini Shekarabi, S. P., Shamsaie Mehrgan, M. and Banavreh, A.2021 Feasibility of superworm *Zophobas morio*, meal as a partial fishmeal replacer in fingerling rainbow trout *Oncorhynchus mykiss*, diet: Growth performance, amino acid profile, proteolytic enzymes activity and pigmentation. *Aquac. Nutr.,* 27(4): 1077-1088. https://doi.org/10.1111/anu.13249.

Ishida, Y., Fujita, T. and Asai, K. 1981 New detection and separation method for amino acids by high-performance liquid chromatography. *J. Chromatogr. A,* 204: 143-148. https://doi.org/10.1016/S0021-9673(00)81650-7.

Liao, I. C, and Chien, Y.-H. 2011. The Pacific white shrimp, *Litopenaeus vannamei,* in Asia: The world's most widely cultured alien crustacean. In: *The wrong place-alien marine crustaceans: Distribution, biology and impacts,* pp. 489-519. https://doi.org/10.1007/978-94-007-0591-3_17.

Olaniyi, C. and Babasanmi, G. 2013. Performance characteristics of African catfish (*Clarias gariepinus*) fed varying inclusion levels of silkworm pupae (*Anaphe infracta*). *Bangladesh J. Anim. Sci.,* 42(1): 76-80. https://doi.org/10.3329/bjas.v42i1.15787.

Panini, R. L., Pinto, S. S., Nobrega, R. O., Vieira, F. N., Fracalossi, D. M., Samuels, R. I. and Amboni, R. D. 2017. Effects of dietary replacement of fishmeal by mealworm meal on muscle quality of farmed shrimp *Litopenaeus vannamei. Food Res. Int.,* 102: 445-450. https://doi.org/10.1016/j.foodres.2017.09.017.

Rahimnejad, S., Hu, S., Song, K., Wang, L., Lu, K., Wu, R. and Zhang, C. 2019. Replacement of fish meal with defatted silkworm (*Bombyx mori L*.) pupae meal in diets for Pacific white shrimp (*Litopenaeus vannamei*). *Aquaculture,* 510: 150-159. https://doi.org/10.1016/j.aquaculture.2019.05.054.

Rangacharyulu, P., Giri, S., Paul, B., Yashoda, K., Rao, R. J., Mahendrakar, N. and Mukhopadhyay, P. 2003. Utilization of fermented silkworm pupae silage in feed for carps. *Bioresour. Technol.,* 86(1): 29-32. https://doi.org/10.1016/S0960-8524(02)00113-X.

Richardson, A., Dantas-Lima, J., Lefranc, M. and Walraven, M. 2021. Effect of a black soldier fly ingredient on the growth performance and disease resistance of juvenile Pacific white shrimp (*Litopenaeus vannamei*). *Animals,* 11(5): 1450. https://doi.org/10.3390/ani11051450.

Samocha, T. M., Davis, D. A., Saoud, I. P. and DeBault, K. 2004. Substitution of fish meal by co-extruded soybean poultry by-product meal in practical diets for the Pacific white shrimp, *Litopenaeus vannamei*. *Aquaculture,* 231(1-4): 197-203. https://doi.org/10.1016/j.aquaculture.2003.08.023.

Sathishkumar, G., Felix, N. and Prabu, E. 2021. Growth performances and nutrient utilization efficiency of GIFT tilapia reared in floating net cages fed with bioprocessed silkworm pupae meal. *Aquac. Nutr.,* 27(6): 2786-2797. https://doi.org/10.1111/anu.13408.

Seema, L., Yahya, B. and Rewa, L. 2011. Replacement of fishmeal with locally available ingredients in diet composition of *Macrobrachium dayanum. Afr. J. Agric. Res.,* 6(5): 1080-1084.

Tacon, A. G. 1993. Feed ingredients for crustaceans: Natural foods and processed feedstuffs. *FAO Fisheries Circular No. 866.* Food and Agriculture Organisation of the United Nations, Rome, Italy.

Trivedy, K., Kumar, S. N., Mondal, M. and Kumar Bhat, C.2008. Protein banding pattern and major amino acid component in de-oiled pupal powder of silkworm, *Bombyx mori* Linn. *J. Entomol.*, 5(1): 10-16. https://doi.org/10.3923/je.2008.10.16.

Wang, G., Peng, K., Hu, J., Mo, W., Wei, Z. and Huang, Y. 2021. Evaluation of defatted Hermetia illucens larvae meal for *Litopenaeus vannamei*: Effects on growth performance, nutrition retention, antioxidant and immune response, digestive enzyme activity and hepatic morphology. *Aquac. Nutr.,* 27(4): 986-997. https://doi.org/10.1111/anu.13240.

Worthington, C. 1991. *Worthington enzyme manual: Enzymes and related Biochemical.* Freehold, New Jersey, USA, pp. 212-215.

Xu, X., Ji, H., Yu, H. and Zhou, J. 2018. Influence of replacing fish meal with enzymatic hydrolysates of defatted silkworm pupa (*Bombyx mori* L.) on growth performance, body composition and non-specific immunity of juvenile mirror carp (*Cyprinus carpio* var. *specularis*). *Aquac. Res.,* 49(4): 1480-1490. https://doi.org/10.1111/are.13603.

Yildirim-Aksoy, M., Eljack, R., Beck, B. H. and Peatman, E. 2022. Nutritional evaluation of frass from black soldier fly larvae as potential feed ingredient for Pacific white shrimp, *Litopenaeus vannamei. Aquac. Rep.,* 27: 101353. https://doi.org/10.1016/j.aqrep.2022.101353.

Zhu, H., Gong, G., Wang, J., Wu, X., Xue, M., Niu, C. and Yu, Y. 2011 Replacement of fish meal with blend of rendered animal protein in diets for Siberian sturgeon (*Acipenser baerii* Brandt), results in performance equal to fish meal fed fish. *Aquac. Nutr.,* 17(2): e389-e395. https://doi.org/10.1111/j.1365-2095.2010.00773.x.