



Supplementation of coated and uncoated crystalline amino acid mix in formulating a low-fishmeal diet for *Penaeus monodon* (Fabricius, 1798): Effect on growth, digestibility, body composition, haemolymph indices and nitrogen metabolism

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Abstract

A sixty-day feeding trial was conducted to elucidate the effect of coated and uncoated crystalline amino acid mix (CAA) in *Penaeus monodon*. The control diet (CNT) was formulated with 250 g/kg fishmeal, and three more test diets were prepared by replacing 50% (125 g/kg) of fishmeal (w/w) using a blend of plant proteins with no supplementation of CAA (AA-NL), supplementation of uncoated (AA-NC) and coated (AA-CT) CAA. The AA-CT diet showed ($p < .05$) lower leaching of amino acids (1.13%) than other diets (2.78%–3.04%). DGC was high ($p < .05$) in shrimp fed on CNT and AA-CT (1.48%–1.49%/day) than other groups (1.32%–1.37%/day). Of all the treatments, FCR was poor ($p < .05$) in AA-NL (2.25). The dietary treatments did not influence survival ($p = .841$) and whole-body composition. The peak of free amino acid was synchronized (60 min) between AA-CT and CNT groups. The control group showed a higher nitrogen intake, oxygen consumption and O:N ratio and lower ammonia-N excretion. Haemolymph indices varied ($p < .05$) among the dietary groups. As a result of the N-metabolism study, the experimental diets were ranked as CNT>AA-CT>AA-NC. In conclusion, a blend of CAA could be effective in formulating low-fishmeal diets and could replace 50% (125 g/kg) of dietary fishmeal using a plant protein mix in *P. monodon*.

KEYWORDS

ammonia-N excretion, black tiger shrimp, free amino acid, O:N ratio, oxygen consumption, shrimp

1 | INTRODUCTION

Aquaculture is ensuring the food security globally with its fast growth rate (Naylor et al., 2021). Shrimp culture is one of the high value aquaculture systems, and among them, black tiger shrimp, *Penaeus monodon*, alone accounts for 12%–15% worldwide and is expected to increase in further years due to its relatively high price and stable markets. In addition, *P. monodon* is picking up nowadays,

mainly in Asian countries (Indonesia, Thailand, Viet Nam, Malaysia and Bangladesh) due to the availability of specific pathogen-free (SPF) seeds. As a result of this expansion and faster aquaculture growth, industrial aquafeed production has also increased from 7.6 mt in 1995 to 57 mt in 2020 (Jannathulla, Rajaram, et al., 2019; Aquafeed Market, 2021). Fishmeal is often included at the rate of 250–500 g/kg in the commercial formulation due to its highest palatability, digestibility and unique nutritional composition (Bulbul

et al., 2015; Jannathulla et al., 2018). However, the scenario of reduced/stagnant availability and increased price lead to replacement of considerable quantity of fishmeal for profitable aquaculture. Plant proteins, particularly oilseed meals/cakes, are the viable alternatives due to their availability and suitability. They have earlier been evaluated individually (Dayal et al., 2011; Jannathulla, Dayal, et al., 2019; Rajaram et al., 2012; Xie et al., 2016) and in combination (Bulbul et al., 2013; Dayal et al., 2020; Richard et al., 2011). However, the results are not fully encouraging, which is mainly due to the imbalanced profile of essential amino acids. In an attempt to balance the dietary amino acids, crystalline amino acids (CAA) are supplemented in shrimp feeds (Alam et al., 2004; Bulbul et al., 2015; Nunes et al., 2019).

In general, both methionine and lysine are the most limiting amino acids in most plant-based protein sources and their supplementation is vital when the inclusion level of plant proteins exceeds certain level. The CAA is also recognized as a feeding stimulant and is very effective in increasing the palatability of plant protein-based diet for aquatic species, including fish and shrimp (Kader et al., 2012). Supplementation of CAA enhanced the utilization of protein in various shrimp species such as kuruma shrimp, *P. japonicus* (Bulbul et al., 2015), whiteleg shrimp, *P. vannamei* (Huai et al., 2010; Nunes et al., 2019; Xie et al., 2014) and *P. monodon* (Liou et al., 2005). However, the effect of amino acid utilization was not optimistic due to their higher leaching, which resulted in poor/lower growth. In addition, it is very important to consider the asynchronism in absorption while supplementing CAA in aquafeeds because supplementing CAA as such is not efficiently utilized by the farmed animals due to its rapid absorption. These asynchronous absorption rates may lead to the presence of free amino acids in an imbalanced proportion at tissue level thereby affecting their utilization pattern. Coating techniques are attempted to prevent rapid absorption of CAA. Various studies have been reported the beneficial effects of coating CAA in different shrimp species such as *P. vannamei* (Chi et al., 2011) and *P. japonicus* (Alam et al., 2005).

In crustaceans, the metabolic pathways involved in nitrogenous excretion are catabolism of amino acid (including certain amides), deamination of purine nucleotides with the formation of ammonia, uricolysis and OUC (the ornithine-urea cycle) with the formation of urea, and the degradation of nucleic acid with the formation of uric acid (Claybrook, 1983; Regnault, 1987). The OUC initiates with the fixation of the metabolic product of NH_4^+ and HCO_3^- , which is formed from CO_2 through carbonic anhydrase. Walsh et al. (1989) have demonstrated that carbonic anhydrase supplies bicarbonate for urea synthesis in toadfish *Opsanus beta* hepatocytes. *P. japonicus* following exposure to ammonia-N at 10 mg/L increased its urea excretion nine-folds and its organic-N excretion 10-folds, which indicates the formation of urea and other nitrogenous compounds. The fact that the specific activity of arginase, which is involved in the last step of the OUC, increased with an increase in ambient ammonia-N suggests that shrimp spent energy to produce urea and other organic nitrogenous waste to avoid the toxic accumulation of ammonia. Hence, the present study is aimed to investigate that

how the coated amino acids are better than the uncoated ones in formulating low-fishmeal diets for *P. monodon* in terms of growth and digestibility. In addition, the supplemental effects of CAA on nitrogen metabolism in terms of oxygen consumption in relation to ammonia-N excretion were determined further in sub-adult shrimp. This baseline data would ensure the availability of dietary nutrients to the cultured species, which in turn helps to formulate the most cost-effective feed for shrimp.

2 | MATERIALS AND METHODS

2.1 | Preparation of coated CAA

Individual amino acids of lysine, methionine, arginine, isoleucine and leucine were purchased from Sigma-Aldrich (Code No: L5626, M9625, A0884, I2752 and L8000, respectively). A blend of CAA was prepared by mixing them at the rate of 0.8, 0.4, 0.4, 0.2 and 0.2 g, respectively, to fulfil their dietary requirement as like control diet (CNT). A double-coating methodology was performed according to Alam et al. (2004). In brief, a blend of CAA was mixed with deionized water, to which carboxymethylcellulose (CMC) was added (0.5 g) and the content was mixed manually until obtaining a dough-like substance. The CMC-coated CAA was further coated with gelatinized casein-gelatin (0.5 g) and was freeze-dried, ground and sieved through a 250 μm sieve and then stored at -18°C until use. However, a blend of CAA was mixed with filler and used as an uncoated one.

2.2 | Experimental diets

Prior to preparing experimental diets, a plant protein mix was prepared by mixing four different plant proteins such as groundnut oil cake, rapeseed meal, sesame oil cake and corn gluten meal at the ratio of 1:1:1:3 based on the result of our previous study (Rajaram, 2010). The nutrient composition plant protein mix along with the fishmeal used in the present study is depicted in Table 1. A control diet (CNT) was formulated to contain 400 g/kg crude protein (% as fed basis) using locally available ingredients listed in Table 2. Subsequently, three more iso-nitrogenous test diets were prepared, where 50% (125 g/kg) of fishmeal present in CNT diet was replaced with a plant protein mix (w/w). In Diet-2 (AA-NL), only fishmeal was replaced with a blend of plant proteins, but there was no supplementation of CAA, while in Diet-3 (AA-NC) and Diet-4 (AA-CT), uncoated and coated CAA, respectively, was included at the rate of 30 g/kg along with fishmeal replacement. The preparation of experimental diets was previously described by Dayal et al. (2011); briefly, all the dry ingredients were grounded and passed through a 250 μm sieve. To which, additives including uncoated and coated CAA, vitamin-mineral mix, vitamin-C (as an anti-oxidant), binder and chromic oxide (as an inert marker) and were added one by one. In addition, liquids such as fish oil and soy lecithin were added as a lipid source. All were



TABLE 1 Proximate and essential amino acid composition of fishmeal and plant protein mix used in the present study

Particulars	Test ingredients	
	Fishmeal	PP-Mix ^a
Proximate composition (g/kg as dry weight basis)		
Crude protein	637.4	551.9
Ether extract	105.1	17.9
Crude fibre	5.3	71.0
Nitrogen free extract ^b	62.7	313.6
Total ash	189.5	45.5
Essential amino acid composition (g/16 g N)		
Arg	6.86	5.55
His	2.65	2.28
Ile	4.64	3.86
Leu	7.97	11.15
Lys	7.90	5.19
Met	2.98	2.05
Phe	4.31	5.29
Thr	4.53	3.37
Trp	1.11	0.77
Val	5.41	4.37

^aPlant protein mix contains groundnut oil cake, rapeseed meal, sesame oil cake and corn gluten meal (1:1:1:3).

^bCalculated by a difference: 100 – (crude protein + ether extract + crude fibre + total ash).

mixed manually and transferred to a mixer and then homogenized for 20 min. To this, approximately 500 ml of deionized water per kg of homogenized mash was slowly added to make soft dough and the mixture was steamed at atmospheric pressure for 5 min. The semi-moist material was cooled and pelletized in a table-top pelletized in two different dies (0.8 and 1.6 mm) and was dried at 60°C to bring the moisture content <100 g/kg. The dried pellets were placed in a plastic container and stored in a refrigerator until used. Proximate and essential amino acid compositions of experimental diets are presented in Table 2.

2.3 | Leaching test of the experimental diets

Leaching loss of amino acids was checked in experimental diets after immersing the diets in experimental water containing a salinity of $24.16 \pm 1.31\text{‰}$ at two different time intervals (10 and 30 min) according to Smith et al. (2007) with slight modification. In brief, about 1 g of each experimental diet was placed in a beaker (500 ml) containing 400 ml of water and was gently shaken with the reciprocal shaker at room temperature. After which, the diet suspensions were filtered through a nylon filter membrane (0.45 µm, dia 47 mm) and rinsed with deionized water. Amino acids in the filtrate were quantified in the HPLC model LC-10 gradient system (Shimadzu Corp).

2.4 | Experimental shrimp

Fifteen-day-old postlarvae of *P. monodon* were procured from a government India certified hatchery located near Chennai, India, and were transported to the Muttukadu Experimental Station, ICAR-Central Institute of Brackishwater Aquaculture (CIBA), Chennai, India in an oxygen-filled polythene cover. Shrimp were reared in a net cage measuring 4 m² (2 m × 2 m) in the brackishwater lagoon of the institute. They were maintained on a commercial larval feed containing about 600 g/kg of crude protein and 150 g/kg ether extract. A part of the shrimp was taken after reaching approximately 0.5 g size for the feeding trial, and others were kept in the cage itself until getting sub-adult shrimp (>10 g) for nitrogen metabolism study. During this time, feed was changed according to the size of the shrimp. Before the transfer of shrimp from the lagoon to the indoor laboratory, it was confirmed that they were free from all the OIE-listed pathogens to rule out the disease risk.

2.5 | Feeding trial

A week prior to the experiment begins, healthy and uniform sized shrimp were transferred from the cage to a 1000 L flat base (43.3-inch bottom diameter) circular fibreglass reinforced plastic (FRP) tank and acclimatized for indoor laboratory conditions with a control diet (CNT, 0.8 mm) used in the present study. Twelve oval-shaped 500 L (1.31 × 0.64 × 0.73 m) FRP tanks were used in the present study, and all were equipped with continuous aeration and covered with fibre sheets to prevent escapees and to minimize the disturbance. Post-acclimatization, a total of 240 number shrimp with an average body weight of 0.51 ± 0.03 g were randomly separated into four triplicate groups/treatments and each replication had twenty numbers of shrimp. They were fed with the respective experimental diet (0.8 mm) by hand thrice a day (7.30 am, 12.30 pm and 5.30 pm) and allowed for feeding an hour. After which, uneaten feed particles (if any) were collected and the bottom of the tank was kept clean to collect the faeces for the determination of digestibility parameters. After feeding time, aeration was stopped and faeces was siphoned out immediately after defaecation, to prevent the leaching of nutrients, using a clean Falcon tube in a silk fabric. They were rinsed with deionized water and transferred to a filter paper using clean forceps, dried and then frozen immediately at –20°C till the analysis of digestibility parameters (Jannathulla et al., 2017). After collecting faeces, again aeration was started and the work was routine throughout the experimental period of 60-days. Meanwhile, the collected uneaten feed particles were rinsed with deionized water and dried at 60°C in a hot air oven to measure the feed intake on a daily basis. Diets were given at the rate of 8% of total biomass and later were adjusted based on intake. Shrimp were maintained in this system under a normal photoperiod throughout the experimental period. At the end of the experimental period, CAA supplementation was evaluated by its effect on growth performance in terms of daily growth coefficient (DGC), feed conversion ratio (FCR), protein efficiency ratio, apparent

TABLE 2 Ingredient and nutrient composition (proximate and essential amino acids) of experimental diets used in the present study

	Experimental diets				
Particulars	CNT	AA-NL	AA-NC	AA-CT	R ^a
Ingredient composition (g/kg as fed basis)					
Fishmeal ^b	250	125	125	125	
PP-Mix ^c	–	125	125	125	
CAA ^d	–	–	30	30	
Acetes ^e	120	120	120	120	
Squilla meal ^f	114	114	114	114	
Squid meal ^g	50	50	50	50	
Soybean meal	200	200	200	200	
Wheat gluten meal	–	36	–	–	
Wheat flour	200	164	170	170	
Fish oil ^b	20	20	20	20	
Soy lecithin ^h	10	10	10	10	
Vit-Min mix ⁱ	20	20	20	20	
Vitamin C	1	1	1	1	
Binder ^j	10	10	10	10	
Chromic oxide ^k	5	5	5	5	
Proximate composition (g/kg as fed basis)					
Moisture	92.3	99.6	96.0	92.4	
Crude protein	408.4	406.4	409.2	409.7	
Ether extract	82.1	79.1	75.9	75.2	
Crude fibre	29.9	42.8	43.2	42.1	
Total ash	173.0	140.5	142.0	149.9	
Nitrogen free extract ^l	214.3	231.6	233.7	230.7	
Essential amino acid composition (g/16 g N)					
Arg	6.32	5.85	6.01	5.88	5.3
His	2.25	2.52	2.22	2.27	2.2
Ile	3.5	3.71	3.52	3.44	2.7
Leu	7.39	8.99	7.8	7.84	4.3
Lys	6.42	4.93	6.3	6.37	5.2
Met	2.62	2.02	3.15	3.05	2.4
Phe	4.21	4.68	4.13	4.17	3.7
Thr	3.5	4.01	3.59	3.54	3.5
Trp	0.88	0.9	0.81	0.85	0.5
Val	4.04	4.86	4.06	3.98	3.7

Note: All other ingredients are purchased in local markets.

^aDietary essential amino acid requirements of *P. monodon* (Dayal et al., 2011).

^bBismi Fisheries.

^cPlant protein mix contains groundnut oil cake, rapeseed meal, sesame oil cake and corn gluten meal (1:1:1:3).

^dCrystalline amino acids contain lysine, methionine, arginine, isoleucine and leucine (0.8, 0.4, 0.4, 0.2 and 0.2 g, respectively in 1 g filler/coating agent) obtained from Sigma-Aldrich.

^eOm-Sai Aqua.

^fBlueline Foods India Pvt. Ltd.

^gKhaja Mohammed Store.

^hReal Soy Enterprises.

ⁱThiamine hydrochloride (25.50 g), riboflavin (25.00 g), pyridoxine hydrochloride (50.00 g), cyanogobalamine (0.10 g), menadione (5.00 g), all-trans tocopherol acetate (99.00 g), retinyl acetate (10.00 g), vitamin D (50 g), nicotinic acid (101.00 g), D-Ca-pantothenate (61.00 g), biotin (25.00 g), folic acid (6.25 g), inositol (153.06 g), ferric citrate (13.70 g), ZnSO₄.7H₂O (28.28 g), MgSO₄.7H₂O (0.12 g), MnSO₄.H₂O (12.43 g), CuSO₄.5 H₂O (19.84 g), CoC₁₂.6H₂O (4.07 g), KIO₄ (0.03 g), KCl (15.33 g), Na₂SeO₃ (0.02 g).

^jPegabind, Bentoli Agri-Nutrition Asia Pvt. Ltd.

^kSigma-Aldrich.

^lCalculated by a difference [1000 – (g/kg of moisture + crude protein + ether extract + crude fibre + total ash)].

protein utilization (APU) and survival as follows. Digestibility parameters such as apparent dry matter, crude protein and essential amino acid digestibility were calculated according to Carvalho et al. (2016).

$$\text{DGC (\% /day)} = 100 \times \text{Final weight (g)}^{1/3} - \text{Initial weight (g)}^{1/3} / \text{No. of days}$$

$$\text{FCR} = \text{Dry feed consumed (g)} / \text{Wet weight gain (g)}$$

$$\text{PER} = \text{Weight gain (g)} / \text{Protein intake (g)}$$

$$\text{APU (\%)} = \text{Protein gain (g)} / \text{Protein intake (g)} \times 100$$

$$\text{Survival (\%)} = (\text{Final number} - \text{Initial number}) / \text{Initial number} \times 100$$

2.6 | Study for nitrogen metabolism

Based on the result of the growth trial, a further 3-week study was conducted to assess the effect of CAA supplementation on nitrogen metabolism in sub-adult *P. monodon*. For that, shrimp with an average body weight of 10.27 ± 0.37 g were transferred from the cage to a 1000 L flat base circular FRP tank for acclimatization for a week before commencing the experiment. During acclimatization, shrimp fed with the control diet (CNT, 1.6 mm) to domesticate them to the experimental conditions as in the growth trial. Three triplicate groups of shrimp (CNT, AA-NC and AA-CT) were subjected in the particular study and were maintained individually in oval shape 100 L ($0.26 \times 0.13 \times 0.17$ m) FRP tanks. The shrimp were hand-fed with the respective diet (1.6 mm diameter) at the rate of 4% of the body weight and allowed for feeding by 15–20 min until gut filling. Then, they were transferred, without any deformities, to a respirometry metabolic system of a sealed chamber containing a 5-inch bottom diameter. Water samples were collected from each respiratory chamber for the analysis of oxygen and nitrogen content on a daily basis. Simultaneously, a blank was carried out with fresh experimental water collected from the animals' free-respirometry chambers. After which, shrimp were again shifted to the respective experimental FRP tanks and this work was routine throughout the experimental period. Meanwhile, daily intake was measured as mentioned previously in the feeding trial. Haemolymph samples were obtained from eighteen shrimp (inter-moult) in each treatment (six shrimp/replication) through the ventral sinus in the first abdominal segment using a 26-gauge hypodermic needle on a 1-ml syringe with 2-mm thickness containing 0.3-ml anti-coagulant solution. Other shrimp not used for haemolymph collection were used for analysing the body composition.

2.7 | Water quality parameters

Ultraviolet-treated water was used in both experiments after filtering using a 5- μ m cartridge filter. About 80% of the water was exchanged daily before the first feeding, and the water quality parameters were measured periodically by a standard method of APHA (2012). The mean values of analysed parameters are given in Table 3.

TABLE 3 Mean value of water quality parameters analysed during the experimental periods of 60 days

Particulars	
Temperature (°C)	27.16 ± 1.47
Salinity (‰)	24.16 ± 1.31
pH	8.11 ± 0.47
Dissolved oxygen (mg/L)	7.86 ± 0.84
Turbidity (NTU)	17.13 ± 1.87
Total alkalinity (mg/L as CaCO_3)	147.77 ± 4.13
Total hardness (mg/L as CaCO_3)	2137.14 ± 59.47
Total ammonia (mg/L)	0.08 ± 0.01
Nitrite-N (mg/L)	0.03 ± 0.01
Nitrate-N (mg/L)	0.04 ± 0.01
Phosphate (mg/L)	0.14 ± 0.04

2.8 | Biochemical analysis

Proximate composition of both experimental diets and shrimp whole body was carried out using a standard method of AOAC (1997). Samples were oven-dried at 105°C and incinerated at 540°C to constant weight for analysing moisture and total ash, respectively. Crude protein was estimated using a Tecator Kjeltect system (Kjeltect™-8100) after multiplying with a common empirical factor of 6.25. Scocs Plus-SCS:6 and FOSS-2022, Tecator™ were used for the determination of ether extract and crude fibre, respectively, while nitrogen-free extract (NFE) was calculated by a difference [$1000 - (\text{g/kg of moisture} + \text{crude protein} + \text{crude lipid} + \text{crude fibre} + \text{total ash})$]. Chromium content was analysed in both diets and faeces according to Furukawa (1966) using a UV visible spectrophotometer (UV-1800, Shimadzu).

The amino acid composition was analysed using an HPLC model LC-10 gradient system (Shimadzu Corp) after hydrolysing the samples with 6 N hydrochloric acid in a sealed tube filled with nitrogen for 22 h at 110°C in a vacuum oven (Finlayson, 1964), while free amino acids (FAA) were measured using the method outlined by Mente et al. (2002) with slight modification. Briefly, the sample taken from the tail muscle was homogenized in absolute ethanol and was centrifuged to precipitate the proteins. The collected supernatant was dried and reconstituted in sodium citrate-perchloric acid sample diluent (pH 2.20), and it is filtered through a 0.2 μ m membrane filter. Individual amino acids were separated by Shimpack column (ISC-07/S1504 Na) packed with a strongly acidic Na^+ type cation exchange resin (Styrene-divinyl benzene copolymer with sulfinic group) under gradient elution at a flow rate of 0.3 ml/min by using buffer A (sodium citrate-perchloric acid, pH:3.2) and buffer B (boric acid sodium citrate-sodium hydroxide; pH:10.0). Amino acids were qualified and quantified by a fluorescent detector (RF-20AXS) after postcolumn derivatization, using the amino acid mixer as an external standard (Sigma-Aldrich, Inc.) and nor-leucine as an internal standard (Jannathulla et al., 2018). Tryptophan, being labile to acid hydrolysis, was measured after alkali hydrolysis using a spectrophotometric

method at 500 nm (Sastry & Tammuru, 1985). The partial oxidation of sulphur-containing amino acids such as cysteine and methionine during acid digestion was prevented by adding 0.1% of phenol (Jannathulla, Dayal, Ambasankar, & Muralidhar, 2018).

Oxygen content in the experimental water sample was analysed by Winkler (1888) method, and the analysed value as oxygen consumption by shrimp was reported as $\mu\text{g/g}$ of dry matter/h. Ammonia-N in water was estimated according to the method of Chaney and Marbach (1962), and the value as ammonia-N excretion by shrimp was reported as $\mu\text{g/g}$ of dry matter/h. From these values, O:N ratio was computed. The activity of glutamate dehydrogenase (GDH) assay was performed on crude homogenates of gill tissue following the method proposed by King et al. (1985). Arginase was estimated using a commercial kit obtained from Sigma-Aldrich (Code No: MAK112) in a UV spectrophotometer (UV-1800-Shimadzu Corp.) according to the accredited methodology given by Sigma-Aldrich (<https://www.sigmaaldrich.com/content/dam/sigmaaldrich/docs/Sigma/Bulletin/2/mak112bul.pdf>). The haemolymph indices such as glucose, total protein, ammonia and urea were estimated using commercial kits obtained from Sigma-Aldrich (Code No: GAHK20, TP0100, AA0100, MAK006) in a UV spectrophotometer (UV-1800-Shimadzu Corp.) according to the accredited methodology given by Sigma-Aldrich ([https://www.sigmaaldrich.com/content/dam/sigmaaldrich/docs/Sigma/Bulletin/2/\(CODE:NO\).pdf](https://www.sigmaaldrich.com/content/dam/sigmaaldrich/docs/Sigma/Bulletin/2/(CODE:NO).pdf)). Haemocyanin and haemolymph lipid content were estimated according to Antonini and Brunori (1974) and Bligh and Dyer (1959), respectively.

2.9 | Statistical analysis

All the experimental data were subjected to statistical analysis using one-way analysis of variance (ANOVA) in the statistical package of SPSS ver.16.0 for Windows. Values were presented as a mean of three replications with the respective SEM and p -values. The significant difference between the means was evaluated by Tukey's test following ANOVA. Regression analysis was performed on oxygen consumption, ammonia-N excretion and O:N ratio against nitrogen intake to find a correlation among them. Prior to statistical evaluation, data were checked for homogeneity of variances after ascertaining the normal distribution. Probabilities of $p < .05$ were considered as significant.

3 | RESULTS

3.1 | Composition of experimental diets

All the experimental diets had an almost similar level of moisture (92.3–99.6 g/kg) and crude protein (406.4–409.7 g/kg). The level of ether extract and total ash was high in CNT compared with test diets (AA-NL, AA-NC and AA-CT) where 50% (125 g/kg) of fishmeal was replaced with PP-Mix, and the reverse was true for crude

TABLE 4 Leaching of total amino acids (% dry weight basis) of the experimental diets after immersion in experimental water for 10 and 30 min

Particulars	Immersion time	
	10 min	30 min
CNT	2.89 ^a	5.84 ^a
AA-NL	3.04 ^a	6.01 ^a
AA-NC	2.78 ^a	5.49 ^b
AA-CT	1.13 ^b	2.24 ^c
SEM ($\pm$)	0.015	0.017
p -value	<.001	<.001

Note: All the values are mean of three replications. Mean bearing the same superscript within the column do not differ significantly ($p > .05$).

fibre and NFE (Table 2). The level of essential amino acids was not affected due to dietary change and was more than the dietary requirements of *P. monodon*. The values reported in the present study are similar to those reported by Dayal et al. (2011) for the same species.

3.2 | Leaching loss of amino acids from experimental diets

The leaching loss of total amino acids from experimental diets is depicted in Table 4, and the result showed that the diet containing coated CAA (AA-CT) had significantly ($p < 0.05$) lower leaching (1.13%) than the other three experimental diets of CNT (2.89%), AA-NL (3.04%) and AA-NC (2.78%) after 10 min. A similar trend was observed event after 30 min of immersion; however, a diet supplemented with uncoated CAA showed better results compared with the diets of CNT and AA-NL.

3.3 | Effect of CAA on digestibility parameters

The effect of CAA supplementation on the apparent digestibility coefficients of dry matter (ADMD), crude protein (ACPD) and essential amino acids is shown in Table 5. ADMD was significantly ($p < .05$) high in shrimp fed with CNT diet (79.47%) followed by AA-CT (76.77%), and it was in the range of 74.33%–75.12% in the groups fed with AA-NC and AA-NL diets. Similarly, ACPD was found to be high ($p < .05$) in CNT groups (89.44%) and was reduced by 8.78%, 7.78% and 5.04% in AA-NL, AA-NC and AA-CT, respectively. Shrimp reared on CNT diet showed the highest digestibility for most of the essential amino acids, including Arg, Lys, Phe and Val, and shrimp with coated CAA supplementation (AA-CT) had a better value in Ile, Met and Phe, while there was no significant difference between CNT and AA-CT diets in Thr digestibility. Interestingly, His had significantly ($p < .05$) higher digestibility in shrimp fed with no CAA supplemented diet (AA-NL). Similarly, it was Leu and Trp of the AA-NC fed group showed a



Particulars	Experimental diets				SEM ($\pm$)	p-value
	CNT	AA-NL	AA-NC	AA-CT		
ADMD ¹	79.47 ^a	74.33 ^d	75.12 ^c	76.77 ^b	0.020	<.001
ACPD ²	89.44 ^a	80.66 ^d	81.66 ^c	84.40 ^b	0.011	<.001
Arg	91.83 ^a	88.46 ^b	86.59 ^c	86.93 ^c	0.253	<.001
His	87.42 ^b	89.03 ^a	83.92 ^c	83.72 ^c	0.262	<.001
Ile	90.57 ^b	86.54 ^c	84.59 ^d	91.71 ^a	0.009	<.001
Leu	81.70 ^c	85.05 ^b	87.44 ^a	85.11 ^b	0.691	.004
Lys	92.32 ^a	85.51 ^b	84.32 ^b	85.27 ^b	0.257	<.001
Met	89.67 ^b	82.93 ^d	86.17 ^c	90.69 ^a	0.027	<.001
Phe	92.42 ^a	86.55 ^b	81.60 ^c	90.86 ^a	0.372	<.001
Thr	86.46 ^a	83.41 ^b	83.61 ^b	85.94 ^a	0.185	.001
Trp	88.97 ^c	89.46 ^c	91.24 ^a	90.47 ^b	0.068	.001
Val	90.59 ^a	82.91 ^d	83.25 ^c	88.61 ^b	0.009	<.001

All the values are mean of three replications. Mean bearing the same superscript within the row do not differ significantly ($p > .05$).

¹Apparent dry mater digestibility.

²Apparent crude protein digestibility.

TABLE 5 Apparent digestibility coefficients (%) of dry mater, crude protein and essential amino acids of experimental diets in *P. monodon*

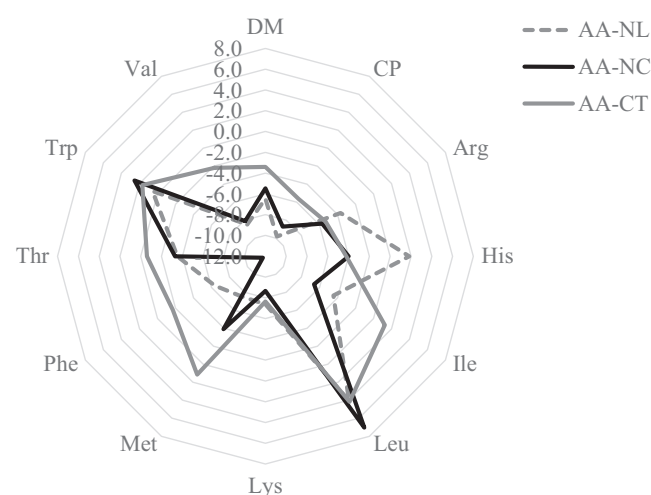


FIGURE 1 Per cent variation in digestibility parameters of test diets compared with control diet in *P. monodon*

higher digestibility among the test diets. The per cent variation was comparatively reduced by 3.4% and 5.6% for ADMD and ACPD, respectively, in the AA-CT diet compared with the CNT diet, and the reduction was 5.5%–8.7% in the AA-NC diet and 6.5%–9.8% in the AA-NL diet. Digestibility of Leu and Trp was increased by 4.1%–7.0% and 0.6%–2.6%, respectively, in test diets than the control diet, while the reverse trend was observed for all other amino acids except Ile, Leu and Met in AA-CT and His in AA-NL. The per cent variation of digestibility parameters was numerically high in shrimp fed no CAA supplemented diet (AA-NL) compared with control and was in the range of 3.5–9.8, while it was 3.3–8.7 and 1.7–5.6 in uncoated (AA-NC) and coated (AA-CT) CAA fed groups, respectively (Figure 1).

3.4 | Effect of CAA on growth performance

Growth performance and feed utilization of *P. monodon* fed different experimental diets are depicted in Table 6. The results revealed that there was no significant difference in the initial weight of the shrimp ($p = .236$), while other measured parameters were significantly affected by the dietary change. Shrimp fed with the CNT and AA-CT diets had the highest DGC (1.49% and 1.48%/day, respectively) and was significantly ($p < .05$) reduced to 1.37%/day in those fed a diet supplemented with the uncoated CAA (AA-NC), whereas it was 1.32%/day in shrimp reared with no CAA supplementation (AA-NL). AA-NL diet showed a very poor FCR as evidenced with the highest value (2.25) compared with others (1.68–1.93); however, the control diet performed better than CAA supplemented diets, including both coated and uncoated. No significant difference was observed in PER between CNT and AA-CT fed groups (1.6) and was significantly ($p < .05$) reduced to 1.44 in AA-NC and 1.23 in AA-NL. Shrimp reared on the AA-CT diet had the highest value of APU (30.65%), while the least value was noticed with the group fed no CAA supplementation diet (AA-NL). The dietary difference did not influence survival among the experimental groups ($p = .841$) and was in the range of 93.33%–97.78%.

3.5 | Effect of CAA on body composition

The whole-body composition of *P. monodon* fed experimental diets is given in Table 7 and was presented as a % wet weight basis. Moisture, crude protein, ether extract and total ash content were in the range of 777.9–783.2 g/kg, 158.8–162.7 g/kg, 16.5–17.9 g/kg and 22.2–30.9 g/kg, respectively, among the experimental groups.

TABLE 6 Effect of crystalline amino acid supplementation on growth performance in *P. monodon*

Particulars	Experimental diets				SEM ($\pm$)	p-value
	CNT	AA-NL	AA-NC	AA-CT		
Initial weight (g)	0.51 ^a	0.47 ^a	0.51 ^a	0.51 ^a	0.001	.236
Final weight (g)	4.91 ^a	3.97 ^b	4.23 ^b	4.83 ^a	0.074	.037
DGC (%/day) ¹	1.49 ^a	1.32 ^c	1.37 ^b	1.48 ^a	0.002	.023
FCR ²	1.68 ^d	2.25 ^a	1.93 ^b	1.74 ^c	0.001	<.001
PER ³	1.60 ^a	1.23 ^c	1.44 ^b	1.61 ^a	0.001	<.001
APU (%) ⁴	29.91 ^b	23.09 ^d	26.09 ^c	30.65 ^a	0.047	<.001
Survival (%)	93.33 ^a	95.56 ^a	97.78 ^a	95.55 ^a	20.667	.841

Note: All the values are mean of three replications. Mean bearing the same superscript within the row do not differ significantly ($p > .05$).

¹Daily growth coefficient.

²Feed conversion ratio.

³Protein efficiency ratio.

⁴Apparent protein utilization.

TABLE 7 Effect of crystalline amino acid supplementation on body composition (g/kg wet weight basis) in *P. monodon*

Particulars	Experimental diets				SEM ($\pm$)	p-value
	CNT	AA-NL	AA-NC	AA-CT		
Moisture	783.2	778.9	777.9	780.9	8.6	.424
Crude protein	158.8	162.0	162.7	160.3	5.6	.472
Ether extract	17.9	17.5	17.7	16.5	0.2	.056
Total ash	22.2	30.4	30.9	30.5	19.5	.294
Arg	9.9	10.5	10.3	10.1	0.3	.747
His	2.8	2.9	3.5	3.5	0.1	.501
Ile	6.3	6.4	7.8	7.1	0.3	.154
Leu	13.3	12.9	12.6	12.5	0.2	.324
Lys	8.9	9.9	9.2	9.4	0.4	.493
Met	3.9	3.3	3.6	3.8	0.1	.117
Phe	5.4	6.0	5.6	5.8	0.1	.510
Thr	5.1	5.6	5.2	5.2	0.2	.793
Trp	1.4	1.3	1.5	1.7	0.1	.247
Val	7.3	7.6	7.2	7.5	0.1	.732

Note: All the values are mean of three replications. No significant difference observed among the treatments.

Of all essential amino acids, Leu (1.25%–1.33%) was found to be high followed by Arg (0.99%–1.05%) and Lys (0.89%–0.99%), while the least value was noticed for Trp (0.13%–0.17%) irrespective of the dietary groups, but no significant effects were found in whole-body composition due to dietary change ($p = .117$ –.793). However, a significant ($p < .05$) difference was observed in free amino acid (FAA) content in the tail muscle of *P. monodon* (Table 8). Among the essential amino acids, except Trp ($p = .522$), all were significantly ($p < .05$) affected by dietary change. In FAA, all the essential acids were significantly ($p < .05$) high in shrimp fed with coated CAA supplemented diet (AA-CT) compared with other experimental groups. In which, Arg was found to be predominant (36.93–42.39 $\mu\text{mol/g}$ of tissue) followed by Leu (11.10–15.07 $\mu\text{mol/g}$) and Val (9.19–10.29 $\mu\text{mol/g}$). After AA-CT, shrimp on the CNT diet had a higher value of FAA than those fed on AA-NL and AA-NC diets, except His, which was high in

AA-NL. The least value of Arg, His and Lys was found with AA-NC, but for Met, it was AA-NL, while there was no significant difference between AA-NL and AA-NC for Ile, Leu and Phe. Of all non-essential amino acids, Gly was found to be predominant (65.58–74.74 $\mu\text{mol/g}$) followed by Ala (30.47–39.92 $\mu\text{mol/g}$), while the least value was noticed in Tyr (2.54–2.97 $\mu\text{mol/g}$) irrespective of the experimental groups. Contrary to the earlier one, the AA-CT diet showed a higher value only for Ala. Asp and Tyr, in addition to Ala, were significantly ($p < .05$) high in AA-NC, while it was Glu and Ser in AA-NL and Gly and Pro in CNT. The peak of the postprandial FAA in haemolymph was attained within 30 min in shrimp fed on uncoated CAA supplemented diet (AA-NC) and was delayed to 60 min in both CNT and AA-CT fed groups (Figure 2), while it was almost constant from 30 to 180 min in shrimp fed with a diet contains no CAA supplementation (AA-NL).



	Experimental diets					
Particulars	CNT	AA-NL	AA-NC	AA-CT	SEM (±)	p-value
Essential amino acids						
Arg	40.87 ^b	37.43 ^c	36.93 ^d	42.39 ^a	0.032	<.001
His	3.46 ^c	3.61 ^b	2.99 ^d	3.86 ^a	0.001	<.001
Ile	4.90 ^b	3.63 ^c	3.43 ^c	5.24 ^a	0.007	<.001
Leu	13.93 ^b	11.60 ^c	11.10 ^c	15.07 ^a	0.066	<.001
Lys	8.77 ^b	6.73 ^c	5.80 ^d	9.95 ^a	0.029	<.001
Met	4.53 ^b	3.87 ^d	4.40 ^c	4.99 ^a	0.001	<.001
Phe	2.68 ^b	2.51 ^c	2.50 ^c	2.73 ^a	0.001	<.001
Thr	8.30 ^b	8.33 ^b	8.49 ^b	9.11 ^a	0.013	.002
Trp	1.24 ^a	1.27 ^a	1.13 ^a	1.18 ^a	0.008	.522
Val	10.29 ^a	9.19 ^c	9.92 ^b	10.43 ^a	0.015	<.001
Non-essential amino acids						
Ala	30.74 ^c	34.07 ^b	39.40 ^a	39.92 ^a	0.136	<.001
Asp	4.17 ^b	3.80 ^c	4.32 ^a	4.09 ^b	0.001	<.001
Glu	14.65 ^b	15.42 ^a	14.23 ^c	14.26 ^c	0.011	<.001
Gly	74.74 ^a	65.58 ^c	66.89 ^c	68.87 ^b	0.470	<.001
Pro	12.99 ^a	11.76 ^c	12.14 ^b	11.79 ^c	0.002	<.001
Ser	6.02 ^c	6.73 ^a	6.11 ^c	6.36 ^b	0.002	<.001
Tyr	2.60 ^c	2.54 ^d	2.97 ^a	2.75 ^b	0.001	<.001

Note: All the values are mean of three replications. Mean bearing the same superscript within the row do not differ significantly ($p > .05$).

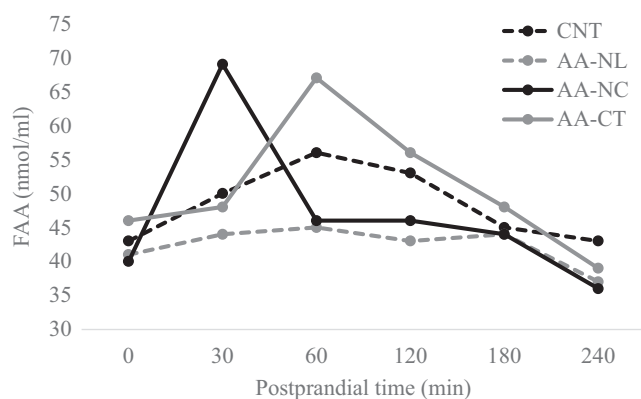


FIGURE 2 Effect of crystalline amino acid supplementation on free amino acid (FAA) levels in haemolymph of *P. monodon*

3.6 | Effect of CAA on nitrogen metabolism

Based on the result of the growth trial, a 3-week study was designed consequently, where a diet with no CAA supplementation (AA-NL) was omitted due to poor performance and the diets with coated and uncoated CAA (AA-CT and AA-NC) were compared with control to elucidate their effect on nitrogen metabolism in terms of oxygen consumption in relation to nitrogen utilization and ammonia-N excretion in sub-adult *P. monodon* (Table 9). Results revealed that shrimp reared on CNT diet showed a nitrogen intake of 1.72 mg (shrimp/meal) and was significantly ($p < .05$) reduced by 23.3% and 25.6% in AA-NC and AA-CT fed groups, respectively, but no significant difference was observed between coated and uncoated one. A similar trend was observed for postprandial oxygen consumption. Shrimp fed on uncoated

TABLE 9 Effect of crystalline amino acid supplementation on the parameters of nitrogen metabolism in *P. monodon*

Particulars	Experimental diets			SEM ($\pm$)	p-value
	CNT	AA-NC	AA-CT		
N intake (mg/shrimp/meal)	1.72 ^a	1.32 ^b	1.28 ^b	0.001	.001
O consumption (μ g/g of DM/h)	600.25 ^a	540.12 ^b	540.03 ^b	236.585	.034
N excretion (μ g/g of DM/h)	19.58 ^c	26.12 ^a	21.14 ^b	0.243	.001
O:N ratio	30.57 ^a	20.67 ^c	25.64 ^b	1.504	.004

Note: All the values are mean of three replications. Mean bearing the same superscript within the row do not differ significantly ($p > .05$).

CAA (AA-NC) showed significantly ($p < .05$) higher ammonia-N excretion than that obtained in coated one (AA-CT) and was still low ($p < .05$) in the control group. Based on O:N ratio, the experimental diets were ranked as CNT > AA-CT > AA-NC. Regression performed on oxygen consumption, ammonia-N excretion and O:N ratio against nitrogen intake revealed a better correlation for oxygen consumption ($R^2 = .7411$), whereas the correlation coefficient found to be poor for both ammonia-N excretion ($R^2 = .0145$) O:N ratio ($R^2 = .4115$) and the equations were $y = 189.99x + 286.05$, $y = -1.5669x + 24.539$ and $y = 10.753x + 10.115$, respectively (Figure 3). The activity of both GDH and arginase was high in shrimp fed with AA-NC diet, but there was no significant among the groups due to dietary change (Figure 4).

3.7 | Effect of CAA on haemolymph indices

The effect of CAA supplementation on haemolymph indices in *P. monodon* is presented in Table 10. Haemolymph protein and haemocyanin were significantly ($p < .05$) higher in shrimp fed with control diet (122.22 mg/ml and 1.45 mM, respectively) compared with other groups, but higher ($p < .05$) values were found with coated CAA than uncoated one. Ammonia level was almost double in test diets (AA-NC and AA-CT) compared with the control. The level of urea and glucose was also affected by the dietary change and was significantly ($p < .05$) lower in AA-NT, while no difference was noticed in the haemolymph lipid content of *P. monodon*.

4 | DISCUSSION

Supplementation of CAA resulted in better performance in monogastric animals, (Selle et al., 2020; Wang et al., 2020), but it is not successful always in aquatic species due to its leaching (Biswas et al., 2007; Chi et al., 2011; Fox et al., 2011). Earlier studies have been reported that supplemented CAA showed a better absorption than the amino acids of intact protein origins in various shrimp species, including *P. monodon* (Simon et al., 2021) and *P. vannamei* (Ji

et al., 2021). In our study, *P. monodon* were reared on low-fishmeal diets supplemented with the CAA (AA-NC and AA-CT) showed a significantly higher growth performance than those reared on without CAA (AA-NL). This result is corroborated with the findings of Richard et al. (2011), who reported that the growth of *P. monodon* was significantly reduced when dietary fishmeal level was decreased from 320 g/kg (control) to 160 g/kg using a mixture of plant proteins. A similar result was reported in *P. japonicus* (Bulbul et al., 2013). This indicates that the supplementation of essential amino acids is often required to improve the nutrient utilization of plant protein-based diets in shrimp species. Biswas et al. (2007) found a positive response in the growth of *P. monodon* fed a diet containing soybean meal as a major protein source that supplemented with 10 g/kg Lys. Similarly, Fox et al. (2011), reported that *P. vannamei* juveniles had an initial weight of 0.22 ± 0.02 g revealed a higher growth with the supplementation of DL-Met, mineral chelates (Cu, Mn, Ca and Zn) of Met and polymerized Met (Novus International, Inc.) than those reared without any supplementation. Similarly, the lower performance of *P. vannamei* due to the replacement of dietary fishmeal using soybean meal was recovered by the supplementation of 1.5–2.0 g/kg Met (Guo et al., 2020). This is in an agreement in *P. japonicus* (Alam et al., 2005) and *P. vannamei* (Forster & Dominy, 2006). From the above results, it is evident that supplementation of CAA is effective in improving the quality of the diet in terms of enhancing amino acid balance and palatability. However, shrimp growth was significantly retarded even after supplementing uncoated essential amino acid (AA-NC) in the present study, which might be due to the faster absorption of uncoated CAA. Consumption of CAA favours a rapid absorption from the gastrointestinal tract, which in turn leads to a fast supply of the plasma amino acid pool and exchange with other amino acid pools, and also to an intensified amino acid catabolism and high ammonia excretion (Liou et al., 2005). Similarly, Chi et al. (2011) observed a lower performance in penaeid shrimp even after supplementing an optimal level of CAA. These controversial results might be due to the variation in leaching loss and absorption (Huai et al., 2010), and/or supplement level might not be sufficient enough to fulfil the dietary requirement of the cultured species (Bulbul et al., 2015). In our study, the leaching loss was very high in the diet supplemented with uncoated CAA after 10 and 30 min, which would have resulted in poor performance in shrimp fed with uncoated amino acids in which leaching is comparatively low (Table 4) than the coated one. Similarly, the highest leaching rate of Arg was reported by Teshima et al. (2004) when it was supplemented in the diet of *P. japonicus*. These results indicated that the necessity of the use of specific binders or coating materials for minimizing the leaching of CAA thereby ensures the availability of essential nutrients, particularly amino acids and resulted in better growth performance of aquatic species.

The comparable shrimp performance when fed with coated amino acids and control diet indicating that the juveniles of *P. monodon* are highly capable of utilizing coated CAA. This result is in agreement with Alam et al. (2004) in *P. japonicus* and Chi et al. (2011) in *P. vannamei*. The authors suggested that the improved performance

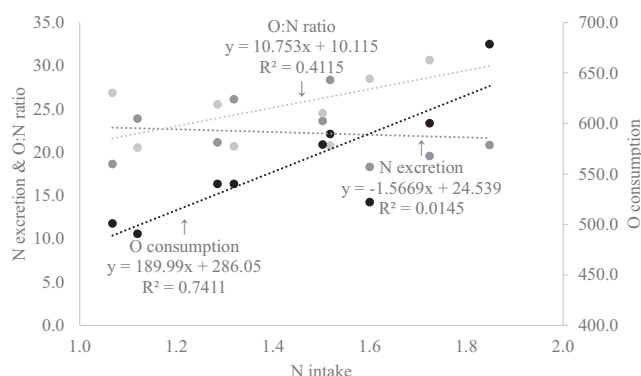


FIGURE 3 Regression on oxygen consumption, nitrogen excretion and O:N ratio against nitrogen intake in *P. monodon* fed various experimental diets supplemented with crystalline amino acids

with coated CAA might be due to the enhancement in their utilization by reducing leaching into the rearing water. However, Alam et al. (2005) reported that supplementation of even coated Lys or coated Met individually failed to show any appreciable positive effect on shrimp growth, which might be due to insufficient quantity of these amino acids than the dietary requirements of shrimp. A blend of amino acids and coating process would help to achieve the best results in terms of growth and feed utilization, even after replacing 50% (125 g/kg) of dietary fishmeal, in our study. A diet of AA-CT showed the highest DGC (1.48%/day) among the fishmeal replaced diets, which is not statistically different from the control one (1.49%/day), indicating that a double-coating of CAA with CMC and the casein-gelatin mixture was more effective coating technique. A similar observation was reported by Millamena et al. (1996) in *P. monodon* exposed to a high amount of CAA coated with CMC and k-carrageenan. The lower leaching rate of CAA after coating (Table 4) would be a possible reason for obtaining a higher growth in the shrimp fed coated diet (AA-CT) in the present study. But in contrast, Lopez-Alvarado et al. (1994) reported the poor result with a higher leaching rate even after coating CAA with alginate, carrageenan or zein. This infers that the beneficial effects of CAA are not only influenced by the retardation or reduction in leaching loss but also influenced by the agents used for the coating process. In addition, Lopez-Alvarado et al. (1994) also reported that coating method,

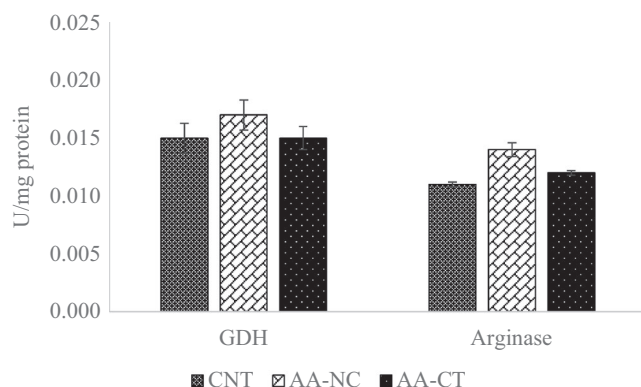


FIGURE 4 Effect of crystalline amino acid supplementation on glutamate dehydrogenase (GDH) and arginase activity in haemolymph of *P. monodon*

coating time, coating machine and coating wall influence the product performance and the utilization of CAA.

A better FCR was observed in shrimp fed on a diet supplemented with the coated CAA (AA-CT) than those fed on uncoated and no supplemented CAA diets (AA-NC and AA-NL), which might be attributed to the attractant characteristics of CAA. Though both coated and uncoated CAA were included at the same level (Table 2), the poor FCR observed with uncoated CAA-based diet than the coated one might be due to the highest leaching rate, indicating the necessity of coating process for supplementing amino acids in shrimp feeds (Huai et al., 2010). Similarly, no improvement was observed due to the supplementation of uncoated Met and Lys in FCR of *P. japonicus* fed a practical diet (Alam et al., 2005), who suggested that a large part of both the amino acids could be lost via leaching before consumption. Our result is also corroborated with the findings of Chen and Leu (1992), who also found a poor FCR in *P. monodon* fed a diet with encapsulated Arg than those fed the diets with no supplemented Arg or uncoated Arg. However, shrimp reared on the control diet (CNT), where no CAA supplementation was done, have shown a significantly better FCR compared with the AA-CT diet. The higher FCR in the AA-CT fed group might be due to the higher replacement of dietary fishmeal (50%; 125 g/kg). Protein efficiency measures in terms of PER and APU were significantly higher in AA-CT fed groups even compared with control. Xie et al. (2014) stated that the efficiency of protein was increased with the supplementation of amino acid in shrimp and was more pronounced after coating in the present study. This would also be an evidence for using coated CAA (AA-CT), which had a balanced nutritional composition, even after replacing 50% (125 g/kg) dietary fishmeal using a blend of plant proteins, as like the control diet. Dietary change did not affect the survival of shrimp among the treatments, indicates the possibility of replacement of 50% (125 g/kg) dietary fishmeal in the diet of *P. monodon*. The results of apparent digestibility coefficients of dry matter, crude protein and essential amino acids exhibited no much of difference between the control group and the group fed with a diet supplemented with CAA, while a wide variation was observed in AA-NL and AA-NC fed groups, which are consistent with the results obtained in the feeding trial.

Whole-body composition of proximate and essential amino acids was not affected by the dietary change in our study, and this

Particulars	Experimental diets			SEM ($\pm$)	p-value
	CNT	AA-NC	AA-CT		
Protein (mg/ml)	122.22 ^a	90.44 ^c	100.79 ^b	0.208	<.001
Lipid (mg/dl)	189.74 ^a	194.88 ^a	188.78 ^a	4.201	.097
Glucose (ml/dl)	23.88 ^b	24.50 ^a	20.51 ^c	0.025	<.001
Haemocyanin (mM)	1.45 ^a	1.14 ^c	1.24 ^b	0.001	<.001
Ammonia (mg/L)	2.11 ^c	4.56 ^a	3.99 ^b	0.002	<.001
Urea (mg/L)	11.32 ^b	12.54 ^a	8.61 ^c	0.001	<.001

TABLE 10 Effect of crystalline amino acid supplementation on haemolymph indices in *P. monodon*

Note: All the values are mean of three replications. Mean bearing the same superscript within the row do not differ significantly ($p > .05$).

result was corroborated with the findings of Alam et al. (2005) in *P. japonicus* fed a diet supplemented with coated Met and Lys, but these findings are not consistent in *P. vannamei* fed coated and uncoated Met (Chi et al., 2011) and in *P. japonicus* fed a blend of amino acids coated with different coating agents/binder (Alam et al., 2004). However, there was a significant difference in free amino acids tested in the tail muscle of *P. monodon*. The values were reported in the study were almost similar to those reported in carapace shrimp, *P. kerathurus* (Torres, 1973), *P. esculentus* (Dall & Smith, 1987), *P. monodon* (Dayal et al., 2011) and *P. vannamei* (Fox et al., 2011; Mente et al., 2002). All the essential amino acids were significantly higher in shrimp fed with the AA-CT diet followed by the control group, while there was no specific trend for non-essential amino acids. Of all the essential amino acids, Arg was the most predominant in shrimp tail muscle. Hird (1986) suggested that this might be due to its prime metabolic role as a precursor to the important phosphagen and phosphoarginine. The coating process might have improved the utilization of CAA by minimizing the leaching loss as in *P. vannamei* (Huai et al., 2010) and slow absorption rate of free amino acids to form a more balanced amino acid pool beneficial for protein synthesis (Murai et al., 1980). A similar result was found in *P. vannamei* fed a low-fishmeal diet supplemented with glycine (Xie et al., 2014). Though some variations were observed in individual free amino acids, the total of the same was remained stable in all the experimental groups, suggesting that intracellular amino acid pools are not determined by the passive movements of amino acids, but rather they are regulated by active trans-membrane transport (Mente et al., 2002). The pattern of free amino acids in the present investigation also proves that the peak of free amino acid absorption in haemolymph of *P. monodon* fed with coated CAA (AA-CT) almost synchronized with the control group at 60 min. This similar tendency obtained between control and AA-CT diets might also be a reason for replacing 50% (125 g/kg) dietary fishmeal using a blend of plant proteins, whereas the peak was attained at 30 min in shrimp fed on the uncoated CAA diet (AA-NC).

Frisk et al. (2013) stated that the rate of oxygen consumption and ammonia-N excretion has a direct relation to the utilization of dietary protein as it is a potential indicator to study the protein metabolism in crustaceans. In this context, a second experiment was performed with sub-adult shrimp to elucidate the effect of coated and uncoated CAA in shrimp fed low-fishmeal diets (AA-NC and AA-CT) while comparing with a group fed a control diet (CNT), where no fishmeal level was reduced. Nitrogen intake was significantly lower in shrimp fed low-fishmeal diets supplemented with coated and uncoated CAA compared with the control, indicating that the acceptability of the control diet by *P. monodon* was comparatively high due to the presence of a higher content of dietary fishmeal. Lemos et al. (2001) documented that the metabolic rate of environmental conditions affected is by various environmental factors by influencing oxygen consumption. Though all the levels have been maintained optimal and constant level (Table 3) in our study, the oxygen consumption rate was significantly higher in *P. monodon* fed a control diet than that obtained in AA-NC and AA-CT, which might be due to

a higher metabolic rate required for a diet containing high fishmeal that attributed to higher feed intake as reflected in Table 9. Though iso-nitrogenous formulation was carried out in the present study, postprandial ammonia-N excretion was significantly varied among the experimental group. The higher excretion of ammonia-N was observed in shrimp fed on a diet with uncoated CAA might be due to its poor quality of protein by a means of imbalance of essential amino acids. The O:N ratio was significantly higher in coated CAA than the uncoated one, which infers that shrimp fed on coated CAA able to use the feed effectively than its counterpart as a low-cost energy source for their metabolic process and exploit the same as a structural component for growth (Rosas et al., 2001). However, fishmeal replacement using a blend of plant protein decreased O:N ratio even after supplementation CAA in our study.

Glutamate dehydrogenase was assayed in the present study to find a correlation between ammonia excretion and catabolism of amino acids against dietary proteins with different qualities. Though there was no significant difference in GDH activity among the experimental groups, almost a similar trend was observed between AA-CT and control diets, indicating that the protein quality/balanced amino acid profiles are similar in both diets. An almost similar trend was observed in the activity of arginase. The higher haemolymph protein and haemocyanin in shrimp fed coated CAA than uncoated one, which indicates the higher nutritional status of the respective diet and their relationship have already been reported in shrimp (Djangmah, 1970). Hagerman (1983) stated that feed with poor quality may cause rapid haemocyanin catabolism, which might be a reason for obtaining a lower level of haemocyanin content in shrimp fed with a diet containing uncoated CAA in our study. Pascual et al. (2006) reported that the haemolymph glucose level was not affected with the dietary protein level (50 and 400 g protein/kg feed) in shrimp. Contrary, haemolymph glucose level was significantly varied from 20.51 to 24.50 ml/dl in our study, though diets were formulated as iso-nitrogenous. Haemolymph lipid is a metabolic variable, which varies depending upon the dietary sources and the ability of the shrimp to utilize and the digestibility of the diet, but it was not affected due to dietary change. Ammonia is formed by the catabolism of amino acids, deamination of amides and deamination of adenylate following the purine nucleotide cycle (Claybrook, 1983; Regnault, 1987), and its trend was similar as reflected in ammonia-N excretion (Table 9). Regnault (1987) stated that ammonia-N excretion is affected by various intrinsic (moult cycle, nutritional status and size, and extrinsic (temperature, salinity and ambient ammonia) factors, but all the extrinsic factors were maintained within the optimal range in our study (Table 3).

5 | CONCLUSION

In conclusion, CAA was effectively utilized by the juveniles of *P. monodon* in the practical diet and provides the most promising results after coating rather than using an uncoated one. A blend of lysine, methionine, arginine, isoleucine and leucine at 0.8, 0.4, 0.4, 0.2 and 0.2 g, respectively, could be the most appropriate combination



in formulating a low-fishmeal diet for *P. monodon*. This could replace 50% (125 g/kg) of dietary fishmeal using a blend of plant protein in shrimp feed without compromising the growth performance, feed utilization and whole-body composition. The present results also confirmed the significant effect and advantage of coated amino acids relating to nitrogen metabolism in sub-adult of *P. monodon*. This paves a way to reduce the formulation cost in the face of the volatile commodity market of protein ingredients and the limited supply of fishmeal.

ETHICS APPROVAL STATEMENT

This study was undertaken with the approval of the Institute Animal Ethics Committee (IAEC), ICAR-Central Institute of Brackishwater Aquaculture, Chennai, India.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

AUTHOR'S CONTRIBUTIONS

Dr Rajaram V involved in study design, conducted experiment and analysed all the parameters. Dr Jannathulla R involved in written rough draft of the manuscript. Dr Ambasankar K involved in statistical analysis. Dr Syama Dayal drafted fair draft and overall coordination.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available based on a reasonable request from the corresponding author.

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