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Effect of dietary protein level on fattening and mineral profiles of mud crab, *Scylla serrata*, in individual cages under mangrove ecosystem

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Abstract

A fattening demonstration was carried out in Sorlagondi (15.8640^{0}N, 80.9671^{0}E) mangrove ecosystem in Diviseema region, Krishna district, Andhra Pradesh, India, by adapting a holistic approach of "Integrated Mangrove Fishery Farming System." The local Yanadi tribal communities engaged in mangrove afforestation programmes were provided with 144 individual crab fattening cages (60 × 40 × 30 cm). Two formulated feeds were prepared with two levels of protein (32% and 36%) and were tested on fattening of the marketable size (200–1,000 g) of mud crabs, *Scylla serrata*. Significantly ($p < 0.05$) higher weight gain, feed and protein efficiency measures were observed in crabs fed with CP-36% diet across all size groups. This diet also reduced the time (days) taken for fattening crabs irrespective of the size. Moisture content was very high in soft crabs (>800 g/kg) compared with hard crabs (679.70–688.65 g/kg), whereas the reverse trend was observed for protein, ether extract and ash content. Both diet and size of the crab have not shown much influence on proximate composition. However, cultured hard crabs had higher ether extract (20.10 g/kg) than wild hard crabs (17.06 g/kg). The changes in concentration of mineral values on wet basis indicated the increase in concentration in hard crabs compared with soft crabs, whereas when the percentage change, values on dry matter basis indicated the reduction in concentration of mineral contents of K, Na, P, Cu, Fe and Zn. This higher concentration of minerals in soft crabs could possibly be attributed to the reabsorption of minerals into soft muscle or to the body fluids.

KEYWORDS
dietary protein, fattening, minerals, mud crabs, proximate, *Scylla serrata*

1 | INTRODUCTION

Mud crab, *Scylla serrata* (Forskal) belonging to family Portunidae, commonly inhabits estuaries and mangrove swamps of the Indo-Pacific region. Growing consumer demand from both local and international markets by virtue of its delicacy and unique taste led to the commercial monoculture in a small-to-moderate scale, thereby supporting coastal livelihoods (Bonine, Bjorkstedt, Ewel, & Palik, 2008; Lee, 1991). Mud crab culture in mangroves or tidal flats is practised in Indonesia, Vietnam and China and is considered to be an ecologically friendly economic activity (SEAFDEC Asian Aquac, 1997). Conservation of the mud crab primary habitat, mangrove forests, is critical to support their populations, and creating awareness of their over-fishing is essential. While reviewing

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the measures for capture-based aquaculture of mud crabs, it was suggested that environmentally sustainable farming of mud crabs in mangrove pens and tidal farms is an important tool in both conservation of mangrove forests and expanding the farm production areas (Kador, 1991; Mirera & Mtile, 2009; Shelley, 2008). Reports of the significance of mangrove forests and its afforestation for nursery grounds of mud crabs exist (Walton, Le Vay, Lebata, Binas, & Primavera, 2006).

Commercial demand exists both for freshly moulted soft crabs and for full hard-shelled crabs (Patterson & Samuel, 2005). Commercially, soft crabs are obtained by holding hard-shelled crabs in cages till they moult. These soft crabs are removed from water and need to be processed and frozen within a few hours of moulting (Dassow, 1968). Obtaining this type of soft crabs from nature is extremely difficult. Mud crabs which have moulted and not fully grown to fill their new shells are commonly referred to as soft "empty" crabs. The process of rearing these soft crabs until they are "full" and ready for market is called "fattening." In the wild fishery, approximately 7%–10% of the catch on an average are soft crabs and are usually low priced, but these crabs when fattened for a month to a "full" hard crab increase their average value by over 200%; hence, this can be regarded both as a product improvement technique and as a specific type of value-added aquaculture (Patterson & Samuel, 2005). The fattening experiments in ponds and cages have indicated lower survival in ponds due to cannibalism and escape because of the burrowing habit of mud crabs (Begum, Shah, Mamun, & Alam, 2009). In most of the earlier mud crab farming demonstration trials in Asia and Africa, trash fish, clam meat or animal by-products have been used as feeds (Mirera & Mtile, 2009). This traditional feeding practice, however, is now considered unsustainable due to the issues of availability, necessity of freezing and water quality deterioration, and hence, the development of formulated low-cost diets is widely viewed as a priority issue for mud crab aquaculture (Ali, Dayal, & Ambasankar, 2011; Christensen, Macintosh, & Phuong, 2004; Tuan, Anderson, Luong-van, Shelley, & Allan, 2006).

Nutrient requirement data of any candidate species are crucial for the development of a nutritionally balanced, cost-effective commercial feed (Tacon et al., 2002). In our earlier studies, formulated feeds were successfully tested for both fattening and culture in farms (Ali et al., 2008), and also the positive effect of dry pellet form of feeding compared with other physical forms of presentation (Ali et al., 2011). The optimization of dietary protein and amino acids along with the growth and increased nutrient retention by the animal appears to be an ideal approach to reduce the nitrogen loading and positively influence the production cost (Dayal, Ali, Ambasankar, & Singh, 2003). Crude protein requirements of different sized mud crabs, *S. serrata*, were in the range of 32%–45% (Catacutan, 2002; Chin, Gunasekera, & Amandokoon, 1992; Sheen & Wu, 1999; Unnikrishnan & Paulraj, 2010). Most of these nutritional requirement studies have been carried out in clear water systems, where the environmental conditions greatly differ from those found in natural culture systems (D'Abramo & Castell, 1997). Although these studies have provided valuable information regarding the requirements under controlled conditions, the practical application of these data is limited unless it is demonstrated under natural conditions (Moss, Divakaran, & Kim, 2001; Moss & Pruder, 1995; Tacon, 1996).

In the process of moulting, a series of physiological and biochemical changes take place where some of the essential nutrients and minerals are absorbed into the muscle tissues or body fluids from the carapace or from the surrounding water, so that these nutrients will be utilized during the new carapace formation (Mohanty, Mohapatra, & Mohanty, 2009; Paez-Osuna, Perez-Gonzalez, Izaguirre-Fierro, Zazueta-Padilla, & Flores-Campana, 1995). The present study, therefore, evaluated the effect of formulated feeds on the growth and survival of mud crab, *S. serrata*, reared in the mangrove ecosystem with feeds containing two different protein levels (32% and 36%). Further, the effect of these formulated feeds on different size groups of mud crabs was also evaluated in comparison with traditional feeding practices. In order to comprehend the biochemical changes during the fattening process, the proximate and mineral composition of both initial soft and final hard crabs were also analysed.

2 | MATERIALS AND METHODS

2.1 | Pond and cage preparation

The experiment was conducted in Sorlagondi (15.8640^{0}N, 80.9671^{0}E) mangrove ecosystem in Diviseema region of Krishna district of Andhra Pradesh, India. It is a tide-fed farming system where the tidal water enters the pond during high tide and drains out during low tide. The regular exchange of tidal water sustains the water quality by avoiding accumulation of organic load without much infrastructure such as water pumps and air blowers. Hence, farm input costs are low compared to the semi-intensive farming system. Mangrove vegetation was grown along the inner and outer bunds and mounds. The water level in the pond system was maintained by gravitational tidal flow with a minimum of 3 ft depth level in low tide, and it was named as "Integrated Mangrove Fishery Farming System" (M.S. Swaminathan Research Foundation, 2016). The plastic cages (60 × 40 × 30 cm) having 1-cm slits were used for fattening individual crabs for easy movement of water. A total of 144 cages were installed at the bottom of the mangrove-based ponds.

2.2 | Stocking and management

Male water crabs with intact appendages were stocked individually in each cage in the morning, to prevent stress. The body weight of the soft crabs ranged from 200 to 1,000 g. These crabs were classified into four groups viz., medium (200–300 g), large (301–500 g), XL (501–750 g) and XXL (751–1,000 g), as the price of the crabs varies with size. In each size, 36 crabs were stocked per each treatment in three mangrove ponds. The pond water quality parameters such as salinity (28.4 ppt), pH (7.68), dissolved oxygen (5.76 ppm), temperature (27.5°C), total alkalinity (241.7 ppm as $CaCO_3$), total

ammonia-nitrogen (NH_3–N, 0.19 ppm), nitrite-nitrogen (NO_2–N, 0.082 ppm), nitrate-nitrogen (NO_3–N, 0.76 ppm) and phosphate-phosphorus (PO_4–P, 0.03 ppm) were recorded once in ten days (APHA, 2012), and values were found to be within the optimum levels for brackishwater crustacean culture. The crabs were individually checked daily for hardness. Hardened crabs were harvested, and their pincers were restrained by a coir rope to prevent cannibalism and to enable easy handling for weighing and marketing.

2.3 | Feed preparation and feed management

The experiment comprised two treatments and one control. The treatment groups were fed with formulated feeds containing 32% CP (Treatment 1) and 36% CP (Treatment 2). The control was fed with low-valued trash fish. Compounded crab feed as dry sinking pellets was produced using ring-die pellet mill at the ICAR-Central Institute of Brackishwater Aquaculture, Chennai, India. All the coarse ingredients listed (Table 1) were powdered by a two-stage grinding using a hammer mill and micropulverizer and passed through a 0.5-mm screen. All the ingredients along with liquid ingredients (fish oil and lecithin) and additives (vitamin–mineral premix and binder) were mixed in horizontal ribbon mixture and thoroughly homogenized after adding 3 L of water per 100 kg. The mash was pelletized in the ring-die pellet mill with 15%–16% moisture at a temperature of 90°C under steam conditioning (Dayal et al., 2017). Two types of pellets of size 4.5 and 6 mm were prepared to feed <500- and ≥500-g-sized crabs respectively. Initially, trash fish and pellets were given at the rate of 8% and 2.5% of the biomass, respectively, and were adjusted based on the intake. Daily ration was distributed in two equal quantities (6.00 a.m. and 6.00 p.m.) during the fattening period.

2.4 | Crab samples for nutrient analysis

The initial soft crabs and final hardened crabs in all the four size groups such as medium (200–300 g), large (301–500 g), XL (501–750 g) and XXL (751–1,000 g) with three replications were collected for nutrient analysis. The proximate composition of the whole crabs was analysed for all size groups. In addition to the proximate profiles, the mineral composition of the whole crabs from six XXL (>750 g)-sized crabs were analysed from each treatment along with wild initial soft crabs of similar size.

TABLE 1 Ingredient and proximate composition of experimental feeds used for *Scylla serrata* fattening experiment (g/kg as fed basis)

	Trash fish	Experimental diets	
Particulars	**Control**	**CP-32%**	**CP-36%**
Ingredient composition			
Fishmeal[a]		180	200
Acetes[b]		90	110
Soybean meal		150	180
Gingelly oil cake		50	60
Wheat		470	392
Fish oil[a]		20	18
Lecithin		10	10
Pre-mix[c]		20	20
Binder[d]		10	10
Proximate composition			
Moisture	733.70 ± 8.59	90.10 ± 2.21	89.80 ± 0.71
Crude protein	159.20 ± 2.45	321.01 ± 0.98	360.50 ± 1.36
Ether extract	36.10 ± 1.17	55.60 ± 1.63	55.90 ± 0.29
Crude fibre	1.70 ± 0.36	26.50 ± 0.47	28.90 ± 0.68
NFE[e]	8.10 ± 0.57	401.39 ± 4.59	344.80 ± 2.64
Total ash	61.20 ± 1.13	105.40 ± 0.66	120.10 ± 1.13

[a]Bismi Fisheries, Mayiladuthurai, Tamil Nadu, India.
[b]Mantis shrimp used as a protein source.
[c]Pre-mix (g/kg): thiamine hydrochloride (25.50 g), riboflavin (25.00 g), pyridoxine hydrochloride (50.00 g), cyanocobalamin (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), $ZnSO_4 \cdot 7H_2O$ (28.28 g), $MgSO_4 \cdot 7H_2O$ (0.12 g), $MnSO_4 \cdot H_2O$ (12.43 g), $CuSO_4 \cdot 5H_2O$ (19.84 g), $CoC_{12} \cdot 6H_2O$ (4.07 g), KIO_4 (0.03 g), KCl (15.33 g), Na_2SeO_3 (0.02 g).
[d]Pegabind, Bentoli Agri-Nutrition Asia Pvt Ltd, Singapore.
[e]Nitrogen-free extract (Calculated by difference).

2.5 | Biochemical analysis

Proximate composition of feeds and crab samples was analysed as per the method of AOAC (1997). Amino acid profiles were analysed using pre-column HPLC gradient system (Shimadzu Corp, LC-30AD) after hydrolysing the samples with 6 N hydrochloric acid in a sealed tube filled with nitrogen for 22 hr at 110°C in an oven (Finlayson, 1964). The YMC-Triart C18, RRH (1.8 μm, 2.1 × 100 mm) column was used to separate the amino acids after derivatization with mercaptopropionic acid, O-phthalaldehyde and fluorenylmethoxycarbonyl chloride under gradient elution using phosphate buffer (20 mmol as mobile phase A) and combination of acetonitrile: methanol: water (45:40:15 as mobile phase B) at the flow rate of 0.3 ml/min. The gradient was changed by increasing mobile phase B concentration at the rate of 11%–13% at 3 min, 31% at 5 min, 37% at 15 min, 70% at 20 min and 100% at 25 min (Jannathulla, Dayal, Ambasankar, Eugine, & Muralidhar, 2018). Amino acids were qualified and quantified by fluorescent detector (RF-20AXS) using amino acid mixer as an external standard (Sigma Aldrich, Cat. No: AAS18) and norleucine as an internal standard. Tryptophan, being labile to acid hydrolysis, was measured after alkali hydrolysis using spectrophotometric method at 500 nm (Sastry & Tammuru, 1985). The partial oxidation of sulphur-containing amino acids such as cystine and methionine during acid digestion was prevented by adding 0.1% of phenol (Jajic, Krstovic, Glamocis, Jaksis, & Abramovic, 2013).

Lipid was extracted by using chloroform and methanol (2:1) by a method of Folch, Lees, and Sloane Stanley. (1957) method, the respective fatty acid methyl esters (FAME) were prepared according to Metcalfe, Schmitz, and Pelka (1966) method, and finally, FAMEs were extracted into petroleum ether. Routine analysis of methyl esters was performed using a gas chromatograph (GC-2014 Shimadzu) on a RTX-Wax Capillary Column (100 m length × 0.25 mm I.D × 0.2 μm film thickness). Nitrogen was used as carrier gas at a linear velocity of 20.9 cm/s with 3 ml/min of purge flow. The oven temperature was held at 100°C for 4 min and increased to 225°C at the rate of 3°C/min and held for 5 min and further increased to 240°C at the rate of 1°C/min. Operating temperature for injection ports and flame ionization detector was 225 and 250°C respectively. Fatty acids were identified by comparing with the retention times of 37 Component FAME Mix (Supelco—Sigma). The quality of analysis was checked with a blank and a control sample menhaden oil.

The samples were digested using microwave digestion method (Anton Paar microwave system) for mineral analysis. The samples of approximately 1.0 g were digested with 6 ml of HNO_3 and 2 ml of H_2O_2 in microwave digestion system. All digested samples were analysed in triplicates using inductively coupled plasma-optical emission spectrometry, ICP-OES Agilent; model 5100, using 5.2 software for the analysis. The analytical measurements were made with an autosampler equipped with a peristaltic pump, across-flow nebulizer (coupled to a double-pass spray chamber) and Quartz central torch tube injector with an internal diameter of 2 mm. Certified reference material, ICP multi-element standard solution (10 mg/l Merck), was used for calibration (Jannathulla, Dayal, Chitra, Ambasankar, & Muralidhar, 2017).

2.6 | Statistical analysis

Data of production performance and whole crab proximate composition were subjected to a two-way ANOVA. The mineral composition was subjected to a single-factor ANOVA. The descriptive statistics were computed for the main factors and their interactions. Prior to statistical evaluation, data were checked for homogeneity of variance after ascertaining for normal distribution. Data were analysed using SAS. Means were compared at 5% significance level ($p < 0.05$).

3 | RESULTS

The proximate composition of experimental feeds used for fattening trial is presented in Table 1. The crude protein content of the formulated feeds was 321.01 and 360.50 g/kg on fed basis, thereby reflecting higher amino acid contents in diet 2 (Table 2). The essential amino acid content of the pelleted experimental diets was computed by calculating A/E ratios and compared with the essential amino acid requirements of *Penaeus monodon* (Millamena, Bautista, Reyes, & Kanazawa, 1997, 1998; Millamena, Bautista-Teruel, & Kanazawa, 1996; Millamena, Teruel, Kanazawa, & Teshima, 1999) and *P. vannamei* (Cuzon, Lawrence, Gaxiola, Rosas, & Guillaume, 2004) as such requirement data are not available for *S. serrata*. Based on the earlier reports of amino acid composition of the experimental diets used for *S. serrata*, the A/E ratios were compared (Ali et al., 2011; Unnikrishnan & Paulraj, 2010). The essential amino acid index (EAAI) was calculated based on shrimp amino acid requirements (*P. monodon* and *P. vannamei*) and the amino acid composition of diets used for *S. serrata*. The aa/AA ratios are set at 0.01 minimum and 1 maximum for estimation of EAAI (Peñaflorida, 1989). The EAAI ratios of both the diets are above 0.86 indicating that both the feeds could nearly meet the dietary requirements of all essential amino acids (Table 3). The CP-36% diet had higher nonessential amino acids (NEAA) especially in glutamic acid, aspartic acid and proline. The mineral composition of experimental feeds is presented in Table 4. The diet having higher crude protein (CP-36%) has shown higher mineral profiles due to the higher inclusion of fish meal.

Significantly ($p < 0.05$) higher weight gain was observed in crabs fed with CP-36% diet compared to other treatments across all size groups tested (Table 5). The survival was higher in a group fed CP-36% diets, but the difference was not significant among the treatments. The lower feed consumption ratio (FCR) and higher protein efficiency ratio (PER) and apparent protein utilization (APU) were observed in crabs fed with CP-36% diet. The time (days) taken for fattening was also shortened in crabs fed with high CP diet in all the size groups. The size of the crab has influenced significantly ($p < 0.05$) weight gain, % weight gain and the number of days taken for hardening. The absolute weight gain (g) is lower in smaller size crabs, but the weight gain (%) is higher compared to larger sized

TABLE 2 Amino acid and fatty acid composition of experimental feeds used for *S. serrata* fattening experiment

	Trash fish	Experimental diets	
Particulars	**Control**	**CP-32%**	**CP-36%**
Essential amino acids (g/kg as fed basis)			
Arginine	10.24 ± 0.58	18.95 ± 1.23	21.22 ± 2.71
Histidine	3.10 ± 0.36	9.52 ± 0.67	10.91 ± 0.34
Isoleucine	5.10 ± 0.41	17.66 ± 0.44	19.62 ± 0.47
Leucine	8.30 ± 0.56	23.47 ± 0.54	28.34 ± 0.16
Lysine	9.10 ± 0.47	9.61 ± 0.64	10.93 ± 0.45
Methionine	3.40 ± 0.69	5.32 ± 0.44	5.93 ± 0.69
Phenylalanine	7.10 ± 0.91	13.43 ± 0.15	14.86 ± 0.54
Threonine	6.80 ± 0.34	12.80 ± 0.34	14.23 ± 0.34
Tryptophan	2.40 ± 0.84	4.11 ± 0.69	4.61 ± 0.51
Valine	9.80 ± 0.64	12.42 ± 0.64	13.61 ± 0.81
Nonessential amino acids (g/kg as fed basis)			
Alanine	10.83 ± 0.23	16.50 ± 0.69	19.10 ± 0.67
Aspartic acid	12.08 ± 0.43	26.50 ± 0.46	31.20 ± 0.46
Cystine	1.28 ± 0.64	4.80 ± 0.51	5.60 ± 0.91
Glutamic acid	13.04 ± 0.26	44.80 ± 2.63	52.60 ± 1.67
Glycine	10.08 ± 0.91	19.50 ± 0.42	24.50 ± 0.73
Proline	6.92 ± 0.32	13.20 ± 0.47	15.70 ± 0.94
Serine	5.61 ± 0.42	14.20 ± 0.73	17.30 ± 0.49
Tyrosine	5.41 ± 0.39	10.70 ± 0.34	12.10 ± 0.62
Fatty acids (% of total fatty acids)			
14:0	5.52 ± 0.23	5.83 ± 0.17	5.93 ± 0.69
16:0	19.92 ± 0.75	22.99 ± 0.22	22.61 ± 0.46
18:0	8.46 ± 0.64	6.64 ± 0.64	6.45 ± 0.77
16:1*n*−7	8.75 ± 0.62	5.57 ± 0.49	5.56 ± 0.61
18:1*n*−9	13.89 ± 0.46	11.72 ± 0.51	11.41 ± 0.97
18:1*n*−7	4.40 ± 0.39	2.84 ± 0.55	2.83 ± 0.15
18:2*n*−6	6.83 ± 0.91	15.99 ± 0.67	16.36 ± 0.64
20:4*n*−6	6.32 ± 0.42	2.25 ± 0.72	2.22 ± 0.27
α 18:3*n*−3	0.64 ± 0.73	0.17 ± 0.61	0.20 ± 0.32
20:5*n*−3	6.32 ± 0.26	9.11 ± 0.49	9.03 ± 0.32
22:6*n*−3	6.89 ± 0.94	8.58 ± 0.64	8.73 ± 0.81

crabs. The number of days taken for hardening is proportional to the size of the crab. Nutrient retention (g) in terms of protein, fat and energy is higher in crabs fed with CP-36% diet.

The proximate composition of mud crabs is shown in Table 6. The soft crabs had very high (>800 g/kg) moisture content, whereas the crude protein (87.81), ether extract (12.90) and total ash content (65.70 g/kg) were significantly ($p < 0.05$) lower compared to average of all the treatments of the hard crabs 685.7, 126.3, 18.8 and 125.0 g/kg respectively. Both diet and size of the crab have not influenced the proximate composition, whereas cultured hard crabs exhibited higher ether extract (1.94%) compared to wild hard crabs (1.71%). During the fattening process, the moisture content decreased by 14% and the crude protein increased by 43%. The maximum increase was observed for total ash (88%) in hardened crabs compared with the soft crabs. Similarly, significant ($p < 0.05$) increase in mineral content was also observed in hardened crabs and diet did not influence mineral composition (Table 7).

4 | DISCUSSION

The price of mud crabs varies with size and also with the moulting stage. Both soft crabs and hard crabs have commercial value. Soft crabs are produced by holding hard-shelled crabs in specific cages or floats until they moult. These soft crabs have to be removed from the water within a few hours of moulting and are to be graded, processed and frozen (Benjakul & Sutthipan, 2009). The fattening is a value-added aquaculture and the price increase is over 200% (Patterson & Samuel, 2005). Based on the available reports, it is very much needed to improve fattening techniques of mud crab to get a higher production with pelleted feeds. Hence, in the present study a holistic approach of "Integrated Mangrove Fishery Farming System" (M.S. Swaminathan Research Foundation, 2016) was adapted by involving the local Yanadi tribal community who are engaged in mangrove afforestation programmes by providing individual crab fattening cages. The fattening of mud crabs adopted in the present study is a significant improvement to reduce cannibalism as it is the main cause of mortality resulting up to 90% loss of the stock (Mann, Asakawa, Kelly, Lindsay, & Paterson, 2007; Ut, Le Vay, Nghia, & Hong Hanh, 2007). The performance of mud crabs during fattening in mangrove ecosystem has shown better performance with CP-36% feed compared with CP-32% feed and traditional feeding with trash fish. Since the size of the crab is having significant effect on protein requirement in the present study, wide marketable-size groups (200–1,000 g) were tested and the results indicated no significant difference in protein requirement in the tested size range. The present results are in agreement with the values of crude protein requirement reported in mud crabs viz. 35%–40% in ~600 g size group (Chin et al., 1992). Catacutan (2002) reported a dietary protein level ranging from 32% to 40% in 9-g-sized crabs with 6%–12% lipid level, whereas 47% in 0.25 g size juveniles was reported by Unnikrishnan and Paulraj (2010). The CP-36% diet also reduced the time (days) taken for fattening in crabs irrespective of the size, and this improved performance can be attributed not only to the higher optimal proportion of essential amino acids (Table 2) but also because of the availability of higher macro minerals such as potassium and phosphorous and trace elements such as iron, manganese, selenium and zinc (Table 4). The presence of these minerals in higher quantities could have helped faster mineralization of crabs fed with CP-36% diet. Our earlier results have shown the positive effect of dry pellet form of feeding compared with other physical forms of presentation (Ali et al., 2011). The growth recorded in the present study is higher than that reported in an earlier fattening demonstration in cage and pond (Begum et al., 2009), and this could possibly be attributed to the size of the crabs used for the experiment, diet

TABLE 3 A/E ratio of pelleted feeds and amino acid requirements of *Penaeus monodon*, *Penaeus vannamei* and *Scylla serrata*

Particulars	Experimental diets		Dietary requirements			
	CP-32%	CP-36%	*P. monodon*[a]	*P. vannamei*[b]	*S. serrata*[c]	*S. serrata*[d]
A/E ratio						
Arginine	13.96 ± 0.54	13.93 ± 0.89	15.82	19.19	13.96	19.94
Histidine	7.01 ± 0.34	7.16 ± 0.37	6.57	5.05	5.10	5.54
Isoleucine	13.01 ± 0.42	12.88 ± 0.43	8.06	9.09	8.46	12.42
Leucine	16.55 ± 0.67	16.64 ± 0.69	12.84	16.16	15.10	14.95
Lysine	12.40 ± 0.29	12.59 ± 0.61	15.52	16.16	15.77	4.84
Methionine	5.58 ± 0.41	5.73 ± 0.64	7.16	6.06	4.77	3.55
Phenylalanine	9.89 ± 0.33	9.76 ± 0.52	11.04	9.09	8.26	14.46
Threonine	9.43 ± 0.82	9.34 ± 0.28	10.45	6.06	8.99	10.16
Tryptophan	3.03 ± 0.67	3.03 ± 0.36	1.49	3.03	8.79	2.38
Valine	9.15 ± 0.29	8.94 ± 0.74	11.04	10.10	10.81	11.77
Essential amino acid index (EAAI)						
Based on *P. monodon*[a]	0.90 ± 0.09	0.90 ± 0.27				
Based on *P. vannamei*[b]	0.93 ± 0.06	0.93 ± 0.26				
Based on *S. serrata*[c]	0.86 ± 0.27	0.86 ± 0.43				
Based on *S. serrata*[d]	0.90 ± 0.31	0.89 ± 0.51				

Note: Essential amino acid index (EAAI): Calculated based on *P. monodon* shrimp, amino acid requirements (% Protein) and aa/AA ratios are set at 0.01 minimum and 1 maximum (Peñaflorida, 1989).
[a]Millamena et al. (1996), Millamena, Bautista, Reyes, and Kanazawa (1997), Millamena, Bautista-Teruel, Reyes, and Kanazawa (1998) and Millamena et al. (1999).
[b]Cuzon et al. (2004).
[c]Unnikrishnan and Paulraj (2010).
[d]Ali et al. (2011).

and also the rearing system. The balancing of essential amino acids is crucial as per the requirement of the species and stage of growth. In the present investigation, all the marketable-sized crabs were tested in actual field conditions with locally available feed ingredients by meeting the A/E, EAAI for crab (>0.85). The present study has utilized diets formulated using locally available ingredients and also tested in natural mangrove ecosystem by involving local tribal population, with a protein source very close to the amino acid profiles of *S. serrata*. The results discussed in the present study have direct field application and it could replace the fresh feeds being used currently (trash fish, molluscan meat, slaughterhouse by-products, etc.), which have limitations in quality, quantity and sustained supply.

The results of proximate analysis of different size groups of cultured and wild *S. serrata* are shown in Table 6. The soft crabs have very high (>80%) moisture content, whereas the crude protein, ether extract and total ash contents are significantly ($p < 0.05$) lower compared to hard crabs. Similar results were observed when soft and hard crab muscle were analysed for chemical constituents (Benjakul & Sutthipan, 2009). Both diet and size of the crab have not influenced the proximate composition except crude lipid. The effect of diet was not conspicuous in lipid values, but the larger size crabs have shown significantly ($p < 0.05$) higher lipid content. The lipid content of mud crabs observed in this study is similar to those reported in other crustaceans (Catacutan, 2002). The values of higher lipid level in cultured crab observed in this study are in agreement with the findings of Yanar, Göçer, Yanar, and Kücükgülmez, (2011) in *Penaeus*

TABLE 4 Mineral composition of experimental feeds used for *Scylla serrata* fattening experiment

Particulars	Trash fish	Experimental diets	
	Control	CP-32%	CP-36%
Macro minerals (g/kg as fed basis)			
Calcium	13.22 ± 0.67	20.16 ± 1.02	24.89 ± 0.99
Magnesium	0.76 ± 0.03	2.96 ± 0.08	3.23 ± 0.33
Phosphorus	8.78 ± 0.41	10.11 ± 0.73	12.30 ± 0.49
Potassium	1.70 ± 0.07	4.25 ± 0.61	4.61 ± 0.17
Sodium	1.34 ± 0.11	2.82 ± 0.16	3.16 ± 0.23
Micro minerals (mg/kg as bed basis)			
Copper	2.24 ± 0.27	27.24 ± 0.66	22.63 ± 0.73
Iron	142.86 ± 3.35	139.21 ± 5.67	144.26 ± 1.69
Manganese	6.71 ± 0.61	140.18 ± 2.39	144.72 ± 6.47
Selenium	0.75 ± 0.04	0.89 ± 0.23	0.98 ± 0.04
Zinc	33.17 ± 1.27	44.34 ± 0.61	51.33 ± 0.89

TABLE 5 Effect of different diets and size groups on weight gain, feed and protein conversion efficiencies in *Scylla serrata* during the fattening experiment

Particulars	Growth performance						No. of days for hardening
	Weight gain (g)	Weight gain (%)	Survival (%)	FCR[1]	PER[2]	APU[3]	
Different diets (A)							
Trash fish (1)	146.63^{b}	30.13^{b}	85.42^{a}	6.22^{a}	1.01^{c}	22.87^{b}	26.33^{b}
CP-32% (2)	151.02^{b}	29.78^{b}	89.58^{a}	2.71^{b}	1.15^{b}	33.55^{a}	28.00^{a}
CP-36% (3)	167.36^{a}	33.99^{a}	93.75^{a}	2.16^{c}	1.28^{a}	32.21^{a}	24.33^{c}
Different size groups (B)							
Medium[4] (1)	88.47^{v}	36.96^{s}	94.44^{s}	3.66^{s}	1.15^{s}	27.78^{s}	19.78^{v}
Large[5] (2)	129.16^{u}	32.42^{t}	86.11^{s}	3.76^{s}	1.14^{s}	29.92^{s}	22.89^{u}
XL[6] (3)	174.05^{t}	28.76^{u}	91.67^{s}	3.69^{s}	1.15^{s}	31.47^{s}	27.67^{t}
XXL[7](4)	228.32^{s}	27.05^{u}	86.11^{s}	3.67^{s}	1.16^{s}	29.00^{s}	34.56^{s}
Interactions							
A 1 B 1	85.95	35.32	83.33	6.06	1.04	19.01	19.00
A 1 B 2	121.28	31.14	91.67	6.43	0.98	18.75	22.33
A 1 B 3	158.25	27.31	91.67	6.16	1.02	27.00	29.00
A 1 B 4	221.05	26.75	75.00	6.20	1.01	26.71	35.00
A 2 B 1	84.72	34.16	100.00	2.75	1.14	37.12	22.00
A 2 B 2	121.80	29.79	83.33	2.65	1.18	34.72	24.00
A 2 B 3	171.70	28.20	91.67	2.77	1.13	30.75	28.67
A 2 B 4	225.85	26.95	83.33	2.65	1.18	31.61	37.33
A 3 B 1	94.75	41.39	100.00	2.18	1.28	27.21	18.33
A 3 B 2	144.40	36.34	83.33	2.20	1.26	36.28	22.33
A 3 B 3	192.20	30.77	91.67	2.14	1.30	36.65	25.33
A 3 B 4	238.07	27.45	100.00	2.15	1.30	28.69	31.33
p-Value							
A	0.006	0.008	0.359	<0.001	<0.001	0.001	<0.001
B	<0.001	<0.001	0.505	0.443	0.743	0.636	<0.001
A × B	0.838	0.587	0.408	0.183	0.415	0.130	0.173
SEM	124.19	6.25	112.08	0.012	0.001	21.70	1.55
CV (%)	9.46	10.51	15.55	3.94	3.84	20.75	6.25

Note: Mean bearing same superscript letters in a column within main effects and interactions between the categories do not differ significant ($p > 0.05$).
[1]Feed conversion ratio.
[2]Protein efficiency ratio.
[3]Apparent protein utilization.
[4]200–300 g.
[5]301–500 g.
[6]501–750 g.
[7]751–1,000 g.

semisulcatus. Higher lipid content in farmed crab could mainly be attributed to the type of food consumed. Compared to the natural feeds consumed by wild crabs, formulated pellets offered to the farmed crabs were nutrient dense. Grigorakis, Alexis, Taylor, and Hole (2002) observed a similar situation of fat accumulation in farmed fish (European seabass), and they attributed this to energy consumption in the form of dietary carbohydrates and reduced activity (Alasalvar, Taylor, Zubcov, Shahidi, & Alexis, 2002) compared to wild fish which were also prone to periods of starvation (Haard, 1992). The earlier comparative studies on proximate composition of soft and hard crabs were mostly on muscle composition. The soft-shelled claw muscle had as high as 94.76% of moisture and as low as 3.05% of protein, whereas hard-shelled claw muscle had moisture and protein content of 84.38% and 14.31% respectively. More interestingly, the ash content of the soft crab claw muscle was higher (2.17%) than that of hard-shelled claw muscle (1.67%) indicating the selective reabsorption of mineral contents into the muscle portion at time of moulting from exuvia (Benjakul & Sutthipan, 2009).

TABLE 6 Proximate composition (g/kg on wet basis) of mud crab, *Scylla serrata*

Particulars	Proximate composition					
	Moisture	Crude Protein	Ether extract	Crude fibre	NFE[1]	Total ash
Different diets (A)						
Soft crab (1)	804.51^{a}	87.81^{c}	12.90^{c}	12.11^{c}	16.93^{a}	65.70^{c}
Fattened-control (2)	688.65^{b}	118.85^{b}	19.05^{a}	18.80^{ab}	23.07^{a}	131.55^{a}
Fattened-CP-32% (3)	679.70^{b}	132.32^{a}	20.10^{a}	17.03^{b}	31.60^{a}	119.23^{b}
Fattened- CP-36% (4)	687.75^{b}	127.45^{a}	19.00^{a}	19.46^{a}	25.39^{a}	120.93^{b}
Fattened-wild (5)	686.57^{b}	126.51^{ab}	17.06^{b}	18.53^{ab}	23.24^{a}	128.06^{a}
Different size groups (B)						
Medium[2] (1)	715.08^{a}	118.44^{a}	16.96^{b}	17.83^{a}	21.70^{a}	109.96^{a}
Large[3] (2)	709.53^{a}	119.44^{a}	17.69^{ab}	15.77^{a}	24.99^{a}	112.56^{a}
XL[4] (3)	710.21^{a}	118.11^{a}	16.58^{b}	17.44^{a}	22.66^{a}	114.98^{a}
XXL[5] (4)	702.93^{a}	118.38^{a}	19.26^{a}	17.71^{a}	26.82^{a}	114.88^{a}
Interactions						
A 1 B 1	815.93	85.73	12.57^{j}	10.57	14.27	60.93
A 1 B 2	810.07	87.30	12.70^{ij}	11.30	15.40	63.23
A 1 B 3	808.57	87.03	12.87^{ij}	13.40	12.63	65.50
A 1 B 4	783.50	91.20	13.50^{hiij}	13.20	25.43	73.17
A 2 B 1	712.33	110.57	20.20^{abcde}	17.87	14.47	124.57
A 2 B 2	690.43	112.07	20.73^{abcd}	18.03	22.40	136.33
A 2 B 3	683.57	124.97	17.27^{defffg}	20.43	21.37	132.40
A 2 B 4	668.27	127.83	18.03^{bcdef}	18.87	34.07	132.93
A 3 B 1	669.97	144.47	21.33^{ab}	20.40	19.20	124.63
A 3 B 2	672.57	132.13	22.63^{a}	15.63	37.90	119.13
A 3 B 3	681.63	125.10	15.70^{fghiij}	15.80	42.43	119.33
A 3 B 4	694.67	127.60	20.73^{abcd}	16.30	26.87	113.83
A 4 B 1	694.03	127.17	13.80^{ghiij}	19.33	28.97	116.70
A 4 B 2	675.97	137.97	17.63^{cdefff}	17.67	29.20	121.57
A 4 B 3	691.20	128.13	20.90^{abc}	19.33	14.73	125.70
A 4 B 4	689.83	116.53	23.67^{a}	21.53	28.67	119.77
A 5 B 1	683.17	124.27	16.93^{efffgh}	21.00	31.63	123.00
A 5 B 2	698.63	127.73	14.77^{fghiij}	16.23	20.07	122.57
A 5 B 3	686.10	125.33	16.17^{fghi}	18.23	22.17	132.00
A 5 B 4	678.40	128.73	20.40^{abcde}	18.67	19.10	134.70
p-Value						
A	<0.001	<0.001	<0.001	<0.001	0.197	<0.001
B	0.626	0.982	0.007	0.196	0.767	0.271
A × B	0.644	0.058	<0.001	0.545	0.505	0.337
SEM	366.538	52.325	2.639	4.847	121.033	35.826
CV (%)	3.552	8.027	12.130	16.856	6.965	60.207

Note: Mean bearing same superscript letters in a column within main effects and interactions between the categories does not differ significant ($p > 0.05$).

[1]Nitrogen-free extract.

[2]200–300 g.

[3]301–500 g.

[4]501–750 g.

[5]751–1,000 g.

TABLE 7 Mineral composition of mud crab, *Scylla serrata*

Particulars	Treatments				*p*-Value	*SEM*
	Soft crab	Fattened-control	Fattened-CP-32%	Fattened-CP-36%		
Macro minerals (g/kg on wet basis)						
Calcium	26.24[b]	48.16[a]	48.17[a]	49.52[a]	<0.001	6.964
Potassium	1.58[b]	1.75[ab]	1.90[a]	1.77[a]	0.030	0.005
Magnesium	2.40[b]	3.57[a]	3.42[a]	3.64[a]	<0.001	0.016
Sodium	3.95[b]	4.46[a]	4.57[a]	4.58[a]	0.020	0.021
Phosphorus	2.57[b]	3.28[a]	3.18[a]	3.25[a]	<0.001	0.006
Micro minerals (mg/kg on wet basis)						
Copper	27.01[b]	31.57[a]	30.48[a]	30.13[a]	0.018	0.862
Iron	189.9[a]	177.67[a]	187.66[a]	189.38[a]	0.263	33.285
Manganese	45.82[b]	73.43[a]	73.80[a]	74.10[a]	<0.001	8.637
Selenium	10.60[b]	34.10[a]	31.69[a]	32.56[a]	<0.001	2.471
Zinc	48.94[b]	58.77[a]	58.43[a]	60.11[a]	0.016	5.661

Note: Mean bearing same superscript letters in a row do not differ significant ($p > 0.05$).

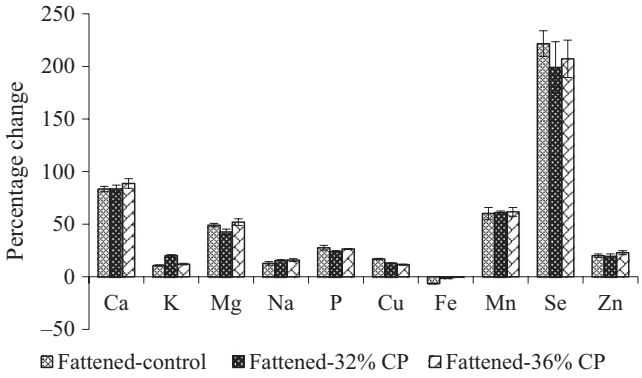

FIGURE 1 Change (%) in mineral composition in fattened crabs compared with initial soft crabs (wet basis)

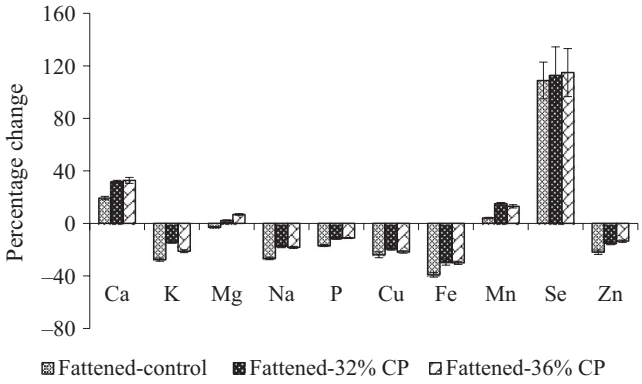

FIGURE 2 Change (%) in mineral composition in fattened crabs compared with initial soft crabs (dry basis)

The mineral content values were higher ($p < 0.05$) in hard-shelled whole crabs compared with those of soft-shelled crabs (Table 7). In order to understand the mineralization pattern, the changes of mineral contents in hard crabs fed control diet and test diets containing CP-32 and 36% were compared with soft crab on both wet basis and dry matter basis (Figures 1 and 2 respectively). On wet basis changes, comparisons indicated the increase in concentration of almost all the mineral content in hard crabs compared with soft crabs, whereas when the percentage change values were compared on dry matter basis, this indicated the reduction in concentration of mineral contents of K, Na, P, Cu, Fe and Zn. The higher concentration of minerals in soft crabs indicated the reabsorption of minerals into soft muscle or to the body fluids (Bergey & Weis, 2007; Engle, 1987; Keteles & Fleeger, 2001; Mohapatra, Rautray, Patra, Vijayan, & Mohanty, 2009). Salinity of the free body fluid recovered from the meropodites of the soft snow crab was significantly higher than that of the hard snow crab (Mizuta, Kobayashi, & Yoshinaka, 2001). In contrast to other minerals, the concentration of Ca was increased on both wet basis and dry basis in hard crabs compared with soft crabs which would be due to the limited storage ability for Ca in marine species and their ability to obtain bulk of their requirement from the surrounding water (Greenway, Arias-Rodriguez, Diaz, & Tobler, 2014).

5 | CONCLUSION

From the present investigation, it could be concluded that pelleted feed with 36% crude protein and 5.59% ether extract is optimal for fattening across all the marketable sizes of the crab. The present demonstration, by involving the local Yanadi tribes, provides alternative livelihoods to them by fattening the crabs in individual cages. The nutrient and mineral profiles of both soft and hard crabs indicated the selective reabsorption of certain minerals. The diet does not seem to have an influence on the crab mineralization process.

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CONFLICT OF INTEREST

Authors declare that there is no conflict of interest.

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REFERENCES

Alasalvar, C., Taylor, K. D., Zubcov, E., Shahidi, F., & Alexis, M. (2002). Differentiation of cultured and wild sea bass (*Dicentrarchus labrax*): Total lipid content, fatty acid and trace mineral composition. *Food Chemistry*, *79*, 145–150. https://doi.org/10.1016/S0308-8146(02)00122-X

Ali, S. A., Dayal, J. S., & Ambasankar, K. (2011). Presentation and evaluation of formulated feed for mud crab *Scylla serrata*. *Indian Journal of Fisheries*, *58*, 67–73.

Ali, S. A., Dayal, J. S., Ambasankar, K., Kathirvel, M., Pandian, S. K., Balasubramanian, C. P., … Rami Reddy, P. (2008). Farming of mud crabs. First ever application of feed pellets with reassuring results. *Fishing Chimes*, *28*(1), 143–145.

AOAC (1997). *Official methods of analysis* (18th ed.). Washington, DC: Association of Official Analytical Chemists, Benjamin Franklin Station.

APHA (2012). *Standard methods for the examination of water and waste water* (22nd ed.). New York, NY: American Public Health Association.

Begum, M., Shah, M. M., Mamun, A. A., & Alam, M. J. (2009). Comparative study of mud crab (*Scylla serrata*) fattening practices between two different systems in Bangladesh. *Journal of the Bangladesh Agricultural University*, *7*, 151–156.

Benjakul, S., & Sutthipan, N. (2009). Muscle changes in hard and soft shell crabs during frozen storage. *LWT-Food Science and Technology*, *42*, 723–729. https://doi.org/10.1016/j.lwt.2008.10.003

Bergey, L. L., & Weis, J. S. (2007). Molting as a mechanism of depuration of metals in the fiddler crab, *Uca pugnax*. *Marine Environmental Research*, *64*, 556–562. https://doi.org/10.1016/j.marenvres.2007.04.009

Bonine, K. M., Bjorkstedt, E. P., Ewel, K. C., & Palik, M. (2008). Population characteristics of the mangrove crab *Scylla serrata* in Kosrae, Federated States of Micronesia: Effects of harvest and implications for management. *Pacific Science*, *62*, 1–19.

Catacutan, M. R. (2002). Growth and body composition of juvenile mud crab, *Scylla serrata*, fed different dietary protein and lipid levels and protein to energy ratios. *Aquaculture*, *208*, 113–123. https://doi.org/10.1016/S0044-8486(01)00709-8

Chin, H. C., Gunasekera, U. P. D., & Amandokoon, H. P. (1992). Formulation of artificial feeds for mud crab culture: A preliminary biochemical, physical and biological evaluation. In C. A. Angell (Ed.), *Report of the seminar on the mud crab culture and trade, Thailand* (pp. 179–184). Madras, India: Bay of Bengal Programme for Fisheries Development.

Christensen, S. M., Macintosh, D. J., & Phuong, N. T. (2004). Pond production of the mud crabs *Scylla paramamosain* (Estampador) and *S. olivacea* (Herbst) in the Mekong Delta, Vietnam, using two different supplementary diets. *Aquaculture Research*, *35*, 1013–1024. https://doi.org/10.1111/j.1365-2109.2004.01089.x

Cuzon, G., Lawrence, A., Gaxiola, G., Rosas, C., & Guillaume, J. (2004). Nutrition of *Litopenaeus vannamei* reared in tanks or in ponds. *Aquaculture*, *235*, 513–551. https://doi.org/10.1016/j.aquaculture.2003.12.022

D'Abramo, L. R., & Castell, J. D. (1997). Research methodology. In L. R. D'Abramo, D. E. Conklin & D. M. Akiyama (Eds.), *Crustacean nutrition, advances in world aquaculture* (pp. 3–25). Baton Rouge, LA: World Aquaculture Society.

Dassow, J. A. (1968). Characteristics of frozen shellfish: Factors affecting quality changes during freezing and storage. Part 1. Crabs and lobsters. *The Freezing Preservation of Foods*, *2*, 197–208.

Dayal, J. S., Ali, A. S., Ambasankar, K., & Singh, P. (2003). Effect of dietary protein level on its *in vitro* and *in vivo* digestibility in the tiger shrimp *Penaeus monodon* (Crustacea: Penaeidae). *Indian Journal of Marine Sciences*, *32*(2), 151–155.

Dayal, J. S., Ambasankar, K., Jannathulla, R., Kumaraguruvasagam, K. P., Kailasam, M., & Vijayan, K. K. (2017). Polyculture of mullets in brackishwater using compounded feed: Proximate and mineral profiles in comparison with wild mullets. *Indian Journal of Fisheries*, *64*, 50–57. https://doi.org/10.21077/ijf.2017.64.4.69810-07

Engle, D. W. (1987). Metal regulation and molting in the blue crab, *Callinectes sapidus*: Copper, zinc and metallothionein. *Biological Bulletin*, *172*, 69–82.

Finlayson, A. J. (1964). Amino acid recovering in the analysis of some feed samples. *Canadian Journal of Plant Science*, *45*, 184–188.

Folch, J., Lees, M., & Sloane Stanley, G. H. (1957). A simple method for the isolation and purification of total lipids from animal tissues. *Journal of Biological Chemistry*, *226*, 497–509.

Greenway, R., Arias-Rodriguez, L., Diaz, P., & Tobler, M. (2014). Patterns of macroinvertebrate and fish diversity in freshwater sulphide springs. *Diversity*, *6*, 597–632. https://doi.org/10.3390/d6030597

Grigorakis, K., Alexis, M. N., Taylor, K. A., & Hole, M. (2002). Comparison of wild and cultured gilthead sea bream (*Sparus aurata*); composition, appearance and seasonal variations. *International Journal of Food Science and Technology*, *37*, 477–484. https://doi.org/10.1046/j.1365-2621.2002.00604.x

Haard, N. F. (1992). Control of chemical composition and food quality attributes of cultured fish. *Food Research International*, *25*, 289–307. https://doi.org/10.1016/0963-9969(92)90126-P

Jajic, I., Krstovic, S., Glamocis, D., Jaksis, S., & Abramovic, B. (2013). Validation of an HPLC method for the determination of amino acids in feed. *Journal of the Serbian Chemical Society*, *78*, 839–850. https://doi.org/10.2298/JSC120712144J

Jannathulla, R., Dayal, J. S., Ambasankar, K., Eugine, A. C., & Muralidhar, M. (2018). Fungus, *Aspergillus niger*, fermented groundnut oil cake as a fishmeal alternative in the diet of *Penaeus vannamei*. *Aquaculture Research*, *49*, 2891–2902.

Jannathulla, R., Dayal, J. S., Chitra, V., Ambashankar, K., & Muralidhar, M. (2017). Growth and carcass mineralisation of Pacific white leg shrimp *Penaeus vannamei* Boone 1931 in response to water salinity. *Indian Journal of Fisheries*, *64*, 22–27. https://doi.org/10.21077/ijf.2017.64.2.59404-04

Kador, A. (1991). Mud crab-A potential aquaculture resource of Bangladesh. In C. A. Angel (Ed.), *The mud crab: A report on the seminar convened in Surat Thai* (pp. 95–102). Chennai, India: Bay of Bengal Programme (BOBP).

Keteles, K. A., & Fleeger, J. W. (2001). The contribution of ecdysis to the fate of copper, zinc and cadmium in grass shrimp, *Palaemonetes pugio* Holthius. *Marine Pollution Bulletin*, *42*, 1397–1402. https://doi.org/10.1016/S0025-326X(01)00172-2

Lee, S. Y. (1991). Herbivory as an ecological process in a *Kandelia candel* (Rhizophoraceae) mangal in Hong Kong. *Journal of Tropical Ecology*, *7*, 337–348. https://doi.org/10.1017/S0266467400005605

M.S. Swaminathan Research Foundation. (2016). *Diversification of livelihoods in transforming socio-economic and gender relations: A case study of Yanadi tribes in AP*. Retrieved from http://www.mssrf.org/?q=-content/diversificationlivelihoodstransformingsocioeconomicandgenderrelationscasestudy downloaded on 12/08/2016

Mann, D. L., Asakawa, T., Kelly, B., Lindsay, T., & Paterson, B. (2007). Stocking density and artificial habitat influence stock structure and yield from intensive nursery systems for mud crabs *Scylla serrata* (Forsskål 1775). *Aquaculture Research*, *38*, 1580–1587. https://doi.org/10.1111/j.1365-2109.2006.01626.x

Metcalfe, L. D., Schmitz, A. A., & Pelka, J. R. (1966). Rapid preparation of fatty acid esters from lipids for gas chromatographic analysis. *Analytical Chemistry*, *38*, 514–515. https://doi.org/10.1021/ac60235a044

Millamena, O. M., Bautista, M. N., Reyes, O. S., & Kanazawa, A. (1997). Threonine requirement of juvenile marine shrimp *Penaeus monodon*. *Aquaculture*, *151*, 9–14. https://doi.org/10.1016/S0044-8486(96)01486-X

Millamena, O. M., Bautista-Teruel, M. N., & Kanazawa, A. (1996). Methionine requirement of juvenile tiger shrimp *Penaeus monodon* (Fabricius). *Aquaculture*, *143*, 403–410. https://doi.org/10.1016/0044-8486(96)01270-7

Millamena, O. M., Bautista-Teruel, M. N., Reyes, O. S., & Kanazawa, A. (1998). Requirements of juvenile marine shrimp, *Penaeus monodon* (Fabricius) for lysine and arginine. *Aquaculture*, *164*, 95–104. https://doi.org/10.1016/S0044-8486(98)00179-3

Millamena, O. M., Teruel, M. B., Kanazawa, A., & Teshima, S. (1999). Quantitative dietary requirements of postlarval tiger shrimp, *Penaeus monodon*, for histidine, isoleucine, leucine, phenylalanine and tryptophan. *Aquaculture*, *179*, 169–179. https://doi.org/10.1016/S0044-8486(99)00160-X

Mirera, D., & Mtile, A. (2009). A preliminary study on the response of mangrove mud crab (*Scylla serrata*) to different feed types under drive-in cage culture system. *Journal of Ecology and the Natural Environment*, *1*, 7–14.

Mizuta, S., Kobayashi, Y., & Yoshinaka, R. (2001). Chemical and histological characterization of raw muscle from soft and hard crabs of snow crab *Chionoecetes opilio*. *Journal of Food Science*, *66*, 238–241. https://doi.org/10.1111/j.1365-2621.2001.tb11324.x

Mohanty, R. K., Mohapatra, A., & Mohanty, S. K. (2009). Assessment of the impacts of a new artificial lake mouth on the hydrobiology and fisheries of Chilika Lake, India. *Lakes and Reservoirs: Research and Management*, *14*, 231–245. https://doi.org/10.1111/j.1440-1770.2009.00406.x

Mohapatra, A., Rautray, T. R., Patra, A. K., Vijayan, V., & Mohanty, R. K. (2009). Trace element-based food value evaluation in soft and hard shelled mud crabs. *Food and Chemical Toxicology*, *47*, 2730–2734. https://doi.org/10.1016/j.fct.2009.07.037

Moss, S. M., Divakaran, S., & Kim, B. G. (2001). Stimulating effects of pond water on digestive enzyme activity in the Pacific white shrimp, *Litopenaeus vannamei* (Boone). *Aquaculture Research*, *32*, 125–131.

Moss, S., & Pruder, G. (1995). Characterization of organic particles associated with rapid growth in juvenile white shrimp, *Penaeus vannamei* Boone, reared under intensive culture conditions. *Journal of Environmental Marine Biology and Ecology*, *187*, 175–191.

Paez-Osuna, F., Perez-Gonzalez, R., Izaguirre-Fierro, G., Zazueta-Padilla, H. M., & Flores-Campana, L. M. (1995). Trace metal concentrations and their distribution in the lobster *Panulirus inflatus* (Bouvier, 1895) from the Mexican pacific coast. *Environmental Pollution*, *90*, 163–170. https://doi.org/10.1016/0269-7491(94)00103-K

Patterson, J., & Samuel, V. D. (2005). Participatory approach of fisherwomen in crab fattening for alternate income generation in Tuticorin, Southeast coast of India. *Asian Fisheries Science*, *18*, 153–158.

Peñaflorida, V. D. (1989). An evaluation of indigenous protein sources as potential component in the diet formulation for tiger prawn, *Penaeus monodon*, using essential amino acid index (EAAI). *Aquaculture*, *83*, 319–330. https://doi.org/10.1016/0044-8486(89)90043-4

Sastry, C. S. P., & Tammuru, M. K. (1985). Spectrophotometric determination of tryptophan in protein. *Journal of Food Science and Technology*, *22*, 146–147.

SEAFDEC Asian Aquaculture (1997). *Aquaculture Department, South East Asian Fisheries Development Center*. Tigbauan, Iloilo, Philippines: SEAFDEC Asian Aquaculture.

Sheen, S. S., & Wu, S. W. (1999). The effects of dietary lipid levels on the growth response of juvenile mud crab *Scylla serrata*. *Aquaculture*, *175*, 143–153. https://doi.org/10.1016/S0044-8486(99)00027-7

Shelley, C. (2008). Capture-based aquaculture of mud crabs (*Scylla* spp.). *Capture-based aquaculture. Global overview* (pp. 255–269). FAO Fisheries Technical Paper-508.

Tacon, A. G. (1996). Nutritional studies in crustaceans and the problems of applying research findings to practical farming systems 1. *Aquaculture Nutrition*, *2*(3), 165–174. https://doi.org/10.1111/j.1365-2095.1996.tb00056.x

Tacon, A. G., Cody, J. J., Conquest, L. D., Divakaran, S., Forster, I. P., & Decamp, O. E. (2002). Effect of culture system on the nutrition and growth performance of Pacific white shrimp *Litopenaeus vannamei* (Boone) fed different diets. *Aquaculture Nutrition*, *8*, 121–137. https://doi.org/10.1046/j.1365-2095.2002.00199.x

Tuan, V. A., Anderson, A., Luong-van, J., Shelley, C., & Allan, G. (2006). Apparent digestibility of some nutrient sources by juvenile mud crab, *Scylla serrata* (Forskal 1775). *Aquaculture Research*, *37*, 359–365. https://doi.org/10.1111/j.1365-2109.2005.01433.x

Unnikrishnan, U., & Paulraj, R. (2010). Dietary protein requirement of giant mud crab *Scylla serrata* juveniles fed iso-energetic formulated diets having graded protein levels. *Aquaculture Research*, *41*, 278–294.

Ut, V. N., Le Vay, L., Nghia, T. T., & Hong Hanh, T. T. (2007). Development of nursery culture techniques for the mud crab *Scylla paramamosain* (Estampador). *Aquaculture Research*, *38*, 1563–1568. https://doi.org/10.1111/j.1365-2109.2006.01608.x

Walton, M. E., Le Vay, L., Lebata, J. H., Binas, J., & Primavera, J. H. (2006). Seasonal abundance, distribution and recruitment of mud crabs (*Scylla* spp.) in replanted mangroves. *Estuarine, Coastal and Shelf Science*, *66*, 493–500. https://doi.org/10.1016/j.ecss.2005.09.015

Yanar, Y., Göçer, M., Yanar, M., & Kücükgülmez, A. (2011). Differences in nutritional composition between cultured and wild green tiger shrimp (*Penaeus semisulcatus*). *Italian Journal of Food Science*, *23*, 1–6.

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