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EXPLORING PROTEIN IMPACT ON MUD CRAB CONDITIONING: A NUTRITIONAL ANALYSIS

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Abstract: Mud crabs, belonging to the genus Scylla, are prized edible crustaceans found in the tropical Indo-West Pacific region. They include species like Scylla serrata, Scylla tranquebarica, Scylla olivacea, and Scylla paramamosain, each distinct in its own right. These crabs are not only known for their delicious flavor but also for their nutritional value, boasting essential amino acids, proteins, polyunsaturated fatty acids, and vital minerals like calcium, iron, zinc, potassium, and phosphorus. Mud crab aquaculture is crucial for small-scale fisheries in the Indo-Pacific region, contributing to commercial demand. One method employed is crab fattening, which involves rapidly increasing the crab's weight and size. This process can take anywhere from 15 to 60 days and is carried out in various enclosures, including earthen ponds, bamboo enclosures, net cages, floating cages, and plastic boxes. Successful crab fattening relies on factors such as providing essential crab nutrition, maintaining water flow and quality, monitoring soil quality, and managing crab density within the ponds. Proper attention to these variables ensures that "water crabs" or "empty crabs" can attain the desired size and meet market demand.

Keywords: Mud crabs, Scylla species, crab fattening, aquaculture, nutritional value

1. Introduction

Mud crabs are crustaceans who belong to the genus *Scylla* and are widely distributed in mangrove swamps and other coastal habitats of the tropical Indo-West Pacific region, mainly throughout the South and SouthEastern Asian regions. They are also known as mangrove crabs, which are edible and large. In Sri Lanka, "*kalapu kakuluwa*" is the common name for mud crabs. *Scylla serrata*, *Scylla tranquebarica*, *Scylla olivacea,* and *Scylla paramamosain* are four species belongs to the genus *Scylla*, which are distinct from each other respectively (Macintosh et al., 2002). *S. serrata*, is the most abundant species in the Asian-Pacific region (Ikhwanuddin et al., 2010). Mud crabs are considered a quality food due to their flavor, texture, and nutritional value (Rodriguez et al., 2003). The meat of these crabs' rich in essential amino acids, proteins, polyunsaturated fatty acids (PUFAs), as well as it is composed of essential minerals like Calcium, Iron, Zinc, Potassium, and Phosphorus (Sarower et al., 2013). Mud crab aquaculture plays an important role in small-scale fisheries of Indo-Pacific countries.

Mud crab fattening is the process that increases the weight or size of the crab within a short period for enhancing the commercial demand. The time taken for the fattening process may vary with the method which is used. However, most probably it takes 15-60 days. Crab fattening carries out in encircled earthen ponds, bamboo enclosures, net cages, floating cages, or galvanized wire nets, and usage of plastic boxes is the most recent practice (Sivasubramaniam & Angell, 1992). Through the process of fattening, "water crabs" or "empty crabs" are allowed to grow up to a certain size, which is demanding in the market. While practicing fattening methods, feeding with essential crab nutrition, maintenance of water flow and water quality, soil quality and the density of crabs stocked in a pond should be maintained under required levels are vital factors for achieving the desired growth.

Trash fish is widely used for feeding mud crabs in fattening practices as trash fish are highly available and cost-effective compared to other feeding materials. Although that is cost-effective, it is insufficient for providing the entire nutrient requirement of mud crabs, and as a consequence of that, it is difficult to acquire the desired outcome. These traditional feeding practices associate with freezing and these practices may lead to water quality reduction, especially the nutrient contamination of natural water bodies which leads to the negative impacts on natural aquatic environment (Darshana Senarathna et al., 2020). Therefore, lack of sufficient knowledge on practices has influenced the sustainability of mud crab aquaculture. Although there are a lot of studies conducted on the feeding of shrimps, the knowledge on the nutrition of *Scylla sp.* is poor. (Dayal et al., 2019). Chin and Gunasekara (1992) have studied Scylla feeding with a formulated diet comprised of 35-40 percent crude protein. A report has been published by Sheen and Wu (1999) regarding the growth response of juvenile Scylla while feeding with formulated feed composed of dietary lipid levels ranging from 5.3% to 13.8%. Moreover, According to Catacutan (2002), optimum growth of mud crabs was shown with feeds formulated as dietary protein level ranging from 32% - 40% with a 6% - 12% lipid level.

While preparing formulated feeds farmers have to invest considerably at a high cost as most of the ingredients are not locally available. Therefore, they have to export. It would be profitable if the exported materials are replaced with locally available materials at a low cost. Another thing is some formulated feeds have several drawbacks such as unattractive, less palatability, low stability, Therefore, Preparing the dry pellets with locally available ingredients makes a lot of benefits. For instance, when there is a global issue, like a pandemic, difficulties may arise while supplying ingredients that are imported. The importing process may disrupt and their cost may vary considerably due to the dropping down of the economy. Thus, it is important to prepare feeds in dry form, as well as with the materials from locally available ingredients.

This study was performed to develop a cost-effective feeding product with balanced nutrients for mud crab fattening, by using locally available materials such as fish meal and shrimp head meal, especially which are removed from seafood processing plants as waste materials. Here, attempts were made to reduce current issues on balancing nutrients of the diet associated with the mud crab fattening process. Moreover, this study was aimed to determine the applicability of locally available materials on mud crab feeding. The suitability of the formulated feeding product was determined comparing with control feed via growth rate, survival, and utilization of feed and the biochemical composition of mud crab meat when using formulated diet vs control feed. The economic benefit over formulated product vs control feed was determined by the cost of feed to produce 1 kg of each feed.

2. MATERIALS AND METHODS

2.1. Ingredient preparation

Trash fish were received from fishing ports and new shrimp heads were obtained from a Seafood Processing plant. Herring and sardines were selected as common trash fish. Soy foods, coconut oil cake, soy lecithin, canola oil, wheat flour, vitamins, and mineral mixtures were purchased at the local market. Unwanted parts were removed from the fish and shrimp heads and washed with boiling water for 15 minutes. After that they were dried under the sunlight for 3 days to reduce the humidity to less than 10%. All dried ingredients were grounded separately and passed through a 0.5 mm filter to obtain fine particles. Ingredients for the prepared feed
(FM and SHM) were analyzed in close composition according to the standard methods of AOAC (2000) (Table 1).

2.2. Feed formulation

The feed was made according to dietary protein, lipid, and the energy requirement of mud crabs. According to a previous study in other countries, mud crabs show excellent growth with a diet containing 32-40% protein, 6-

12% lipid and food intake from 14.7 to 17.6 MJ / kg (S. Ahamad Ali et al., 2011). Feed formulation was done with PRO-4SE (version 1.0) software.

2.3. Feed preparation

The feed types were prepared by mixing dry ingredients in proportions made using an electric mixer and soy lecithin, vitamins, and mineral mixtures mixed with oil and then added to the mixture, followed by the addition of gelatinized starch (300 ml of water per 1kg of feed mixture). The compositions of the tested feeds are given in Table 1. The dough was passed through a mincer machine with a 3.0 mm diameter disk. The lead threads were broken by hand into short pieces (8 mm). After that the pellets were steamed for 5 minutes and dried in the oven for 2 hours at 65℃. Dried pellets were stored in polythene bags and stored at 4℃ - 8℃ until use (Catacutan, 1991)(S. Ahamad Ali et al., 2011). As a pre-limitation feed experiment, five feeds were prepared by mixing ingredients at different ratios and selecting three feeds (feeds 01, 02 and 03) based on the acceptance of the mud crabs, feed stability of the feed and keeping quality of feeds.

Table 1: The composition of experimental feeds (g / 100 g dry feed).

Ingredients	**Feed 01**	**Feed 02**	**Feed 03**
Fish meal	12	12	36
Shrimp head meal	28	37	6
Soybean meal	7	13	30
Coconut oil cake	13	7	6
Wheat flour	34	24	16
Canola oil	4	5	4
Soy lecithin	1	1	1
Vitamin and mineral premix	1	1	1

2.4. Proximate composition of feed

The three selected feeds and control feed were analyzed for the proximate composition in accordance with the standard method of AOAC (2000) (Table 3.2).

2.5. Experimental study (Design and procedure)

Mud crabs (*S. serrata*) (Weighted around 60.30 g ± 9.76) were selected and a total of 24 mud crabs were used for this experiment. Vertical culture racks were used for this experiment. It contained rectangular plastic boxes, containing about 23 L of brackish water in one box. One box contains water inlet and an outlet. The system was designed for automatic water circulation. Completely Randomized Design (CRD) was used as the statistical design. One box was used as a single test unit. It was made up of four repetitive feeding modes (feed 01, 02, 03 and control feed) as 6 replicates. Each treatment was randomly assigned to each box. Trash fish were used as the control feed as it the common feed material for crab fattening. All crabs were fed with a daily allowance equal to 8% of body weight. Feeding was done twice a day in equal doses, at 9.00 A.M. and 3.00 P.M. (S. Ahamad Ali et al., 2011). The supply of pellet feed was done by hand and feeding behavior and acceptability of the feed was observed.

The containers were siphoned daily after two hours of food intake. The uneaten feed was oven dried at

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600℃ and the weights were taken. Water temperature, salinity, pH, Nitrogen, and Nitrite in water intake were measured three times a week using a digital thermometer, refractometer and laboratory pH meter respectively. Nitrogen was rated according to color gradient and Nitrite was rated by N × 3.3. Boxes were checked daily for mortalities and molting. The testing procedure was performed for 30 days.

2.6. Analysis of mud crab (S. serrata) meat

Proximate composition of juvenile mud crabs (*S. serrata*) meat fed with selected three formulated feeds and control feed were analyzed according to the standard AOAC (2000) methods. Bodyweight, carapace width and length of crabs were measured at the end of every week and calculated the body weight gain (BWG) percentage and specific growth rate (SGR). Feed utilization was measured by weekly feed intake, feed conversion ratio (FCR), feeding efficiency (FE), protein efficiency (PE), and lipid efficiency (LE).

2.7. Determination of water stability by feed pellets

About 5 g of feed pellet was weighed with a 250ml water into a beaker. The condition of the pellets was closely monitored over time while stirring the water with a glass rod. At the end of one hour, a water sample was taken from each beaker and the turbidity was measured visually. Water is filtered through a blotting cloth and pellets were collected, dried and weighed (S A Ali et al., 2005).

2.8. Statistical analysis

Statistical analysis was done using one way ANOVA with 95% confidence interval and the Duncan's multiple range test was used to identify significant differences between the each treatment.

3. Results and Discussion

3.1. Proximate composition of feed ingredients

Fish meal and shrimp head meal have been used as major feed ingredients in this mud crab feed experiment. Raw materials were the waste of the seafood processing sector for the preparation of both ingredients and therefore the product was a cost-effective mud crab feed.

Table 2: Proximate composition of fish meal and shrimp head meal (Dry matter basis)

	Fish meal	Shrimp head meal
Dry matter %	93.16 ± 0.44^a	94.91 ± 0.33^a
Moisture %	5.77 ± 0.38^a	4.11 ± 0.12^a
Crude protein %	44.76 ± 1.00^a	48.41 ± 1.02^a
Crude Fat %	9.72 ± 1.22^a	3.89 ± 0.39^b
Crude Fiber %	0.98 ± 0.11^a	16.15 ± 0.46^b
Ash %	32.13 ± 0.33^a	29.81 ± 0.33^b
Nitrogen Free Extract%	2.99 ± 0.95^a	1.87 ± 0.13^b
Energy (J/g)	15994 ± 30.970	14555.6 ± 23.73

Means in the same row with different superscripts are significantly difference ($p < 0.05$)

Table 2 presents the proximate composition of the feed ingredients. The fish meal and the shrimp head meal are usually rich in protein. According to the results of the shrimp head meal showed a higher protein content than the fish meal, but there was no significant difference between the two ingredients in terms of crude protein content. The highest content ($p < 0.05$) of crude oil was found in fish meals. However, the shrimp head meal showed a relatively high content of raw fiber ($p < 0.05$). Both ingredients have shown high energy content.

The moisture content of dry feed ingredients should be less than 10 -12%. A high-quality fish meal usually contains 60-72% crude protein produced in the conventional method of wet pressing. It consists of several steps; cooking, pressing, drying, and grinding (Miles R.D and Chapman F., 2015)(Miles R.D and Chapman F., 2015). In this experiment, the fish meal was made with the direct sunlight method. Therefore, the low protein content may be reflected in a sun-dried fish meal over a meal produced by the standard wet pressing method. There is no commercial way to produce a shrimp head meal. Shrimp head powder is, therefore, a cheap and good source of protein for the aquaculture sector. In the shrimp's head meal, a portion of the complete nitrogen/crude protein is provided by chitin, N-acetylated glucosamine which is the main constituent of the exoskeleton of shrimp. Fish lipids are highly digestible by all species of animals and are good sources of essential PUFAs; both n3 PUFAs and n-6 PUFAs. However, fish meal contains n-3 PUFAs rather than n-6 PUFAs. The normal lipid content of the fish meal and shrimp head meal respectively is 6-10% and 3-8% (Ariyawansa et al., 2000). Generally, both ingredients contain a high amount of PUFAs. However, shrimp heads include digestive glands that contain many TAGs and often contain low levels of n-3 PUFAs (Fox et al., 1994). So the high-fat content of the shrimp head meal is lower than the fish meal. However, about 10% of fat is cholesterol in the shrimp's head meal (Suprayudi et al., 2012). Crude fiber is considered to be an insoluble residue of acid hydrolysis followed by alkaline and this residue contains cellulose, hemicellulose, and lignin (Carrillo et al., 1995). Therefore, the determination of crude fiber is basically for feed ingredients are based on plant origin. Thus a fish meal shows a very low level of raw fiber content because fish is low or no fibrous carbohydrates. However, the shrimp head meal shows a high level of crude fiber content because the shrimp has an exoskeleton made of chitin. Chitin is a fibrous polysaccharide made up of converted glucose chains, N-acetylated glucosamine.

In general, a high-quality fish meal contains 17-25% of ash content. More ash presents a high level of mineral content, especially calcium, phosphorus, and magnesium. Phosphorus in plant nutrients grown in plants is not readily available to monogastric animals, as it is mainly in the organic state, known as phytate. However, phosphorus in a fish meal is highly available to many animals (Miles R.D and Chapman F., 2015). The mineral composition in the shrimp head meal is calcium, phosphorus, magnesium, potassium, sodium, and iron. Among them, calcium has a very high content in the shrimp head meal, as calcium is one of the key components of the crustacean's exoskeleton. The shrimp's head meal is therefore considered a mineral-rich source. Both ingredients showed a high level of ash content than the optimum level since both were prepared by the direct sun-drying method. This technique is frequently carried out under unhygienic conditions and it leads to the high microbial load in the final product. In addition, this method is likely to lower the protein, lipid, and nutritional value of the final product. High levels of chitin/fiber and ash content in shrimp head meal can lead to low pellet stability.

Therefore, fiber and ash content can be reduced by converting shrimp head waste into silage by the fermentation process (Fagbenro & Bello-Olusoji, 1997).

3.2. Proximate composition of experimental feeds

Table 3: Proximate composition of experimental feeds (Dry matter basis)

Feed 01			Feed	02	Feed 03		Control feed	
Dry matter %	98.49	± 0.41^a	96.65	± 0.91^a	91.69	± 0.11^b	31.90	± 0.24
Moisture %	3.22	± 0.50^a	3.98	± 0.95^a	6.89	± 0.15^b	77.42	± 0.01

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Crude Protein	31.32	± 0.42^{c}	34.11	± 1.53^{b}	39.25	± 1.80^{a}	57.01	± 0.11
%								
Crude Fat %	11.22	± 0.37^{a}	11.99	± 0.02^{a}	10.18	± 0.11^{a}	9.80	± 0.20^{a}
Crude Fiber %	5.91	± 0.42^{b}	9.90	± 1.00^{a}	2.90	± 0.11^{c}	1.90	± 0.01^{c}
Ash %	12.99	± 0.04^{b}	16.49	± 0.69^{a}	15.16	± 0.33^{ab}	12.62	± 0.31^{c}
Nitrogen free extract %	37.82	± 1.15^{a}	26.87	± 0.25^{b}	27.07 ± 1.04^{b}		3.33	± 0.10
Energy (J/g)	17880.5	± 40.1^{a}	17350.5	± 35.5^{b}	17100.5 ± 40.5^{c}		16190.5	± 16.45

Means in the same row with different superscripts are significantly difference ($p < 0.05$)

Table 3 express the dry matter basis proximate compositions of the three feed verities and the control feed. Sardine was given as the control feed. All three feeds were prepared as dry pellets where the moisture content of each feed was controlled to be less than 10%. Feed 03 had a high moisture content ($p<0.05$) than feed 02 and feed 01. However, there was no significant difference between feed 01 and feed 02 for moisture content. The highest crude protein content (39.25%) was available in feed 03 among the three formulated dry pellet feeds and all three formulated feeds were significantly different from each other for the crude protein content. However, there was no significant difference among the crude fat content of the developed feeds. The highest crude fiber content (9.09 %) was found in feed 02 and the lowest crude fiber content (1.90 %) was found in the control feed. However, feed 01 and 02 ($p<0.05$) showed significantly higher crude fiber content than feed 3 and the control feed ($p>0.05$). Formulated feeds showed a significantly higher ash percentage than the control feed. The lowest value of Nitrogen free extract (NFE) was 3.33 %, for the control feed and the highest value of NFE is 37.82 %, for feed 01. However, feed 02 and feed 03 did not show a significant difference for nitrogen-free extract.

Analyzing the moisture content is crucial when formulating any type of dry pellet feed. According to previous research studies, the moisture content in dry pellet feed should be around 8-12% (Clayton et al., 2007). The moisture content of the dry feed is related to its water activity (aw). Water activity is considered to be an essential factor for the microbial growth within the feed and it can be defined as the amount of available water in the feed by microorganisms. High water activity leads to the rapid growth of microorganisms, especially bacteria. The dry feed has a comparatively lower water activity than the moist or semi-moist feed. However high moisture content (>12%) in the dry feed may trigger mold growth while shortening the shelf life of the particular feed. Low moisture content increases the shelf life during storage. Protein in the feed drives the growth of animals and it is a nitrogen-based organic compound that is considered to be essential for life. Proteins consist of one or more long polypeptide chains that are composed of more amino acids connected by peptide bonds. However, the protein requirement of animals varies with species, growth stage, gender, and body functionality. Therefore, the protein content in the feed should be able to fulfill the protein requirement of the targeted animal. Unnikrishnan and Paulraj (2010) studied the best growth performance in mud crabs (*S. Serrata*) fed with 40-45% crude protein in the diet. Therefore, in terms of the crude protein content, feed 02 is the most suitable feed for mud crab fattening. Arginine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, and valine are the essential amino acids in the crustacean's feeds. Fish meal, shrimp head meal, soybean meal, and coconut oil cake are contributed to crude protein content in the experimental feeds (Table 3). Undeniably, the animal protein sources predominantly influence the crude protein content in feeds than plant protein sources.

Lipids can be classified into 3 major groups; simple lipids (fats and oil), complex lipids or compound lipids (phospholipids, glycolipids, lipoproteins), and derived lipids (such as sterols, cholesterol). As previously mentioned, fish meal and shrimp head meal contain more polyunsaturated fatty acids (n-3 PUFAs, n-6 PUFAs). Mud crabs require polyunsaturated fatty acids for their growth, development, and survival as essential fatty acids, particularly EPA and DHA. They could not also be synthesized by mud crabs (Suprayudi et al., 2012). Hence, those fatty acids should be supplied by feeds. However, canola oil was used in a high amount when preparing feed 02 than other formulated feeds (Table 1). Therefore, feed 02 has shown a high crude fat content. Crude fiber and nitrogen-free extract are mainly from the carbohydrates in the feed that left after the determination of the moisture, crude protein, crude fat, and ash. The nitrogen-free extract comprises sugars (monosaccharides and disaccharides), starches, oligosaccharides, pectins, and β-glucans. According to feed compositions shrimp head meal, soybean meal, and coconut oil cake were caused by crude fiber content in each diet. Normally, the crude fiber content of soybean meal and coconut oil cake is respectively 6–8 % and 12–13 % (Ravindran, 1992). In this feed experiment, shrimp head meal was prepared one and crude fiber content is 9.90 % (Table 3). A high amount of shrimp head meal was used in feed 01 and feed 02 (Table 3). Therefore, the feeds showed high crude fiber content than the other one. Shrimp head meal predominately influences crude fiber content in each feed.

Soybean meal, wheat flour, and coconut oil cake significantly influence NFE content in the feeds. Fish meal and shrimp head meal poorly influence the NFE content in the feeds. Because those ingredients are derived from animals and have shown low NFE values. NFE content of soybean meal, coconut oil cake, and wheat flour is respectively 38–41%, 50–52%, and 69–71% (Ravindran, 1992). However, feed 1 showed the highest NFE value (37.82%). Because feed 01 comprised a high amount of wheat flour, and coconut poonac, than others. Feed 03 belongs to the optimum crude fiber level (Table 3). A high level of crude fiber in raw materials may be caused to reduce the binding capacity and water stability of the pellet and a high fiber level in the feed may be caused to increase the fecal production and consequently pollute the water environment. Therefore, crustacean species have a preference for feed with little fiber and NFE.

The ash or the part left-over after incineration contains minerals and trace elements. Fish meal and shrimp head meal showed more ash level (Table 3). In prepared feeds, the total ash content was between 12–16%. However, minerals and trace minerals play an important role in various metabolic processes in the crustacean body like the formation of the exoskeleton, molting, developing cofactors for enzymatic activities, osmotic regulation, growth, and development. Aquatic animals, inclusion crustaceans are also able to absorb minerals and trace elements from water. Major minerals in the crustaceans feed are calcium, phosphorus, sodium, potassium, magnesium, chlorine, and trace elements are iron, copper, iodine, zinc, manganese, and selenium (Cuzon et al., 1994).

Table 3 has shown the gross energy values for each feed. Gross energy is the energy that is released when nutrients; lipids, proteins, and carbohydrates are completely oxidized in a bomb calorimeter. Feed 1 showed the highest gross energy value (17880.5 J/g) because a comparatively high amount of energy sources were used in feed 1, such as wheat flour, shrimp head meal, and canola oil. Crude fat has a high gross energy value (39.6 KJ/g) than crude proteins (23.65 kJ/g) and carbohydrates (17.5 kJ/g). The recommended dietary energy level for the crustacean feed is 16790 – 17090 J/g (Cuzon et al., 1994). According to the experiment, results feed 03 contains the optimum energy level when compared with the others.

3.3. Utilization of feeds and analysis of growth performances of mud crabs

The average growth pattern of the juvenile mud crabs fed on developed diets for 30 days has been presented in table 4. The first week of the feed experiment was considered as the acclimatization period and the performances were not clear to observe during this period. The growth response was measured by final body weight, final carapace length, width, body weight gain, and specific growth rate. Feed utilization was measured by weekly feed intake, feed conversion ratio, feeding efficiency, protein efficiency, and lipid efficiency.

Table 4: Growth study and feed utilization of juvenile mud crabs (S. serrata)

Parameters	Feed 01		Feed 02		Feed 03		Control feed	
Initial BW (g)	48.63	± 2.60^{d}	56.02	± 2.22^{c}	67.61	± 2.11^{b}	68.13	± 1.30^{a}
Final BW (g)	67.50	± 2.52^{b}	88.19	± 12.49^{a}	90.29	± 15.82^{a}	81.24	± 2.73^{a}
Initial CL (cm)	4.71	± 0.66^{b}	4.98	± 0.41^{ab}	5.13	± 0.03^{ab}	5.39	± 0.21^{a}
Final CL (cm)	5.25	± 1.02^{b}	5.81	± 0.51^{ab}	5.82	± 0.33^{ab}	6.12	± 0.66^{a}
Initial CW(cm)	6.60	± 0.22^{c}	7.04	± 0.27^{b}	7.17	± 0.12^{b}	7.39	± 0.20^{a}
Final CW (cm)	6.99	± 0.28^{b}	7.69	± 0.52^{a}	7.84	± 0.65^{a}	7.95	± 02^{a}
Weight gain (g)	18.8	6± 0.82^{ab}	32.16	± 4.83^{a}	22.68	± 7.01^{b}	13.11	± 0.81^{c}
SGR %	1.09	± 0.04^{ab}	1.52	± 0.71^{a}	0.96	± 1.02^{b}	0.58	± 1.00^{c}
Feed Intake (g)	28.72	± 0.73^{b}	33.30	± 2.20^{a}	21.49	± 0.93^{c}	20.91	± 0.78^{c}
FCR	1.52	± 0.34^{a}	1.04	± 1.10^{b}	0.95	± 1.33^{b}	1.59	± 0.50^{a}
FE	0.22	± 0.03^{b}	0.29	± 0.22^{b}	0.38	± 0.27^{b}	0.75	± 0.08^{a}
PE	0.69	± 0.03^{b}	0.91	± 0.10^{ab}	1.00	± 0.80^{ab}	1.42	± 0.33^{a}
LE	1.92	± 0 .07^{b}	2.40	± 1.00^{b}	3.55	± 3.71^{b}	7.90	± 0.80^{a}

Molting frequency 0.000 16.677 16.677 0.000
Mortality % 0.000 16.677 16.677 0.000
BW: body weight, CL: carapace length, CW: carapace width, SGR: specific growth rate, FCR: feed conversion ratio, FE: Feeding efficiency, PE: Protein efficiency, LE: Lipid efficiency
Moulting frequency = (No. of crabs moulted / total number of crabs in treatment) × 100 Mortality % = (No. of crabs dead / total number of crabs in treatment) × 100
Means in the same row with different superscripts are significantly difference ($p < 0.05$)

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According to the data, the bodyweight of mud crabs in all treatment modes has been increased with time.
There was a statistically significant difference in the initial weight of the mud crabs stored in these four treatments. However, the final body weight in feed 01 was lower (p <0.05) than in other feeds. Feed 02 has shown higher weight gain over time than other feeds. There were no statistically significant differences between feed 02 and feed 03 in the final and final carapace lengths of mud crabs. However, feed 01 and the control showed a significant difference (p <0.05). There were no statistically significant differences between feed 02 and feed 03 in the first and last carapace widths of mud crabs. However, feed 01 and the control showed a significant difference (p <0.05) in the initial carapace diameter of mud crabs. However, the final carapace diameter in feed 01 was lower (p <0.05) than in other test feeds. Moreover, the highest BWG % showed in feed 02 and lower BWG % showed in the control feed. However, BWG % did not show a statistically significant difference between feed 1 and feed 02 as well as feed 01 and feed 04. However, BWG % showed a significant difference ($p<0.05$) between feed 02 and feed 03. Feed 02 has shown the highest specific growth rate and it is produced a higher percentage ($p<0.05$) than feed
04 and control feed. However, there was no statistically significant difference between feed 01 and feed 02 in terms of SGR %. The highest FCR value has shown in control feed-fed mud crabs and the lowest FCR value has shown in feed 4 fed mud crabs. However, the lowest FCR value has shown in feed 4 fed mud crabs and it is not showing a statistically significant difference with feed 02. Weekly feed intake was not significantly different between feed 04 and control feed. However, feed 02 showed a higher feed intake ($p<0.05$) than feed 01.
According to the feeding efficiency (FE), protein efficiency (PE), and lipid efficiency (LE) data of juvenile mud crabs, all formulated feeds were presented with lower mean values for feeding efficiency, protein efficiency, and lipid efficiency ($p<0.05$) than the control feed. However, there was no statistically significant difference among the formulated feeds in terms of FE, PE, and LE.
The present study showed that 29 % and 33 % of dietary protein levels show better body weight gain and specific growth rates at different energy levels (Table 4). According to Akiyama *et al* (1991) reported dietary protein level in the feed for fattening mud crabs (*S. Serrata*) is between 30–35 % with contains all essential amino acids. The high level of dietary protein may lead to the lower retention of protein in the body and reduce the voluntary feed intake. Therefore, 38 % and 55 % dietary protein levels may result in lower body weight gain and specific growth rate. However, excess protein may lead to the high ammonia level in the hemolymph in crustaceans as an excretory product. The accumulation of ammonia in hemolymph may negatively affect metabolic processes and growth response. As previously mentioned a high level of dietary protein level may be caused to reduce the voluntary feed intake. Therefore, 33 % dietary protein level (feed 02) shows higher feed intake than other protein levels and highest body nutrient gains. It is known that crabs avoid eating nutritionally unbalanced feeds. Loss of appetite and the reduction of feed intake explain that they do not meet their nutritional requirements. However, the dietary protein above the optimum level showed a negative correlation with the growth response of mud crab juveniles in the present study.
FCR is a ratio that indicates the animal converts the feed into the desired output. Regarding fattening mud crabs, FCR is indicated the body mass gained by the crabs for meat development. In the present study, FCR is varied from 3.9 to 5.1 and the lowest value was found in feed 2 and the highest value was found in the control feed. The lower FCR is an animal that uses fewer feeds to produce the desired output, which is good for the farmers and lower FCR is also indicates a good quality feed. The FCR of the control feed with a dietary protein level of 55.46 % was higher than other dietary protein levels. Comparatively, both feed 02 and feed 03 ($p<0.05$) showed the best FCR in the present study.
The highest protein efficiency value was found in the control feed and the lowest value was found in feed 01. Protein efficiency can be defined as the bodyweight gain of fattening mud crabs regarding their dietary protein intake. Protein efficiency has expressed the quality of protein in the feed and it depends on the availability of

essential amino acids profile in the feed. If the essential amino acid requirement has been sufficiently supplied by the feed, the available protein in the feed will be more efficiently utilized by mud crabs. In the present study, protein efficiency has increased with 29%, 33%, and 38% dietary protein levels. Feed 01 and control feed showed a 100 % survival rate and feed 02 and 03 showed similar molting frequencies during the 30 days experimental period. The dietary protein level did not significantly influence the mortality and may be caused by incomplete molting, high ammonia level, and disease condition.

3.4. Proximate composition of juvenile mud crabs (S. serrata) meat

Table 5: Proximate composition of juvenile mud crabs (S. serrata) meat

M–1			M–2		M–3		M–C	
Dry matter %	25.41	± 0.62^{ab}	20.89	± 0.81^{c}	22.46	± 0.25^{bc}	26.05	± 1.05^{a}
Moisture %	76.36	± 0.72^{ab}	78.01	± 0.01^{c}	75.35	± 0.44^{bc}	74.02	± 0.87^{a}
Crude Protein	64.22	± 0.98^{b}	70.01	± 1.55^{a}	64.19	± 2.01^{b}	59.02	± 2.91^{c}
%								
Crude Fat %	4.66	± 0.73^{ab}	3.88	± 0.78^{bc}	5.60	± 0.21^{a}	3.29	± 0.01^{c}
Ash %	14.21	± 0.95^{ab}	14.99	± 0.12^{a}	14.70	± 0.74^{a}	12.57	± 0.42^{b}

DM = Dry matter; crude protein, crude fat, and ash were determined in dry weight basis; M – 1, M – 2, M – 3 and M – C have indicated respectively crab meat from feed 1, 2, 3 and control

Means in the same row with different superscripts are significantly difference ($p < 0.05$)

The proximate composition of juvenile mud crabs meat fed with newly developed formulated dry pellet feeds and control feed are given in Table 5. Fresh flesh was obtained from the abdomen and chelipeds for analysis. The highest crude protein content (64.22%) and lowest crude protein content (59.02%) were identified respectively in crabs fed with feed 02 and control. However, there was no significant difference between the crabs fed with feed 01 and feed 03 for crude protein content. According to Akiyama *et al*., (1991), protein content in the feed for fattening mud crabs is between 30– 35 %. The average meat protein in the mud crabs directly related to the protein level of their feed.

The highest crude fat content was found in the crab meat fed with feed 03 (5.60 %), and the lowest crude fat content was identified with the crabs fed with the control feed. The highest ash content was found in the crab meat fed with feed 02 (14.99 %) and the lowest ash content was found related to the control feed C (12.57 %). However, the crab flesh fed with formulated pellet feeds showed high ash content ($p<0.05$) than the control one. The biochemical composition of an animal depends on the size of the animal, gender, age, stage of maturity, season, nutrient availability, and environmental conditions. The results of this study clearly showed the biochemical composition of mud crabs varies significantly with the feed composition. Table 6 shows the meat composition of natural and fattened mud crabs. Therefore, feed 2 fed mud crabs have significantly shown a better biochemical composition than the other three diets fed mud crabs.

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Table 6: Meat composition of natural and fattened mud crabs (S. serrata)

	Normal range	Natural mud crab	Fattened mud crab	References
Moisture %	77.7 % – 80.19 %	83.64 %	81 %	(Sarower et al., 2013), (Prasad & Neelakantan, 1989)
Crude protein%	16.8 % – 20.11 %	22.77 %	27.43 %	(George & Gopakumar, 1987), (Prasad & Neelakantan, 1989)
Crude fat%	1.65 % – 1.07 %	1.35 %	1.92 %	(Sarower et al., 2013), (Prasad & Neelakantan, 1989)
Ash	1.23 % - 5.09 %	2.09 %	3.54 %	(Sarower et al., 2013), (Prasad & Neelakantan, 1989)

3.5. Analysis of water stability of formulated feeds

Table 7: Water stability of formulated dry pellet feeds in water at the end of one hour

Feed 1				Feed 2	Feed 3	
Initial wt. of dry pellet (g)	6.89	± 0.05	6.34	± 0.05	6.18	± 0.05
Final dry wt. of pellet (g)	5.14	± 0.05^a	4.55	± 0.03^a	4.88	± 0.08^a
Turbidity rank		2		3		1
Dry pellet water stability rank		2		3		1

Turbidity and water stability of dry pellet were ranked visually by eyes. Turbidity rank 1: low turbidity in water and rank 3: high turbidity in water. Water stability rank 1: high water stability of dry pellet and rank 3: low water stability of dry pellet.

Means in the same row with different superscripts are significantly difference ($p < 0.05$)

Table 3.5 presents the water stability of formulated dry pellet feeds. The stability of pellets were remained in a satisfactory level even after one hour. There was no significant difference ($p<0.05$) among the feeds for mean final dry weights.

According to the data, water stability of dry pellet feeds have decreased progressively with the time. Feed 02 has the lowest water stability from three developed feeds. Feed 01 showed the highest water stability at the end of one hour. The water stability of aquaculture feed can be used to determine the overall performance of feed. Water stability ensures minimum disintegration of the pellet when exposure to water and manipulation by the crabs during feeding.

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3.6. Water quality parameters

Table 8: Water quality parameters during experimental period

Water quality parameters	Values	
PH	7.25	± 0.05
Temperature (ºC)	31.35	± 1.05
Salinity ((ppt)	24.18	± 0.05
Nitrogen (mg/L)	0.03	± 0.03
Nitrite (mg/L)	0.12	± 0.05

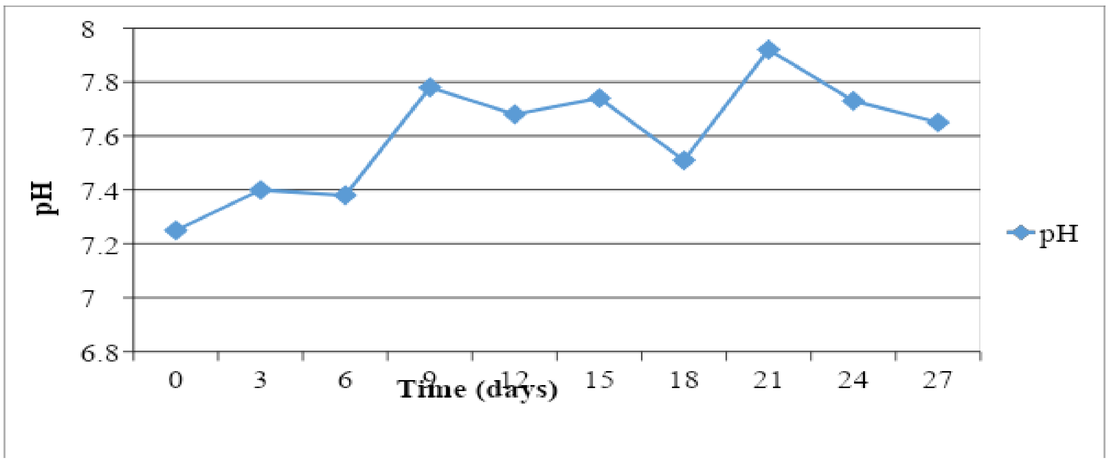

Figure 1: pH of brackish water

Figure 1 presents the variation of pH in brackish water intake during 30 days experimental period. Pedapoli and Ramudu (2014) recorded pH range from 7.2 to 7.4 is suitable for culturing the mud crabs in brackish water.

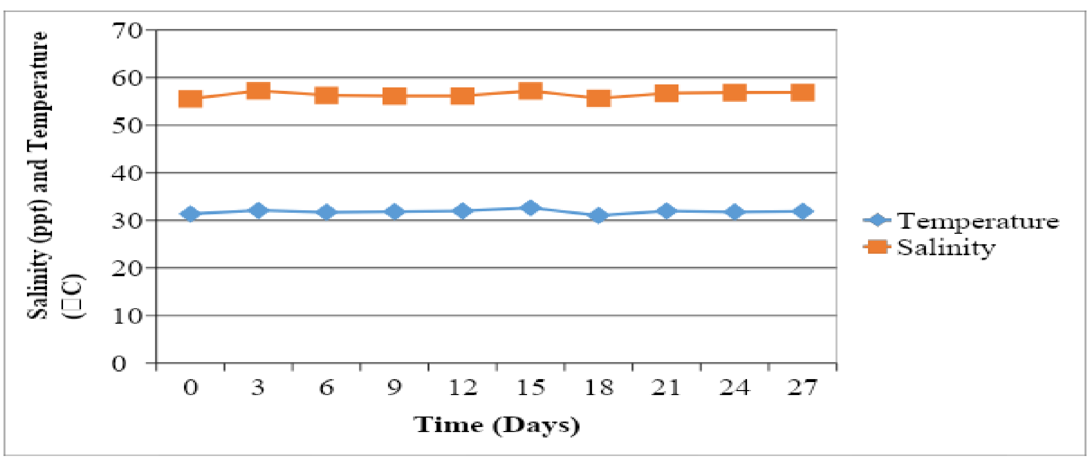

Figure 2: Temperature and salinity of brackish

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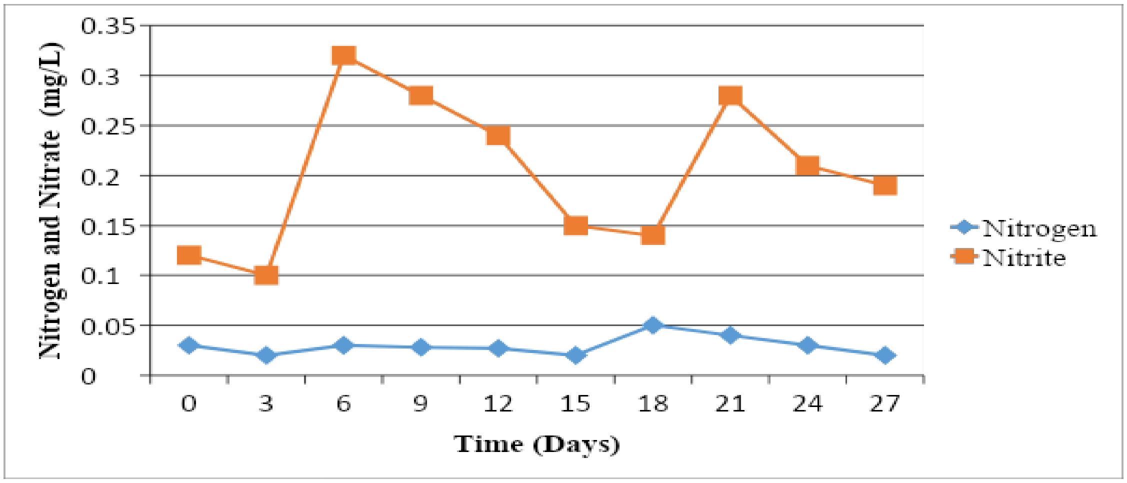

Figure 3: Nitrogen and Nitrite level in brackish water

Figure 2 reveals the variation of temperature and salinity in brackish water intake during 30 days experimental period. In the present study, temperature ranged from 28.8 °C to 32.4 °C (mean: 30.10 °C ± 1.07) and salinity ranged from 23 to 24 ppt (mean: 23.12 ppt ± 0.18). Pedapoli and Ramudu (2014) recorded high survival rate of mud crabs at approximately 25 ppt of salinity and temperature range from 28 °C to 30 °C. Moreover, low temperatures disturb on the moulting procedure of mud crabs (Paterson & Mann, 2011).

Figure 3 presents the variations of nitrogen and nitrite level in brackish water intake during the 30 days experimental period. Pedapoli and Ramudu (2014) recorded nitrite range from 0.015 mg/L to 0.025 mg/L is suitable for culturing the mud crabs in brackish water. In the present study, nitrogen ranged from 0.015 mg/L to 0.08 mg/L (mean: 0.04 mg/L ± 0.01) and nitrite ranged from 0.070 mg/L to 0.222 mg/L (0.16 mg/L ± 0.05).). However, nitrite showed wide range variation during experimental period.

3.7. Economic evaluation of feeds Table 9: Approximate feed cost

Feed	Cost (US$, For 100 kg)
Feed 1	54.00
Feed 2	69.00
Feed 3	61.00
Control Feed	49.00

Table 9 elaborated the estimated feed costs to produce 100kg of each diet. The cost analysis has shown that the formulated pellets are expensive than the control diet. Among them, feed 01 is the cheapest one and diet 02 is the most expensive one in terms of the cost of feed. However, diet 02 performed well compared to other experimented feed. The economic analysis following the cost of ingredients in Sri Lanka pointed out that the cost of fish meal, canola oil, and vitamin-mineral premix are high out of all the raw ingredients. In practice, cheaper feeds could be developed by substituting the available resources for expensive ingredients. This can be done by utilizing more of the nonconventional feed ingredients including earthworm meal, poultry by-products, snails, and slaughterhouse waste. According to the present study cost of fish, meal varies with an off-season of fish. However, the costs of commercial mud crab feed depend on a range of factors, including ingredients and production cost, transport cost, negotiation, and discounts.

4. CONCLUSION

According to the study, there was no statistically significant difference between the prepared fish diet and the fish's head diet in crude protein content. So fish meal can be replaced by shrimp head meal as a cheap and good source of animal protein in the aquaculture industry. However, there was a statistically significant difference between locally prepared fish feed or control feeds in terms of survival rate, growth response, and conversion rate of mud crab feeds. The feed prepared with 12 % fish meal and 37 % shrimp head meal has a very good growth response and the mud crab meat fed with this feed contains a high level of protein. Prepared feeds with 36% fish meal and 6% shrimp head meal have a very low rate of feed conversion ratio and high water stability. However, according to this study trash fish or control feeds showed better feed efficiency, protein efficiency, and lipid performance than prepared feeds. The feed having the best growth response and best water stability presents a high feed cost compared to the trash fish. Therefore it can be concluded that the use of trash fish meal is economically viable than the use of newly formulated feeds for the mud crab fattening. However, in the case of convenience, water quality management, and the feasibility of feeding in an epidemic situation or after a long-term storage period, the formulated feed is more beneficial than the wet feed.

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