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A Comparative Study of the Natural and Formulated Feeds to Promote Crab Aquaculture Industry in Pakistan

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Abstract
In Pakistan, the aquaculture industry is still in its early stages, and no significant work has been reported in crab aquaculture. A few local fishermen are practicing non-scientific farming of mud crabs, using naturally available feed. This study was conducted to initiate crab fattening practices along the coastal regions of Pakistan; for this purpose, Portunid crabs (*Scylla serrata*) were selected due to their muddy bottom habitat. The experiment was designed in which two groups of crabs (*S.serrata*), designated Group A and Group B, were fed different diets, and their body tissues were analyzed using biochemical techniques. Group A was given a natural diet (trash fish), while Group B received a formulated feed. Biochemical analysis of Group A's tissues revealed that protein content (20.82±0.98 mg/g) was higher than lipid (0.49±0.34 mg/g) and carbohydrate (1.02±0.16 mg/g) levels. Similarly, the analysis of Group B indicated that protein levels (19.47±2.00 mg/g) were higher than lipids (0.72±0.25 mg/g) and carbohydrates (1.17±0.32 mg/g). The biochemical comparison of both groups showed that Group A had a higher protein content, while Group B had higher lipid and carbohydrate levels. Growth assessment parameters such as feed conversion ratio (FCR), carapace length (CL), carapace width (CW), and mass increment were better in Group A, which was fed a natural diet. However, the survival rate (SR) was higher in Group B, which received the formulated feed. It was concluded that continuous research is required to improve feed formulation to enhance the growth of crabs and build the aquaculture industry in Pakistan.

Keywords: Mud crab fattening, *Scylla Serrata*, natural and formulated feed, Biochemical assessment,

Highlight:
- The fattening experiment has been carried out using the portunid crab *Scylla Serrata*
- Natural feed (trash fish) and formulated feed were used in the experiment.
- A high level of proteins reflects the increment in morphometric characteristics of crabs.

1.0. Introduction
Crabs and their by-products are in high demand worldwide (Rao et al., 1973). Commercially important crab, *S.serrata* (Forskall: 1775) of the Portunidae family (Samuel and Soundarapanian. 2009), is distributed throughout the Indo-Pacific region (Stephenson and Campbell, 1959). *S. serrata* is a habitant of muddy bottoms, mangrove marshes and estuaries (Motoh, 1979). Mud crabs can survive longer without water at minimum temperatures and are easily exported to other countries (Lalramchhani et al., 2019). These crabs are carnivores, preying on small invertebrates such as molluscs, crustaceans, polychaetes, detritus, and plant material consumed as food (Eldredge and Smith, 2001). Mud crabs can digest protein, carbohydrates and lipids from a variety of sources, such as mollusks, fish, crustaceans and plants (Catacutan et al., 2003; Tuan et al., 2006; Truong et al., 2009). Crab meat contains many nutrients and is an excellent source of high-quality proteins, vitamins and minerals. Mud crab export can help to generate foreign exchange in developing countries like China, the Philippines and Indonesia (Akbar et al., 1988; Alverson, 1971; Rahman et al., 2020; Macintosh et al., 2002; Quinitio, 2017).

The crab aquaculture industry is growing fast as formulated feed is available along with natural feed. Natural feed, such as trash fish, crustaceans, and mollusks, is available seasonally (FAO, 2011); hence, the formulation of feed for the fattening of crabs is highly focused on meeting market demand. The term "fattening" is applied when low-weight and soft-shelled crabs are stocked and raised for a specific duration to be fattened (Rahman et al., 2020; Sujan et al., 2021). Soyabean, containing a balanced nutritive protein, readily available and reasonably priced, is now used in formulated feed (Amaya et al., 2007). Recent studies showed that replacing fishmeal with soybean as a protein source increases the growth rate of mitten Crab (*Eriocheir sinensis*) (Chen et al., 2000). Introducing formulated feed in crab aquaculture requires further research to improve nutritional value and cost-effectiveness (Tacon et al., 2009; FAO, 2011).

All over the world, approximately 4500 species of crabs have been discovered so far. Two hundred species of crabs are found in the coastal waters of Pakistan (Rasheed and Mengal, 2024). Mud crab is one of the fastest-growing and most predicted aquaculture species ((Meng et al., 2017; Yusof et al., 2019. Islam et al., 2022).

In Pakistan, there are no significant records about the crab aquaculture industry. A few local fishermen are farming mud crabs on naturally available feed. Therefore, this study intends to provide the practical fattening practice and growth assessment of mud crabs using formulated and natural feeds.

2.0. Materials and Methods

2.1 Preparation and biochemical assessment of feed

In this study, trash fish was used as natural feed for a fattening experiment and was purchased from the local fish market. However, the formulated feed was prepared in the laboratory. Different animal and plant ingredients were purchased from the local market to prepare feed (Table 1). These ingredients, including dry fish, cockle's meat, Alfa alfa (lussan), corn and soybean, were powdered using a feed mill (micro pulverizer) with a mesh size of 0.5mm. Afterwards, vitamins and minerals were added to powdered ingredients and mixed properly through an electrical blender. Fish oil, water and binder were added as per formulation. A mince machine was used to form 4 mm pallets. These pellets were sun-dried and stored in a dry paper bag (FAO 2008, FAO 2011).

The crude protein was estimated using the Kjeldhal method, and a conversion factor of 6.25 was used to convert total Nitrogen into crude protein (Whida et al., 2024). Crude Fat was quantified through the Soxhlet extraction technique using hexane (65-70 °C) as the solvent. The crude fiber was determined by acid and base hydrolysis. The sample's moisture was estimated by weighing it and then dried in the oven at 67 °C for 24 h. After that, the sample was reweighed, and the difference in weight was taken as the moisture. The sample was burned at about 600-700 °C for 5-8 hours in the furnace, and then the remaining material was determined as ash. Nitrogen-free extract (NFE) was determined by the difference between the original weight of the sample and the sum of the weights of its moisture, crude protein (CP), crude fat (CF), ash and crude fiber (CFb) as determined by appropriate analytical method according to AOAC (2000) methods.

Crude protein (%) = Nitrogen (%) × conversion factor (6.25 for animal and plant origin ingredients)

Crude Fat (%) = Corrected weight of fat/weight of original sample × 100

Moisture (%) = wet weight – dried weight

NFE (%) =100-(CP+ CF+ ash+ Moisture+ CFb)

2.2 Feeding trails and growth assessment

Mud crabs (*S. serrata*) were purchased from local fishermen. Crabs were acclimatized for one week by a gradual change of salinity of seawater from 30 to 20 ppt. For the experiment, crabs were divided into two groups. Group-A was provided trash fish as natural feed, while Group-B was kept on formulated feed for six weeks. Each crab was placed in a plastic tank with well-aerated seawater (4L; 20 ppt) at 20 °C to 26 °C under a 12/12 light/dark illumination cycle. Water was changed every second day throughout the experiment. All the crabs were fed 10% of their biomass.

For growth assessment, morphometric parameters such as carapace length, CL (mm), carapace width, CW (mm) and weight (gm) were recorded fortnightly. These parameters were used to evaluate the optimal growth of crabs. Moreover, a biochemical assessment of the tissue of the fattened crab has been carried out. Protein content was estimated using the Lowry method (Lowry et al., 1951), and BSA (Bovine serum albumin) was used as a standard. The total lipid content has also been determined (Folch et al., 1957). The Phenol-sulfuric acid method (Dubois et al., 1956) was used for carbohydrate content and D-glucose as a standard.

Feed conversion ratio (FCR) = $F / (W_f - W_o)$,

Where,

F = the weight of food supplied to crab at the beginning of the study period;

Wo = The live weight of the crab at the beginning of the study period;

Wf = the live weight of the crab at the end of the study period.

T: maintenance time in days.

Survival rate (SR) = Nt / No × 100

Where,

SR: the survival rate (%)

Nt: the number of live crabs at the end of rearing (tails)

No: the number of crabs at the beginning of rearing (tails).

3.0 Results and Discussion

Feeding crabs with formulated or natural feeds is a practical approach to promote the aquaculture industry for growth, health, and sustainability. The study was undertaken to promote the crab aquaculture industry in Pakistan. In the present work, natural and formulated feeds were used to feed the mud crab for comparison. Formulated feed containing animal and plant ingredients was prepared in a laboratory (De Silva et al. 2009), while the percent values of ingredients are reported in Table (1).

S.No.	Ingredients	Values (%)	S.No	Ingredients	Values (%)
1	Fish Meal	30	**8**	Di calcium phosphate	2.0
2	Meat (Cockles)	10	**9**	Lime Stone	5.0
3	Corn	20	**10**	Vitamin & Minerals	0.4
4	Lusan (Dry)	5.0	**11**	DL Methanine	0.15
5	Soyabean Meal	20	**12**	L-Lysin	0.25
6	Soyabean Oil	2.5	**13**	Binding Agent	2.0
7	Cod Liver Oil	2.5	**14**	Preservative	0.25

Table 1: Composition (%) of formulated feed

Results demonstrate the proximate biochemical composition of formulated feed consists of crude proteins (35.63%), crude fiber (9.20%), crude lipids (7.08%), Nitrogen free extract (17.06%), moisture (19.75%), and ash (11.28%).In contrast, the natural feed contains a high value of moisture (37.32%), whereas other macromolecules, crude proteins (30.46%), crude fiber (0.67 %), crude lipids (8.32%), Nitrogen free extract (7.55%) and ash (15.68%) were shown in Table (2).

Composition	Formulated feed (%)	Natural feed (%)
Crude Protein	35.63	30.46
Crude fiber	9.20	0.67
Crude lipid	7.08	8.32
Nitrogen free extract	17.06	7.55
Moisture	19.75	37.32
Ash	11.28	15.68

Table 2: Proximate composition of formulated and natural feed.

The outcomes of the experiment after six weeks, showed that Group A, having a natural feed, showed a 15.83% increase in CL ($F= 367.40$; $P< 0.05$), 13.44% CW ($F =237.62$; $P< 0.05$) and 31.37% body mass ($F = 138.29$; $p< 0.05$). Conversely, Group B consumed formulated feed showed increments of 7.5% in CL ($F= 9.13$ $P< 0.05$), 7.20% CW ($F= 8.85$ $P< 0.05$) and 14.12% body mass ($F=5.61$ $P< 0.05$). Group B has a higher survival rate (90%) than Group A (87.50%) (Table 3).

Growth Parameters	Formulated feed (%)	Natural feed (%)
Survival rate (SR)	90.00	87.50
Carapace length(CL)	7.50	15.83
Carapace width (CW)	7.20	13.44
Mass	14.12	31.37
Feed conversion ratio (FCR)	3.20	3.90

Table. 3: Survival rate and growth parameters for two feeds used for fattening of Mud Crab.

For crabs fed with natural feed (Group A), there is a strong and linear relationship of body weight with carapace length ($r2 = 0.9973$) and carapace width ($r2 = 0.9974$) (Figure 1). Sex-based comparison indicated a strong relationship between body weight and carapace length and width. In females, the regression line demonstrated a positive and robust relationship of body weight with CL ($r2 = 0.999$) and CW ($r2 = 0.995$) (Figure 2), whereas in male crabs CL ($r2 = 0.994$) and CW ($r2 = 0.993$) were observed (Catacutan et al., 2003).

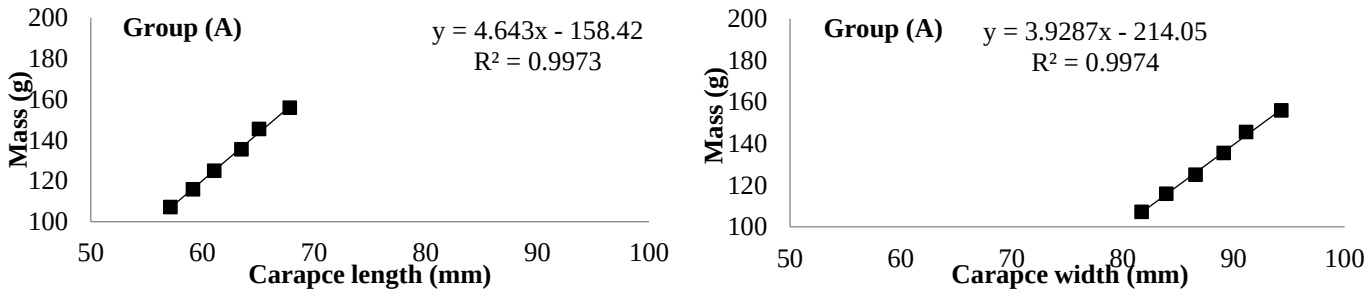

Figure 1: Relationship between body mass with carapace width and body mass and carapace length of crabs fed on natural feed during feeding trails (6 weeks).

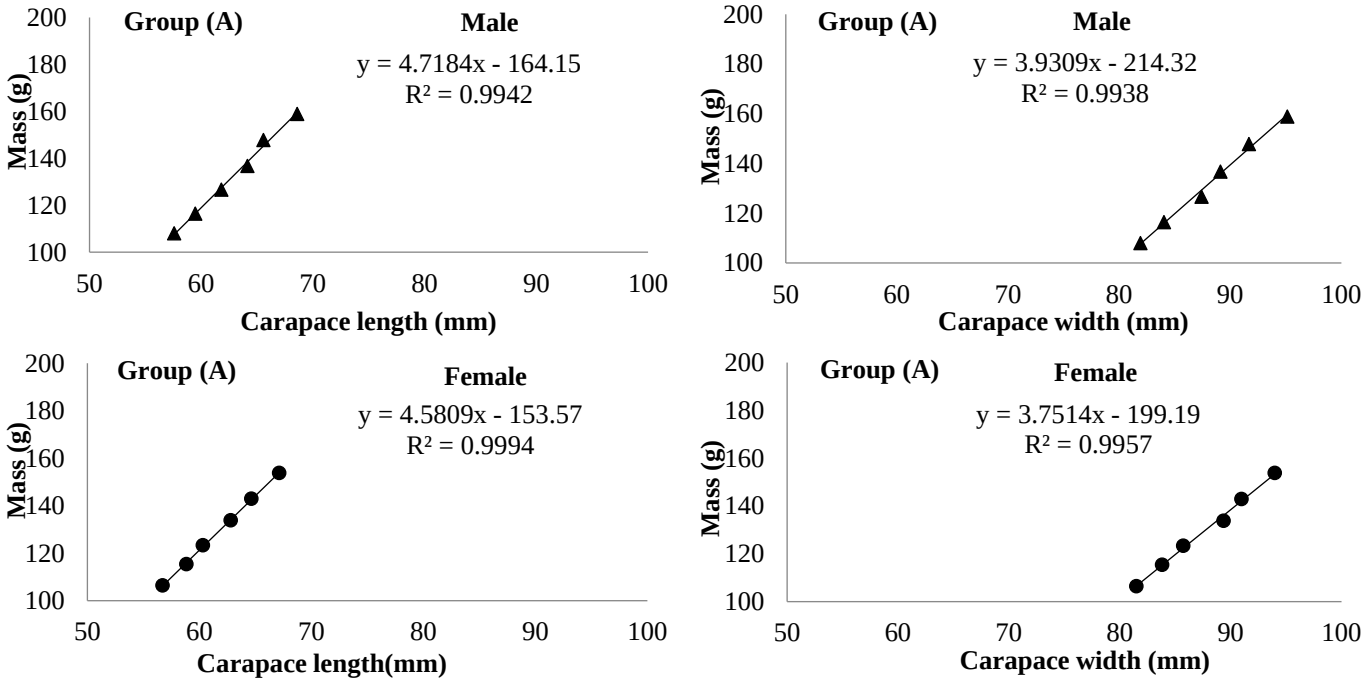

Figure 2: Relationship between body mass with carapace length and between body mass and carapace width of both male and female Crabs fed on natural feed during feeding trials (6 weeks).

Similar to that of Group A, Group B also have potent body weight relationships with CL (r^2= 0.992) and CW (r^2= 0.964) (Figure 3). Sex-specific observations revealed a strong relationship of body weight with CL and CW for both males and females (Christina et al., 2019). In male crabs, the relationship of body mass with CL (r^2= 0.986) and CW (r^2= 0.971). Whereas, in female crabs CL (r^2= 0.978) and CW (r^2= 0.916) (Figure 4) with body weight describe linear and positive trends, which was higher when compared to the reported work of Bello Olusoji et al. (2009) who observed that the r^2 = 0.81, provide a combined average of length and weight for the whole crab population of male and female. The results of the present investigation are similar to the reports of How-Cheong et al. (1992), who reported that the fattening of mud crabs is related to a nutritious diet that can promote the aquaculture practice.

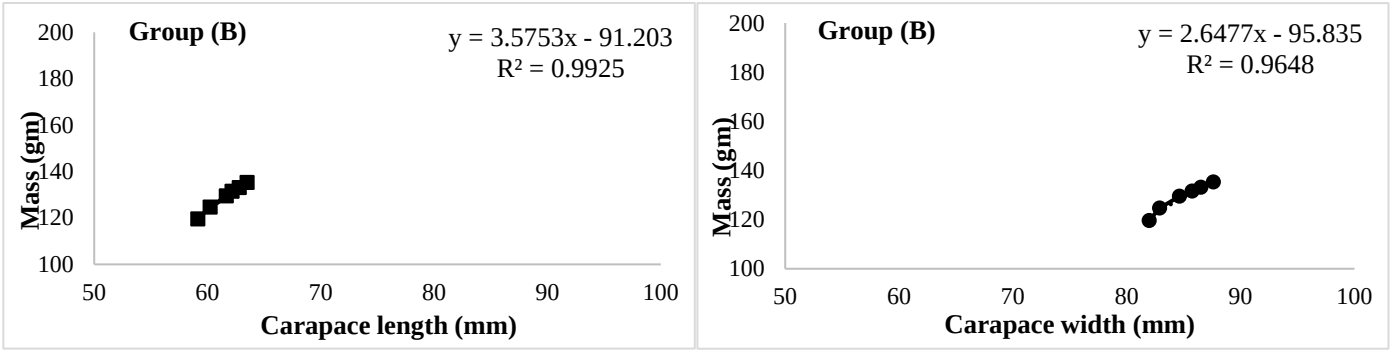

Figure 3: Relationship between body mass and carapace length and body mass and carapace width of Crabs fed on formulated feed during feeding trials (6 weeks).

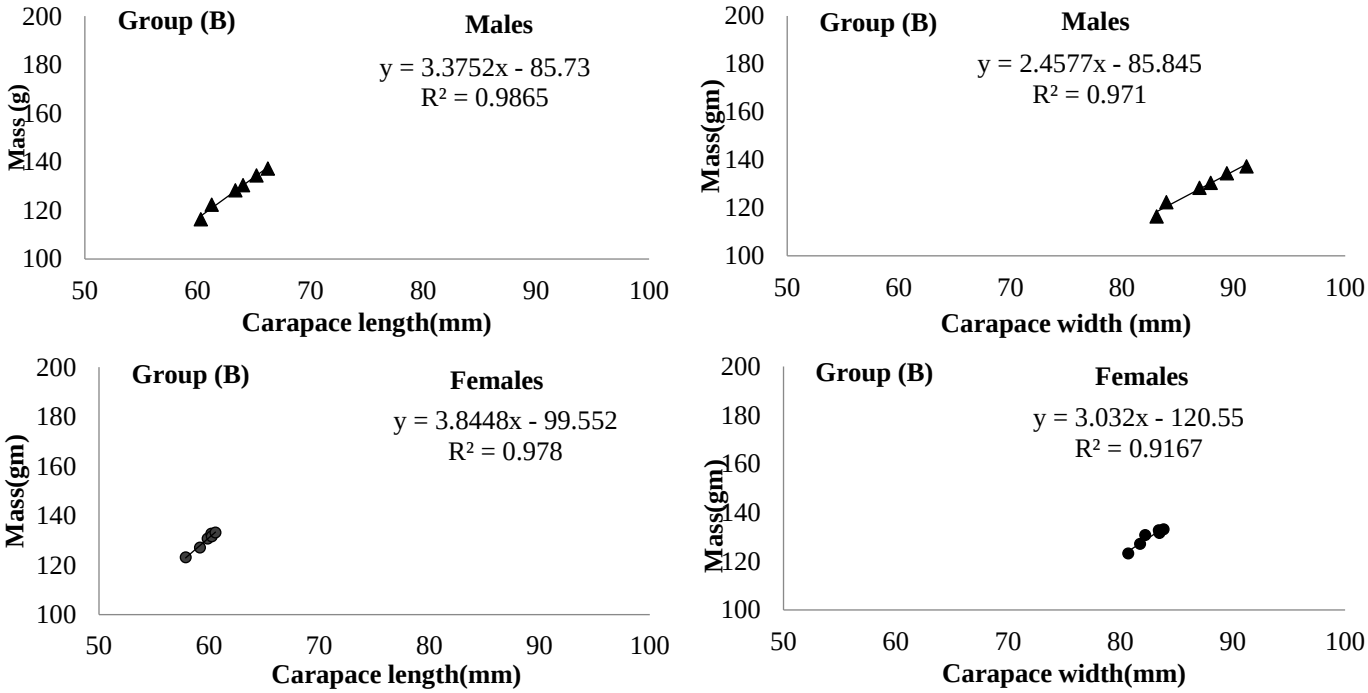

Figure 4: Relationship between body mass and carapace length and between body mass and carapace width of both male and female Crabs fed on formulated feed during feeding trials (6 weeks).

Biochemical analysis demonstrated that the quantity of proteins (20.82±0.98 mg/g) is more than that of lipids (0.49±0.34mg/g) and carbohydrates (1.02±0.16 mg/g) in Group A. Similarly, results for Group B indicate that the level of proteins is higher (19.47±2.00 mg/g) as compared to the lipids (0.72±0.25 mg/g) and carbohydrates (1.17±0.32 mg/g). A comparison of the biochemical compositions of both groups showed that the level of proteins is higher in Group A, while lipids and carbohydrate levels are higher in Group B (Table 4) (Khan 1992).

GROUPS	Protein (mg/g) mean ±S.D.	Lipid(mg/g) mean ±S.D.	CHO(mg/g) mean ±S.D.
Group A	20.82±2.00	0.49±0.34	1.02±0.16
Group B	19.47±0.98	0.71±0.25	1.17±0.32

Table 4: **Biochemical composition of Group A fed with (Natural feed) and Group B (Formulated feed).**

Body tissues of female and male crabs in Group A have 21.47 (mg/g) and 20.17(mg/g) of proteins, respectively and for Group B, 19.91 (mg/g) in female crabs and 19.02 (mg/g) in male crabs (Figure 5(a). In Group A, the quantity of lipids is (0.61mg/g) and (0.82 mg/g) in females and males, respectively. In Group B quantity of lipids in females is (0.77 mg/g) while in males, it is (0.21mg/g) depicted in Figure 5(b). The level of carbohydrates in Group A females is 0.96 mg/g, while in males, it is 1.083 mg/gm. Group B carbohydrate level is 1.43 mg/gm for females and 0.903 mg/gm for males (Figure (c). The biochemical analysis in the present study showed lower values than the report of Manivannan et al. (2010), who observed the biochemical analysis based on the two feeds, Acetes sp. and Clam meat, with high protein, carbohydrate and lipids.

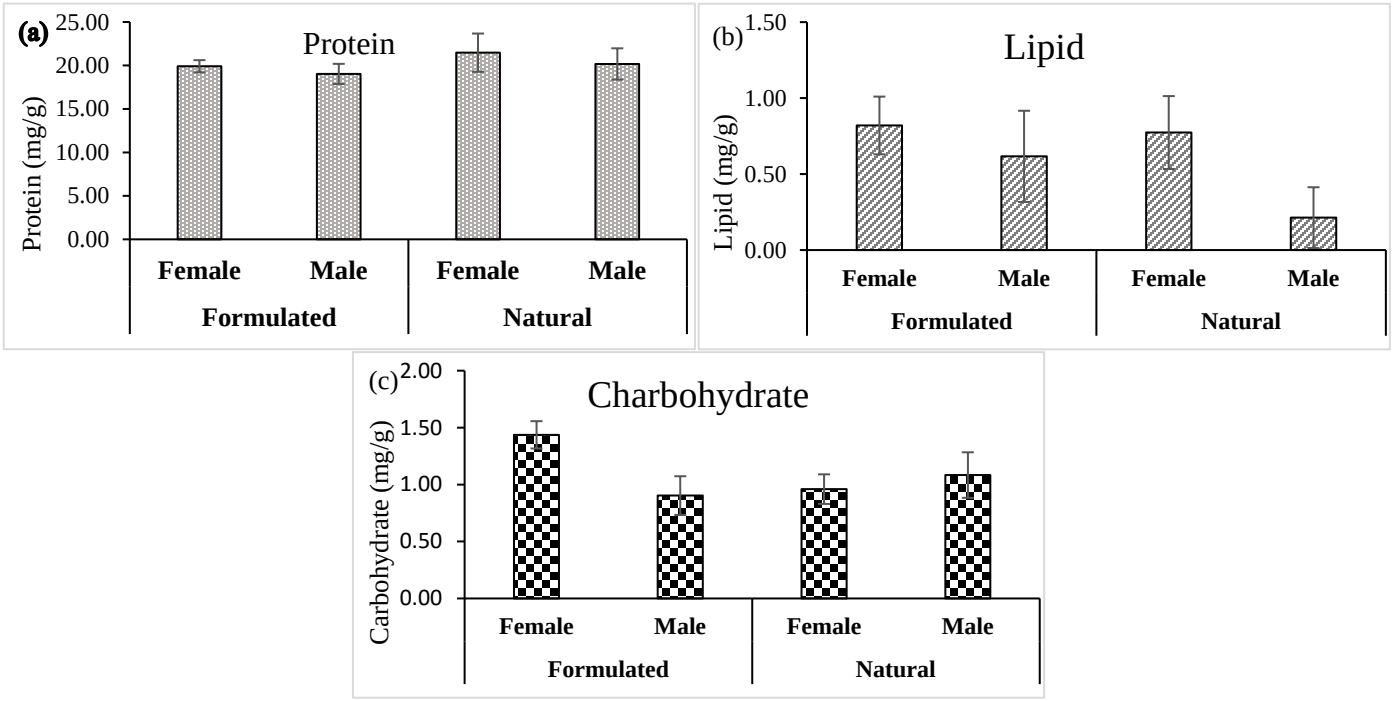

Figure. 5: Biochemical composition of crab body tissue fed with formulated and natural feed in male and female specimens.

Conclusion

Results describe that in this fattening experiment, mud crabs fed with formulated feed accomplished less growth than Crabs fed with natural feed. The nutritional levels of both fattened groups were found to be almost similar. Therefore, improvement n feed formulation is still required to enhance the growth of crabs in captivity, and it is recommended that more researches should be undertaken to improve the growth and nutritional level of the crabs to strengthen the aquaculture industry at the international level.

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Conflict of Interest

It is declared that there is no conflict of interest among authors.

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