Analysis of Growth and Molting Performance of Tank-Raised *Metapenaeus dobsoni* Supplemented with Chicken Eggshell

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A thesis submitted in the partial fulfillment of the requirements for the degree of Master of Science in Fish Biology and Biotechnology

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June 2023

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This is to certify that we have examined the above Master's thesis and have found that is complete and satisfactory in all respects, and that all revisions required by the thesis examination committee have been made

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Table of Contents

List of figures

ABSTRACT

Marine crustacean species are expanding their contribution to the export economy of Bangladesh as a result of rising demand. Shrimp aquaculture is growing almost everywhere in the world, and it has been practiced in Bangladesh for a very long time. Therefore, improved aquaculture and export output require sustainable shrimp cultivation methods. This study examines the development and molting abilities of shrimp of the species *Metapenaeus dobsoni* which were reared in tanks with the supplementations of chicken eggshells. The goal was to assess how adding eggshells to their diet may affect their growth rates and molting frequency. In the experiment, shrimp were fed feed with various amounts of eggshell in a controlled tank setting. Over a predetermined time period, growth factors including increase in weight and length were observed, and molting occasions were noted. The findings suggest that adding chicken eggshells to the food of *M. dobsoni* has a good effect on their molting and growth. This effect might be caused by the eggshell's calcium content, which could help the formation of the shell. The frequency of molting may also be impacted by this supplementation. This research advances our knowledge of aquaculture practices and sustainable shrimp production by revealing possible advantages of employing chicken eggshells as a food supplement to improve the development and molting performance of tank-raised *M. dobsoni*.

Keywords: Eyestalk ablation, Growth, Molting, Mortality, *Metapenaeus dobsoni*

INTRODUCTION

Bangladesh has an abundance of water resources, including ponds, lakes, canals, rivers, estuaries, and natural depressions (haors and beels). It ranks third in the world in terms of water resources just behind China and India. In the form of ponds, beels, lakes, canals, small and big rivers, and estuaries, these extensive and enormous water resources occupy an area of 18,290 sq. km and are dispersed throughout the country (FAO, 2014). In Bangladesh, the inland capture, inland culture, mariculture (artisanal fisheries), and marine industrial fisheries are the four main sub-sectors of the fisheries industry. The fisheries sector contributed roughly 23.12% to the agricultural gross domestic product (GDP), 3.69% to the overall GDP, and 2.01% to the nation's export revenue in 2013–14 (Islam et al., 2016). Given that it supplies over 60% of the animal protein needs and employs more than 11% of the total population either directly or indirectly in Bangladesh. The sector's economic impact on the country is substantially greater than its GDP share of 4.39% (DOF, 2013). According to estimates from 2012–2013, there was a total fish production of 3.41 million tons, of which 83% and 17% of the total catch came from inland and marine fisheries, respectively (DOF, 2014). Fish exports from Bangladesh rank third in terms of profitability and are increasing by 5–8% annually. Fish and fish products exports brought approximately USD 630.24 million for Bangladesh in FY 2013–14 (EPB, 2014).

Due to the abundance of shallow water bodies that offer a special chance for prawn and shrimp production, Bangladesh is also regarded as one of the world's ideal nations for prawn and shrimp farming (Ahmed et al., 2008; Islam, 2008). A "blue revolution" has been used to describe the rapid growth of prawn farming in southwest Bangladesh during the 1990s (Ahmed et al., 2010). In Bangladesh, shrimp farming takes place over a little more than 275,000 hectares of land. In the years 2018–2019, Bangladesh produced over 2 lakh tons of shrimp, and from exports in 2020, it realized a profit of 348 million US dollars (DOF, 2020). This industry has seen about a 3.5% yearly increase in labor employment (DOF, 2013). Despite having an appropriate level of output in the fisheries industry, aquaculturists are always working to create farming-friendly conditions by lowering feed costs, developing novel treatments for viral diseases, etc.

Shrimp, which makes up about 70% of all agricultural exports from Bangladesh, is one of its vital exportable items from Bangladesh. Because of this, shrimp is frequently called "White Gold" in Bangladesh. In Bangladesh, 56 species of shrimp have been identified, of which 37 are freshwater species and the remaining 12 are brackish water species. The marine fisheries industry of Bangladesh, which is seen to be a key component of the country's economy, produces a wide range of species that are essential for both domestic and international trade. Bangladesh recently regained access to 1.10 million square kilometers of marine water in the Bay of Bengal (DOF, 2019), which has benefited the nation's coastal and marine fisheries as well as its natural diversity and economic growth. According to the Ministry of Fisheries and Livestock (MoFL) (2002), shrimp farming is an important component of Bangladesh's economy. Shrimp production currently accounts for 2.5% of worldwide output, and the shrimp business is Bangladesh's second-largest export sector. In Bangladesh, the bagda (*Penaeus monodon*) and golda (*Macrobrachium rosenbergii*) farms employ 600,000 people and provide US$301 million in revenue yearly (US$243 million from bagda alone). The marine ecology of the nation generated 64688 MT of shrimp in the 2019–20 year (DoF, 2020). The country's favorable climate, availability of resources including feed, seed, water, and a cheap labor force have all contributed to the growth of shrimp aquaculture there. Although there are dangers associated with raising shrimp, including salt intrusion, natural catastrophes, contamination of the culture area or Gher, viruses and infections. Despite a steep decline in shrimp fry output, it still had a positive impact on the socioeconomic situation of the underprivileged fishing community (Islam, 2003). Therefore, sustainable shrimp farming techniques are crucial for the coastal population to improve and strengthen overall quality of life.

Shrimp farming is expanding in practically every part of the world and it has a long history in Bangladesh as well. The primary reasons for the 34% increase in global shrimp production between 2002 and 2008 (FAO, 2008) are the intensification of farming patterns and technological developments (Lebel et al., 2002), as well as the global decline in fish catch from marine sources (Bailey, 1988; Naylor et al., 2000; Erondu and Anyanwu, 2005). In the tropical and subtropical nations, it has grown to constitute a significant portion of the economy. However, there are certain negative impacts of

shrimp farming that come from poor planning and management strategies. The most significant of these is the high cost of feed and dangerous feeding practices.

Crustaceans molting, a biological process that is essential for growth, gonad development, and reproduction, demands a significant amount of energy (Panganiban et al., 1995; Jung et al., 2013; Huang et al., 2015). In crabs, molting involves the shedding of the old exoskeleton and the synthesis of the new exoskeleton, which is necessary for the constantly expanding body size and controls the somatic growth of the animal. Shrimp molt as they grow, and this is perfectly a natural process. It indicates adequate growth and excellent health if they are molting more regularly. It appears that they are not molting if they are experiencing issues in developing and are not doing so appropriately. Under ideal circumstances, adult shrimp can molt once approximately every three to four weeks, although young shrimp molt more frequently. Shrimps have the ability to molt as part of the natural molt cycle (It takes this cycle over 120 days) which varies depending on the species and season.

Induced molting is currently used to shorten the length of the molt cycle in many crustacean species. Eyestalk ablation, which means eyestalks are ablated unilaterally or bilaterally, is one of the traditional techniques used to induce molting. The eyestalk ablation stops all molting-inhibiting hormones, enabling the animal to molt. Unilateral eyestalk ablation can also be utilized in shrimp to decrease the molt interval and boost gonad development (Lin et al., 2002). In various species, unilateral eyestalk ablation has been used to induce ovarian development and spawning with variable degrees of effectiveness (Simon John and Sivadas, 1979; Brown et al., 1980; Zaib Un Nisa Ahmed, 2001). The removal of the eyestalk causes an increase in bodyweight and a decrease in hemolymph osmolality (Heit and Fingerman, 1975). Though molting appears to be a brief and infrequent intermission, it has a far-reaching impact on the whole life cycle, and the time between molts is one of continual morphological and physiological change of a species.

In order to meet the need for energy, growth, and survival, farmers are employing high-quality formulated feed that contains several multivitamins and minerals, including Dicalcium phosphate (DCP), a crucial source of calcium. The use of these commercial

vitamins and minerals increases the cost of shrimp feed, which ultimately increases the cost of farming. Sometimes commercial feed may be affected by microplastic exposure to the animal body which is more harmful for humans also. So we have to think about the substitution of commercial feed. In that case, as an alternative, eggshell powder may save cost besides playing role in the development and survival rates for shrimp. Eggshell powder can also mitigate the problem by supplying proper nutrition to ensure a better molting rate of shrimp including minimal feed cost. In addition, along with ensuring better growth and survival rate eggshells can also reduce environmental pollution which will give a better environment to us.

Eggshell supplementation as a source of minerals is the preferable choice for fish farmers because commercially prepared meals are quite expensive in the view of the farmers. As an alternative to crustaceans, calcium extracted from eggshells is a useful source of dietary calcium (Suguro et al., 2000). Additionally, Ca intake from egg shells was more readily absorbed than $CaCO_3$ from a mercantile source (Swiatkiewicz et al., 2015). In addition to calcium, egg shells also contain trace quantities of the following microelements: Mg, B, Mn, Mo, S, Co, Fe, Si, and Zn (Nakano et al., 2003). Therefore, Egg shells are a rich source of minerals and are used in a variety of products, including pharmaceutical excipients, base materials for the creation of dental and medical preparations, food additives and calcium supplements, diluents for solid dosage forms, agricultural fertilizer, and bone implants (Murakami et al., 2007). Eggshells can easily gather waste products from fast food restaurants, residences, and hatcheries (Amu et al., 2005; Phil and Zhihong, 2009). The total quantity of eggshell trash produced worldwide in 2018 was roughly 2.3 million tonnes (Hincke et al., 2012; Laca et al., 2017; Ahmed et al., 2019). In 2018, China generated 458,448 million eggs (FAO, 2020), with 137,534 million eggs being broken in that time. 825,204 tonnes of eggshell waste rich in minerals are produced by plants. The disposal of eggshell trash leads to environmental contamination which causes problems in cost, site accessibility, odor, fly infestation, and abrasiveness (Phil and Zhihong, 2009). So, the utilization of eggshells could be a suitable option to avoid the above-mentioned circumstances.

Metapenaeus dobsoni (Kadal shrimp) are widely distributed throughout the Indo-West Pacific, from the west coast of India to the Philippines and New Guinea. This species

prefers to reside in mangroves, muck, and amid shells and can survive in a wide variety of salinities, from 3−43 ppt. On the south and southwest shores of India, *M. dobsoni* establishes a substantial inshore and trawl fishery (FAO, 1980). It is also known to be a species with great commercial value in the Persian Gulf near Kuwait, the most common species caught as shrimp along Sri Lanka's east coast, and the main species in fisheries Indonesia's south coast of Java (FAO, 1988). To date, there is no information on the utilization of chicken eggshells on the growth performance and molting of *M. dobsoni* from the Bay of Bengal, Bangladesh. Therefore, this study is designed to investigate the effect of chicken eggshell supplementation on the growth performance, molting and survival of *M. dobsoni* in aquarium conditions.

Figure 01: *Metapenaeus dobsoni*

1.1 Objectives:

The objectives of the proposed research are as follows:

- To determine the growth, molting performance and survival rate of *M. dobsoni* fed with chicken egg shell as feed supplement
- To validate the availability of low-cost feed in sustainable shrimp aquaculture

REVIEW OF LITERATURE

2.1 Eggshell:

The main component of chicken eggshells is calcium carbonate, which is a natural byproduct of egg production. Eggshells have drawn interest due to their potential uses in a variety of industries, such as agriculture, medicine, and environmental engineering. Animal models have been used to study the calcium bioavailability from eggshells, and the results show that eggshell calcium may be effectively absorbed and used (Cao et al., 2015). In addition, it has been demonstrated that calcium supplementation from eggshells promotes bone health and guards against osteoporosis in people (Pacheco et al., 2022).

Applications for eggshells in biomedicine have shown potential. The production of biomaterials utilizing calcium carbonate from eggshells has been the subject of research. The potential for bone tissue engineering and drug delivery systems has been investigated using hydroxyapatite, a substance generated from eggshells that is comparable to the mineral component of bones (Mehrali et al., 2017). Joe et al. (2021) examined the eggshell crystal structure, they discovered a distinctive aragonite structure with distinct crystal orientations. When added to the soil, eggshell powder increased soil fertility and served as a calcium supply that is released slowly (Hess et al., 2018).

According to Arduini et al. (2018), eggshells are a highly concentrated source of calcium since they have a weight-based calcium content of between 37 and 40 percent. Research has also shown that of adding calcium from eggshells to animal feed can increase production, eggshell quality, and bone strength (Huang et al., 2019). In addition, it has been discovered that supplementing eggshell calcium increases egg production and hatchability in laying hens (Mikulski et al., 2012). Eggshell comprises about 9–12% of the total weight of an egg and is mostly made of calcium carbonate (94%), with a small amount of magnesium carbonate. Thus, the organic matrix must not have *Salmonella* where calcium phosphate is deposited on it (Bowero J., 1992). In addition to being a great substitute for vital crustacean shells, eggshell calcium is a rich source of dietary calcium (Suguro et al., 2000). The eggshell is made up of dry stuff (98%), and just 2% of it is water, chemically speaking. 5% crude protein and 93% ash make up the dry matter (Waheed et al., 2019). Proteins make up the majority of the organic material in eggshells

and shell membranes, with modest quantities of lipids and carbohydrates (Burley and Vadehra, 1989). Calcium is the main microelement found in eggshells, along with trace levels of magnesium, copper, iron, manganese, boron, sulfur, silicon, molybdenum, and zinc (Bee, 2011). The best natural calcium supply is probably found in eggshells, which have a 90% absorption rate (Bee, 2011).

Biochemical and immunological experiments have demonstrated that hydroxyproline, which is found in eggshell membrane hydrolysates, is a component of collagen (Wong et al., 1984). According to research by Long et al. (2004), eggshell membrane collagen has a very low incidence of autoimmune and allergic responses and a high level of biosafety. Average mineral content levels in various areas of the egg and egg shell (Yasothai et al., 2014). Numerous researchers have been attempting to find a way to use the leftover eggshells as a calcium source for human nutrition (Schaafsma et al., 2000). Despite being non-edible byproducts with minimal commercial value, eggshells and shell membranes may nonetheless contain physiologically active substances (Nakano et al., 2003).

A two-year project titled "Separating Eggshell and its Membrane to turn Eggshell Waste into Valuable Source Materials" was funded in 2012 with funding from the European Commission and the Seventh Framework Program (FP7) (Shellbrane, 2012). The development of eggshell powder as a functional food ingredient and funded projects for the repurposing of eggshell waste protein as potential medical and health-care spinoffs (Cordeiro and Hincke, 2011; Poultry Innovations Conference, 2013). This project was intended to help reduce the environmental impact of eggshell waste. Approximately one-third of the food that is deemed fit for human consumption and is edible is wasted each year, amounting to over 1.3 billion tones (FAO, 2014).

2.2 Molting:

Crustaceans naturally undergo a process known as molting, in which a new exoskeleton is created and periodically shed from the body. It permits further weight gain or growth. It is a continuously recurring crisis in the life of the decapod crustaceans because it is both risky and expensive. The most crucial and difficult stage of a crustacean's life cycle is the molt cycle. During molting, the old exoskeleton is completely replaced by a new exoskeleton, including appendages. The process varies depending on the species and the

season and is mediated by ecdysteroids released by the Y-organ (YO) (Srinivasa et al., 2016). This process is necessary for the constant expansion of body size. Every stage of the shrimp life cycle involves molting, which is the process of replacing the old shell with a new one. This is so because shrimp are crustaceans and have an exoskeleton called a cuticle covering their whole body surface. Molting causes different changes in the crustaceans' overall behavior in addition to development. Molting warrants documentation since it drastically alters the routine behavior of crustaceans.

Mineral deficiencies are another prevalent cause of mortality in shrimp after molting. If water quality, dissolved oxygen (DO), mineral content, and pH are kept stable, shrimp can molt successfully. The fact that calcium levels change over the molt cycle shows how crucial this mineral is for the development of the exoskeleton. Depending on their state of molting, crustaceans require varying amounts of calcium. Similar to other crustaceans, freshwater prawns obtain a major portion of their calcium from the exoskeleton.

2.3 Eggshell supplementation:

Eggshells typically include $CaCO_3$ (94%) with some $MgCO_3$ and $Ca_3(PO_4)_2$ deposited on the organic matrix (Bowero, 1992). Egg shells make up about 9–12% of the total egg weight. As an alternative to crustaceans, calcium extracted from eggshells is a useful source of dietary calcium (Suguro et al., 2000). Additionally, Ca intake from egg shells was more readily absorbed than $CaCO_3$ from a mercantile source (Swiatkiewicz et al., 2015). Eggshells are a rich source of calcium, thus researchers have been exploring different ways to include leftover eggshells into the diets of people to meet their calcium needs (Ray et al., 2017). According to Wellman-Labadie, Picman and Hincke (2007), pharmaceutical firms also include eggshells in a variety of supplements to strengthen bone structures and eliminate radioactive materials. In addition, it was shown that calcium derived from eggshell powder had a higher bioavailability than commercially available calcium carbonate in rat experiments (Swiatkiewicz et al., 2015).

Due to economic effectiveness and improved dietary acceptability, egg consumption per capita has grown recently, especially in emerging nations. The demand for eggs has increased, which has increased egg production. Egg production has increased by more than 150% over the last three decades (FAO 2020), and the accompanying eggshell

waste, which often ends up in landfills, poses major risks to both the environment and human health (Ajala et al., 2018). Human bone mass growth is positively impacted by the use of eggshell powder (Schaafsma and Pakan, 1999). It significantly raises the bone density of the femoral neck (Schaafsma et al., 2002). In a study, Sakai et al. (2017) discovered that eggshell calcium increased women's bone mass more effectively than calcium carbonate, suggesting the possibility of using it as a calcium supplement in the human diet.

2.4 Feedstuff supplementation in shrimp:

Diverse elements of growth analysis in tank-raised *Penaeus monodon* have been studied in research investigations. Rahman et al. (2013) looked at how different feeding schedules affected *P. monodon*'s growth performance, they discovered that a balanced diet greatly accelerated growth rates. Additionally, research has examined the effects of environmental conditions, stocking density, and water quality parameters on the growth of *P. monodon* (Bhuyan et al., 2017). Growth performance and variables influencing growth in *Litopenaeus vannamei* grown in tanks have been examined in growth analysis studies. Islam et al. (2016) studied how different feed formulations affected *L. vannamei* growth and survival rates and found that feed composition had a substantial impact on growth rates.

According to studies, creating nutritionally balanced diets that are rich in vital nutrients can encourage ideal development rates and improve the effectiveness of molting (Guo et al., 2019). In order to enhance the development and molting performance of *M. dobsoni*, the use of feed additives, such as probiotics and growth-promoting vitamins, has also been investigated by Liu et al. (2018).

When Huang et al. (2019) examined how dietary probiotics affected *Litopaeneus vannamei* growth rates, they discovered that probiotic supplementation dramatically enhanced growth performance. In addition, research has looked at the use of lipid supplementation and other protein sources to enhance the development and feed consumption in *L. vannamei* (Liu et al., 2021).

Several studies have investigated the molting patterns and frequencies of tank-raised *M. dobsoni*. The average molting frequency was reported to be around every 30 days by Chen

et al. (1995) and differences in molting patterns across individuals, which accounting variables like nutrition, temperature, and tank conditions may affect the frequency of molting. In a growth analysis of *Litopaeneus vannamei* performed in various rearing conditions, Liu et al. (2018) found that water temperature, salinity, and feed composition had a substantial impact on growth rates.

According to Zhang et al.'s (2019) investigation into the impact of various feeding regimens on growth performance, a well-balanced diet considerably increased growth rates in *Penaeus monodon*. When Wang et al. (2016) studied how temperature affected *Penaeus japonicas* growth performance, they discovered that a temperature range of 25–30°C produced the best growth rates. In order to improve growth and immunological responses in *Penaeus japonicas,* the use of functional feed additives, such as plant extracts and immune stimulants, has also been investigated (Wang et al., 2020). Aquaculturists can establish efficient techniques for boosting development and enhancing overall production efficiency in various shrimp species using the data from growth analysis studies as a reference.

M. dobsoni also known as the greasyback shrimp, is a species of significant economic importance in the aquaculture sector. Liu et al. (2017) obtained *M. dobsoni* developed quickly in tanks, gaining an average of 1.5–2.0 grams in weight per month. Similar to this, throughout a six-month raising period, Zhang et al. (2019) saw considerable increases in the length and weight of tank-raised *M. dobsoni*. These experiments demonstrate the possibility of obtaining significant growth in regulated tank settings. To improve shrimp farming techniques and increase production effectiveness, it is essential to comprehend the growth and molting behavior of *M. dobsoni* which has been grown in tanks. However, to date, very little is known about eggshell supplementation in any commercial shrimp farming. Therefore, the aim of this study is to know the dietary supplementation of chicken eggshells on the molting and growth processes of *M. dobsoni* under laboratory conditions.

MATERIALS AND METHODS

3.1 Collection of samples:

Juvenile *Metapenaeus dobsoni* (4.64 ± 0.51 cm and 0.62 ± 0.06gm) were collected from the local fishermen from the Dorianogor beach of Cox's Bazar. The shrimp were transported using large plastic buckets to the rearing tanks of the Institute of Coastal Biodiversity, Marine Fisheries and Wildlife Research Centre of Chattogram Veterinary and Animal Sciences University (CVASU), Bangladesh. About 200 individuals of *M. dobsoni* were collected for this research purpose. Full strength of seawater (33ppt) was collected from the near shore area of Dorianogor beach of the Bay of Bengal and transported using a large carboy of 5 tons' capacity. The salinity for the *M. dobsoni* was adjusted to the desired level (17 ppt) by adding aerated and filtered fresh water. The shrimp was fed once a day with commercial pellet feed *ad libitum* until further experiment. The unused food was removed daily after 6 to 8 hours of providing feed.

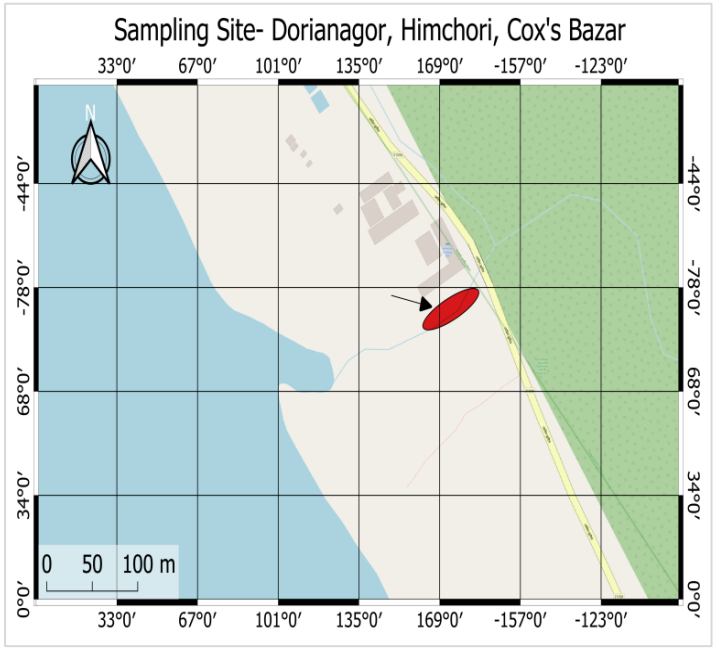

Figure 02: Sampling site in the Dorianagor, Cox's Bazar

3.2 Experimental design:

To examine the effects of chicken eggshell supplementation on *M. dobsoni* three different formulated feeds were prepared on the basis of source of calcium, including (1) 100%

DCP (Di-Calcium Phosphate), (2) 50% DCP and 50% eggshell, and (3) 100% egg shell. Feed formulation was carried out by following standard methodology with a fixed protein percentage (35%) for the treatment groups. The indoor control trials were carried out at the Institute of Coastal Biodiversity, Marine Fisheries and Wildlife Research Centre of Chattogram Veterinary and Animal Sciences University (CVASU), Bangladesh.

Figure 03: Preparation of experimental tanks or aquarium with adequate requirements

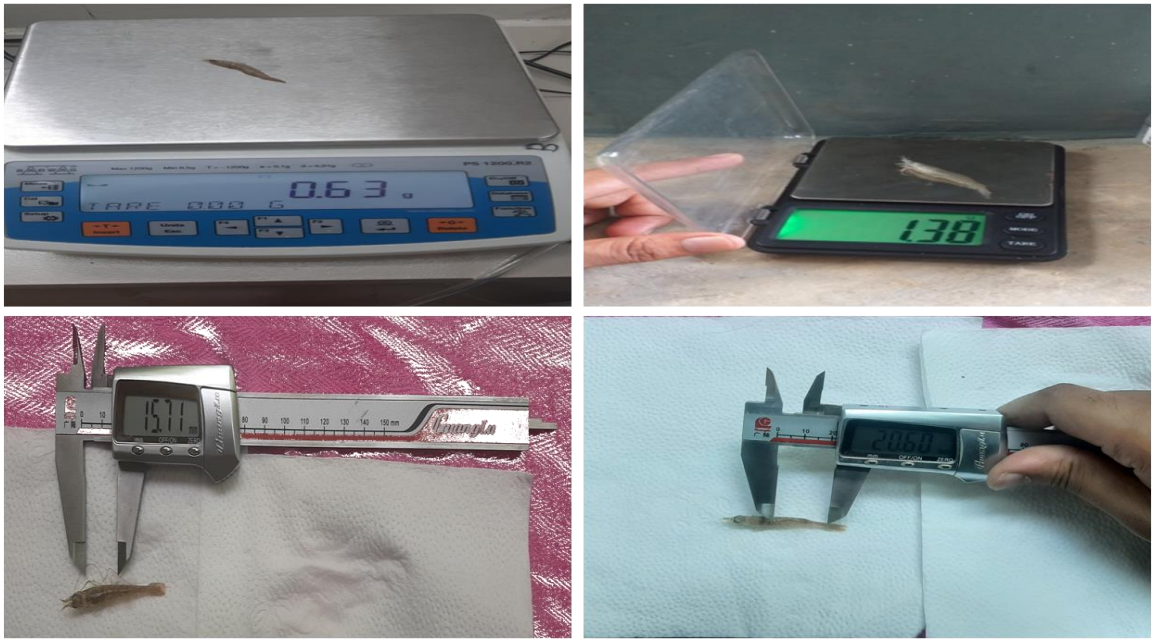

Figure 04: Measuring weight and length of *M. dobsoni*

During this experiment, we set up nine tanks about 18 × 12 × 10 cm^3 in size containing 10 liters of saline water. Water was filtered before using it in the experiment and an adequate amount of saline water was kept in storage to use at the time of necessity. Water

quality parameters were maintained in a planned way to keep it at an optimum level which ensured that the requirement for happening experiment was successful. A good amount of aerators was used to keep the water oxygenated proportionate to its requirements.

3.3 Feed formulation:

Three isonitrogenous affordable diets (35% protein) with varying doses were developed by combining fish meal, shrimp meal, white flour, mustard oil cake, rice bran, and soybean meal as sources of proteins and fish oil as a fat source (Table 2). All of the ingredients were crushed into powder and sieved using a 60-mm mesh grinder before being properly combined and blended with oil. Water was added at an amount of 200 mL/kg diet, and the paste dough was then put through a pelletiser (Geepas Meat Grinder, GMG-765, Dubai, UAE) and air-dried to roughly 8% moisture. All diets were wrapped in plastic bags after drying and stored at 20°C until use.

Figure 05: Formulated feed

Table 2: Feed formulation for the eggshell supplementation in the diet of *Metapeneaus dobsoni*

Ingredients	CP%	Inclusion (%)	Protein (%)
Soybean Meal	44.0	25.00	
Fish Meal	60.0	20.00	
Shrimp Meal	40.0	10.00	34.38
Wheat Flour	15.0	15.00	
Mustered Oil Cake	30.0	15.00	
Rice Bran	12.5	5.00	

DCP	-	1.20
Salt	-	1.00
SQUARE Aqua Mix (Premix)	-	2.00
Molasses	-	0.75
Enzyme	-	0.05
Fish Oil	-	2.00
Binder (Carboxymethyl Cellulose)	-	3.00
Total		100.00

3.4 Size and weight measurements:

Different lengths of the shrimp, like standard length (SL), rostrum length (RL), carapace length (CL), pre-carapace length (PCL), telson length (TL) was measured by digital vernier calipers (model-W150, China). The weight of the shrimp was taken using an electronic balance (WT-X series table balance, China) after keeping the *M. dobsoni* on filter paper.

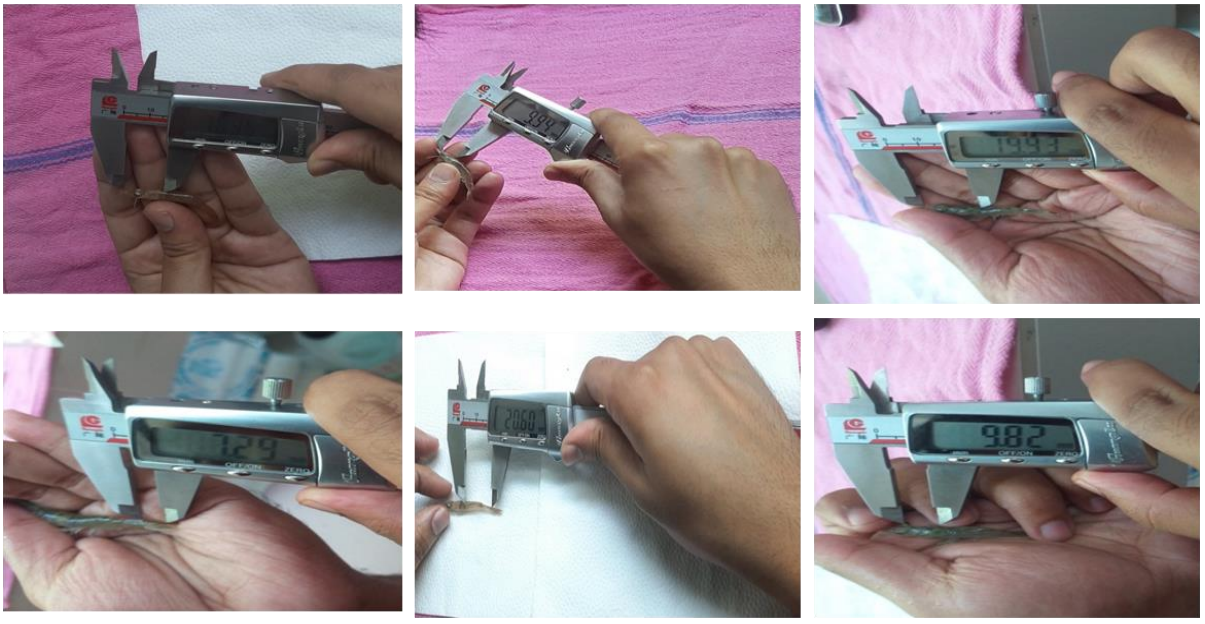

Figure 06: Different length of *M. dobsoni*(standard length, total length, rostrum length, carapace length, telson length)

3.5 Estimation of the growth performances:

Over the experiment, the net yield, specific growth rate (SGR%/day), feeding efficiency (FE) and survival rate (%) were calculated on the basis of the following formulas:

- (SGR%/day) = $100\times(\text{In } W_n - \text{In } W_{n-1})/t$
- FE (%) = $100\times (W_n - W_{n-1})/C$
- Survival (%) = 100× (final no. of shrimp/ initial no. of shrimp)
- FCR = Amount of feed fed/ Total weight gain
- FCE = Total weight gain/ Amount of feed fed
- No of Molting = Total number of molting / no of treatments

3.6 Observation of molting:

Molting number, molting frequency, and survival of shrimp in the aquarium were observed 4−5 times a day for a period of thirty days. Most of the molting was observed during the early morning. Molting was confirmed by observing the old exoskeleton and soft body of the molted shrimp. Old exoskeletons sometimes remained floated in the aquarium; the number of floated exoskeletons confirmed the number of molting shrimps in the aquarium. The deaths of the shrimp were confirmed by observing unmoved appendages. The dead shrimp were also counted and the final weight (gm) was recorded

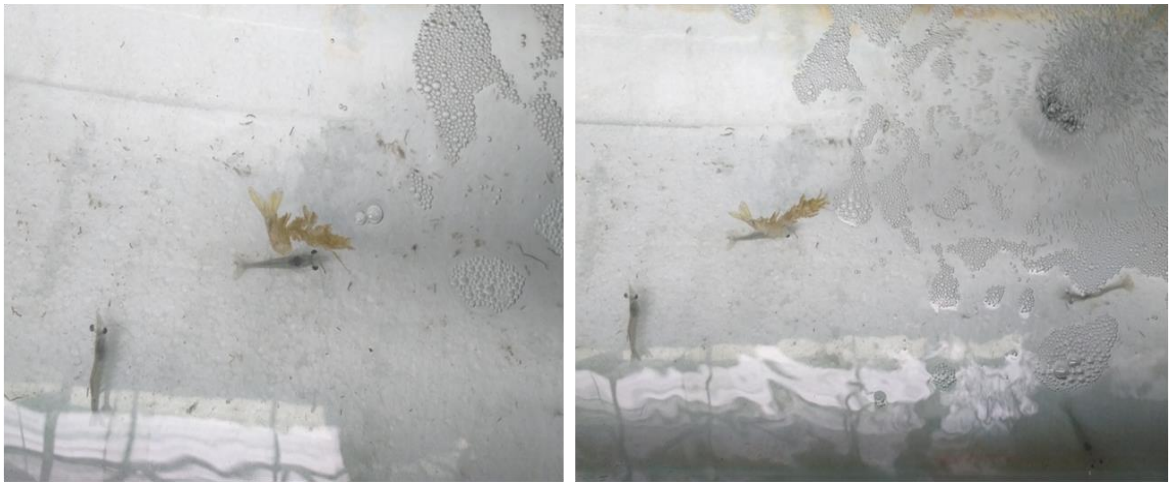

Figure 07: Observed Molting of *M. dobsoni*

3.7 Water quality parameters:

Indicators of water quality include physical, chemical, and biological characteristics that were regularly observed in accordance with the intended water parameters of concern. Several parameters were routinely collected or monitored to determine the quality of the water, including temperature, conductivity, salinity, ORP, dissolved oxygen, pH, and turbidity. In addition, seawater was regularly exchanged to keep the aquarium clean and to provide a suitable environment for the shrimp

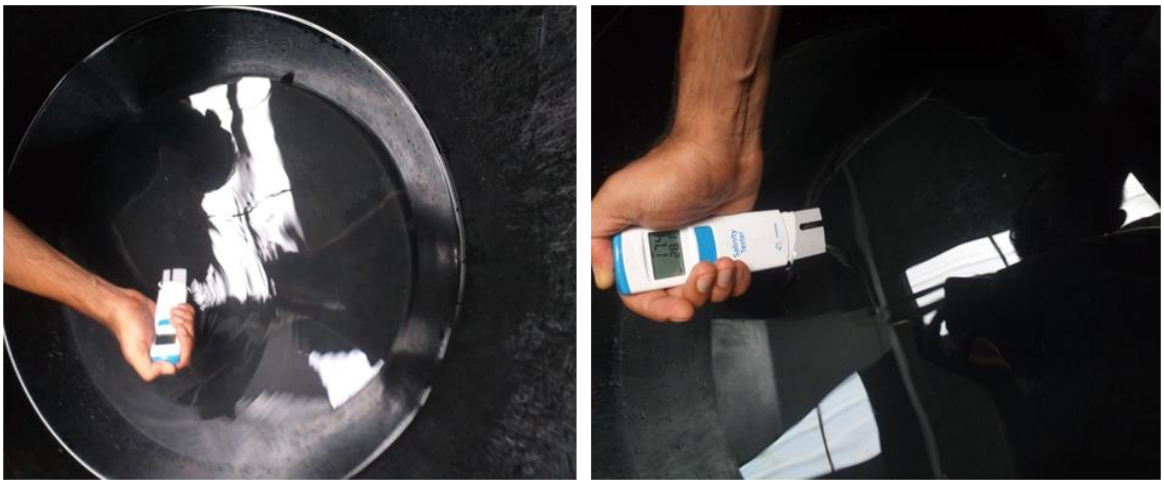

Figure 08: Water quality parameter measurement

3.8 Statistical analysis:

Data sets for the measured variables was presented as mean ± standard deviation (SD). Data was subjected to one-way analysis of variance (ANOVA) followed by the comparison of means by Tukey's HSD post hoc test using SPSS version 26.0 (SPSS Inc., Chicago, IL) to assess the statistically significant difference among the different feeding treatment. Significant differences were indicated by p value < 0.05. A principal component analysis was carried out for the different measured length to find out the major component that differentiated the different feeds.

RESULTS

4.1 Protein percentage in different eggshell:

In this study, the protein percentage of eggshell were measured following standard methods. Protein content were estimated from the boiled eggshell and omelette eggshell. Results showed that omelette eggshell contains 7.20 ± 0.18 % (mean ± SD) protein while the boiled eggshell contains 4.71 ± 0.18 % (Figure 9). A statistically significant differences was also observed between two groups of protein percentage ($p < 0.001$).

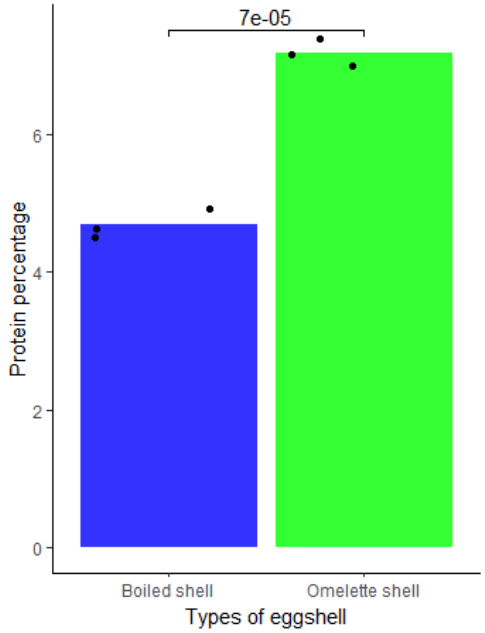

Figure 09: Protein percentage of boiled and omelette eggshell collected from local restaurant (n = 3 restaurant, each sample comprises of 50 eggshells). Values are presented as mean ± standard deviation of the mean.

4.2 Effect of egg shell supplementation of the moulting percentage in *Metapenaeus dobsoni*:

Dietary supplementation of chicken omelette eggshell at different concentrations had significant effect on the moulting number in *M. dobsoni*. Results showed that *M. dobsoni* supplemented with chicken eggshell had significantly higher moulting number compared to the control groups (T1) (Figure 10). However, there was no significant differences in the moulting number between T2 (diet having chicken egg shell with DCP) and T3 (diet having chicken eggshell) (Figure 10).

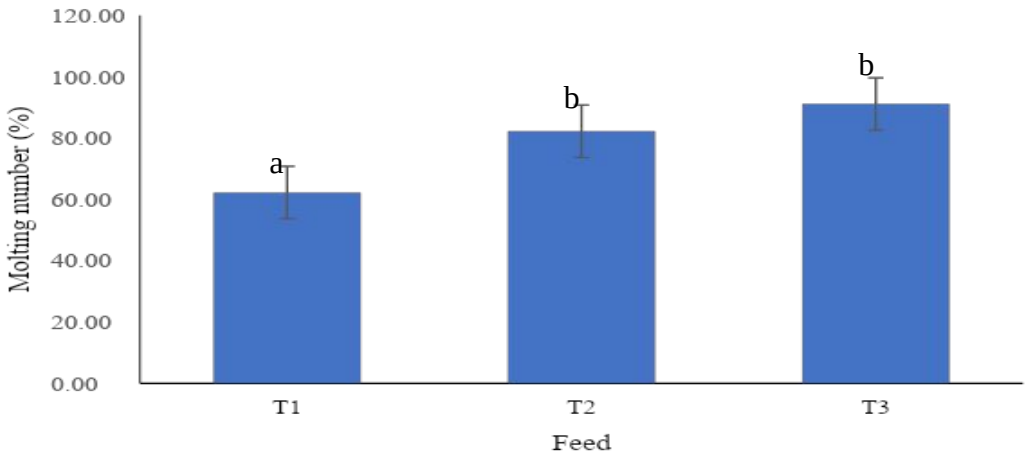

Figure 10: Moulting percentage in *M. dobsoni* after 30 days supplemented with different concentration of chicken eggshell (T1- diet having 100% DCP, T2- diet having 50% chicken eggshell and 50 % DCP and T3- diet having 100% chicken eggshell as a source of calcium). Values are presented as mean ± standard deviation of the mean (SD). Different subscript of alphabets is statistically significant at $p < 0.05$ (n = 15 in each treatment group).

4.3 Effect of egg shell supplementation of the moulting events in *Metapenaeus dobsoni*:

Dietary supplementation of chicken eggshell also has significant impact on the moulting frequency in *M. dobsoni*. *M. dobsoni* fed with chicken eggshell enriched diet had significantly higher moulting frequency compared to the control during 30 days feeding period (Figure 11). However, there was no significant differences between diet 2 (diet having chicken egg shell with DCP) and diet 3 (diet having 100% chicken eggshell) (Figure 11).

4.4 Effect of egg shell supplementations on the growth performance in *Metapenaeus dobsoni*:

Chicken eggshell supplementation increased the growth performance of *M. dobsoni* under aquarium conditions for a period of 30 days. Results showed that *M. dobsoni* supplemented with chicken eggshell with DCP (T2) and chicken eggshell (T3) had higher weight gain, % weight gain, and SGR in compared to control (feed supplemented without chicken eggshell) (Table 3). The lowest FCR was recorded in diet 2 (feed supplemented

with 50% eggshell and 50% DCP) in compared to the diet 3 (chicken eggshell) and diet 1 (no chicken eggshell). The survival rate was 100% in all feeding conditions.

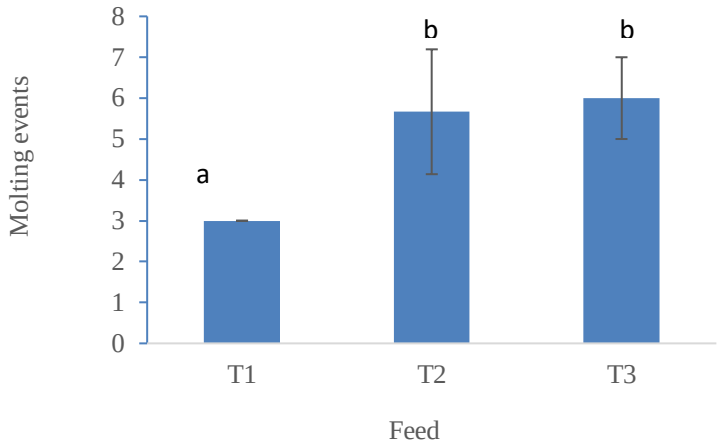

Figure 11: Moulting events in *M. dobsoni* after 30 days supplemented with different concentration of chicken eggshell (T1- diet having no eggshell, T2- diet having 50% chicken eggshell and 50 % DCP and T3- diet having chicken eggshell). Values are presented as mean ± standard deviation of the mean (SD). Different subscript of alphabets is statistically significant at $p < 0.05$ (n =15 in each treatment group).

Table 3: Growth performance of *M. dobsoni* feeding with different concentration of eggshell (T1- diet having 100% DCP, T2- diet having 50% chicken eggshell and 50 % DCP and T3- diet having 100% chicken eggshell) for a period of 30 days. Values are presented as mean ± standard deviation of the mean (SD) (n =15 in each treatment group).

Growth parameters	**T1**	**T2**	**T3**
Initial weight (g)	0.60 ± 0.03	0.63 ± 0.10	0.62 ± 0.03
Final weight (g)	0.74 ± 0.08	0.84 ± 0.08	0.84 ± 0.08
Weight gain (g)	0.15 ± 0.06	0.21 ± 0.04	0.22 ± 0.06
% weight gain	24.2 ± 10.2	33.8 ± 12.0	35.8 ± 7.9
SGR (%/day)	0.31 ± 0.12	0.42 ± 0.13	0.44 ± 0.08
FCR	1.88 ± 0.96	1.11 ± 0.23	1.30 ± 0.32
FCE	0.07 ± 0.03	0.10 ± 0.02	0.09 ± 0.02
Survival (%)	100	100	100

4.5 Principal component analysis (PCA) for three treatments in *M. dobsoni*:

Principal component analysis using different lengths (total length, standard length, rostrum length, pre-carapace length, telson length, highest body depth, lowest body depth) and body weight showed that the first (PC1) and second (PC2) principal components accounted for 45.7% and 12.7 % of total variance respectively (Figure 4). PCA dispersion showed a vast divergence in the rostrum length, highest body depth, lowest body depth and carapace diameter among different feeding trial. The other measured growth variables were almost similar in distribution among three different feeding trial.

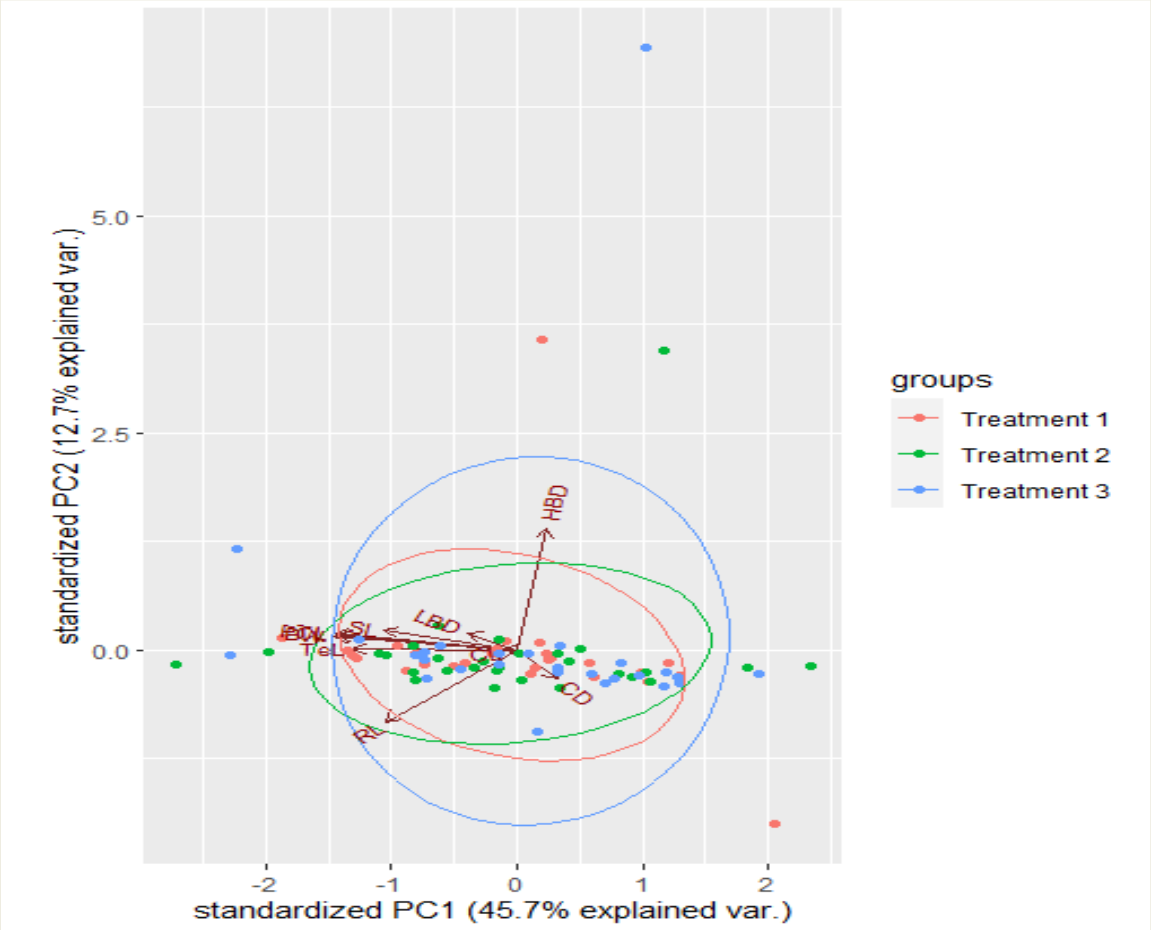

Figure 12: Principal component analysis (PCA) for the three different feeding groups for *M. dobsoni*. (TL-total length, SL-standard length, RL-rostrum length, PCL-pre-carapace length, TL-telson length, HBD-highest body depth, LBD-lowest body depth, BW- body weight, CD-carapace diameter).

DISCUSSION

This study focused on the effects of chicken eggshells to development and molting abilities *M. dobsoni* (commonly known as banana shrimp). This study provides new research directions for maximizing the growth and development of aquaculture organisms, thereby enhancing the sustainability and financial success of shrimp farming operations. The remarkable improvement in the development of *M. dobsoni* raised in tanks by adding chicken eggshells to their diet was one of the main successes of the present study.

5.1 Protein percentage in different eggshells:

In this experiment, boiled eggshells and omelette eggshells obtained from local restaurants were used to determine the protein level of the diet during formulation. Additionally, a statistically significant difference showed that the eggshell used to make omelettes had more protein than that of boiling eggshells (Figure 9). The boiling process may significantly result in a loss of protein in the case of boiled eggshells which may result in a significant loss of the protein present in the eggshell if we use them in preparing feed. However, in the case of omellette eggshells, there may be a chance of microbial assault because eggs have a high nutrient content and are an excellent medium for bacterial development. As a result, the eggshell should be properly sterilized in order to obtain the highest possible protein content.

5.2 Effect of eggshell supplementation of the molting percentage and molting events in *M. dobsoni*:

The research revealed that *M. dobsoni* fed with chicken eggshell had considerably more molts than the control groups (Treatment 1, Figure 10). However, there were no appreciable variations in the molting rate between Treatment 2, (feed including 50% chicken egg shells along with 50% DCP) and Treatment 3 (feed with entirely chicken eggshell, Figure 10). This finding indicates that adding chicken eggshells to the shrimp diet improved molting performance as compared to commercial feed or DCP supplementation. A large amount of calcium in chicken eggshells aided in enhanced development and molting performance.

The events of molting in *M. dobsoni* is significantly influenced by dietary additions of chicken eggshells. During the 30-day feeding period, *M. dobsoni* fed a diet supplemented with chicken eggshells exhibited a noticeably greater molting events than the control (Figure 11). Between treatment 2 (a diet containing a chicken eggshell with DCP) and treatment 3 (a diet containing 100% chicken eggshell as a source of DCP), there were no appreciable changes (Figure 11). Therefore, it may be claimed that adding eggshell powder to the shrimp diet can cause molting to occur more frequently and that this indicates that *M. dobsoni* is growing more quickly.

5.3 Effect of eggshell supplementation on the growth performance in *M. dobsoni*:

It was also observed that *M. dobsoni* supplemented with chicken eggshell (treatment 3) or chicken eggshell with DCP had higher weight gain, % weight gain, and SGR in comparison to control (feed supplemented without chicken eggshell) (Table 3). The lowest FCR was recorded in treatment 2 (feed supplemented with eggshell and DCP) compared to treatment 3 (entirely chicken eggshell) and treatment 1 (no chicken eggshell). According to Zhang et al. (2019), investigation into the impact of various feeding regimens on *Penaeus monodon*, the growth performance, and a well-balanced diet considerably increased growth rates. The rostrum length, peak body depth, lowest body depth, and carapace diameter among several feeding trials were all significantly different, according to PCA dispersion. The distribution of the additional growth indicators across the three separate feeding trials was quite consistent.

Additionally, studies have demonstrated that supplementing animal diets with calcium from eggshells can boost growth, eggshell quality, and bone density (Huang et al., 2019). In order for crustaceans to molt, shrimp feed must have a suitable amount of calcium, as we learned in a prior notion about how eggshells are made of this mineral like Ca. In addition to calcium, egg shells also contain trace quantities of the following microelements: Mg, B, Co, Fe, Mn, Mo, S, Si, and Zn (Nakano et al., 2003). In the current study, shrimp in treatment 3 whose feed is entirely comprised of eggshell as a calcium source performed better in terms of growth than shrimp in treatments 1 and 2. The survival rate was 100% in all feeding conditions. It was also observed that treatment 3, where shrimp were solely fed chicken eggshell rather than DCP, had frequent molting.

As a consequence, treatment 3 produced better results than the other treatments in terms of growth and molting performance. So, the results of this study may be useful for developing a sustainable shrimp industry in Bangladesh's coastal region.

It can be concluded that shrimp feed may be made from egg shells, reducing waste and the requirement for costly calcium supplements. This is a more cost-effective choice. Due to its cost-saving quality, eggshell supplementation is a preferred option for farmers, especially in regions where access to commercial feed additives may be limited. Shrimp diets that contain chicken eggshells have positive impacts that also apply to environmental sustainability. By reducing the amount of waste generated by the chicken industry and utilizing egg shells as a feed component, the initiative supported the concept of a circular economy in aquaculture. Additionally, the use and production of calcium supplements can have a less negative impact on the environment if artificial chemicals are used less frequently.

CONCLUSIONS

Promising and favorable results are obtained from the investigation of the growth and molting performance of tank-raised *M. dobsoni*. Through careful observation and data gathering, it has become clear that good tank management and circumstances have led to impressive growth and molting successes in these shrimp populations. The results indicate that elements like good nutrition (eggshell supplementation), a healthy diet, and appropriate habitat are crucial in promoting *M. dobsoni's* general health and development. These discoveries have important consequences for the aquaculture sector as well as the larger scientific community. Researchers and practitioners may improve and put into practice ways that promote responsible aquaculture, reduce environmental impact, and contribute to the supply of high-quality seafood by acquiring knowledge on the growth and molting performance of tank-raised *M. dobsoni*. Overall, this analysis highlights the need for thorough research in achieving successful outcomes in aquaculture ventures and highlights the possibility for additional improvements in shrimp farming methods.

RECOMMENDATIONS

The effects of chicken eggshell supplementation on *M. dobsoni's* growth and molting are both fascinating and challenging to examine. We must be conscious of the complexities as we research this field and strive for complete information that considers the full range of factors influencing crustacean physiology in a controlled environment. We become closer to understanding the potential benefits of such cutting-edge ideas in aquaculture and marine science through continuous research and an open mind. Some suggestions for future research can be taken in order to get better results.

Some recommended areas are listed as follows:

- If the experiment had been conducted on a bigger scale, the outcome could have been more favorable.
- Larger tanks might accommodate more samples.
- The research should be conducted throughout several seasons of the year.
- Longer periods of time may result in higher growth.
- Use shrimp from a variety of age ranges.

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