Dietary Effects of Seaweed (*Hypnea musciformis*) on Growth Performance and Blood Parameters in Mice

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Department of Applied Food Science and Nutrition

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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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DEDICATION

DEDICATED TO MY RESPECTED AND BELOVED PARENTS AND TEACHERS

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List of Abbreviations

Abbreviation	Elaboration
ADFI	Average Daily Feed Intake
ADG	Average Daily Growth
ANOVA	Analysis of variance
AOAC	Association of Official Analytical Chemists
BFRI	Bangladesh Fisheries Research Institute
DHA	Docosa Hexaenoic Acid
dl	Deciliter
DM	Dry Matter
EPA	Eicosa Pentaenoic Acid
FAO	Food and Agriculture Organization
FCR	Feed Conversion Ratio
g	Gram
HDL	High Density Lipoprotein
LDL	Low Density Lipoprotein
mg	Miligram
MoEF	Ministry of Environment and Forests
NACA	The Network of Aquaculture Centers in Asia-Pacific
ppt	Parts per thousand
PUFA	Poly Unsaturated Fatty Acids
RDA	Recommended Daily Allowance
RNI	Reference Nutrient Intake

Sd	Standard deviation
SPSS	Statistical Package for Social Science
TG	Triglyceride
USDA	United States Department of Agriculture

Abstract

This study was designed to observe the dietary effects of *Hypnea musciformis* on growth performance and blood parameters in mice. A total of 27 Swiss Albino male mice were divided into three dietary treatments group: T_0 = Control (basal diet), T_1 = 0.5% seaweed (basal diet + 0.5% seaweed on DM basis), T_2 = 1.0% seaweed (basal diet + 1.0% seaweed on DM basis). A completely randomized design was settled possessing three replications having three mice in each replicate group for a 28 days trial. The results showed that live weight at the final week differed significantly (p<0.001) compared with the control group. A significant variation was observed in average daily feed intake (ADFI) (p<0.001) among treatment groups for every week of the experiment. *Hypnea musciformis* contains high amount of crude protein (15.33±0.02%), crude fiber (8.34±0.01%) and ash (15.18±0.04%). It also contains noticeable amount of minerals, Ca (122.93±2.17 mg/100g), Na (94.37±2.75 mg/100g), K (31.33±1.04 mg/100g), Fe (15.05±0.73 mg/100g), and P (21.23±1.04 mg/100 g). No significant (p>0.05) impact was found in dietary seaweed supplementation on the blood glucose level for control vs other treatment groups. A noticeable reduction in serum cholesterol and triglyceride level (p<0.001) was found in the treatment groups compared to control group. There was no significant difference found in serum HDL and LDL levels of treatment group comparing with the control group (p>0.05). The total protein and calcium content of blood increased significantly (p>0.05) while phosphorus content remains non-significant (p>0.05) in treatment groups compared to control group. Hence, seaweed based diet showed beneficial dietary effects in controlling body weight and lowering cholesterol and triglyceride level in mice.

Keywords: *Hypnea musciformis*, swiss albino mice, seaweed, minerals, lipid profile.

Chapter-1: Introduction

Seaweeds are macroscopic marine algae that has morphological characteristic to hitch and colonize over the hard substratum and shallow water zone of the seashore which is suitable for massive growth of them. Seaweeds are the species of marine plants and algae that grow in the coastal area as well as in rivers, lakes, and other water bodies. Seaweeds are the eukaryotic organisms that live in salty water and recognized as a potential source of bioactive natural products. Seaweeds have been used as food, fodder, fertilizer and as source of medicine since ancient times. Today, seaweeds are being used as the raw materials for many industrial productions like agar, algin and carrageenan but they continue to be widely consumed as food in Asian countries. In the sea, 3 types of plants occur and they are phytoplanktons, seaweeds or marine algae and seagrasses. Phytoplanktons are microscopic and free-floating forms; they are the primary producers of the sea. They form important marine living renewable resources. They are primitive plants without any true root, stem and leaves. They are belonged to the division of Thallophyta in plant kingdom. seaweeds or Marine algae are classified into four groups namely Chlorophyceae (green algae), Phaeophyceae (brown algae), Rhodophyceae (red algae) and Cyanophyceae (blue-green algae) based on the type of pigments, morphological, anatomical and reproductive structures (Kolanjinathan *et al*., 2014).

Seaweeds are plant like ocean organisms that are botanically categorized as macrophytic marine algae. Edible seaweeds are often called "sea vegetables". Seaweeds are found in all around the world's oceans and are of beautiful shapes, colors and sizes. They're most abundant in shallow rocky coastal areas, especially where they're exposed at low tide. Coastal people around the world are harvesting and eating sea vegetables since the beginning of time. In the US and Europe, increasing numbers of people are learning that eating sea vegetables can provide a broad range of health benefits. Seaweeds contribute to primary production of the ocean and hence seaweed beds are considered highly productive and dynamic ecosystem.

Since, at least fourth century in Japan and sixth century in China, seaweeds have been an important dietary component. In Bangladesh, naturally growing seaweeds are in the littoral and sub-littoral zones of St. Martin's Island. The only government organization engaged in seaweed study is the Directorate of Fisheries which is conducting this work through the

Marine Fisheries Survey, Management and Development Project based at Chittagong and Cox's Bazar. Skrovankova (2011) indicated that seaweed vitamins are very important for their biochemical functions and antioxidant activity as well as it has other health benefits such as vitamin C helps in decreasing blood pressure; beta-carotene role in the prevention of cardiovascular diseases or reducing the risk of cancer (vitamins E and C, carotenoids). Seaweed could be a potential source of food for human beings (Oliveira *et al*., 2009). Seaweed is well known for its high carbohydrates, proteins, fiber, vitamins, and minerals contents and low fat content (Delaporte *et al*., 2003; El-Banna *et al*., 2005)

In Bangladesh, the natural abundance of seaweeds is reported from the south-eastern part of the mainland and offshore island, the Saint Martin Island have rocky substratum which is suitable for natural growth of seaweeds. Most common seaweed in Bangladesh coast *Hypnea sp*. (Sarker, 1992) is a red algae which is inhabited in shallow tropical and subtropical marine environments (Guist *et al*., 1982). This species is harvested in Vietnam, Senegal, Brazil, Burma, India, Philippines, USA and Bahamas (Boer, 1981). *Hypnea* cultivation has been initiated in many countries (Humm and Kreuzer, 1975; Mshigeni, 1976; *Guist et al.,* 1982) due to its great tolerance over a wide range of water temperatures, salinities and light intensities (Dawes *et al*., 1976). In India, *Hypnea musciformis* culture was adopted in the lagoon of Krusadai Island (Rao and Subbaramaiah, 1980). Tropical countries with coastlines are searching for seaweed cultivation as a sustainable alternative livelihood for coastal people. Mindanao, Philippines adopted seaweed farming as their major source of livelihood (Bardach *et al*., 1972).

In Bangladesh, seaweed exploitation is very rare except utilization by Mog or Rakhyine tribal community and seaweed collectors of St. Martin's Island (Majumder, 2010; Sarkar, 2015). The Bangladeshi coastal areas are one of the most unreached areas of the world within the field of phycology. There is lack of information and statistics regarding seaweeds distribution, total seaweeds and commercially important species available, abundance, seasonal availability, status and approaches for utilization in Bangladesh (Majumder, 2010; Khan, 1990).Without these information and statistics, commercial utilization of seaweed would be near to impossible. *Gracilaria* spp. from Penang, Malaysia leaded the country toward agar production and utilization through this species (Doty and Fisher, 1987). *Hypnea musciformis* is a highly expedient invader which is well known for

its large floating blooms. This algae is easily identified by the flattened, broad hooks at the end of many branches. *H. musciformis* is vulgur on calm intertidal and shallow subtidal reef flats, tidepools and on rocky intertidal benches which is mostly found low intertidal to shallow subtidal reef flats, attached to sandy flat rocks, or frequently epiphytic on Sargassum and other algae. In bloom stage, may be found free-floating.Whereas the mass people of Bangladesh do not know that the seaweeds can be used as human food. Information on chemical composition and its nutritive value is essential, to grow interest on seaweeds.

Seaweeds can have almost 10 times the amount of calcium as milk does. Seaweed based products will help to recover the nutritional problem. Various researchers worked on several seaweeds to see its effects on rat. A case-control study on seaweed consumption and the risk of breast cancer was done by (Yang *et al*., 2010). He found that consumption did not have any significant associations with breast cancer. These results suggest that high intake of seaweed may decrease the risk of breast cancer. Another researcher work on the effects of edible seaweeds on the metabolic activities of intestinal micro flora in rats (Gomez *et al.,* 2012). Similar studies were done by various researchers to see the effect of seaweed on rat. This study was conducted to evaluate the dietary effects of seaweed (*Hypnea musciformis*) on growth performance and blood parameters in mice.

Objectives of this study:

i. To observe the growth performance of mice after seaweed supplementation
ii. To assess the blood glucose and lipid profile in dietary seaweed supplementation
iii. To observe the dietary effects of seaweed on total protein, calcium and phosphorus level on animal model

Chapter-2: Literature review

2.1 History of seaweed

Seaweeds have been used for several food and non-food purposes around the world for thousands of years. Traditionally, the people of China, Korea and Japan uses seaweed as food for more than 2000 years. Seaweed is used to make "nori" from Porphyra species in Japan. Nori is a dried sheet of seaweed which is used in the preparation of sushi. Seaweeds are eaten fresh as salad in Malaysia and Indonesia. South East Asian countries have a long history of application of seaweeds in food. On the other hand, non-food application of seaweed is highly practiced in the western countries. Seaweeds were used as animal feed in Greece as early as 100 BC. Red seaweeds were used for medicinal purposes in Mediterranean countries. In Ireland and Scotland, farmers used seaweeds for agricultural applications, such as, mulch for soils. In Europe, seaweed is harvesting of natural stocks whereas in Asian countries seaweeds are cultivated for various applications (Tiwari and Troy, 2015).

Seaweeds are often overlooked or neglected but have significant academic, biological, environmental, and economic roles in the coastal ecosystem. The term seaweeds("sea" and "weed") often invokes an image of smelly and rotting masses found on beaches that does not present a positive image in various western countries. "Kaiso" is a common Japanese term for all varieties of edible seaweeds that derived from the term "kia" (ocean), which can represent water, plants, and trees – a more acceptable term representing photosynthetic organisms from oceans (Nisizawa, 2002).

Followed by Japan and Korea, China is the major producer of seaweeds. Most of the people around the world knowingly or unknowingly use seaweed or products derived from seaweeds in various forms, including processed dairy, meat, and fruit products as well as domestic commodities like paint, toothpaste, cosmetics, solid air fresheners, and pharmaceuticals (Dhargalkar and Pereira, 2005).

Nowadays, in Europe, the seaweed processing industry is comprised of several sectors including biopolymers, cosmetics, agrifood, and functional food additives with various health properties. In the European Union, seaweeds are generally used for the commercial production of additives for both food and nonfood applications (e.g., alginates). Like any processing industry, the production of additives from algae generates several waste and by-

products that are usually discarded. Dumping of these by-products is not justified from the social, economic, and environmental perspective, given the fact that these by-products contain valuable bioactives (e.g., health-promoting biochemicals), fine biochemicals (e.g., dyes and pigments), and biomolecules (e.g., proteins, oils, etc.). Bio actives are obtained from seaweed processing waste which possess several biological activities such as (i) antimicrobial activity (disinfection), (ii) antioxidant activity (potential replacement for chemical antioxidants used in the food industry), and (iii) inhibition of lipid peroxidation, antiproliferative activity, antidiabetic effect, and anti-inflammatory substances for various pharmaceutical and nutraceutical applications. Seaweeds have also been investigated for fuel applications. Comprehensive bio refinery solutions will allow sufficient scale to enable the economic production of fuel from seaweeds.

2.2 Intervention of seaweed to enhance human nutrition

The key to wellness of human being depends on the acquirement of a good mental and physical health through optimum nutrition. The nutrients in our regular diet or those synthesized in the human body using the precursor molecules play an important role in regulating the bodily functions, essential for normal growth and development. Major nutrients- carbohydrates, proteins, lipids and vitamins are supplied to the human body through different food sources. Seaweeds are also a good source of the above nutritional components like other terrestrial plants. In comparison with many common vegetables, high levels of fiber, minerals, ω-3 fatty acids and moderate concentrations of lipids and proteins available in most of the edible seaweed. This is why seaweeds are considered as an important source for human nutrition. However, the available amounts of nutrients that are mentioned above may vary basically depending on the variety, climate and the area of production (Murata and Nakazoe, 2001).

2.2.1 Carbohydrates

Seaweeds contain a large amount of carbohydrate as structural, storage and functional polysaccharides. The total carbohydrate content may ranges from 20% to 76% of dry weight depending on the species (Holdt and Kraan, 2011). The carbohydrate content in seaweed is considerably high but its greater portion is available as polysaccharide dietary fiber, which is not taken up by the human body. Therefore, seaweed is not a good source

of carbohydrate in terms of bioavailability. Vary little, but absorbable, forms of carbohydrate is present in seaweed which comprises glucose, mannose and galactose.

2.2.2 Proteins

Seaweed protein is rich in arginine, glycine, alanine and glutamic acid and contains all the essential amino acids. The levels of these proteins are comparable to those of the FAO/WHO requirements of dietary proteins (Anonymous, 2006). However, seaweed contains limited amount of lysine and cysteine while comparing with other protein-rich food sources. With respect to the protein level and amino acid composition, red seaweeds contain higher amino acid score and essential amino acid index comparing with those in green and brown seaweed (Holdts and Kraan, 2011). The amino acid score of the proteins in some red seaweeds namely *Porphyra* spp. and *Undaria* spp. was 91 and 100, respectively which was seemed to be the same as that in animal-derived foods (Murata and Nakazoe, 2001). A comparative study was carried out with the protein content of red seaweeds and brown seaweeds which revealed that protein content of red seaweed species *Porphyra palmate* and *Porphyra tenera* ranged from 21% to 47% and that in brown seaweeds *Laminaria japonica* and *Undaria pinnatifida* ranged from 7% to 16% (Marsham *et al.,* 2007). Therefore, most of the edible red seaweeds can be considered as a good source of protein to be included in the diet. However, brown seaweed contains larger amount of aspartic and glutamic acid than red seaweed, that exhibit interesting properties in flavor development. In addition, Spirulina, the blue-green alga, is well known for its very high protein content which is close to 70% of the dry matter. The *in vivo* digestibility of seaweed proteins is not well documented. However, the extractability and the *in vitro* digestibility of seaweed protein attain more than 80% irrespective of the species (Fleurence, 1999).

2.2.3 Lipids

Seaweeds contain a very little lipid content (1% to 5% of dry matter) (Khotimchenko, 2005). All seaweeds contain neutral lipids and glycolipids. Seaweed has higher essential fatty acid than in land plants. In cold climates seaweed synthesizes higher amounts of polyunsaturated fatty acids (PUFAs) and the total lipid content is elevated during the hot seasons (Narayan *et al.,* 2006). However, the lipid content and the composition of fat can be greatly varied depending on the species of seaweed. ω-3 fatty acids are the major

component of PUFAs in seaweed. Eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) are the two important fatty acids of marine origin belonging to ω-3 fatty acids that are greatly beneficial for human health. α-Linolenic acid is the precursor of both EPA and DHA which is not synthesized in mammalians. However, α-linolenic acid can be converted into EPA and DHA upon consumption by the human. EPA is the major PUFA in most seaweed (30% of the total fatty acid content). All seaweeds are a balanced source of ω-3 and ω-6 acids but the red seaweeds are rich in EPA and ω-6 fatty acids such as arachidonic acid. Therefore, seaweeds are a good source of health-promoting PUFA compared to the other animal and plant foods. The amount of phospholipids in seaweed is about 4-10% of the total lipid. In the diet, phospholipids act as an emulsifier and ease the digestion and absorption of fatty acids. Phospholipids enhance the nutritive value of the food. Moreover, seaweed contains many essential fatty acids, which may add to their efficacy as a part of a balanced diet.

2.2.4 Vitamins

Seaweed contains both water soluble (vitamin B and C) and fat soluble vitamins (vitamin A and E) at varying levels. *U. pinnatifida*, a brown seaweed, contained 14.5 mg/100g of vitamin E which was much higher than the vitamin E content (10mg/100g) in peanut (Anonymous, 2004). This high vitamin E content of seaweed helps to protect PUFA in seaweed so as to maintain their nutritional benefits. Red and brown seaweeds contain good amount of carotenes (provitamin A) and vitamin C (20 to 170 ppm and 500 to 3000 ppm, respectively). Seaweeds are also a good sources of vitamin $B_{12,}$ which is found in very little amount in some vegetables (Bender, 1980).

2.2.5 Minerals

Seaweed contains high ash content which indicates the presence of appreciable amounts of minerals in seaweed. Seaweed contains about 36% mineral content on dry weight basis. Mineral macronutrients include sodium, potassium, calcium, magnesium, chlorine, sulfur and phosphorus and the micronutrients include zinc, copper, iodine, iron, selenium, molybdenum, fluoride, manganese, boron, nickel and cobalt. Calcium holds 4-7% of dry matter of seaweed. At 7% calcium, a typical daily portion size of seaweed (8g dry weight) provides 560 mg of calcium. The RDA of calcium is 800-1000 mg. (Anonymous, 2004).

In seaweeds, calcium is available as calcium phosphate which is the more bioavailable than the form of calcium in milk (calcium carbonate).

Seaweed is a primary source of iodine. In some seaweed, iodine content exceeds its dietary minimum requirement (150 µg/day). Brown seaweed contains highest amount of iodine (1500-8000 ppm). Red and green seaweeds have lower iodine contents. Seaweeds can be considered as the best inexpensive source to fulfill the iodine requirement of human since animal and plant foods are very low in iodine. Seaweed contains considerably high amounts of iron and copper compared to food sources though meat and spinach are very renowned to contain those minerals (Holland *et al.,* 1993). In addition, a normal portion size of brown seaweed, *Laminaria* spp and *Undaria* spp, provides more than 50% of the recommended daily allowance of magnesium. Therefore, the requirements of the most important minerals of the body can be fulfilled with the supplementation of seaweed as food.

2.3 Nutritive value of seaweed

Seaweeds contain numerous minerals due to their marine habitat, and the diversity of the minerals they absorb is wide and this mineral content in seaweeds is much higher when compared to the edible terrestrial vegetables (Indegaard and Minsaas, 1991; USDA, 2001). Mineral content has been shown to vary according to species, geographical place of harvest, seasonal, environmental and physiological factors, and type of processing and method of mineralization (Honya *et al.*, 1993; Mabeau and Fleurence, 1993; Yoshie *et al*., 1994).

Calcium, accumulates in seaweeds at much higher levels than in terrestrial foodstuffs. This is illustrated as in an 8 g portion of *Ulva lactuca* (sea lettuce), which provides 260 mg of calcium, equaling approximately 37% of the RNI of calcium for an adult male (Committee on Medical Aspects of Food and Nutrition Policy, 1991). Calcium content ranged from 476 to 1,093 mg/100 g in fishes from inland waters of Bangladesh. As would be expected, calcium content was much higher in species in which bones are commonly consumed and included in the edible parts. In developed countries, dairy products tend to be the primary source of dietary calcium; however, this is not the case in Bangladesh where frequency of dairy consumption is very low (Belton *et al.*, 2014). The content of calcium in seaweed is

not only up to 10 times higher than that in cow's milk but is also much easier for the body to assimilate (Leyman, 2002).

2.4 Available seaweed species

About 193 seaweed species of 94 genera belonging to only three major divisions i.e. Chlorophyta-green algae, Phaeophyta-brown algae, Rhodophyta-red algae are available in Bangladesh (Sarkar *et al*., 2016).

2.5 Commercially important seaweed species

Among the available seaweed species, 19 species of 14 genera are considered as economically important (Sarkar *et al.,* 2016). (Table 1)

Table 1. Commercially important seaweed species of Bangladesh (Sarkar *et al*., 2016)

Sl No	Genus	Species	Type
1	Caulerpa	*Caulerpa racemosa*	Green Seaweed
		Caulerpa sertularioides	
2	Enteromorpha	*Enteromorpha intestinalis*	Green Seaweed
		Enteromorpha moniligera	
3	Gelidiella	*Gelidiella tenuissima*	Red Seaweed
4	Halymenia	*Halymenia discoidea*	Red Seaweed
5	Hypnea	*Hypnea pannosa*	Red Seaweed
		Hypnea valentiae	
		Hypnea musciformis	
6	Hydroclathrus	*Hydroclathrus clathratus*	Brown Seaweed
7	Sargassum	*Sargassum oligocystum*	Brown Seaweed
		Sargassum coriifolium	
8	Gelidiella	*Gelidiella tenuissima*	Red Seaweed
9	Gelidium	*Gelidium pusillum*	Red Seaweed
10	Padina	*Padina tetrastromatica*	Brown Seaweed
11	Catenella	*Catenella* spp.	Red Seaweed
12	Porphyra	*Porphyra* spp.	Red Seaweed
13	Gelidium	*Gelidium amansii*	Red Seaweed
14	Codium	*Codium fragile*	Green Seaweed

2.6 Natural production of seaweeds

Approximately, 5,000 metric ton seaweed biomass is annually available throughout the whole Bangladeshi coast from October to April (sarkar *et al*., 2016).

2.7 Seasonal variation in seaweeds' availability

Seasonal variation in seaweeds availability are basically due to variation in water quality. Geographical and vertical distribution and growth of seaweed are governed by various factors like water temperature, salinity, pH, dissolved oxygen, water transparency, nutrients etc. (Luning, 1990). Specific water quality parameters are required for growth and propagation of seaweeds (Round, 1970) and that's why seaweeds can only be found in those season or months of the year where water quality is favorable. Seaweeds in Bangladesh are available in winter, summer and spring seasons. As, the water quality parameters remain in peak in respect of favorable conditions of seaweeds, the highest abundance of seaweeds found from January to March. Similar finding was also reported by FAO/NACA, 1996. Salinity can be considered as an example of physical parameter of water. Heavy rainfall during the monsoon season lowers the salinity of coastal region than the other seasons of year. For growth and propagation 20-34 ppt salinity is required by seaweeds. This range or around this range is available only from October to April. Abundance of seaweed is also influenced by pollution, disturbance etc. The present day populations of marine algal flora are very different from what they were in 1960s and even 1980s, and this degradation may be ascribed to continuous disturbance of inter-tidal rocks, particularly for construction and household use, is an impediment to growth of marine algae. The dragging of seine nets across the inter-tidal zone adversely affects seaweed settling. Pollution is also an issue: waste entrance to coastal waters may affect marine algae growth (MoEF, 2001; Thompson and Islam, 2010).

2.8 Proximate composition

Table 2. Proximate composition of different seaweed species

Species	CP (%)	Lipid (%)	Fibre (%)	Ash (%)	Moisture (%)	Reference
Eucheuma cottonii	9.76	1.10	5.91	46.19	10.55	Matanjun *et al.* (2008)
Gracilaria cervicornis	22.96	0.43	5.65	7.72	14.33	Marinho-Soriano *et al.* (2006)
Hypnea japonica	19.00	1.42	53.2	22.10	9.95	Wong and Cheung (2000)
Hypnea charoides	18.40	1.48	50.3	22.80	10.90	Wong and Cheung (2000)
Gracilaria changgi	6.90	3.30	24.7	22.70	_	Norziah and Ching (2000)
Gelidium pristoides	11.80	0.90	_	14.00	_	Foster and Hodgson (1998)
Gracilaria cornea	5.47	_	5.21	29.06	_	Robledo and Freile-Pelegrin (1997)

Porphyra tenera	34.20	0.70	4.80	8.70	_	Arasaki and Arasaki (1983)
Hypnea pannosa	16.31	1.56	40.59	18.65	12.35	Siddique *et al.* (2013)
Hypnea musciformis	18.64	1.27	37.92	21.57	11.54	Siddique *et al.* (2013)

2.9 Micronutrients contents

The mineral contents of some green, brown and red seaweeds are represented in Table 3. *Hypnea sp., J. rubena and S. oligocystum* contains higher calcium (2,289−228 mg/100 g) and potassium (98−60.8 mg/100 g) contents. The major constituent of the investigated seaweeds was calcium and sodium which formed the bulk of total minerals. The present result clarified that calcium (2,289 mg/100 g), potassium (71 mg/100 g) and sodium (161 mg/100 g) values of *J. rubena* was actually high. Fe content ranged from 12.5 to 28.7 ppm among the studied seaweeds except for *J. rubens* (4.6 mg/100 g). Presence of low concentration of Zn (0.1−0.8 mg/100 g) was recorded from the investigated seaweeds (Khan *et al*., 2016).

Table 3. Micronutrient contents of different seaweed species (Khan *et al*., 2016)

Seaweed species	**Class**	**Minerals (mg/100 g)**				
		Ca	K	Na	Fe	Zn
Caulerpa racemosa	Green	202.0±0.1	25.8±0.4	106.6±0.5	13.3±1.0	0.8±0.2
Enteromorpha intestinalis	Green	103.8±0.3	35.0±0.1	51.6±0.1	21.7±0.3	0.7±0.3
Padina tetrastromatica	Brown	279.4±1.1	41.4±0.3	4.7±0.3	28.7±0.2	0.1±0.5
Sargassum oligocystum	Brown	228.0±0.4	60.8±1.0	144.4±1.2	21.0±0.3	0.2±0.4
Hypnea musciformis	Red	140.7±0.2	30.8±0.2	110.3±0.5	14.2±0.5	0.5±0.1
***Hypnea* sp.**	Red	102.1±1.0	98.0±0.8	150.0±0.1	12.5±0.6	0.4±0.2
Jania rubens	Red	2,288.9±0.6	71.0±0.5	161.0±0.4	4.6±1.1	-

2.10 Nutrition-related health benefits of seaweed

In addition to the above discussed nutrients, seaweed contains diverse amount of phenolic molecules which are classified under different groups of phytochemicals. Those molecules do not act as nutrients and proven to have different bioactive properties associated with enhancing physical fitness to refrain from diseases or to exert therapeutic effects against certain illnesses. Non communicable diseases such as diabetes, obesity and cardiovascular diseases have a strong relationship with dietary habits and nutritional profiles of the food.

2.10.1 Reduction of obesity by bringing down the caloric value of the diet

Now a days, obesity is one of the most widely occurring nutritional health problems in most of the developed nations in the western world. The dietary fiber in seaweed helps to control weight gain in different ways. Adding seaweed to the diet of individual keeps them feel fuller and reduces appetite for further eating. Moreover, most of the dietary fiber in seaweed is not taken up by the human body and provides a low caloric value to the diet. In addition, this soluble fiber forms a viscous mass in the gut and traps digestive enzymes and some other nutrients, slowing down the digestibility of food and the absorption of nutrients in the intestine. A recent study carried out with a drug developed using alginic acid revealed that volunteers who were 25-30% overweight significantly decreased their body weight after treating with the drug (Zee, 1991). In addition to the dietary fiber, polyphenols in the seaweed extracts of *Ascophyllum* and *A. nodosum* inhibited α-amylase and α-glucosidase activities (Nwosua *et al.,* 2011).

2.10.2 Reduction of lipid absorption and cardiovascular diseases

Consumption of seaweed helps in the reduction of the risk of cardiovascular diseases. Seaweed shows its modifying effects on the GI tract such as emulsification of bile acid and interfering with lipid micelle formation, dilution of lipase concentration, binding with cholesterol and slowing down of lipid absorption. Studies carried out using rats reported that cholesterol level is decreased with the action of alginic acid and is often coupled with an increase in the fecal cholesterol content and a hypocholesterolemic response (Dumelod *et al.,* 1999). Other studies also concluded with the fact that higher level of hyper tension

and blood cholesterol is lowered significantly with the action of porphyran which secures the cardiac health (Noda, 1993).

2.10.3 Influence on glycemic control

Seaweed fiber has an action on diluting and slowing down the action of carbohydrates in the gut which shows a positive impact on regulating the blood glucose level. Therefore, controlled starch digestion can help in the control of blood glucose in type II diabetes. A study was conducted with the administration of 5 gms of sodium alginate to the daily diet of type II diabetic patients was found to prevent a postprandial increase of glucose and insulin, and to slow down gastric transit (Torsdottir *et al*., 1991). Hydrolysates of agar resulted in agaro-oligosaccharides possessing an activity against α-glucosidase (Chen *et al*., 2005). Moreover, Ascophyllum extracts at 50 mg/ml completely inhibited amylase activity. A meal supplemented with 5% alginates from brown seaweed decreased glucose absorption balance over 8 h in pigs and much similar studies have been done on rats and humans (Vaugelade *et al*., 2000). The above findings suggest that seaweed fiber has an effective influence in inhibiting starch digestive enzymes at a very low level and maintains glycemic control *in vivo*.

Taking all the above discussed dietary functions of seaweed into consideration, it can be concluded that seaweed is a potential food to be added to the diet to enhance the human nutrition and digestive health.

2.11 Uses of Seaweeds

Seaweeds contain different vitamins, minerals, trace elements, protein, iodine and bioactive substances. Seaweeds are the only source for the production of phytochemicals such as agar (China grass), carrageenan and algin. Some red algae such as Gelidiella, Gracilaria, Gelidium and Pterocladia are used in the extraction of agar. Some other red algae viz., Bucheuma Chondrus, Hypnea and Gigartina are used for carrageenan production. Sargassum, Turbinaria, Cystoseira, Lalllinaria, Macrocystis and Ascophyllum – these brown algae are used in the production of algin. These phytochemicals were used as gelling, stabilizing and thickening agents in food, pharmaceutical, confectionary, dairy, textiles, paper, paint, varnish industries etc. Some chemical products such as mannitol,

iodine, laminarin, fucoldin are also obtained from marine algae (Kolanjinathan *et al*., 2014).

Ulva sp., *Enteromorpha* sp., *Caulerpa* sp., *Codium* sp., *Monostroma* sp., *Sargassum* sp., *Hydroclathrus* sp., *Laminaria* sp., *Undaria* sp., *Macrocystis* sp., *Porphyra* sp., *Gracilaria* sp., *Eucheuma* sp., *Laurencia* sp. and *Acanthophora* sp. are highly rich in protein and are used as human food in Japan, China, Korea, Malaysia, Thailand, Indonesia, Philippines and other South East Asian countries in the form of soup, salad, curry, etc. The people of Japan, China and Korea, uses *Ulva* sp., *Enteromorpha* sp., *Monostroma* sp. and *Porphyra* sp. in soup and *Undaria* sp. and *Laminaria* sp. are eaten in dried form. In Philippines, people consume *Caulerpa lentiifera* as salad and *Codium tomentosum*, *Eucheuma denticulatum* and *Kappaphycus alvarezii* in the form of curry. Seaweed can be used in the production of commercial food products such as jelly from *Gelidiella* sp. and *Gracilaria sp*; jam from *Ulva* sp. and *Enteromorpha* sp*;* pickle from *Gracilaria* sp., *Hypnea* sp., *Acanthophora* sp. and *Laurencia* sp. can be prepared and marketed (Kolanjinathan *et al*., 2014).

The food value of seaweed depends on the minerals, trace elements, proteins and vitamins present in them. Marine algae contains all essential amino acids needed in the human diet, which are not available in vegetable food materials. In India, seaweeds are not eaten except the jelly prepared from agar and porridge prepared from *Gracilaria edulis* in the coastal areas of Ramanathapuram District. Agar is added in the preparation of following foodstuffs – ice cream, tomato sauce, jelly, marmalade, blancmange and lime jelly (Kolanjinathan *et al*., 2014).

Seaweeds are cheap source of minerals and trace elements. Hence, meal could be prepared by grinding the cleaned and washed seaweeds. It can also be mixed with fishmeal and used in different parts of the world as fertilizer for various land crops. The high amount of water-soluble potash, other minerals and trace elements present in seaweeds are readily absorbed by plants and they control deficiency diseases. The carbohydrate and other organic matter present in the marine algae alter the nature of soil and improve the moisture retaining capacity. The liquid seaweed fertilizer is used as foliar spray for inducing faster growth and yield in leafy and fleshy vegetables, fruits, orchards and horticultural plants. Seaweeds

possess several medicinal properties. Seaweeds were considered to be of medicinal value in the Orient as early as 3000 B.C. In China and Japan seaweeds were used in the treatment of goiter and other glandular diseases. The Romans considered seaweed as useless product but they also applied them to heal wounds, bums, scurvy and rashes. *Porphyra* sp. was used to prevent scurvy during long voyages, in Britain (Kolanjinathan *et al*., 2014).

2.12 Utilization of naturally occurring seaweed in Bangladesh

2.12.1 Conventional utilization

Most of the people of Bangladesh has no knowledge about seaweeds. Only the tribal community (Mog or Rakhyine) and the local people of Saint Martin's island utilizes seaweed as their food item. Traditionally, the Mog respects seaweed as a marine plant. They term seaweed as 'Hejla'. They take seaweed as other non-conventional food items. Using seaweed Mog people prepare salad and sauce. Mog people use a black color seaweed; resemble shape like a thin thread. Seaweeds are most significantly utilized in St. Martin's Island. The harvested and processed seaweeds are exported to Myanmar from St. Martin's island. Seaweeds are also utilized there as medicinal food for young ladies and post-pregnant females. Traditionally adult female takes boiled seaweeds for good health. Rotten seaweeds are used there as plant manure for vegetable production (Sarkar *et al*., 2016).

2.12.2 Approaches for seaweed utilization by government organization

Marine Fisheries and Technology Station, BFRI, Cox's Bazar has established a seaweed processing lab. Seaweed based food products such as salad, soup, pickle, cake, chanachur, jelly sauce etc. has manufactured by them (Sarkar *et al*., 2016).

2.12.3 Approaches for seaweed utilization by private entrepreneur and non-government organization

Several seaweed foods, functional and personal care products have been developed by Jahanara Islam, a private entrepreneur. The post-harvest handling procedure followed by that private entrepreneur is presented at Figure 1. A local NGO named COAST Trust also prepared different value added food and functional food products (Sarkar *et al*., 2016).

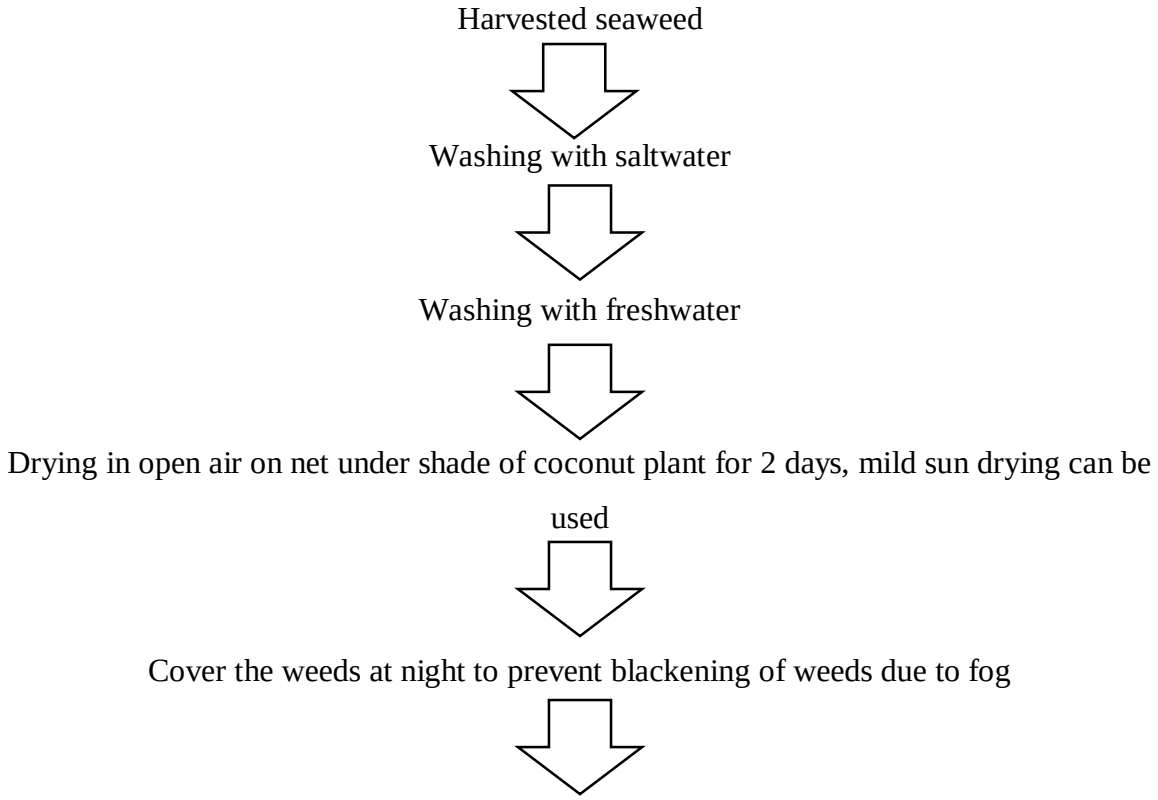


Figure 1. Post-harvest management flow chart

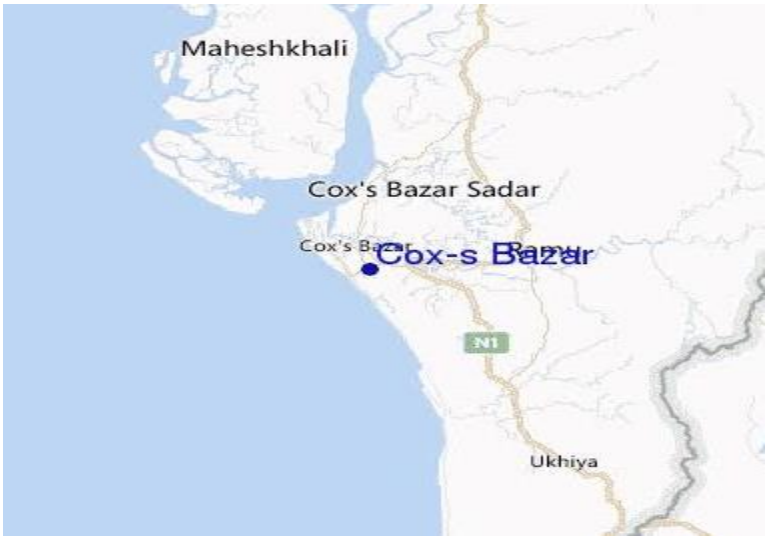
Chapter-3: Materials and methods

3.1 Study area and period

The present study on "dietary effects of seaweed on growth performance and blood parameters of mice" was carried out at Chattogram veterinary and animal sciences university during February to September, 2019. The experimental mice shed under the Department of Animal Science and Nutrition was used for animal trial and different analysis were conducted at Physiology and PRTC laboratories of Chattogram veterinary and Animal Sciences University, Chattogram. The study consists of collection of seaweed, preparation of seaweed powder, preparation of mice feed, designing experimental animals, growth observation, collection of blood, proximate analysis of seaweed and mice feed and biochemical analysis of blood serum of mice.

3.2 Collection of seaweed

Seaweed (*Hypnea musciformis*) was collected from coxsbazar region, Chattogram.



3.2.1 Processing of seaweed

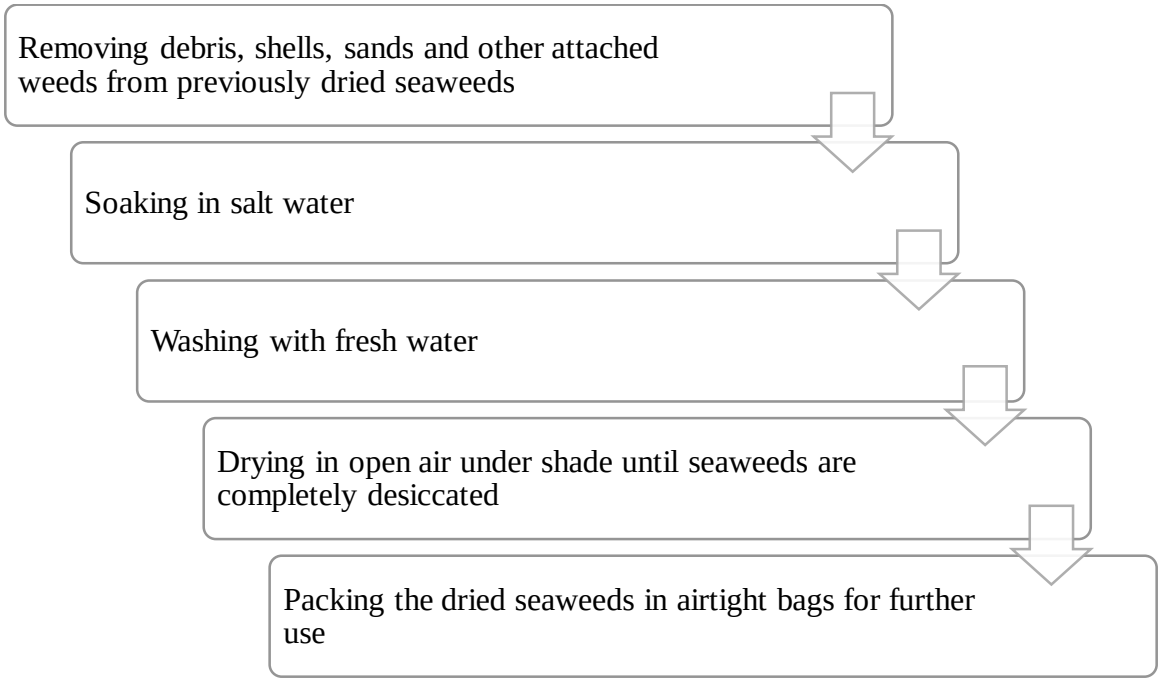


Figure 2. Seaweed processing flow chart

3.3 Collection of raw materials for feed

All the ingredients were collected from the local market of Jhautola Bazar, Chattogram.

3.4 Preparation of mice ration

Table 4. Composition of mice feed (%)

Ingredients	**Treatments**		
	T_0	**T_1**	**T_2**
Ground maize	22.00	22.00	22.00
Rice Polish	3.00	3.00	3.00
Wheat Flour	26.33	26.33	26.33
Full fat soybean	12.00	12.00	12.00
Soybean meal	12.00	12.00	12.00
Vegetable oil	6.00	6.00	6.00
Full cream milk powder	13.00	13.00	13.00
Mustard oil cake	4.00	4.00	4.00
Salt	0.25	0.25	0.25
Vitamin-mineral premix	0.25	0.25	0.25
DCP	1.00	1.00	1.00
Lysine	0.01	0.01	0.01
Methionine	0.05	0.05	0.05
Enzyme	0.01	0.01	0.01
Choline chloride	0.10	0.10	0.10
Seaweed powder		0.50	1.00
Total	100.00	100.50	101.00

T_0=Control (Basal diet); T_1=0.5% seaweed (basal diet + 0.5% seaweed on DM basis); T_2=1.0% seaweed (basal diet + 1.0% seaweed on DM basis)

3.5 Collection of mice

Mice were brought from Biology department of Jahangirnagar University, Savar, Dhaka.

3.6 Experimental Model

The experimental model was a completely randomized model with 3 treatments, each treatment was repeated 9 times with 3 replication so that there was total 27 experimental units.

Table 5. Experimental model

Dietary treatment group	Replications	Number of mice per replication	Number of mice per treatment
T_0= control (basal diet)	R_1	3	9
	R_2	3	
	R_3	3	
T_1= 0.5% seaweed (basal diet + 0.5% seaweed on DM basis)	R_1	3	9
	R_2	3	
	R_3	3	
T_2= 1.0% seaweed (basal diet + 1.0% seaweed on DM basis)	R_1	3	9
	R_2	3	
	R_3	3	
Total			27

3.7 Preparation of shed

The shed was washed and cleaned with tap water and caustic soda using brushes and scrapers. Animals were placed in a well-ventilated room. 24/7 air supply was ensured. All the cages, racks, ceiling, corners, feed containing mug and fans were given extra attention to. Lighting was provided at night time. There was a water feeder attached with each case of experimental mice. Water was changed every alternate day so that the mice could consume fresh water. Wood shavings was provided as the bedding of mice. Wood shavings was changed every week so that the mice could stay free from any kind of infection.

3.8 Observation of growth performance

Growth performance was determined per replication on weekly basis using weight balance. At the last day of the experiment final weight gain was recorded. In addition, feed consumption for each replication was determined by deducting the feed residue from supplied feed. Feed conversion was calculated as the weight of feed consumed divided by body weight gain.

3.9 Live weight gain

The live weight was measured by weighing in digital weight balance. The live weight gain is calculated from the difference between live weight and initial weight. The weight gain per day was calculated using following formula:

$$Average\ daily\ gain\ (g) = \frac{\text{final weight} - \text{initial weight}}{\text{number of days}}$$

3.10 Feed intake

Feed intake is determined by subtracting the refusal feed collected every morning before supplying of feed from the weighed feed provided to the mice for ad-libitum feeding. The average daily feed intake was calculated using the formula:

$$Average\ daily\ feed\ intake = \frac{\text{weight of supplied feed} - \text{weight of refused feed}}{\text{number of mice}}$$

3.11 Feed conversion ratio (FCR)

The feed conversion ratio was determined as average daily feed intake divided by average daily gain.

3.12 Proximate analysis of seaweed and mice feed

Proximate analysis of seaweed, mice feed for control group and two treatment group was carried out for moisture content, crude protein, crude fat, fibre, and ash are expressed in percentage. The whole process of proximate analysis was done in PRTC laboratory, CVASU.

3.12.1 Determination of Moisture content

Principle: Moisture is always present in food staffs. Estimation of moisture is done simply by heating at 104-105°C for 3-4 hours in the oven and is cooled in a desiccator to absorb moisture. The process is repeated for several times until the constant weight shows by the sample. (AOAC, 2005)

$$\text{Moisture \%} = \frac{\text{initial weight} - \text{final weight}}{\text{sample weight}} \times 100$$

3.12.2 Determination of Ash content

The enamel disc or crucible was dried in a hot air oven regulated at 105°C which was cooled in a desiccator and weighted. 5 g of dry seaweed sample, seaweed biscuits, local biscuits, and control biscuits were weighted into the crucibles and burned up to no smoke in heater. The crucible with sample was cooled and transferred to the muffle furnace. Then the sample was ignited at 550-600°C for 6-8 hours until white ash. The furnace was cooled at 150°C and the samples were transferred to desiccator and weighted (AOAC, 1995).

$$\%\text{Ash} = \frac{\text{weight of crucible and ash} - \text{weight of crucible}}{\text{weight of sample}} \times 100$$

3.12.3 Determination of Crude Fiber (CF)

2 g of dry seaweed samples was weighted and taken into a beaker. 125 ml of 1.25% H_2SO_4 was added into each beaker. Then it was fitted in condenser and placed on heater. After that it was cooled and filtered through filtering cloth. The samples were washed until it was free from acid. Residues of samples were transferred into same beaker. 125 ml of 1.25% NaOH was added there and again fitted in condenser and placed on heater. It was boiled for 30 minutes and removed from heater which was cooled and filtered through filtering cloth. The samples were washed until they were free from alkali. The residue of samples was transferred in previously weighted crucibles. The crucibles were placed into the muffle furnace and ignited at 60˚C temperature for 5 hours. Then samples were weighted after cooling. (AOAC, 2005)

$$\%\text{CF} = \frac{\text{wt of crucible with dry sample} - \text{wt of crucible with ash}}{\text{wt of sample}} \times 100$$

3.12.4 Determination of Crude Protein (CP)

0.5 g sample was weighted and one spoonful of catalyzer mixer (KOH, NaOH, Se) was added there. 10 ml concentrated H_2SO_4 was added and the digestion flask was placed in Kjeldahl Digestion Set. After that heat was increased gradually and continued up to clear residue (45 min to 1 hr). The flask was removed and cooled. 10 ml 2% boric acid solution, 2 drops mixed indicator were taken in a conical flask. The conical flask was fitted in the collection arm of distillation set. 50 ml distilled H_2O was added in the digestion tube and

fitted in the distillation flask. 40 ml of 40% NaOH was added there and the distillation was continued up to 100 ml of distillate. The distillate was titrated against 0.1 N HCl. Titration was continued until the color changed into pink. Then the titration volume was calculated (AOAC, 1990).

$$\%CP = \frac{(titre-blank)\times Normality\ of\ HCL \times 14.007 \times 6.25}{wt\ of\ sample} \times 100$$

3.12.5 Determination Ether Extract

The Ether extract content of dried seaweeds was determined by Soxhelt method (AOAC, 1995) using petroleum ether (60-80ºC) as solvent in a SOCS PLUS –SCS system. For the fat extraction approximate 2.0 g finally grinded sample was placed in a cellulose thimble paper and fat extraction was carried out using Hexase in a 250 ml Soxhlet extractor for 3 hrs. The sample was removed and air dried. Then sample was placed in oven at 80℃ a constant weight was obtained. Then extractible fat was calculated as percentage ether extract.

$$EE\% = \frac{initial\ wt - wt\ after\ extraction}{wt\ of\ sample} \times 100$$

3.13 Mineral analysis of seaweed

Mineral contents were determined by using biochemical analyzer (Humalyzer 3000). Commercially available biochemical kit (Randox®) was used for biochemical assay. For sample preparation, 1 g of powdered sample was taken into a conical flask. After that, 7.5 ml HNO_3 and 2.5 ml $HClO_4$ was added into the conical flask. Then it as heated over an induction cooker at 200W until complete digestion. Then it was cooled. Finally, deionized water was added up to 100ml. The results were expressed as mg/100g after conversion from mg/dl.

3.14 Biochemical analysis of mice blood

The blood of two mice from each replication was collected in 5 ml syringe using 23 Gauge needle. The blood was immediately transferred to eppendorf tube. The eppendorf tubes were centrifuged at 1000 rpm for about 10 minutes to separate the serum from blood. The

separated serums were then separated using micropipette and collected in another eppendorf tube. The serums were then stored in freezer at –20°C. From these serums different biochemical tests such as total cholesterol (TC), triglyceride (TG), high density lipoprotein (HDL) were determined using biochemical analyzer (Humalyzer 3000, Human® Diagnostics, Germany), glucose, total protein, calcium, phosphorus in the physiology laboratory of CVASU by following the directions supplied with the kits (Randox® Laboratories limited, UK). The low-density lipoprotein (LDL) levels were calculated according to the formula: (Friedewald et al., 1972)

LDL = Total Cholesterol − (HDL+ Triglyceride/5)

3.15 Statistical analysis

All statistical analysis was done using statistical package for social sciences (SPSS) version 16. One-way analysis of variance (ANOVA) was used to evaluate the data. Data are presented as the mean± sd (Standard deviation). Differences in means were compared using the Tukey test. p values ≤ 0.05 were considered significant.

Chapter-4: Results

4.1 Effects on growth performance

4.1.1 Live weight

Data represents the live weight of mice during four week experimental period (Table 6). Live weight of mice showed a significant (p<0.001) decrease in T_1 and T_2 group compared to T_0 group. The highest live weight (41.67 g) found in T_0 group at final week. At 2nd and 3rd week live weight decreased significantly in T_1 and T_2 group (p<0.05).

Table 6. Effect of seaweed on live weight of mice

Parameters (g)	**Treatments**			**p-value**
	T_0	**T_1**	**T_2**	
Initial	17.02±3.17	17.32±0.52	17.99±0.43	0.813
Week-1	21.81±0.31	21.62±0.45	21.83±0.51	0.815
Week-2	24.91±0.15	25.30±0.15	25.31±0.20	0.047
Week-3	34.22±0.51^{a}	32.00±1.46^{ab}	30.89±0.51^{b}	0.013
Week-4	41.67±0.34^{a}	38.44±1.02^{b}	36.33±0.34^{c}	<0.001

^{abc} means with different superscipts in the same row differ significantly.

Data are mean value ± Standard deviation.

Data indicated the mean value of 3 replications with 3 mices per treatment (n=9).

T_0=Control (Basal diet); T_1=0.5% seaweed (basal diet + 0.5% seaweed on DM basis); T_2=1.0% seaweed (basal diet + 1.0% seaweed on DM basis)

4.1.2 Average daily gain

The data presented in Table 7 showed a significant (p<0.05) decrease in overall average daily gain (ADG) in all treatment groups compared to that of control. The highest ADG was observed in T_0 group (0.89 g/m/d). The weekly ADG in 1st and 2nd week showed non-significant (p>0.05) decrease in T_1 and T_2 groups in contrast to T_0 group whereas in 3rd and 4th week the ADG differed significantly (p<0.05) in T_1 and T_2 groups in comparison to T_0 group.

Table 7. Effect of seaweed on ADG in mice

Parameters	**Treatments**			**p-value**
(g/m/d)	**T_0**	**T_1**	**T_2**	
1st week	0.68±0.45	0.61±0.11	0.55±.03	0.821
2nd week	0.33±0.14	0.53±0.09	0.50±0.10	0.157
3rd week	1.33±0.08[a]	0.96±0.23[b]	0.79±0.10[c]	0.012
4th week	1.06±0.06[a]	0.92±0.12[b]	0.78±0.10[c]	0.031
0-4th week	0.89±0.12[a]	0.75±0.06[b]	0.65±0.03[c]	0.025

[abc] means with different superscipts in the same row differ significantly.

Data are mean value ± Standard deviation.

Data indicated the mean value of 3 replications with 3 mices per treatment (n=9).

T_0=Control (Basal diet); T_1=0.5% seaweed (basal diet + 0.5% seaweed on DM basis); T_2=1.0% seaweed (basal diet + 1.0% seaweed on DM basis)

g/m/d= gram per mice per day

4.1.3 Average daily feed intake

The average daily feed intake (ADFI) presented in Table 8 showed significant (p<0.001) variation among all dietary groups throughout the study period. The highest weekly ADFI showed in T_2 throughout the study period of 4 weeks. In the 4th week it showed highest adfi (28.78±1.17) which is highly significant (p<0.001) comparing to the other groups.

Table 8. Effect of seaweed on ADFI in mice

Parameters (g)	**Treatments**			**p-value**
	T_0	**T_1**	**T_2**	
1st week	14.67±.67[b]	19.89±1.07[a]	21.33±0.34[a]	<0.001
2nd week	16.78±0.51[c]	21.33±0.34[b]	23.89±0.84[a]	<0.001
3rd week	18.22±0.51[c]	23.33±1.00[b]	25.78±0.51[a]	<0.001
4th week	20.44±0.51[c]	24.78±0.69[b]	28.78±1.17[a]	<0.001

[abc] means with different superscipts in the same row differ significantly.

Data are mean value ± Standard deviation.

Data indicated the mean value of 3 replications with 3 mices per treatment (n=9).

T_0=Control (Basal diet); T_1=0.5% seaweed (basal diet + 0.5% seaweed on DM basis); T_2=1.0% seaweed (basal diet + 1.0% seaweed on DM basis)

4.1.4 Feed conversion ratio (FCR)

The feed conversion ratio tabulated in Table 9 showed a significant (p<0.001) difference in the FCR in all treatment groups in comparison to control in 2nd and 3rd week. The FCR value at 4th week differed significantly (p<0.001).

Table 9. Effects of seaweed on Feed Conversion Ratio

Parameters	**Treatments**			**p-value**
	T_0	**T_1**	**T_2**	
1st week	53.10±32.14	38.67±8.47	44.31±9.70	0.689
2nd week	12.63±0.50^{b}	22.62±4.21^{a}	30.44±4.29^{a}	0.002
3rd week	17.17±1.14^{b}	25.70±3.94^{ab}	33.47±5.10^{a}	0.006
4th week	23.14±2.92^{c}	33.04±3.21^{b}	44.05±0.11^{a}	<0.001

^{abc} means with different superscipts in the same row differ significantly.

Data are mean value ± Standard deviation.

Data indicated the mean value of 3 replications with 3 mices per treatment (n=9).

T_0=Control (Basal diet); T_1=0.5% seaweed (basal diet + 0.5% seaweed on DM basis); T_2=1.0% seaweed (basal diet + 1.0% seaweed on DM basis)

4.2 Proximate analysis of seaweed

The proximate composition of *Hypnea musciformis* is graphically shown below (Figure 3)

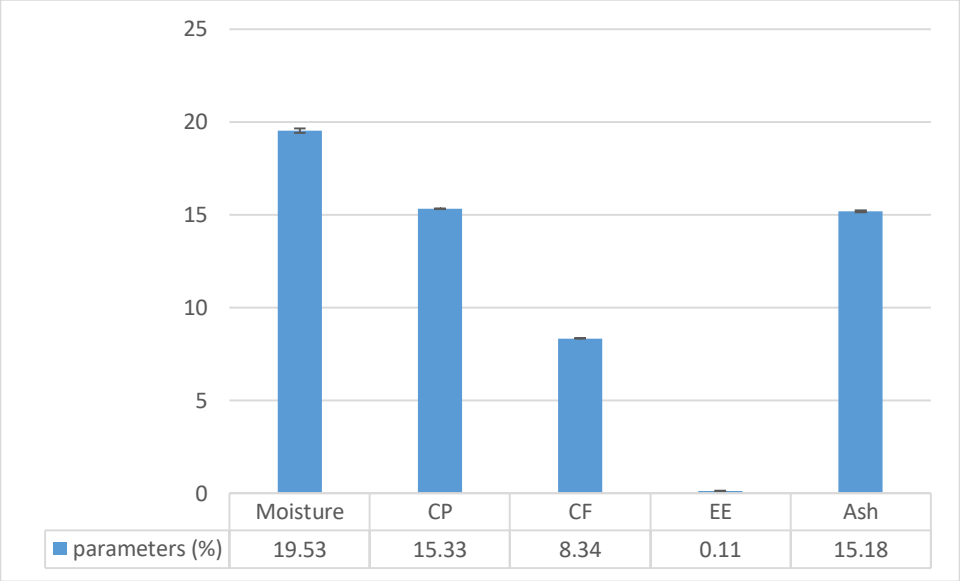


Figure 3. Proximate composition of *Hypnea musciformis*

Data values are mean value ± standard deviation (n=3).

CP= Crude protein, CF= Crude fibre, EE= Ether Extract

4.3 Proximate analysis of mice ration

Data represents the proximate composition of mice ration (Table 10). T_0 group showed significantly (p<0.001) higher moisture content than the other treatment groups. T_2 and T_0 group had significantly (p<0.01) higher crude protein content than T_1 group. Ether extract content differs non-significantly among the treatment groups. There was a significant (p<0.001) variation in crude fiber and ash content among the treatment groups.

Table 10. Proximate composition of mice ration

Parameters (%)	Treatments			p-value
	T_0	T_1	T_2	
Moisture	18.55±0.05[a]	11.50±0.50[b]	11.81±0.50[b]	<0.001
CP	26.61±0.05[a]	26.43±0.01[b]	26.68±0.05[a]	0.001
EE	0.50±0.20	0.73±0.04	0.79±0.02	0.052
CF	5.85±0.05[c]	7.45±0.05[a]	7.15±0.05[b]	<0.001
Ash	10.33±0.02[a]	9.81±0.02[c]	10.11±0.02[b]	<0.001

[abc] means with different superscipts in the same row differ significantly.

Data are mean value ± Standard deviation (n=3).

T_0=Control (Basal diet); T_1=0.5% seaweed (basal diet + 0.5% seaweed on DM basis); T_2=1.0% seaweed (basal diet + 1.0% seaweed on DM basis)

CP = Crude Protein; EE = Ether Extract; CF = Crude Fiber

4.4 Mineral contents of Seaweed

The figure below represents the mineral contents (Cacium, sodium, potassium, iron, phosphorus) of *Hypnea musciformis* (Figure 3)

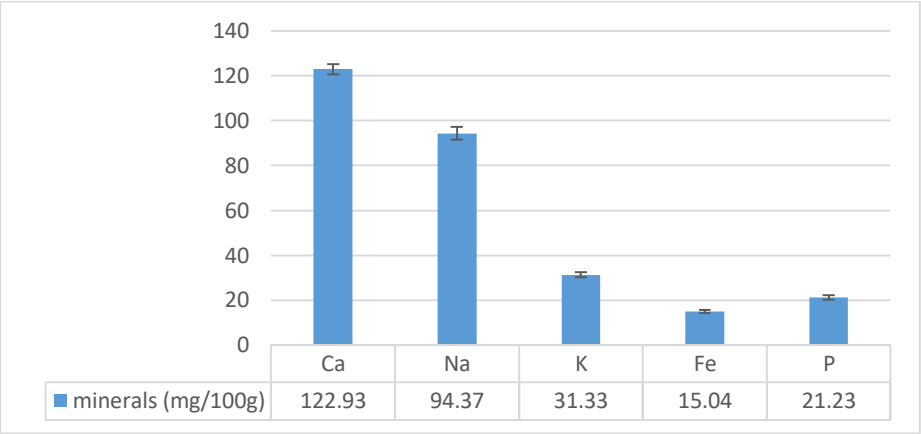


Figure 4. Mineral contents of *Hypnea musciformis*

4.5 Blood parameters

4.5.1 Blood glucose

Blood glucose level shows a non-significant difference in treatment groups comparing to the control group. Highest numerical increase was observed in the T_1 group (114.54±22.73 mg/dl).

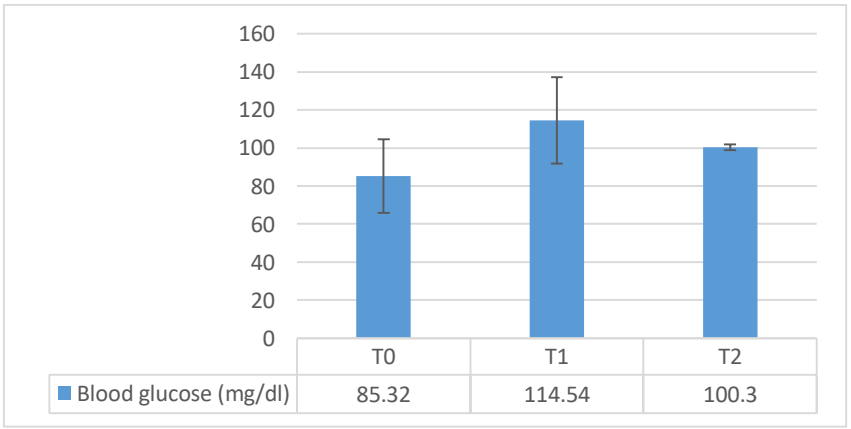


Figure 5. Effect of Seaweed on blood glucose

Data are mean value ± Standard deviation (n=5).

T_0=Control (Basal diet); T_1=0.5% seaweed (basal diet + 0.5% seaweed on DM basis); T_2=1.0% seaweed (basal diet + 1.0% seaweed on DM basis)

4.5.2 Blood Lipid profile

This study shows a significant (p<0.001) difference in blood cholesterol level of control group and the treatment groups. T_2 group shows lowest blood cholesterol level of 89.70±4.31 mg/dl. Triglyceride level is also lowest in the T_2 group (52.28±6.23 mg/dl). HDL and LDL level differs non-significantly (p>0.05) among the treatment groups.

Table 11. Effect of seaweed on blood lipid profile in mice

Parameters (mg/dl)	Treatments			p-value
	T_0	T_1	T_2	
Cholesterol	123.38±9.51[a]	98.70±7.22[b]	89.70±4.31[b]	<0.001
TG	81.24±12.85[a]	73.44±10.95[a]	52.28±6.23[b]	0.002
HDL	72.54±3.42	65.36±12.00	62.56±9.78	0.246
LDL	34.59±7.65	18.65±10.64	16.69±14.41	0.052

[abc] means with different superscipts in the same row differ significantly.

Data are mean value ± Standard deviation (n=5).

T_0=Control (Basal diet); T_1=0.5% seaweed (basal diet + 0.5% seaweed on DM basis); T_2=1.0% seaweed (basal diet + 1.0% seaweed on DM basis)

HDL= High Density Lipoprotein; LDL= Low Density Lipoprotein; TG= Triglyceride

4.5.3 Blood total protein and mineral contents

Data represents the blood mineral contents of different treatment group of mice (Table 12). There was a significant (p<0.001) difference in serum total protein content within the treatment groups. Calcium content also differs significantly (p<0.01) among the treatment groups. Phosphorus level of mice blood show non-significant (p>0.05) difference between the treatment groups.

Table 12. Effect of seaweed on blood total protein & minerals in mice

Parameters (mg/dl)	**Treatments**			**p-value**
	T_0	**T_1**	**T_2**	
Total protein	2.73±0.37^{b}	3.54±0.76^{ab}	3.74±0.36^{a}	0.001
Calcium	11.39±0.67^{b}	12.63±0.69^{ab}	12.81±0.83^{a}	0.008
Phosphorus	12.54±1.64	15.81±2.52	15.68±2.40	0.065

^{abc} means with different superscipts in the same row differ significantly.

Data are mean value ± Standard deviation (n=3).

T_0=Control (Basal diet); T_1=0.5% seaweed (basal diet + 0.5% seaweed on DM basis); T_2=1.0% seaweed (basal diet + 1.0% seaweed on DM basis)

Chapter-5: Discussion

The results of the present study, conducted for investigating the dietary effects of seaweed (*Hypnea musciformis*) on growth performance and blood parameters (blood glucose, lipid profile, total protein, and serum calcium and serum phosphorus) are discussed under this chapter.

While measuring the productive performance it was evident that the dietary supplement of seaweed powder with the basal diet of mice has a significant impact on the growth performance of mice. There was a significant weight loss in the group T_1 and T_2 compared with the T_0 (control) group. The maximum weight loss was observed in the T_2 group which was fed with 1.0% seaweed powder. Similar study was done by Matanjun *et al*. (2010), who reported lowering the body weight of rats with the application of seaweed based diet. In a study it was found that seaweed carotenoid fucoxanthin has anti-obesity effects and can reduce body weight and white adipose tissue, which composed of perirenal and epididymal abdominal adipose tissue (Maeda *et al*., 2005).

Average daily gain (ADG) had also shown a significant difference in the treatment groups. The group treated with 0.5% dried seaweed powder showed the lowest weight gain. Average daily feed intake (ADFI) of this group T_2 was also higher than the other two groups. Similar studies were done by Wong *et al*. (1999) and Amano *et al*. (2005), where the feed intake and weight gain in different treatment group were not significantly different. This variation of result is might be due to the different species of the seaweed used, different formula for feed and due to the climatic condition.

The Feed Conversion Ratio (FCR) showed significant difference between the treatment groups. The T_2 group showed highest FCR at the final week. At the 1st week of the experiment there was no significant change among the treatment groups but at the 2nd and 3rd week there was significant increase in FCR. T_2 group which was treated with 1.0% seaweed supplementation, showed the highest FCR from 2nd week till final week of the experiment. Alaeldein *et al.* (2013), applied seaweed to broiler and found no significant impact in FCR. This variation with the present study might be due to the variation of species of seaweed and the different animal used in that study.

The proximate chemical composition of *Hypnea musciformis* seaweed was evaluated to understand the nutritional value of the species. The result of proximate analysis showed that the species is rich in protein, ash and dietary fiber. Similar study was done by using *Hypnea musciformis* (Siddique *et al*., 2013). The result shows that the species is high in dietary fiber and ash compare to this study. There is a variation between these two studies. This is due to some factors like seasonal variability, location, species variation and others. The proximate chemical composition of mice ration showed significance in moisture content. The T_1 and T_2 group both differed significantly with the control group in case of moisture content. The T_2 group contains significant higher amount of crude protein. In case of ether extract there was non-significant variation among the treatment groups. In case of crude fiber content, T_1 group which was treated with 0.5% seaweed supplementation showed highest amount. The T_0 (control) group showed the highest amount of ash content than the other treatment groups.

Mineral content of *Hypnea musciformis* was found as calcium (Ca)-122.93±2.17 mg/100g, sodium (Na)-94.37±2.75 mg/100g, potassium (K)-31.33±1.04, iron (Fe)-15.05±0.73 mg/100g, phosphorus (P)-21.23±1.04 mg/100g. Khan *et al*. (2016) reported the mineral contents of *Hypnea musciformis*. Iron content of that study is almost similar with the present one. There is some variation with the present study which might be due to seasonal variability, location and others.

Blood glucose level of different treatment group was determined in this study to know the effect of seaweed on blood glucose level of mice. The result of blood glucose test showed no significant variation within the treatment group. There was numerical difference between the treatment groups and control group. Highest blood glucose level was observed in the T_1 group which was treated with 0.5% seaweed powder. In a study it was found that the level of glucose is increased after the administration of *Hypnea musciformis*. This could be a transient increase only, through action on glucagon and could also be attributed to the fact that the *Hypnea musciformis* contain many amino acids, which may form glucose (Najam *et al*., 2010).

In this study, while measuring the lipid profile, a significant decrease of serum cholesterol level was found in the treatment groups compared with the control group. T_2 group which

was administrated with 1.0% seaweed supplementation showed the lowest serum cholesterol level whereas the T_0 group showed highest content of serum cholesterol. Serum triglyceride level was also decreased significantly in the treatment groups compared with the control group. The lowest triglyceride level was observed in the T_2 group while the highest was for T_0. There is numerical difference of HDL and LDL within the treatment groups and the control group. In this study the T_0 group showed higher HDL and LDL level than the both treatment group. The result was in accordance with a similar study where *Hypnea musciformis* was applied on rabbit to see the change in the blood parameters (Najam *et al*., 2010). Literature survey indicates that seaweeds contain unsaturated fatty acids more than saturated fatty acid which are supportive in lowering blood cholesterol level (Aliya *et al*., 1991). Ingestion of diets containing highly unsaturated fatty acids has been shown to depress blood cholesterol level (Grundy, 2004). Ahmed *et al*., 1993, also reported the antihypertensive effect of seaweeds. Some seaweeds and their dietary fiber (particularly polyionic ones) interact with dietary cholesterol, leading to its excretion and subsequently lowering blood cholesterol levels. It has been reported that dietary fiber including seaweed polysaccharides lowers serum lipid levels mainly by interfering with cholesterol absorption in the jejunum and by reabsorbing bile acid in the ileum, resulting in enhanced excretion of cholesterol and bile acid in the feces (Kodama *et al*., 1972; Kiriyama *et al*., 1974).

The total protein content of treatment groups showed significant difference with the control group. The treatment groups showed higher serum total protein. The T_0 group had significantly lower total protein content than the both treatment groups. In a study it was reported that seaweeds have been reviewed favorably as sources of proteins for nutritional purposes (Wong and Cheung, 2000). In another study it was reported that *Hypnea musciformis* containing diet group has a positive impact on increasing the total protein content in blood (Najam *et al*., 2010).

In this study serum calcium and phosphorus level was determined to know the impact of seaweed on blood mineral contents. This showed a positive impact on the serum calcium level. Serum calcium level of the treatment groups were significantly higher in comparing with the control group. The T_2 group possessed the highest amount of serum calcium level.

Serum phosphorus level showed no significant impact on the treatment groups. Numerically the T_1 showed higher serum phosphorus level. This increase in blood mineral with seaweed administration can be explained with a study that seaweeds are high in minerals due to their marine habitat, and the diversity of the minerals they absorb is wide and this mineral content in seaweeds is much higher when compared to the edible terrestrial vegetables (Indegaard and Minsaas, 1991; USDA, 2001).

Chapter-6: Conclusion

The results from this study revealed that *Hypnea musciformis* is rich in protein, dietary fiber and minerals. Among the minerals determined under this study there was good amount of calcium, sodium and iron. The nutritional properties of the red seaweed species suggested that it has potential food value and could be utilized as functional ingredient in our food industry. It will play a vital role to meet up the nutritional demand among people. *Hypnea musciformis* is a very common seaweed species and available & nutrient rich. So this study was undertaken using this specific seaweed. This study was conducted to determine the dietary effects of seaweed on growth performance and blood parameters. Seaweed could be introduced to human diet as it helps in lowering the blood cholesterol level. With respect to the higher level of crude protein, calcium, iron and balanced fiber profile, *Hypnea musciformis* appeared to be an interesting potential source for human consumption. The higher level of protein and fiber content of this red seaweed species has a great food value from the nutritional and biochemical point of view. The result of this study suggested that *Hypnea musciformis* could be utilized as a healthy food item for human consumption.

Chapter-7: Recommendations

Seaweeds have become a very versatile product widely used for food or food supplement in many countries. However, the seaweed industry in Bangladesh is at its initial stage, people in Bangladesh are still not aware of the potentials of seaweed. However, recently seaweeds received increased attention as a potential source of essential nutrients. Seaweeds are rich in protein, minerals, fiber which may fulfill the demand of additional nutrient requirement for pregnant, lactating woman, children and older people. Seaweeds can be administrated to the people suffering with anemia as it contains high amount of iron. Seaweeds have an effect in lowering the blood cholesterol. Therefore hypercholesteromic patients can be suggested to have seaweed based diets. Seaweed cultivation can add a new dimension to the country's economic development.

Further studies should be implemented with different strains of edible seaweed and product development should be conducted using seaweed varieties. With larger sample size and variable, other blood parameters should be determined in further studies.

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Appendix A (Photo gallery)

Figure 6. Preparation of sample

Figure 7. Experimental works

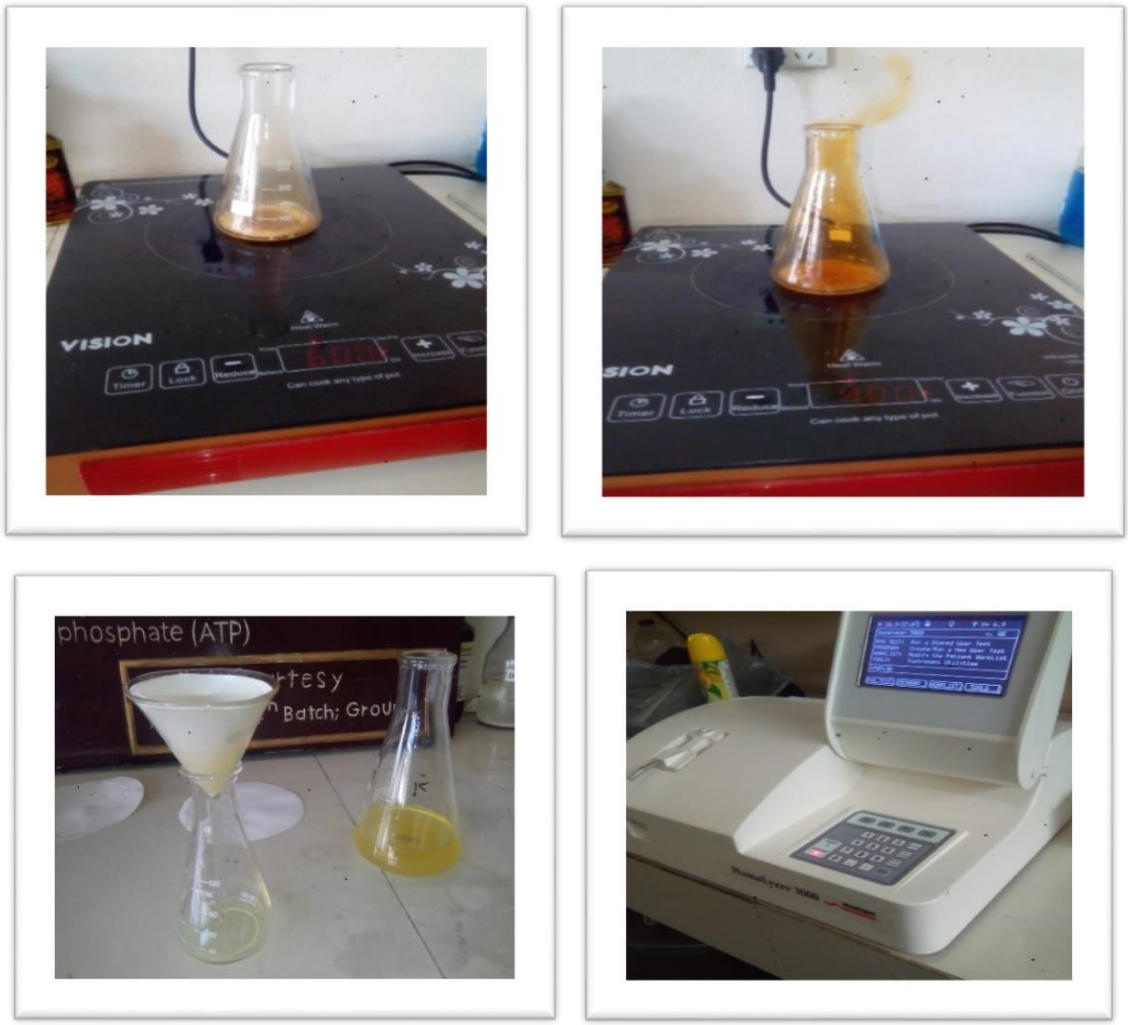

Figure 8. Biochemical analysis

Brief Biography

The author passed the Secondary School Certificate Examination from T. S. P. Complex Secondary School, Chattogram, and then Higher Secondary Certificate Examination from Chattogram College, Chattogram. She obtained her B.Sc. (Hon's) in Food Science and Technology from the Faculty of Food Science and Technology at Chattogram Veterinary and Animal Sciences University, Chattogram, Bangladesh. Now, she is a candidate for the degree of Master of Science in Applied Human Nutrition and Dietetics under the Department of Applied Food Science and Nutrition, Chattogram Veterinary and Animal Sciences University (CVASU). She has an immense interest to work in improving health status of people through proper guidance and suggestions and to create awareness among people about food safety and nutrition.