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Impact of dietary inclusion of *Gracilaria parvispora* on digestibility, growth performance, and health status of lambs

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ABSTRACT

Gracilaria parvispora, a red seaweed belonging to the Rhodophyta phylum, represents a particularly interesting option for ruminant nutrition. The inclusion of seaweed in ruminant diets presents a promising strategy for enhancing animal health and productivity by providing essential nutrients and minerals. This study aimed to characterize the chemical composition and mineral profile of brown (n = 8) and red (n = 3) seaweed species harvested from the Bay of Bengal, Bangladesh, and to evaluate the effects of dietary supplementation with *G. parvispora* on growth performance, nutrient digestibility, and health status in lambs. Eighteen lambs were assigned to three dietary treatments: control (0 g/kg dry matter [DM]), low (21 g/kg DM; 7 g/lamb/day or 2% of DM intake), and high (42 g/kg DM; 14 g/lamb/day or 4% of DM intake) inclusion levels of *G. parvispora*. Results indicated that all seaweeds are nutrient-rich, particularly in CP (88.3-122 g kg-1 DM), Ca (0.06-0.084 g kg-1 DM), Zn (76.1-164 ppm), and Fe (135-216 ppm). Lambs' DM and CP digestibility increased (4.1%) ($p<0.05$), and their average daily gain rose ($p<0.05$) when a low quantity (7 g/lamb/day) of *G. parvispora* was added to their diet. Blood protein, albumin, globulin, triglycerides, low-density lipoprotein, high-density lipoprotein, glucose, phosphorus, urea, uric acid, and creatinine were all unaffected ($p>0.05$) by *G. parvispora* supplementation, according to blood parameter data. However, when *G. parvispora* was added to the diet, fecal levels of coliform bacteria and alkaline phosphatase dropped ($p<0.05$), whereas IgM rose ($p<0.05$). In conclusion, seaweeds are a good source of nutrients for ruminants, according to the CP content, while CP digestibility, growth rate, immunity, and coliform bacterial load were all enhanced by adding *G. parvispora* (7 g/lamb/day) to their diet.

INTRODUCTION

The global livestock industry faces mounting pressure to develop sustainable feeding strategies that enhance animal performance. Traditional feed ingredients, particularly protein sources, are becoming increasingly expensive and environmentally costly, prompting researchers to explore alternative feedstuffs that can maintain or improve animal productivity [1]. Among these alternatives, marine macroalgae have emerged as promising candidates due to their nutritional composition, bioactive compounds, and potential functional properties [1]. Since seaweeds are rich in minerals, vitamins, and crude protein (CP), they have a great deal of promise for use as an alternative feed item in ruminant diets [2] and to reduce methane (CH4) emissions from ruminants [3].

Seaweeds are highly digestible in ruminants, and an *in vitro* study has shown a remarkably high organic matter digestibility (970 g kg^{-1}) of *Saccharina latissima* in sheep [4]. Additionally, seaweed is a significant source of important micronutrients, especially

chelated minerals, which have been shown to improve ruminant fertility and semen quality [2, 5, 6]. Recent research in ruminant nutrition has demonstrated that certain seaweed species can positively influence digestive processes, potentially through their prebiotic effects on rumen microbiota, anti-inflammatory properties, and mineral supplementation capabilities [q]. Ruminant immunity and antioxidant status have been found to be improved by Ascophyllum nodosum meal and extracts [2]. Feeding *A. nodosum* meal (40% of daily feed intake) to lambs and kids improved their immunity, overall health, and protected them against prolonged heat and transportation-induced oxidative stress [8]. Red seaweeds (macro algae) are usually rich in CP, with values ranging from 100 to 290 g kg^{-1} in *Palmaria* and 180 to 500 g kg^{-1} in *Porphyra* and *Pyropia* species [9] on a dry matter (DM) basis. The floridean starch, which is similar to starch but lacks amylose, is one of the main storage polysaccharides found in red seaweeds. Bangladesh is home to about 250 different types of seaweed, with St. Martin's Island, the Teknaf coast, Inany beach, Rezukhal, and Nuniarchara shore serving as the main habitats [10]. 390 MT of seaweed, primarily Hypnea, Gracilaria, and Ulva, is produced annually. High CP (236 g kg-1 DM) content in local Hypnea spp. was recently found by [11]. Like many other countries, the increasing prices and scarcity of traditional feed ingredients are impeding the growth of the large sheep (3.68 million) and cattle (17.3 million) industry in Bangladesh [13,14].

Despite the potential benefits, limited research has specifically examined the effects of *G. parvispora* supplementation in small ruminant diets. Most existing studies have focused on other seaweed species or different animal models, leaving a significant knowledge gap regarding the optimal inclusion levels, processing methods, and long-term effects of these particular red algae in lamb nutrition [15]. Understanding how *G. parvispora* inclusion affects digestibility parameters, growth metrics, and overall health status in lambs could provide valuable insights for sustainable sheep production systems. Considering the above facts, the experiment is conducted to quantify the chemical composition of the brown (n = 8) and red (n = 3) seaweed species cultivated on St. Martin's Island in the Bay of Bengal, Bangladesh. The experiment also quantifies the effects of dietary supplementation of seaweed (*Gracilaria parvispora*) on the growth performance, blood metabolites, DM and nutrient intake, digestibility, and health status of lambs in different inclusion levels.

MATERIALS AND METHODS

Experimental site, seaweeds collection, and processing

Eight brown seaweed species, namely, *Sargassum muticum, S. fluitans, S. polycystum, S. natans, Padina tetrastomatica, Hydroclathrus clatheretus, Spatoglossum asperum* and *Colpomenia sinuosa*, and three red seaweed species, namely, *Hypnea musciformes, G. parvispora, and Asparagopsis taxiformis* were collected from St. Martin's Island of the Bay of Bengal, Bangladesh, in February 2020. During the experimental period, the temperature ranged from 26 to 32°C, and humidity from 49 to 65% (Regional Forecast, Teknaf Upazila, Cox's Bazar Region). Samples of each species were collected from three sublocations, and from each sublocation, three samples were collected from a randomly selected one-meter area. The samples of each species collected from each sublocation were pooled, mixed, and a representative sample was collected. Following sampling, the seaweeds were extensively cleaned in baths of ambient saltwater to remove any remaining dirt, epibionts, related mesograzers, and other invertebrates and insects. Two freshwater washes of the cleansed samples reduced the salinity by up to 30%.

After manually draining the remaining water, the samples were allowed to air dry before being kept at -20°C in plastic zip-lock bags until additional analysis.

Using a Wiley mill (Wiley mill, Arthur H. Thomas, Philadelphia, PA, USA), the samples of each species that had been kept at -20°C were thawed, well mixed, and subsamples were dried in a forced-air oven at 70°C. After that, the samples were processed to fit through a 1 mm screen in a Wiley mill (Arthur H. Thomas, Philadelphia, PA, USA), and their chemical composition was examined. The ground subsamples were stored at 4°C in plastic zipped bags before being analyzed for proximate chemical components and mineral concentrations.

Experimental design and animals

Twenty-seven male lambs (local breed, non-descriptive), weighing 8–10 kg and two to three months old, were divided into three food groups using a randomized complete block design. The age and body weight of the animal were set in the blocks uniformly. A 10-day adaptation time for experimental diets was included in the 52-day experimental period. The Animal Research Ethics Committee (AREC) of (GAU), Gazipur, Bangladesh, granted ethical approval for the study (GAU/DEAN (FVMAS)-23/AREC/2019/6753).

Experimental diets and feeding

In accordance with the Indian Council of Agricultural Research (ICAR, 1994), three experimental diets (Table 1) were developed in order to attain an average daily gain (ADG) of 50 g [16]. The diets were denoted as Diet-A, a control diet; Diet-B, a control diet supplemented with seaweed (*G. parvispora*) powder 21 g kg^{-1} DM (7 g/lamb/day or 2% of DM intake); and Diet-C was a control diet supplemented with *G. parvispora* powder 42 g/kg DM (14 g/lamb/day or 4% of DM intake). In Diet-B and Diet-C, wheat bran was replaced with *G. parvispora*. At 6.00 am and 4.00 pm during the experiment period, the lambs were allowed to be fed, and *ad libitum* water was available. The lambs were fed twice daily at 06:00 and 16:00 h, and clean drinking water was available *ad libitum* throughout the experiment.

Table 1. Ingredients and chemical composition of experimental diets fed to lambs.

Items	Control (Diet-A)	Seaweed (*Gracilaria parvispora*) powder	
		(Diet-B)	(Diet-C)
Ingredients (g kg^{-1} DM)			
German grass (*Echinochloa polystachya*)	650	650	650
Maize	105	105	105
Wheat bran	140	119	98
Til oil cake	88.0	88.0	88
Molasses	14.0	14.0	14
Seaweed	0.00	21.0	42
Salt	3.50	4.00	4
Chemical composition (g kg^{-1} DM)*			
ME (MJ kg^{-1} DM)	9.53	9.44	9.36
DM	440	440	439
CP	114	113	112
Starch	102	99.2	96.7
NDF	562	563	564
ADF	327	329	332
Ether extract	19.2	18.7	18.1
Ash	81.9	88.7	95.1

*Unless otherwise stated; DM, dry matter, ME, metabolizable energy; CP, crude protein; NDF, neutral detergent fiber; ADF, acid detergent fiber; and Cfat, crude fat.

Animal performance

For two days in succession, the lambs' body weight was noted at the beginning of the experiment and then every week before the morning meal. Initial and final weights of individual lambs were used to determine average daily gain (ADG) as follows:

$$\text{Average daily gain (ADG)} = \frac{(\text{Final weight} - \text{Initial weight})}{\text{Research period (days)}}$$

The feed conversion ratio (FCR) was calculated as:

$$\text{FCR} = \frac{\text{ADMI}}{\text{ADG}}$$

Where, ADMI is the average DM intake.

Blood sampling and digestibility trial

Blood samples were collected on day 0, 21, and 42 of the trial from the jugular vein of each animal at 5:30 am before morning feeding. The collected blood samples were analyzed to measure blood metabolites. Fecal samples were collected at days 0, 21, and 42 of the trial and transferred into pre-weighed Whirl-Pak® sampling bags (Cole Parmer India Pvt. Ltd., Mumbai, Maharashtra, India) using sterile scoops to exclude non-fecal contaminants. Transported in an ice-cooler, samples were promptly analyzed upon arrival in the laboratory. For microbial load assessment, each sample was prepared by mixing 1 gram of feces with 9 ml PBS through vortexing. From this initial mixture, 1 ml was transferred to another clean tube for a series of dilutions. Each subsequent dilution step involved transferring 1 ml of the previous dilution to a new tube, achieving dilutions up to $1:10^{10}$. This extensive dilution aimed to facilitate accurate counting of microbial colonies. Next, 60 µl of the appropriately diluted samples were spread onto specific agar plates: MRS agar for Lactobacillus, EMB agar for Coliforms, and PCA agar (HiMedia Laboratories, A-516, Swastik Disha Business Park, Via Vadhani Ind..) for total viable counts. The organisms (colony) grew at 37°C in 48 hours of incubation time. Post-incubation, colonies were enumerated, and colony-forming units per gram of feces (CFU/g) were calculated to assess microbial populations at different time points during the trial.

The *in vivo* digestibility trial was conducted applying partial methods [17], where lambs were placed in a semi-metabolic cage. The trial lasted seven days for data collection and ten days for adaptation. Throughout the collecting period, measurements were made of each sheep's daily feed intake and refusal. Each lamb's feces were physically removed every six hours, weighed, and stored in polythene bags to prevent contamination from urine and dirt and to preserve volatile nitrogen. At the end of each day, feces were pooled, mixed, and about 10% feces of each animal were sampled for DM determination and chemical analysis.

Proximate analysis

Seaweeds and other feed ingredients were subjected to proximate analysis at the Animal Nutrition Laboratory of GAU. The protocols were followed in the analysis of the contents of DM (method # 930.15) and CP (method # 984.13) [18]. In short, an oven drying process at 105°C to constant weight was used to measure the DM content. CP was computed by multiplying the nitrogen content by 6.25, and the nitrogen content was ascertained using the macro Kjeldahl Method. The petroleum ether extraction method utilizing a Soxhlet extractor (P. K. Scientific Works in Ambala, India) was used

to determine the ether extract. The approach was followed to analyze the contents of acid detergent fiber (ADF) and neutral detergent fiber (NDF) [19].

Biochemical analysis

Megazyme International Ltd. (Wicklow, Ireland) supplied the Megazyme test kits (Catalog Nos. K-TSTA, K-AMYL, and K-BGLU) for starch determination. Mineral content was analyzed using Atomic Absorption Spectroscopy (AAS), following the method described in [20]. Each sample was analyzed in duplicate, and the average values were recorded. The commercial kit (Human Diagnostic, 65205 Wiesbaden, Germany) was used to measure the blood samples' levels of total protein, albumin, creatinine, blood urea nitrogen, and glucose. A Randox kit (Kearneysville, West Virginia, USA) was used to assay alkaline phosphatase (ALP), aspartate aminotransferase (AST), and alanine aminotransferase (ALT). Total cholesterol, triglycerides (TG), low-density lipoprotein (LDL), and high-density lipoprotein (HDL) were analyzed with the procedure [21] in Humalyzer 2000 (Human Diagnostic, 65205 Wiesbaden, Germany). A sandwich enzyme-linked immunosorbent assay (ELISA) and ELISA quantification kits (Bethyl Laboratories Inc., Montgomery, TX, USA) were used to measure the quantities of immunoglobulins, IgG, IgM, and IgA.

Microbial analysis

Lactobacillus species were detected in fecal samples using De Man, Rogosa, and Sharpe agar (MRS) (Hi-medium, India) medium, coliform bacteria using Eosin methylene blue (Levine's formulation) agar, and total bacteria using plate count agar (Standard Methods Agar) media.

Statistical analysis

The effects of seaweed species on proximate chemical components and mineral contents were analyzed using the analysis of variance procedure in Statistix 10. The effects of seaweed inclusion levels in the diet on intake, apparent digestibility, and growth performance of indigenous lambs were analyzed using the General Linear Model (GLM) of the ANOVA program of Statistix 10 software. Blood metabolites were analyzed by repeated measures analysis of variance using the Statistix 10 software.

Model used:

$$Y_{ijlm} = \mu + A_i + U_j + T_l + (U \times T)_{jl} + C(_{jm})i + e\ (_{ijlm})$$

where μ is overall mean, Ai the fixed effect of animal (i = 1–27), U_j the fixed effect of feed (j = 1–3), T_l the fixed effect of time after feeding (l = 1–2), $(U \times T)_{jl}$ the fixed interaction effect of U_j and T_l, $C(_{jm})_i$ the random effect of A_i within U_j × period $_m$ × e_{ijlm} the random error. Time after feeding was used as a repeated measure. For parameters with an overall significant ($p<0.05$) effect of treatment/seaweed species, differences among means were compared using Tukey's multiple range test.

RESULTS

Proximate chemical components and mineral contents of seaweeds

Except Cfat, all measured chemical components had a large range among the seaweed species (Table 2). The content of CP ($p<0.005$), DM ($p<0.005$), ADF ($p<0.005$), and NDF ($p<0.001$) varied among the seaweed species. The contents (g kg^{-1} DM) of CP ranged from 88.3 to 111, NDF from 296 to 419, and ADF from 110 to 298 in brown seaweeds. On the other hand, in red seaweeds, the contents (g kg^{-1} DM) of CP varied from 94.3 to 122, NDF from 308 to 436, and ADF from 205 to 249.

G. parvispora, a red seaweed, had the highest CP (122 g kg^{-1} DM) content, and *S. natans*, a brown seaweed, had the lowest CP (88.32 g kg^{-1} DM) content. The lowest ADF (110 g kg^{-1} DM) contents were found in *S. polycystum*, a brown seaweed. In general, all seaweeds had an adequate amount of CP (88.32 to 122 g kg^{-1} DM).

Table 2. Dry matter content and chemical composition of brown1 and red2 seaweed species.

Seaweed type	DM[3] (g kg^{-1} FM)	Chemical components (g kg^{-1} DM)[4]		
		CP	ADF	NDF
Brown Seaweed				
Sargassum muticum	143^{c}	104^{c}	174^{d}	296^{c}
Sargassum fluitans	151^{b}	111^{b}	190^{d}	343^{b}
Sargassum polycystum	133^{d}	101^{c}	110^{e}	310^{c}
Sargassum natans	145^{bc}	88.3^{d}	179^{d}	419^{a}
Padina tetrastomatica	150^{b}	91.2^{d}	202^{c}	412^{a}
Hydroclathrus clatheretus	199^{a}	103^{c}	232^{c}	342^{b}
Spatoglossum asperum	122^{e}	108^{b}	298^{a}	310^{c}
Colpomenia sinuosa	192^{a}	111^{b}	190^{d}	348^{b}
Red Seaweed				
Hypnea musciformes	123^{e}	94.3^{d}	227^{c}	412^{a}
Gracilaria parvispora	129^{d}	122^{a}	249^{b}	436^{a}
Asparagopsis taxiformis	132^{d}	118^{a}	205^{c}	308^{c}
Significance				
SEM[5]	2.36	0.97	4.35	4.74
P value	<0.004	<0.005	<0.005	<0.001

$^{a-h}$ means with different superscripts within the same column vary significantly ($p<0.05$); [1]*Sargassum muticum, S. fluitans, S. polycystum, S. natans, Padina tetrastomatica, Hydroclathrus clatheretus, Spatoglossum asperum* and *Colpomenia sinuosa;* [2]*Hypnea musciformes, Gracilaria parvispora and Asparagopsis taxiformis;* [3]DM, dry matter; FM, fresh matter; [4]CP, crude protein; Cfat, crude fat; ADF, acid detergent fiber; NDF, neutral detergent fiber; [5]SEM, standard error of the mean.

Effect of seaweed on nutrient intake, digestibility, and growth performance

The effects of varying amounts of seaweed (*G. parvispora*) in the feed on lamb intakes and apparent digestibility of DM, CP, ADF, and NDF are displayed in Table 3. Intakes of DM, NDF, and ADF rose when seaweeds were added to the diet. In comparison to Diet-A and Diet-C, Diet-B showed a higher ($P < 0.05$) apparent digestibility of DM and CP (Table 3). At the conclusion of the 42-day feeding study, there were no changes ($p>0.05$) in the final weight of the lambs fed the control diet or diets with low or high amounts of *G. parvispora* (Table 4). However, ADG of lambs increased ($p<0.05$) due to incorporation of *G. parvispora* in the diets, and the highest ($p<0.05$) ADG (31.20 g d^{-1}) was observed for Diet-B. The FCR was lowest in Diet-B (8.32), followed by Diet-C (10.20) and Diet-A (13.61).

Table 3. Impact of seaweed feeding on feed intake and digestibility in lambs.

Item	Experimental diets			Significance	
	Control (Diet-A)	*G. parvispora* powder		SEM	P value
		21 g kg^{-1} DM (Diet-B)	42 g kg^{-1} DM (Diet-C)		
Intake (g day^{-1})^{2}					
DM	258^{b}	274^{a}	268^{a}	3.81	0.003
CP	29.4	30.9	30.0	0.29	0.184
NDF	145^{b}	154^{a}	151^{a}	1.74	0.006
ADF	84.3^{b}	90.2^{a}	88.9^{a}	0.95	0.003
Apparent digestibility (g kg^{-1})^{2}					
DM	731	780	756	1.09	0.120
CP	749^{a}	790^{b}	761^{ab}	2.13	0.002
NDF	663	694	688	3.06	0.134
ADF	571	626	545	2.95	0.126

a, b, means with different superscripts within the same row vary significantly ($p<0.05$). SEM, standard error of the mean; DM, dry matter; CP, crude protein; NDF, neutral detergent fiber; and ADF, acid detergent fiber.

Table 4. Impact of seaweed feeding on growth performance in lambs.

Item	Experimental diets			Significance	
	Control (Diet-A)	*G. parvispora* powder		SEM	P value
		21 g kg^{-1} DM (Diet-B)	42 g kg^{-1} DM (Diet-C)		
IBW (kg)	10.2	10.1	10.0	0.33	0.488
FBW (kg)	11.0	11.4	11.1	0.42	0.863
WG (kg)	0.85	1.31	1.10	0.30	0.431
ADG (g)	20.2^{a}	31.2^{c}	26.2^{b}	0.48	0.012
FCR	13.6^{b}	8.32^{a}	10.2^{ab}	0.15	0.003

$^{a, b}$, means with different superscripts within the same row vary significantly ($p<0.05$). SEM, standard error of the mean; DM, dry matter; IBW, initial body weight; FBW, final body weight; WG, weight gain; ADG, average daily gain; and FCR, feed conversion ratio.

Effect of seaweed on blood metabolites

Table 5 shows the effect of *G. parvispora* in the ration on blood physiological indicators at different inclusion levels. Inclusion of *G. parvispora* in the feed didn't alter any of the measured physiological indicators ($p>0.05$), except ALP ($p=0.031$) and IgM ($p=0.045$). The ALP decreased, and IgM increased with the inclusion of *G. parvispora*. With an increase in the feeding period, blood TG ($p=0.026$), HDL ($p=0.003$), and creatinine ($p=0.017$) decreased, and the AST ($p=0.005$) and ALT ($p<0.001$) increased. Blood creatinine decreased with increasing feeding time in lambs fed with Diet-C, while it was not influenced by Diet-B (Diet × time, $p = 0.024$).

The inclusion level of *G. parvispora* in the diets did not affect the blood protein, albumin, globulin, urea, and phosphorus contents (Table 5). Total blood protein ranged from 5.52 to 6.82 g dL^{-1}, albumin from 1.82 to 2.61 g dL^{-1}, globulin from 2.91 to 4.95 g dL^{-1}, urea from 50.8 to 60.6 g dL^{-1}, and phosphorus from 2.90 and 3.63 g dL^{-1}. Data on the impact of seaweed inclusion in the diet on uric acid, creatinine, alkaline phosphatase, aspartate aminotransferase, and alanine aminotransferase are presented (Table 5). There were no variations ($p>0.05$) in uric acid and creatinine concentration after feeding the seaweed powder to the experimental lambs.

Table 5. Effect of seaweed supplementation on blood metabolites of lamb.

Items	Control (Diet-A)		*(G. parvispora)* powder				SEM	Significance		
			21 g kg^{-1} DM (Diet-B)		42 g kg^{-1} DM (Diet-C)			Diet	Time	Diet × time
	Day 0	Day 42	Day 0	Day 42	Day 0	Day 42				
Glucose (mg/dL)	64.6	71.2	58.4	72.6	61.6	74.7	4.22	0.290	0.144	0.615
Cholesterol (mg/dL)	120	102	111	97.0	118	102	4.31	0.371	0.122	0.914
TG (mg/dL)[1]	66.3^{c}	50.6^{a}	60.4^{b}	49.5^{a}	56.9^{b}	48.4^{a}	1.84	0.721	0.026	0.042
HDL (mg/dL)[2]	51.9	38.4	53.2	38.1	50.9	34.4	1.31	0.178	0.003	0.592
LDL (mg/dL)[3]	55.2	53.1	46.1	49.1	57.2	58.1	3.53	0.092	0.714	0.769
Total protein (g/dL)	5.52	6.14	6.03	6.82	5.91	6.34	1.47	0.276	0.291	0.161
Albumin (g/dL)	2.61	2.12	1.83	1.95	1.82	2.35	0.08	0.655	0.203	0.125
Glubulin (g/dL)	2.91	3.93	4.24	4.95	4.14	4.13	0.29	0.457	0.151	0.076
Urea (mg/dL)	50.8	60.6	55.2	52.6	53.2	53.3	0.50	0.788	0.525	0.087
P (mg/dL)	3.57	3.36	3.35	3.44	3.63	2.90	0.09	0.454	0.189	0.534
Uric acid (mg/dL)	2.51	2.71	2.62	2.53	2.75	2.62	0.08	0.475	0.956	0.426
Creatinine (mg/dL)	0.84	0.91	1.00	0.99	1.30^{a}	0.60^{b}	0.71	0.668	0.017	0.024
ALP (U/L)[4]	80.9^{a}	114^{b}	114^{b}	108^{b}	117^{b}	102^{b}	7.34	0.031	0.073	0.004
AST (U/L)[5]	29.4	36.5	28.6	33.8	27.5	34.4	1.23	0.762	0.005	0.925
ALT (U/L)[6]	6.62^{a}	12.1^{c}	7.60^{a}	12.8^{c}	8.72^{ab}	10.1^{b}	0.97	0.422	0.000	0.015
IgA (mg/L)[7]	2.53	3.13	2.21	3.56	3.54	3.37	0.072	0.562	0.123	0.092
IgM (mg/dl)[8]	207^{b}	175^{a}	264^{c}	178^{ba}	207^{b}	276^{c}	6.341	0.045	0.053	0.023
IgG (gm/L)[9]	14.4	10.1	16.4	14.2	15.9	17.0	1.270	0.342	0.223	0.212

TG – triglycerides, HDL - high density lipoprotein , LDL - low-density lipoprotein, ALP - alkaline phosphatase, AST - aspartate aminotransferase, ALT - alanine aminotransferase, IgA - Immunoglobulin A, IgM - Immunoglobulin M, and IgG - Immunoglobulin G.

Effect of seaweed on fecal microbiota

On day 42 of the trial, the total *lactobacillus* fecal population was higher ($p<0.05$) in lambs fed with Diet-C, as compared to other diets (Figure 1A). The total *lactobacillus* fecal population was decreased ($p<0.05$) from 0 to 3 and 6 weeks of rearing in Diet-B, whereas the control group was unchanged. Inclusion of *G. parvispora* in the diet reduced ($p<0.05$) the prevalence of coliform bacteria in feces when animals were fed with Diet-B compared with Diet-C (Figure 1B). Higher coliform bacteria were observed in lambs fed with Diet-A at the start of week and 3 weeks as compared to lambs fed with diets containing *G. parvispora* (Figure 1B). Total number of bacteria was lower in the control group and increased with inclusion of *G. parvispora* in the diet (Figure 1C), with the highest ($p<0.05$) increase being observed for Diet-C (3.74E+12) on day 42.

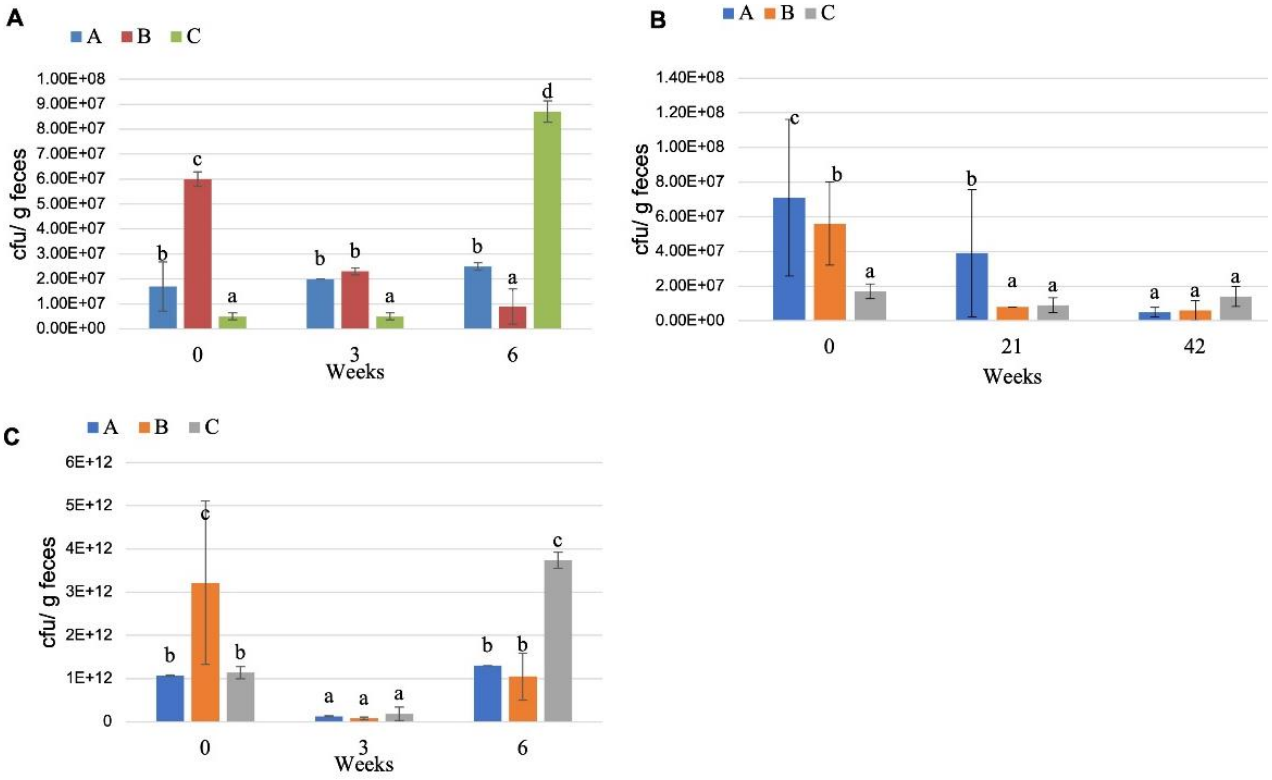

Figure 1. Effect of *Gracilaria parvispora* inclusion levels on A) total Lactobacillus, B) total coliform, and C) total bacteria in the feces of lambs. Diet-A, control; Diet-B, control diet supplemented with 7 g *G. parvispora* /lamb/day; Diet-C, control diet supplemented with 14g *G. parvispora* /lamb/day.

DISCUSSION

According to recent studies, seaweeds are valuable, sustainable alternative feeds for animals, especially as a source of energy, protein, and important nutrients such as chelated micro-minerals. They can be fed to sheep and cattle, both fresh and stored [22]. First time, we quantified the contents of chemical components and mineral contents of eight brown and three red seaweed species in this study. The seaweed species were collected from marine water at St. Martin's Island, Bangladesh, under the same experimental conditions to compare their nutritional value and suitability as a feed ingredient in ruminant diets. Considering the contents of CP and minerals, most species of seaweed in this study can be used as CP, minerals, and energy source feed ingredients in ruminant diets.

The findings of this study also present the first dataset on the effect of dietary supplementation of *G. parvispora*, red seaweed, with superior nutritional value, on nutrient intake, digestibility, growth performance, blood metabolites, immunity, and health conditions of lambs. All seaweed species were a good source of CP (88 to 122 g kg^{-1} DM), and the amount of CP was comparable to that of rice bran, a common feed ingredient for cattle and poultry in Bangladesh [23]. The CP content of the seaweeds studied in this study was above the minimum CP (>70 g kg^{-1} DM) required for support of normal rumen fermentation and microbial growth [24]. One of the primary distinguishing nutritional qualities of seaweeds is their well-established high CP content. The CP content (97.5 to 111 g kg^{-1} DM) of the brown seaweeds in the present study was within the range of CP values (75 to 127 g kg^{-1} DM) reported for brown seaweeds [25]. Mehedi *et al.* (1999) [26] reported CP content of 98 to 109 g kg^{-1} DM for *A. taxiformis*, which is very close to our findings. Nevertheless, lower CP contents for *H.*

clatheretus (30 to 51 g kg^{-1} DM) and *C. sinuosa* (29 to 48 g kg^{-1} DM) was reported [26], and higher CP contents for *Hypnea* spp were reported by [27] (214 g kg^{-1} DM) and [28] (236 g kg^{-1} DM), than the values recorded in current study (Table 2). Furthermore, the current study's red seaweed CP concentrations were lower than previously published results [25]. Further comparisons revealed that the CP content of *G. parvispora*, a red seaweed, was lower than that of Ulva [28] and *Hypne* [27] red seaweeds. The discrepancies in CP values may be due to the differences in seaweed species, as well as in their ecological and growth conditions [29]. Moreover, seasonal variation in the chemical composition of seaweeds is also reported [30].

The NDF content (296 to 419 g kg^{-1} DM) of brown seaweeds in the current study was higher than the NDF values reported for *A. nodosum* (162 g kg^{-1} DM) and *Laminaria digitata* (120 g kg^{-1} DM) brown seaweeds [31]. Moreover, the NDF contents of red seaweed, *H. musciformes* (412 g kg^{-1} DM) and *G. parvispora* (436 g kg^{-1} DM) in the present were also higher than *P. palmate* (320 g kg^{-1} DM) as reported [32]. The discrepancies in NDF values of seaweeds among the studies may be due to the differences in seaweed species, and environmental and growth conditions [30,31].

Notably, the NDF contents of the seaweed were in a lower range. This could be related to the presence of different polysaccharides in seaweed; however, these are not fully characterized so far [33]. The detailed evidence about certain polysaccharides and the true nutritional value of the seaweeds is not provided by the NDF and ADF analyses.

In the current study, the NDF fraction was almost two-fold greater than the ADF fraction, which can be attributed to the presence of the specific polyphenolic compounds that are precipitated in the neutral environment, but not in the acidic environment [19]. The precise polysaccharides of seaweeds, their incorporation in various analytic fractions, and their contribution to nutritional content need to be properly documented and quantified through additional analyses.

Globally, there is an increasing need to find alternative, low-cost, home-grown feeds for ruminant livestock that can decrease the growing food-feed-fuel competition [34]. The chemical composition, mineral profile, and *in vitro* digestibility results showed that seaweed can be used as an alternate feed resource for ruminants. Therefore, we further evaluated the effect of different levels of *G. parvispora* feeding on the intake, growth performance, and health status of lambs. The inclusion of *G. parvispora* in the diet increased DMI of lambs as compared to the control diet. However, reverse finding of feeding seaweed (*A. nodosum* meal) decreased the DMI of Holstein calves [35]. The average DMI of the lambs was 0.266 kg d^{-1} in our study, which was lower than that of Dorper × Brazilian Somali crossbred sheep (0.9 kg d^{-1}) [36]. Similarly, higher DMI of 1.4 kg d^{-1} [42] and 1.33 kg d^{-1} [43] were reported for Dorper × Hu crossbred male lambs. The differences in DMI across studies could be mainly attributed to the variations in growth stages, genotypes, and other dietary components. The apparent DM digestibility was higher in the *G. parvispora*-containing diets as compared to the control, which could be related to the lower NDF intakes of lambs fed with the *G. parvispora*-containing diets (Table 3). Greater DM digestibility can also be related to the presence of specific polyphenolic compounds and floridean starch in *G. parvispora*-containing diets. An increase in NDF digestibility with the inclusion of *A. nodosum* meal in the diet of beef cattle has also been reported [37]. The inclusion of *G. parvispora* in the diets significantly increased the ADG of lambs, and the greatest increase in ADG was observed for lambs fed with a low level of *G. parvispora*. Since the DMI of lambs decreased with the inclusion of *G. parvispora* in diets, the increase in the ADG of the lambs fed with *G. parvispora* could be related to their greater digestibility (Table 3), resulting in more efficient utilization of the dietary nutrients and energy by the growing lambs, and

superior growth performance than those fed with control diet. The greater improvement in diet-digestibility was observed with the inclusion of a lower level of *G. parvispora*, which corresponds to a greater increase in ADG. The improved daily gain of Diet-B corresponded with lower FCR (Table 3), which might be due to higher DM digestibility of feed. Overall, the ADG of the lambs in the current study was 25.90 g, which was 7 times lower than that in pure Dorper sheep [38]. This discrepancy in ADG between the two studies may be due to the differences in the genotypes, feeding regime, and diet composition.

Adding *G. parvispora* to the diets decreased the blood TG, HDL, and creatinine in the current experiment. According to [39, 40], meals containing *Sargassum latifolium* decreased blood cholesterol in goats and lambs, respectively, which is consistent with our findings. These polysaccharides hinder lipid absorption in the gastrointestinal tract, resulting in lower serum cholesterol levels [33] [39] (2007) reported that adding *S. latifolium* to the diet (@ of 4%) increased albumin, globulin, and total proteins, which is consistent with the findings of our study. An essential diagnostic tool for many metabolic illnesses, renal glomerular filtration, and other diseases is the quantity of urea and creatinine in animal blood [42]. Serum levels of ALT, AST, ALP, creatinine, and uric acid were within acceptable limits in the seaweed-treated groups, suggesting appropriate kidney function and animal health. Serum aminotransferase enzymes are sensitive indicators of tissue damage, and an increase in their levels in the blood is suggestive of cellular leaks and a breakdown in the cell membrane's functional integrity [43].

Accordingly, the blood glucose level is considered a precursor for lactose production in the bovine mammary gland and one of the markers of energy status [44]. Non significantly higher blood glucose levels were observed in *G. parvispora*-fed groups during the entire experimental period (Table 5). In agreement with our findings, [45] observed an increase in blood glucose level of dairy cows fed with seaweed, *A. nodosum*. [46] contended that the addition of *G. parvispora* may raise blood glucose levels through the promotion of intestinal microflora growth, which would promote feed digestion and feed nutrient utilization.

The immune system makes antibodies to defend the body from bacteria, viruses, and allergens. In the current study, adding *G. parvispora* to the diet boosted immunological features (IgM), suggesting that seaweed enhanced the animal's growth and overall health. Our results are consistent with previous research that found that supplementing lambs with seaweed (*A. nodosum*) enhanced their immune systems and general health. Studies conducted to date, concerning the use of seaweed in ruminant nutrition, have been focused on the addition of small quantities of different macroalgal species in feed to evaluate their potential as a prebiotic for enhancing animal performance. This is the first study reporting the effect of seaweed feeding on fecal output of *Lactobacillus* spp., *Coliform,* and total bacteria (Figure 1A-C).

The population of *Lactobacillus*, with known health-promoting properties, increased in the GIT of *G. parvispora*-fed groups in comparison to the control group. In contrast, coliform bacteria were reduced in the seaweed groups compared to the control group. Feeding brown seaweed (*A. nodosum*) to feedlot cattle decreased *Escherichia coli* fecal shedding, which is consistent with our findings [47]. Supplementation of Tasco (air-dried *A. nodosum*) also reduced *E. coli* O157:H7 shedding in the feces of beef cattle [48]. Because the meal contains phlorotannin, feeding *A. nodosum* meal at two percent DM for two weeks before slaughter decreased animal excretion of *E. coli* O157:H7 in cattle and lambs [49]. These findings demonstrate that feeding seaweed reduces the population of pathogenic microorganisms and increases beneficial microorganisms in the GIT of lambs without interfering with production performance [51].

Recent studies have shown that seaweeds, particularly *Asparagopsis taxiformis*, have great potential for reducing methane (CH_4) emission from ruminants [52]. Li *et al.* (2016) reported a strong anti-methanogenic effect of *A. taxiformis* in sheep, which triggered enormous research into the exploration of red, green, and brown seaweeds for anti-methanogenic effects. The *A. taxiformis* and *A. armata* have shown greater mitigating effects in *in vivo* experiments with dairy and beef cattle [53]. A decrease between 9 and 98% of CH_4 production has been reported with supplementation of *Asparagopsis*, depending on dose and diet composition [53, 54]. The anti-methanogenic action of *Asparagopsis* relies on the presence of halogenated CH_4 analogues, such as bromoform, a key active compound, which varies (3 to 51 mg/kg DM) greatly in *A. taxiformis* [55]. In methanogenic archaea, the last stage of methanogenesis is blocked by a reaction between methane halogenated analogs and vitamin B12. It is recommended that future studies investigate how these naturally existing resources can help reduce ruminant CH4 emissions.

CONCLUSIONS

The seaweeds were good sources of CP (87.5 to 122 g kg^{-1} DM). The feeding trial showed that adding a small amount (7 g/animal) of *G. parvispora* to the meal greatly improved CP digestibility, decreased the pathogenic microbial load, and enhanced immunological (IgM) status. As a result, native lambs had the highest average daily growth rate (31.2 g/d). Using seaweed, a marine by-product that is readily available in the area, in animal feed might help sustain animal output over the long term and lessen pollution of the marine environment caused by excessive disposal of the same.

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AUTHOR CONTRIBUTIONS

The experiments were conceptualized and designed by ASMS, ZT, and ST. Seaweed collection and experiment execution were assisted by AFMST and MRI, respectively. The data was evaluated by ASMS, ST, and NAK. The manuscript was drafted by ASMS. The manuscript was critically reviewed by RB, SI, MMR, and NAK for significant intellectual content. The final version was approved for publishing by all writers.

CONFLICTS OF INTEREST

There is no conflict of interest among the authors.

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