RESEARCH ARTICLE

Possible use of seaweed (Gracilaria tenuistipitata Var. Liui) to the reduction of enteric methane emissions from dairy cattle

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ABSTRACT

Objective: Gracilaria tenuistipitata var. Liui is a red seaweed, artificially cultivated in Nuniachara sea beach at Cox’s Bazar, primarily utilized as a human diet and nutritional supplement in Bangladesh. It has specific industrial applications as well. The study sought to assess the potential for enteric methane (CH₄) reduction of this seaweed from the cattle industries.

Materials and Methods: To explore the diversified application of this seaweed in livestock feed industries to reduce enteric CH₄ production from ruminants, a feeding trial was done at the Pranisheba research and development cattle farm, Savar, Dhaka. An ambient CH₄ amount was measured with and without the application of seaweed in concentrate feed by a sensor-based Internet of Things device of the remote cowshed monitoring system. Other clinical parameters, like feeding amount, body temperature, and consistency of feces, were physically monitored.

Results: After proximate analysis, the composition of total crude protein, crude fiber, and moisture of G. tenuistipitata var. Liui was 24.09%, 0.18%, and 21.31%, respectively. The quantity of total energy was 2,615 kcal/kg. Adding 1% seaweed to the cattle-concentrated diet on a dry matter basis lowered the average ambient CH₄ concentration from 48.30 ± 4.45 to 41.02 ± 3.41 ppm; the differences were statistically significant (p < 0.05). Average body temperature, amount of daily feed intake, and consistency of feces remained unchanged.

Conclusion: The results show that there is a chance that G. tenuistipitata var. Liui could be used to stop cattle from making enteric CH₄.

Introduction

Seaweeds are primitive plants lacking true roots, stems, and leaves that are found in marine water and come in various forms, sizes, colors, and compositions. They are broadly classified as brown seaweed (Phaeophyceae), red seaweed (Rhodophyceae), and green seaweed (Chlorophyceae). They vary greatly in the composition according to the species, collecting time, and habitat, as well as external factors like water temperature, light intensity, and nutrient content in the water [1]. These are one of the emerging natural items that are considered important components of the development of the blue economy [2]. Because of their low-calorie content and diversity of vital nutrients such as proteins, essential amino acids, vitamins, minerals, soluble and insoluble dietary fibers, and bioactive substances like Omega-3 fatty acids, Omega-6 fatty acids, folic acid, iodine, calcium, magnesium, potassium, copper, iron, zinc, vitamin B, and vitamin K, these foods are considered good for everyone [3]. The potential industrial applications of seaweed in sectors such as biofuel, chemicals, nutraceuticals, medicines, cosmetics, and environmental bioremediation have boosted the demand for seaweed production globally. With the bright prospects and potential use in mind, numerous government and non-government institutions in Bangladesh are trying to focus as well as expand the growth, cultivation, preservation, and diversified use of seaweed as a viable marine farm in the future. According to reports by Hossain et al. [4], in Bangladesh, around 300
households are involved in seaweed production, which will produce 390 tons (wet weight) by 2020. It might raise seaweed output from 5,000 km² of shallow coastal waters to 50 million tons by 2050.

The greenhouse gas methane (CH₄) significantly contributes to global warming. Ruminant animals create roughly 3.3 Gt CO₂ eq. of enteric CH₄ annually worldwide [5]. Only cattle are responsible for 77% of these emissions, making them the most significant direct contribution to global warming. The increased focus on decreasing GHG emissions from the cattle business necessitates the development of improved strategies for reducing and attaining the worldwide aim of reducing enteric CH₄ by 40% by 2030 [6]. In vitro studies have shown that more than 21 seaweeds can lower CH₄ emissions. When fed to a diet at a 5% organic matter inclusion rate, Asparagopsis taxiformis, a tropical or subtropical red seaweed, can reduce CH₄ generation by 95% [7]. Among other seaweeds, Gracilaria tenuistipitata var. Liui is an important one extensively growing on the sand flat of Nuniachara beside Moheshkhali Channel, Cox’s Bazar. It is mainly promoted as human food but is economically very important for other diversified uses that must be explored. Because of this, the main goal of this study was to find out if G. tenuistipitata var. Liui has any effect on how much enteric CH₄ gas live cattle make.

Materials and Methods

Seaweed collection

Under some research and development (R&D) projects, G. tenuistipitata var. Liui seaweed is being artificially cultivated at Nuniachara sand flat southeast of Moheshkhali Channel, Cox’s Bazar, in the form of farming for alternative foodstuffs and livelihood improvement for the people in the coastal area. From there, the seaweed was collected in October 2020 by a local agent through personal contact.

Processing of seaweed as a cattle feed supplement

After collecting the wet seaweed, it was sun-dried on a net scaffold beside the beach. Afterward, 250 gm were filled into plastic bags and brought to the research farm. At the research station, it was again sun-dried and made into powder by a hand grinder. Then it was stored at room temperature in an airtight plastic container and used during concentrate feeding, mixed up regularly.

Proximate analysis of seaweed

The proximate composition of seaweed was analyzed in the QC Lab, the first accredited laboratory of the Department of Livestock Services with ISO 17025:2017 and ISO 9001:2015. Three bags of seaweed were randomly selected for nutritional analysis. Dry Matter (DM), Ether Extract (EE), Crude Protein (CP), Crude Fiber, Neutral Detergent Fiber (NDF), and Acid Detergent Lignin were carried out according to the methods of the AOAC [8].

Research farm and cattle selection

Two sheds at Adorsho PraniSheba R&D farm (23°52’26.8”N, 90°19’40.0”E), Ashulia, Savar, Dhaka were used. One was for treatment and another served as control. A total of 10 Holstein Friesian crossbred, milking, nonpregnant cows having 4–6 years of age and 450 ± 25 kg body weight were used. Their average daily milk production was 12–15 liters. In each shed, five randomly selected cows were kept.

Ration calculation

The daily ration of individual cows was calculated considering their daily milk production and body weight by using the The Food and Agriculture Organization (FAO) Ration Formulation Tool for Dairy Cows [9]. Napier grass as fodder, straw as roughage, and various feed ingredients like wheat bran, rice polish, gram, pulse bran, molasses, oil cake, broken maize, a multivitamin mixture, and calcium supplement as concentrated feed were given regularly as per their recommended amounts. The used feed ingredients for the daily mixture were wheat bran 26.6%, rice polish 11%, gram 7%, pulse bran 21.6%, molasses 3%, oil cake 10%, broken maize 18%, multivitamin mixture 1%, and calcium supplement 1.4%. The average per kg cost of this mixture feed was 39.75 Bangladesh taka. Fresh drinking water was given ad libitum regularly.

The CH₄ measurement device

The Pranisheba Remote Cowshed Environment Monitoring Sensor device was installed in two sheds. Adorsho PraniSheba Ltd., a leading Agrotech company in Bangladesh (https://pranisheba.com.bd), manufactures this device. The description of the device is given below:
The Pranisheba Remote Cowshed Environment Monitoring Sensor is an all-in-one sensor for monitoring environmental changes in your cowshed. A collection of powerful embedded sensors measures ammonia gas, CH₄ gas, the environment’s temperature, and humidity all at the same time. Not only can the device do these things, but it can also detect smoke and alert the user right away (Fig. 1).

Each sensor is managed through the Pranisheba Internet of Things (IoT) app. Users can configure the devices to display sensor data as they desire and set custom alarms based on user-defined multiple thresholds for different hazardous levels. For extreme cases like fire hazards and smoke, the sensor immediately triggers the alarm and notifies the user. Users receive real-time alert notification messages via SMS and push notifications.

Sensor data from the Pranisheba Environment Sensor is visualized in an interactive live chart in the Pranisheba

http://vetresnotes.com/

This device has two variants: Standalone and Mesh. The device in the standalone variant sends data to the remote server via mobile internet (GPRS technology) using IOT Sim and does not require a base station to do so. This feature opens many opportunities to deploy devices in remote areas where Wi-Fi is scarce.

On the other hand, the mesh-enabled variant does not require an internet connection to transmit sensor data. Instead, it transmits sensor data to the base station via 2.4GHz Radio Frequency, and the base station sends all sensor data to the remote server. This feature enables the opportunity to deploy multiple sensor devices using only one base station. This device stores 1 month’s data as a fail-safe option to avoid data loss during transmission and unavailability of the internet.

The device has a built-in alarm system to notify the user of any abnormality or loss of connection. For local visualization, it has one multicolor LED for each type of sensor on the device casing. The device casing is designed to sense smoke, gases, temperature, and humidity independently and without disturbance. Data-sending insights are demonstrated in Figure 2.

![Figure 1. Functions of remote cowshed monitoring device.](image1)

![Figure 2. Daily, monthly, yearly data insight of cowshed environment sensor.](image2)
Data collection

All the data, like ambient CH$_4$, temperature, and humidity, was stored on a cloud-based server. Other clinical and feed-related information was collected manually and recorded in a Microsoft Excel sheet. A manure score (1–5) was set to evaluate the cow dung. A manure score of 1 was of a cream soup consistency, a score of 2 was of cake butter consistency, a score of 3 was like thick pancake batter, a score of 4 was equivalent to peanut butter, and a score of 5 was firm and dry.

Statistical analysis

Regression analysis was done using Microsoft® Excel® for Microsoft 365 MSO (Version 2205 Build 16.0.15225.20172) 64-bit service pack.

Results

Proximate analysis of seaweed

In a proximate analysis of *G. tenuistipitata* var. Liui seaweed in the QC Lab, the following results were found: The nutritional values of *G. tenuistipitata* var. Liui seaweed is mentioned in Table 1. After that, this seaweed was fed to all the animals in the treatment shed, and another shed was kept under control.

The CH$_4$ reduction potential of seaweed

Remote cowshed monitoring devices were installed in both sheds. The device regularly measures the ambient CH$_4$ in the shed and generates the report through a cloud-based data server. The daily average CH$_4$ volume in the air of these sheds is shown in Table 2.

Our experimental setup has reduced ambient CH$_4$ volume from 48.30 ± 4.45 to 41.02 ± 3.41 ppm. We measured the ambient gas in ppm, which does not provide any precision about the integral value of CH$_4$ emitted by each cow. We sampled and calculated the value of CH$_4$ in the atmosphere every 30 sec and calculated a day’s average CH$_4$ volume in the air (Fig. 3). We ran this experiment in two phases; we collected data within an equal timeframe. In the first and second phases, we recorded data without seaweed and with seaweed, each with a time frame of 10 days. We have also calculated the per-day average CH$_4$ value in

**Table 1.** Nutritional values of *G. tenuistipitata* var. Liui.

<table>
<thead>
<tr>
<th>Test parameter</th>
<th>Method</th>
<th>Result value</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crude fat (DM basis)</td>
<td>AOAC 920.39</td>
<td>0.18%</td>
<td>1710-7199-12871, 1710-7199-12872, 1710-7200-12873, 1710-7202-12875, 1710-7207-12880, 1710-7208-12881, 1710-7209-12882, 1710-7210-12883</td>
</tr>
<tr>
<td>Total gross energy</td>
<td>FAO_VOL_14</td>
<td>2615.00 kcal/kg</td>
<td>1710-7199-12871, 1710-7199-12872, 1710-7200-12873, 1710-7202-12875, 1710-7207-12880, 1710-7208-12881, 1710-7209-12882, 1710-7210-12883</td>
</tr>
<tr>
<td>Acid detergent lignin</td>
<td>AOAC 973.18</td>
<td>0.00%</td>
<td>1710-7199-12871, 1710-7199-12872, 1710-7200-12873, 1710-7202-12875, 1710-7207-12880, 1710-7208-12881, 1710-7209-12882, 1710-7210-12883</td>
</tr>
</tbody>
</table>

Figure 3. Daily real-time flow chart of ambient CH$_4$ measurement.
ppm for trial phases 1 and 2 (Figs. 4 and 5) and shown the per-phase average value in ppm in Figure 6. From Figure 4, we see that there is a constant reduction of CH$_4$ after the use of seaweed. At the start of the use of seaweed, the difference was insignificant, but as days increased, CH$_4$ reduction became significant and constant. As each phase was done consecutively, external factors like the cowshed’s cleanliness, airflow, and temperature affected the environment’s CH$_4$ volume. That is the main reason for crossing two lines in Figures 2 and 3. Similarly, in Figure 4, we see an overall increase in the atmospheric CH$_4$ value in trial 2 compared to trial 1. This occurred due to the cowshed’s environmental change over time. Table 2 represents the daily average ambient CH$_4$ values. The difference is inconsistent, as each trial phase was sequential in the time frame, and the weather effect was not neglected. We found a $p$-value of 0.0065 ($p < 0.05$), representing that the CH$_4$ volume reduction between controlled and seaweed feeding is statistically significant at a 95% confidence interval. Thus, we reached our assumption that CH$_4$ reduction is

Table 2. Daily average ambient CH$_4$ values measured in ppm of trial periods.

<table>
<thead>
<tr>
<th>Phase</th>
<th>Day</th>
<th>Treatment (with seaweed) Daily average value (ppm)</th>
<th>Controlled (without seaweed) Daily average value(ppm)</th>
<th>Difference</th>
<th>$p$-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phase-1</td>
<td>1</td>
<td>12.323</td>
<td>12.323</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>15.852</td>
<td>15.923</td>
<td>0.071</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>40.639</td>
<td>17.060</td>
<td>−23.579</td>
<td></td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>20.309</td>
<td>25.002</td>
<td>4.693</td>
<td></td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>28.617</td>
<td>26.372</td>
<td>−2.245</td>
<td></td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>44.484</td>
<td>54.496</td>
<td>10.012</td>
<td></td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>55.484</td>
<td>77.632</td>
<td>22.147</td>
<td></td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>32.409</td>
<td>60.372</td>
<td>27.963</td>
<td></td>
</tr>
<tr>
<td></td>
<td>9</td>
<td>47.138</td>
<td>44.640</td>
<td>−2.497</td>
<td></td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>30.182</td>
<td>74.549</td>
<td>44.366</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>66.640</td>
<td>58.701</td>
<td>−7.938</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>50.093</td>
<td>74.425</td>
<td>24.331</td>
<td></td>
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<tr>
<td></td>
<td>3</td>
<td>40.930</td>
<td>57.563</td>
<td>16.633</td>
<td></td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>36.558</td>
<td>43.425</td>
<td>6.866</td>
<td></td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>34.577</td>
<td>50.341</td>
<td>15.763</td>
<td></td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>37.873</td>
<td>63.407</td>
<td>25.534</td>
<td></td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>47.300</td>
<td>56.344</td>
<td>9.0438</td>
<td></td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>62.810</td>
<td>49.556</td>
<td>−13.253</td>
<td></td>
</tr>
<tr>
<td></td>
<td>9</td>
<td>61.508</td>
<td>62.765</td>
<td>1.257</td>
<td></td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>54.690</td>
<td>41.282</td>
<td>−13.408</td>
<td></td>
</tr>
</tbody>
</table>

Figure 4. Average CH$_4$ (ppm) per day (Phase 1).
possible with seaweed. For accurate measurements of how much CH\textsubscript{4} is being used up, we need a CH\textsubscript{4} chamber with a good air control system.

**Clinical and production output of seaweed**

Other parameters such as body temperature, respiratory rate, milk production, average weight, amount of dung, and consistency were recorded manually. After looking at the data, it was decided that the measuring parameters in Table 3 did not change in an important way.

**Discussion**

Because of its nutritional value, G. tenuistipitata var. Lui is commonly used as a human consumable seaweed. Aziz et al. [10] studied the detailed nutritional values of this seaweed. In the Micro-Kjeldahl method, the total CP, NDF, crude lipid, carbs, ash, and moisture had percentage compositions of 25.55 ± 0.18%, 5.65 ± 0.13%, 0.16 ± 0.03%, 45.93 ± 1.53%, 10.61 ± 0.69%, and 12.10 ± 0.25%, respectively. CP and NDF vary slightly from our findings.
These differences may be due to seasonal variations, collection times, or drying or preservation techniques. Here, the gross energy was 2,615 kcal/kg, which is lower than the previous result of 2,940.56 kcal/kg. The gross energy of seaweeds ranges from 3,500 kcal/kg to 2,200 kcal/kg [11], with variations depending on the cultivation stage, water salinity, turbidity, and other species variation factors [12]. In this sense, the variation of this result is acceptable.

Seaweeds have a long history of being used as livestock feed [13]. It is mainly used in cattle feed today due to its nutritional value, protein content, and other micronutrients [14]. Enteric CH₄ accounts for 16% of worldwide greenhouse gas emissions, thus, mitigating CH₄ output from ruminants is critical for the ruminant industry’s long-term viability [15]. Anti-methanogenic features of certain seaweeds like A. taxiformis, Alaria esculenta, Ascophyllum nodosum, and Chondrus crispus are highly opportune nature-based solutions to reduce CH₄ emissions to combat global climate change [16]. The active anti-methanogenic component, bromoform, in red seaweeds has been shown to eliminate enteric CH₄ [17]. More than 21 seaweeds have been shown to reduce CH₄ emissions in vitro [18;20]. They are usually added as dry powders and included in the diet at levels ranging from 0.1 to 1.5% of the feed. Among them, A. taxiformis was highly effective in decreasing the production of CH₄ with a reduction of 99% at doses as low as 2% organic matter [16]. This species is found somewhere in the coastal zone, especially on St. Martin Island, but its collection is not easy from nature, and it is not cultivated artificially yet [21]. Our experimental seaweed is G. tenuis-tipitata var. Liui due to its availability both naturally and artificially. And it was seen that ambient CH₄ emissions were reduced by using this seaweed. Measuring how much CH₄ is made on a farm is not the same as measuring how much CH₄ is breathed out by livestock, but it does give an idea.

This sensor-based detection method measures CH₄ gas leaks in the industry from a safe distance [22]. Because temperature, humidity, cleanliness, and other environmental variables impact ambient CH₄, some changes in the average daily observations may be noticed by viewing Figures 2 and 3. Seaweed must have a nutritional flavor that will improve its production, but it will take a long time to observe. Our primary objective was to see the anti-methanogenic properties of this seaweed, so the production improvement by feeding seaweed was somehow ennobled. However, parameters like body temperature, milk production, and average weight gain were observed to see if there were any negative effects on health and production [23]. But in our trial, we did not find any significant difference between the trial and the control. Some important features like microbial impact, digestibility, and hematological features should be monitored, but it requires more budgets. Through these trials, it can be concluded that G. tenuis-tipitata var. Liui has anti-methanogenic properties and is capable of reducing enteric CH₄. Further research could be conducted to determine the precise amount of CH₄ reduction and other health benefits in livestock. Other major features, such as production impact, rumen microbial change, manure analysis for digestibility, and so on, were not given much attention because our key objective was to look at the CH₄ reduction potential of this species of seaweed.

Conclusion

Gracilaria tenuis-tipitata var. Liui has anti-methanogenic properties that may be used in the livestock industry of Bangladesh, and it could be a nature-based solution to reduce greenhouse gas emissions, especially enteric CH₄.

Acknowledgments

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List of Abbreviations

CH₄, Methane; CP, Crude Protein; DM, Dry Matter; EE, Ether Extract; FAO, The Food and Agriculture Organization; GHG, Green House Gas; IoT, Internet of Things; ISO, International Organization for Standardization; LED, Light Emitting Diode; NDF, Neutral Detergent Fiber; PPM, Parts per million; R&D, Research and Development; QC, Quality Control.

Conflict of interest

The authors have declared that no competing interests exist.

Author contributions

Conceptualization: MSAS and MSA; methodology: MSAS, MSA, and HIR; validation: HZI; formal analysis: MSAS and HIR; investigation: MSAS and MRA; resources: MSAS, MSA, and MRA; data curation: MSAS and MSA; writing-original drafting preparation: MSAS, writing review and editing.

References