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A Sustainable Approach to Managing Invasive Macroalgae: Assessment of the Nutritional Profile and the Potential for Enteric Methane Mitigation of *Rugulopteryx okamurae*

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Abstract: The expansion of the invasive Asian macroalgae *Rugulopteryx okamurae* along the coasts of the Azores represents a significant challenge for local marine biodiversity. A promising approach to managing the biomass produced by this alien alga is to valorize it in the context of the blue economy. This study characterizes and evaluates the potential of *R. okamurae* biomass for incorporation into cattle feed, with a focus on mitigating enteric methane production. The nutritional value of *R. okamurae*, its digestibility, and its potential as a mitigating agent for enteric methane production were analyzed in vitro. The results indicate that the inclusion of 5% *R. okamurae* in the diet significantly ($p < 0.05$) reduced accumulated methane production by 98% after 24 h of incubation. The addition of 1% algae over the same period resulted in a 38% reduction in methane production. However, a significant decrease ($p < 0.05$) in gas production of 57.02% and 73.5% was also observed in relation to control, with the inclusion of 1% and 5%, respectively, during 96 h. Nutritionally, *R. okamurae* was found to have a crude protein content of 18.68% and fiber (NDF) of 55.71% of DM. It is also worth highlighting the high content of ash (31.86%) that was identified in these brown macroalgae. In conclusion, the fresh biomass of *R. okamurae* could serve as a functional ingredient in cattle feed to mitigate enteric methane production, provided it is used in low percentages. However, it is important to emphasize that high concentrations in the first 12 h did not produce methane, which is also not recommended for enteric fermentation. However, before including it in animal feed, in vivo tests are needed to assess its toxicity.

Keywords: enteric fermentation; methane reduction; ruminants; animal supplement; blue economy; Azorean Sea

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Citation: Nunes, H.P.B.; Maduro-Dias, C.; Carvalho, J.; Borba, A. A Sustainable Approach to Managing Invasive Macroalgae: Assessment of the Nutritional Profile and the Potential for Enteric Methane Mitigation of *Rugulopteryx okamurae*. *Oceans* **2024**, *5*, 662–671. https://doi.org/10.3390/oceans5030038

Academic Editors: Haohao Wu, Yanbo Wang and Na Sun

Received: 2 August 2024
Revised: 28 August 2024
Accepted: 5 September 2024
Published: 10 September 2024

1. Introduction

Ruminant production is essential for ensuring global food security, as these animals are utilized by all economic and social demographic groups. They are also unique in their ability to convert inedible or low-nutritional-value feeds into high-quality proteins for humans [1]. However, ruminants contribute to approximately 16% of global methane emissions, primarily through enteric fermentation. Within the agricultural sector, livestock account for 73% of methane emissions, with beef and dairy cattle representing the largest share (35% and 30%, respectively), followed by small ruminants and buffaloes (15%) [2]. Consequently, ruminant production is under increasing pressure to mitigate these emissions and meet the ambitious target of zero greenhouse gas emissions (GHG) by 2050, aimed at limiting global temperature increases to 1.5 °C above pre-industrial levels [3]. In addition to the adverse environmental impacts, methane emitted by ruminants represents a loss of energy from the feed they consume, ranging from 2% to 12%, depending on various factors, including the type of feed ingested [2,4]. Researchers have been studying various strategies to reduce methane emissions without negatively impacting animal production [5]. Recently, significant attention has been given to seaweed as a nutritional strategy to mitigate enteric methane production [6,7]. Advances have been made in identifying algae species that can reduce methane production by 12–95%, depending on factors such as the type, species, and inclusion rate of the algae in the diet [8–11]. However, due to the potential toxicity of some algae, it is essential to ensure their safety before introducing them as supplements in ruminant diets to maintain the health, welfare, and productivity of the animals. Currently, *Rugulopteryx okamurae*, a brown macroalgae native to East Asia (China, Japan, Korea) [12], was first identified in Europe in 2002 in Lake Thau, France [13]. Since then, the algae have spread to the French coast [14], across the Mediterranean, especially in the south of the Iberian Peninsula and the Strait of Gibraltar [15,16], and across the North Atlantic, including the Azores archipelago [17]. This alga has caused major ecological impacts, including the reduction of marine biodiversity and the accumulation of substrates in the intertidal zones of rocks and beaches [18], where thousands of tonnes of biomass have been extracted, causing serious implications for local fishing activity, tourism, and public health [19–21].

R. okamurae may be a promising source of polyphenolic compounds, called phlorotannins (PT), which have been identified as the main anti-methanogenic compounds in brown algae [22]. However, brown algae exhibit high intraspecies variability in their chemical composition, influenced by factors such as seasonality, geographical variation, light, temperature, nutrient availability, and salinity [23]. This variability may explain the inconsistency in the literature regarding the effects of brown algae on methane production [22]. Consequently, there is a need for more exploratory studies on the methane-mitigating properties of these algae, considering the potential benefits of utilising the biomass produced by these algae for environmental sustainability in ruminant production.

To our knowledge, there are no published studies on the nutritional value of *R. okamurae* or its potential as a mitigating agent in the production of enteric methane when added to the diets of the ruminants. Therefore, the aim of this preliminary study was to determine the nutritional value of *R. okamurae*, as well as its digestibility. Additionally, we intended to evaluate the in vitro effects of this brown macroalgae on gas and methane production, using low and high concentrations of *R. okamurae* in a basal diet for dairy cows.

2. Materials and Methods

2.1. The Experimental Design

Three samples of *R. okamurae* were collected to determine their chemical and biological composition in the laboratory and to measure the production of total gas and enteric methane over 96 h. An in vitro anaerobic fermentation system was employed to simulate rumen fermentation and assess the effect of *R. okamurae* on enteric methane production. The base substrate used in the fermenters consisted of a cattle feed common in the Azores. This basal feed comprised 50% local pasture with a floristic composition of 80% perennial ryegrass (*Lolium perenne*) and 20% clover pasture (*Trifolium repens*). In addition to the pasture, the basal feed included corn silage (25%), grass silage (20%), and concentrate (5%). The chemical composition of the substrate used is presented in Table 1, using the methodology described in Section 2.3.

Table 1. Nutritional composition of basal diet (substrate).

	DM (%)	CP (%DM)	NDF (%DM)	ADF (%DM)	ADL (%DM)	EE (%DM)	Ash (%DM)	DMD (%)	OMD (%)
Basal Diet	10.35	19.79	64.14	33.9	3.34	1.09	14.56	76.03	72.41

Abbreviations: DM—dry matter; CP—crude protein; NDF—neutral detergent fiber; ADF—acid detergent fiber; ADL—acid detergent lignin; EE—ether extract; DMD—dry matter digestibility; OMD—organic matter digestibility.

All samples were analyzed at the Animal Nutrition and Feed Laboratory of the University of the Azores. Two levels of *R. okamurae* supplementation were tested:

- Low level: A1% (1% DM, of the total dry matter of the basal diet);
- High level: A5% (5% DM, of the total dry matter of the basal diet).

The results from these diets were compared to a control diet without *R. okamurae* added. Each test was conducted in triplicate for each *R. okamurae* sample.

2.2. Seaweed Collection

Three distinct samples of the algae *R. okamurae* were collected in December 2023 from three different areas within the Prainha bathing area (38°39′13.5″ N; 27°13′11.3″ W), located in Angra do Heroísmo, Terceira, Azores, Portugal. Approximately 1.5 kg of biomass was collected from each site. The samples were immediately transported to the laboratory, where they were carefully washed with fresh water to remove any sand and other impurities. The samples were then left to drain for 30 min to eliminate excess water. After draining, the biomass was dried in a forced-air oven (Thermo Fisher Scientific, Model XYZ123, Waltham, MA, USA) at 65 °C until a constant weight was achieved, ensuring complete moisture removal.

2.3. Determination of Chemical Parameters of R. okamurae and Basal Diet

To determine the chemical parameters, the sample was dried at 65 °C in an oven with forced air circulation until a constant weight. Subsequently, it was ground using a Retsch mill with a 1 mm sieve. The Weende scheme [24] was used to determine the dry matter (DM, method 930.15), crude ash (CA, method 942.05), ether extract (EE, method 920.39), and crude protein (CP, method 954.01) using the Kjeldahl method. Neutral detergent fiber (NDF), acid detergent fiber (ADF), and acid detergent lignin (ADL) were measured using the methods proposed by Goering and Van Soest [25].

2.4. Determination of Biological Parameters of R. okamurae

The in vitro digestibility of dry matter (DMD) and organic matter (OMD) was determined according to the method described by Tilley and Terry [26], with modifications proposed by Alexander and McGowan [27].

Ruminal fluid used in these determinations was collected following the methodology described by Borba et al. [28] under controlled conditions at a local slaughterhouse.

For each experiment, ruminal fluid was obtained immediately after the slaughter of five healthy dairy cattle (*Holstein* breed), aged between 3 and 5 years, with an average body weight of 550–650 kg. The animals were fed a diet primarily composed of fresh ryegrass (*Lolium multiflorum*) and corn silage, supplemented with a mineral mix to ensure a balanced intake of nutrients. To minimize changes in microbial activity, the ruminal fluid was collected within 10 min post-slaughter [10].

Immediately after collection, the fluid was filtered through four layers of cheesecloth to remove large particles, ensuring a homogeneous sample. It was then transferred into pre-warmed, airtight containers maintained at 39 °C to preserve anaerobic conditions. The fluid was delivered to the animal nutrition laboratory within 30 min of collection to maintain microbial viability and activity for the subsequent fermentation and digestibility experiments.

2.5. Measuring Gas and Methane Production In Vitro

To assess the inhibitory potential of *R. okamurae* on gas production, a hermetically sealed gas production fermentation system was employed, closed with an agitation device, following the methodology described by Nunes et al. [8]. Briefly, in vitro tests were conducted with three different treatments: Control (no algae added); A1% algae; and A5% algae, measuring volumes of total gas and methane. Each trial was replicated three times to ensure statistical precision. Rumen fluid was diluted at a 1:2 (v/v) ratio in buffer solution, with blank samples included to control for rumen variability. Total gas volume was directly measured using a MilliGascounter, while methane volume was determined after CO_2 absorption in a NaOH solution, also using the MilliGascounter. Bioreactors were maintained at 38 °C for 96 h. All data were recorded and automatically processed using specific software (Rigamo v3.1).

2.6. Statistical Analyses

All statistical analyses were performed using *IBM SPSS Statistics* for Windows, Version 27.0. IBM Corp (Armonk, NY, USA). The data were assessed for normality to meet ANOVA assumptions. The results presented include least squares means, standard errors of the means, and *p*-values for the variables analyzed. Significance was tested at $\alpha = 0.05$ to determine the effects of *Rugulopteryx okamurae* addition on substrate gas production, with results considered significant if $p < 0.05$.

3. Results

3.1. Chemical and Biological Composition of *Rugulopteryx okamurae*

The values obtained for the chemical and biological composition of *R. okamurae* are shown in Table 2. The dry matter values range from 9.86% to 10.46%, with an average of 10.11 ± 0.31% and a coefficient of variation of 3.11%, indicating that the results are consistent. In terms of protein content, the variability of the results was moderate, as the CV was 6.97%, yielding an average crude protein value of 18.68 ± 1.30%. Regarding the fiber fractions (NDF, ADF, and ADL), which allow us to assess the quality of the fiber in the seaweed, we found that the average NDF value was 55.71 ± 1.46% of DM, while the average ADF value was 43.28 ± 1.59%, with both parameters showing a low coefficient of variation. The ADL results showed moderate variability (6.29%), with the average value obtained being 18.62 ± 1.17%. The EE content varied between a minimum of 1.64% and a maximum of 1.82%, with the average being 1.68 ± 0.06%. Finally, the crude ash content showed an average of 31.86 ± 3.04% and a high variation coefficient (9.53%), indicating considerable variability in crude ash.

Table 2. Nutritional value of the *Rugulopteryx okamurae*.

	Minimum	Maximum	Mean	Standard Deviation	Coefficient of Variation
Dry Matter (%)	9.86	10.46	10.11	0.31	3.11
Crude Protein (%DM)	17.42	20.02	18.68	1.30	6.97
NDF (%DM)	54.03	56.67	55.71	1.46	2.62
ADF (%DM)	41.96	45.04	43.28	1.59	3.66
ADL (%DM)	17.41	19.75	18.62	1.17	6.29
EE (%DM)	1.64	1.82	1.68	0.06	3.37
Ash (%DM)	29.87	35.36	31.86	3.04	9.53
DMD (%)	16.29	17.65	16.60	0.93	5.60
OMD (%)	9.21	9.76	9.47	0.27	2.85

Abbreviations: DM—dry matter; CP—crude protein; NDF—neutral detergent fiber; ADF—acid detergent fiber; ADL—acid detergent lignin; EE—ether extract; DMD—dry matter digestibility; OMD—organic matter digestibility.

In terms of biological parameters, we found that DMD was higher than OMD, as expected (Table 2). On average, DMD showed low digestibility at 16.60 ± 0.93%. OMD ranged from 9.21% to 9.76%, with an average of 9.47 ± 0.27%. The CV of DMD was 5.60%, indicating considerable variability in dry matter digestibility, which was not the case with OMD, where a coefficient of variation of 2.85% was recorded.

3.2. Total Gas and Methane Production

Total gas production in the control group increased consistently over time, with a cumulative value always higher than that of the A1% and A5% treatments (Table 3).

Table 3. Total gas production (ml g^{-1} DM).

Treatment	6 h	12 h	24 h	48 h	72 h	96 h
Control	29.59 ^{a}	51.95 ^{a}	99.03 ^{a}	147.89 ^{a}	154.53 ^{a}	155.25 ^{a}
A1%	22.18 ^{a,b}	25.35 ^{b}	46.59 ^{b}	66.36 ^{b}	66.49 ^{b}	66.72 ^{b}
A5%	17.68 ^{b}	26.18 ^{b}	40.6 ^{b}	40.65 ^{c}	40.77 ^{c}	40.99 ^{c}
SEM	3.69	2.79	2.93	2.87	1.84	1.99
p-value	0.023	0.002	<0.001	<0.001	<0.001	<0.001

Abbreviations: A1%—1% DM, of the total dry matter of the base diet; A5%—5% DM, of the total dry matter of the base diet. ^{a,b,c} Values within a row with different superscripts differ significantly at $p < 0.05$.

The differences were statistically significant ($p < 0.001$), except at 6 h, where no statistical differences ($p > 0.05$) were observed between the control and A1%.

The addition of 1% of the algae *R. okamurae*, after 24 h of incubation, resulted in more than a 50% reduction in total gas production compared to the control group, with a maximum reduction of 57% at 96 h of incubation. In the A5% treatment, the differences from the control group were even greater than those observed in A1%. At 6 h after incubation, the algae reduced the total volume of gas produced by the base diet by 40%. At 24 h, we can see that the reduction in the total volume of gas was around 60%. However, after 48 h of incubation, the reduction was around 70%. Regarding the methane production, results are shown in Table 4. The control exhibited the highest methane production and was statistically different ($p < 0.05$) from the A5% treatment throughout the trial. It should be noted that in treatment A5%, methane production was not produced in the first 12 h, i.e., there was a 100% reduction in methane compared to the control group.

Table 4. Total methane production (ml g^{-1} DM).

Treatment	6 h	12 h	24 h	48 h	72 h	96 h
Control	12.28 ^{a}	20.78 ^{a}	29.02 ^{a}	37.62 ^{a}	39.16 ^{a}	40.5 ^{a}
A1%	4.53 ^{a,b}	13.91 ^{b}	23.86 ^{a}	23.87 ^{b}	24.24 ^{b}	24.76 ^{b}
A5%	0 ^{b}	0 ^{c}	0.32 ^{b}	0.64 ^{c}	0.96 ^{c}	0.96 ^{c}
SEM	0.18	0.25	0.29	0.30	0.87	0.98
p-value	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

Abbreviations: A1%—1% DM, of the total dry matter of the base diet); A5%—5% DM, of the total dry matter of the base diet. ^{a,b,c} Values within a row with different superscripts differ significantly at $p < 0.001$.

Methane production in the A5% treatment was only detected after 24 h of incubation, producing 0.32 mL·g^{-1} DM, and stabilized at 72 and 96 h with a production of 0.96 mL·g^{-1} DM. However, in the A1% treatment, although the methane values obtained were always lower than those of the control group, there were only statistical differences ($p < 0.05$) at 12 h, 48 h, 72 h, and 96 h, reducing the methane produced by more than 30% at 12 h, and by more than 38% after 72 h.

4. Discussion

4.1. Chemical Composition of Rugulopteryx okamurae

The analysis of the chemical composition of *Rugulopteryx okamurae* has revealed that this alga possesses a suitable nutritional profile for dietary supplementation, with significant levels of crude protein and fiber. The dry matter of brown algae is composed of various components, among which fiber stands out [29]. The obtained data indicate that parameters such as dry matter, NDF, and ADF show little variation among samples, reflecting consistency in their properties. The fiber content of *R. okamurae* was determined based on NDF, ADF, and ADL, indicating the content of structural fibers present in the algae. The fiber levels of the studied macroalgae ranged from 54.03% to 56.67% for NDF and 41.96% to 45.04% for ADF. The average values obtained for dry matter, NDF, and ADF are consistent with the values referenced in the literature for other brown macroalgae [30,31]. Structural carbohydrates such as cellulose and hemicellulose of macroalgae vary according to the growth phase [32]. However, since the macroalgae were all collected at the same growth phase, the fiber levels of this macroalgae, particularly the structural carbohydrates, showed minimum variation, providing a stable ruminal environment, which is fundamental for ruminant digestion [33]. The high levels of lignin (ADL) present in this macroalgae can reduce digestibility due to the complex and resistant structure of lignin. However, this is a relatively new area of research, given the uniqueness of seaweed carbohydrates, which are slightly different compared to terrestrial plants, with still a limited amount of work done to date on the application of seaweed fibers in ruminant diets.

The crude protein (CP) content of the *Rugulopteryx okamurae* samples ranged from 17.42% to 20.02%. These values are higher than the ranges observed in other brown algae (5 to 10%) [34], such as *Laminaria digitata*, which generally has a lower crude protein content in the range of 12–15% [35], indicating that this alga could serve as a protein source for ruminants, like other macroalgae found in the Azores Sea [8]. The levels of crude protein found in various macroalgae in the Azores Sea may be primarily associated with factors such as species, growth conditions, water salinity, water temperature, or even processing methods [36,37]. It is noteworthy that the algae in this study originated from wild populations of marine macroalgae, collected when these algae washed ashore in December. However, according to Campbell et al. [38], the ideal period, in terms of peak CP content, for brown macroalgae for use in animal diets is between January and April, but further studies are needed to assess the seasonality of the biological availability of proteins in *Rugulopteryx okamurae* in ruminant diets. The high ash content (31.86% DM) suggests that this macroalgae could be an important source of minerals. However, the ash content showed considerable variability (CV = 9.53%) among the collected samples, which may be attributed to several factors that could significantly influence further analyses. Firstly, the sampling site was a sandy area, and although the algae were thoroughly washed to minimize contamination, it is possible that fine sand particles adhered to the surface of the algae, particularly in crevices or irregular surfaces, were not completely removed. This residual sand could artificially elevate the ash content, introducing variability that might obscure the true mineral composition of the algae itself. Studies have shown that algae washed up on the beach tend to accumulate more minerals due to the deposition of extraneous materials, such as sand, compared to those attached to natural substrates [39]. Secondly, the presence of *Spirorbis borealis*, small tube-building organisms that use the algae as a transport platform, could also contribute to the variability in ash content. The exoskeletons of these organisms are composed of calcium carbonate, a mineral that significantly adds to the ash content when the algae are analyzed. If the density of *Spirorbis borealis* varies among the samples, it could lead to inconsistent ash content readings, further complicating the assessment of the algae's mineral profile.

Regarding the biological parameters, we observed that the DMD and OMD of this macroalgae are low, which compromises its use in ruminant diets [40]. Additionally, the potential of *R. okamurae* as a protein source for ruminants, as previously mentioned, is also limited due to the low digestibility of this macroalgae [36]. Vizcaíno et al. [41] also report that this macroalgae has low digestibility, but it is promising as a functional ingredient in aquatic feeds for European seabass, when pre-treated to improve nutrient availability and reduce harmful metabolites.

4.2. Addition of Rugulopteryx okamurae to Total Gas and Methane Production In Vitro

The addition of *Rugulopteryx okamurae* to the feed substrate simulating the diet of dairy cows (control) resulted in a reduction in total gas production at concentrations of 1% and 5%, compared to the data obtained in the control group. The obtained data indicate a noticeable overall decrease in ruminal fermentative activity, possibly due to the interference of bioactive compounds from *Rugulopteryx okamurae* with the ruminal microbiota [42]. This significant reduction ($p < 0.05$) in total gas production is a negative indicator, suggesting that this alga may decrease microbial activity in the rumen, thus reducing the efficiency of the digestive process in ruminants. Other studies with brown algae, such as *Sargassum* *fusiforme*, also indicate reductions in total gas production when added to animal diets, but the effects are less pronounced than those observed with *R. okamurae* [43], suggesting this seaweed possesses unique characteristics that affect fermentation. Regarding the effect of *Rugulopteryx okamurae* addition on methane production, we observed a reduction in methane production, especially at higher concentrations (5%), which eliminated methane production during the first 12 h of incubation and maintained very low levels over the 96 h incubation period. When the alga was added at the lower concentration (1%), there was a reduction in methane production, but it was not as intense as when the higher concentration was added. This result is consistent with other studies [18,44,45], suggesting that these bioactive compounds present in brown marine macroalgae can inhibit the activity of methanogenic archaea (microorganisms responsible for methane production in the rumen). This methane reduction can be attributed to the presence of polyphenolic compounds called phlorotannins (PT). These have been proposed as the main antimethanogenic compounds in brown algae [22]. PTs are analogous to condensed tannins found in terrestrial plants, but little is known about their properties as they have not been extensively studied [46]. However, some studies on brown algae have shown that extracts of the polyphenolic fraction possess direct inhibitory actions against microbial activity associated with methane production, as well as indirect anti-methanogenic effects by forming complexes with nutrients (i.e., proteins and carbohydrates) in ruminant diets [42,45]. Furthermore, *Rugulopteryx okamurae* exhibited a high inorganic content, which may have an indirect adverse effect on methane production in the rumen [31], interfering with the anaerobic fermentation process [47]. The mechanism of action in brown algae for the inhibition of methanogens differs from that observed in red algae, where the main methane mitigating properties derive from the presence of another group of secondary metabolites, namely halogenated compounds such as bromoform [22]. The results clearly showed that the addition of *Rugulopteryx okamurae* at 1% and 5% of DM influences ruminal fermentation, with a reduction in total gas production and enteric methane production.

5. Conclusions

Rugulopteryx okamurae, an invasive brown macroalga in the Azores Sea, showed a nutritional profile suitable to become a potential protein source for ruminant feed. It also demonstrated consistency in parameters such as NDF and ADF, reflecting a stable content of structural fibers, essential for ruminant digestion. However, the high lignin content limited the digestibility of dry matter and organic matter, which constrains the direct application of this alga in ruminant diets. The addition of *Rugulopteryx okamurae* to a base diet for ruminants resulted in a significant reduction in total gas and methane production *in vitro*. This decrease in ruminal fermentation and methane production highlights the potential of *Rugulopteryx okamurae* as a functional supplement to mitigate enteric methane emissions, contributing to more sustainable animal feeding practices. Thus, despite the challenges related to digestibility, *Rugulopteryx okamurae* shows promise as a dietary supplement, particularly due to its high protein content and ability to reduce methane production.

Future studies should focus on the toxicity of this alga and processing methods that increase digestibility, as well as evaluating the efficacy and safety of its prolonged use in ruminant diets. Additionally, research should consider its application under different seasonal and environmental conditions to ensure the viability of *Rugulopteryx okamurae* as a strategic dietary supplement to mitigate enteric methane production.

Author Contributions: Conceptualization, H.P.B.N. and A.B.; Formal analysis, C.M.-D. and J.C.; Investigation, H.P.B.N., C.M.-D. and J.C.; Data curation, H.P.B.N.; Writing—original draft, H.P.B.N.; Writing—review & editing, C.M.-D. and A.B.; Supervision, A.B. All authors have read and agreed to the published version of the manuscript.

Funding: This work was supported by the project "ROKAMURAE AZ—Estudo Multidisciplinar para o Conhecimento da Alga Invasora *Rugulopteryx okamurae* na Região Autónoma dos Açores".

Institutional Review Board Statement: The ethical approval was waived because the ruminal fluid mentioned in Section 2.4 was obtained from healthy cows that were already present at a local slaughterhouse for consumption purposes. These cows were not slaughtered specifically for the experiment; the ruminal fluid was simply utilized for the study.

Data Availability Statement: Data are contained within the article.

Conflicts of Interest: The authors declare no conflicts of interest.

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