

Article

In Vitro Fermentation Characteristics of Pelagic Sargassum for Inclusion in Integral Diets for Ruminants

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Abstract

Pelagic *sargassum* arriving in the Mexican Caribbean is a mixture of brown macroalgae containing polysaccharides, minerals, and secondary metabolites with potential in ruminant diets. The objective of the present study was to evaluate the effect of the inclusion of sargassum in integral diets (ID) on in vitro fermentation characteristics. A completely randomized design was used. The treatments were different levels of sargassum (ICD: 0%, ID10: 10%, ID20: 20% and ID30: 30%) added to a basal substrate (a mixture of *Pennisetum purpureum* Vc. CT-115 hay, corn, soybean, and molasses). Rumen fluid was obtained from five male lambs with a body weight of 40 ± 3 kg. In vitro gas production (IVGP) as well as dry matter degradability (DMD) and organic matter degradability (DOM) increased linearly ($p < 0.0001$) as the proportion of sargassum increased at 24, 48, and 72 h. Rumen fluid pH decreased ($p < 0.05$) with 30% inclusion at 48 h, while protozoan concentration was similar ($p > 0.05$) in all treatments with respect to the control at all evaluation times. These results indicate that the inclusion of pelagic *sargassum* in integral concentrated diets improves fermentative parameters, and its inclusion in diets for ruminants is feasible. This opens up a window of opportunity for its study as a novel additive or unconventional supplement. However, in vivo studies are necessary to rule out harmful effects on animal health and performance.

Keywords: brown algae; secondary metabolites; ruminants; integral diets; fermentation parameters



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1. Introduction

In some parts of the world, several species of seaweeds have been used as feed for farm animals because of their high nutritional value and their beneficial properties on health, yield, and quality of derived products [1,2]. However, despite being an abundant resource and not competing for water and soil with conventional crops used as raw material in

the preparation of diets, they have been little studied [3]. In this regard, every year large amounts of pelagic sargassum (a mixture of brown algae, mainly: *Sargassum natans* and *Sargassum fluitans*) arrive on the coasts of the Mexican Caribbean from West Africa [4], which represent a threat to coastal ecosystems, tourism, and the local and national economy [5–7]. Further, the annual cost of removing sargassum from beaches is more than \$300,000 per kilometer of beach [8–10].

Brown seaweeds are rich in macro and micro minerals (Ca, P, K, Mg, S, Fe, I, Cu, Mn and Se, mainly) highly bioavailable to ruminants compared to inorganic sources [11]; they contain polysaccharides and polyphenolic compounds such as phlorotannins and other molecules that can modify the ruminal microbiome helping to reduce CH₄ emissions [1,11–17]. The Phlorotannins selectively affect the abundance of cellulolytic bacteria and reduce methanogenic archaea without compromising feed fiber utilization [18,19]. Consequently, the production of volatile fatty acids (VFA), DMD, and DOM increases due to a redirection of H₂ to other metabolic pathways [19,20]. Therefore, the management and sustainable use of sargassum as a potential raw material in diets for ruminants would help to reduce its negative impact on the economy and the environment in the region [8,21,22].

Among the limitations of the use of pelagic sargassum in ruminant feed are: (a) its low DM (10 to 12%) and OM content (50 to 60%) and a high concentration of minerals that reaches 40% of the DM and (b) the presence of heavy metals, among which arsenic (As) stands out with levels higher than 500 mg kg^{−1} DM [23–25]. However, the concentration of As in algae can be reduced by more than 50% using techniques involving hot water, citric acid, and fermentation with lactobacillus, although secondary metabolites are also altered [26]. According to in vitro studies with other algae species, it is feasible to include high levels in concentrated diets without negative effects on ruminal fermentation [20,27]. Based on the above, we hypothesize that the inclusion of pelagic sargassum as part of concentrated diets for ruminants improves the in vitro fermentation parameters, while also revealing the extent to which brown algae could improve animal productivity. Thus, the objective of this study was to determine the effect of the inclusion of sargassum in integral diets on in vitro fermentation parameters, in order to evaluate its potential in ruminant diets.

2. Materials and Methods

2.1. Location

The study was carried out in the laboratory of Advanced Agroecosystems Studies of the Technological Institute of the Mayan Zone, Quintana Roo, Mexico, located at coordinates 18°30′57.20″ N 88°29′21.04″ W, at 7 masl [28], with an Aw₁ climate [29].

2.2. Animal Management

The management and care of the animals were according to the Mexican Official Standard-062-Z00 1999 [30], and according to the animal handling and welfare standards of the Technological Institute of the Mayan Zone. Five hair lambs weighing 35 ± 3 kg body weight were used as rumen fluid donors, under grazing conditions on star grass (*Cynodon nlemfuensis*); after grazing, they were housed in a pen (5 × 5 m) where they had free access to water and 200 g of commercial “Engorda Ovinos” feed (Campi[®], Yucatan, Mexico city, Mexico; 15% CP) per animal/day. The lambs were dewormed with Closantel 50 mg/mL (5% Closantel[®], Wyeth LLC, Madison, NJ, USA) at a dose of 10 mg kg^{−1} body weight.

2.3. Sample Preparation

The basal substrate used for the in vitro trial was an integral diet for growing sheep according to NRC [31], based on corn, soybean, Taiwan grass hay (*Pennisetum purpureum* Vc. CT-115,

harvested at 60 days of regrowth) and molasses with different levels of *sargassum* inclusion: 0/100 (ICD: 0%), 10/90 (ID10: 10%), 20/80 (ID20: 20%) and 30/70 (ID30: 30%), formulated to contain the same concentration of CP (12%, respectively). The chemical composition of the mixtures is presented in Table 1. The *sargassum* was collected in May of 2023 on the coast of Quintana Roo, Mexico, located at coordinates 18°57'55.93" N latitude and 87°36'43.72" W longitude. Once collected, the *sargassum* was partially sundried for 8 h and subsequently dried in a forced-air oven at 60 °C for 48 h. The CT-115 grass hay, corn, soybean, and *sargassum* were ground to a particle size of 1 mm using a Wiley mill (Thomas Wiley® Laboratory Mill, Swedesboro, NJ, USA).

Table 1. Ingredients and chemical composition of the dietary treatments.

Components	Sargassum	Treatments (Inclusion Level of Sargassum)					
		ICD	ID10	ID20	ID30	SEM	p Value
Ingredients and levels of inclusion (%)							
CT-115 grass		64.2	47.95	31.7	15.46		
Corn		22.27	26.67	31.07	35.47		
Soybean		3.54	5.38	7.23	9.07		
Molasses		10	10	10	10		
Sargassum		0	10	20	30		
Chemical composition (%)							
DM	94.11	94.05 ^a	93.52 ^a	92.62 ^a	91.42 ^a	0.440	0.0834
OM	58.89	90.09 ^d	88.48 ^c	86.62 ^b	83.93 ^a	0.170	0.0005
CP	6.73	12.00 ^a	11.99 ^a	12.05 ^a	12.00 ^a	0.070	0.7134
aNDF	45.04	52.04 ^c	42.97 ^b	37.06 ^a	32.35 ^a	0.840	0.0016
ADF	30.93	31.94 ^c	26.19 ^b	23.03 ^b	19.02 ^a	0.510	0.0013
Lignina (sa)	5.32	2.06 ^a	2.24 ^a	2.47 ^a	2.49 ^a	0.300	0.8144

Different letters in the same row indicate significant differences ($p < 0.05$). SEM: standard error of the means; n = 3. Treatments: ICD = Control (integral diet); ID10 (integral diet with 10% *sargassum*); ID20 (integral diet with 20% *sargassum*); ID30 (integral diet with 30% *sargassum*); DM: dry matter; OM: organic matter; CP: crude protein; aNDF: neutral detergent fiber assayed with a heat stable amylase and expressed inclusive of residual ash; ADF: acid detergent fiber expressed inclusive of residual ash; lignin (sa): determined by solubilization of cellulose with sulphuric acid.

2.4. In Vitro Trial and Experimental Design

Ruminal fluid (solid and semisolid phases) was obtained through an esophageal probe prior to morning grazing [32] and conserved at 39 °C in a thermos to transport it to the laboratory, where it was filtered in four layers of gases and saturated with CO₂. The inoculum was prepared by mixing the rumen fluid with a buffer solution in a ratio of 10:90 [33]. Once the inoculum was prepared, 90 mL was added to 48 amber flasks with a capacity of 120 mL in which 1 g of substrate had been previously placed. In addition, 24 flasks were used as blanks (only with ruminal fluid) to correct the gas production.

A completely randomized design with four treatments, four replicates (n = 4), and three incubation periods (24, 48, and 72 h) was used, for a total of 12 amber flasks per treatment. In vitro gas production (IVGP) was recorded at 0, 3, 6, 9, 12, 18, 24, 30, 48, and 72 h of incubation.

2.5. In Vitro Gas Production

Volume of gas was measured according to the procedure proposed by Theodorou et al. [34]. To measure pressure changes, a pressure transducer was used. The kinetics of gas production were evaluated using the Gompertz model [35]:

$$Y = A \exp \{-\exp [1 + be/A (LAG - t)]\},$$

where:

Y = total production of gas accumulated (mL);

A = theoretical maximum gas production (mL);

b = maximum rate of gas production (mL/h), which occurred at the point of inflection of the curve;

LAG = lag time (h), defined as the intercept of the time-axis of the tangent line at the point of inflection; and

t = time.

The parameters a, b, and LAG were estimated using a non-linear regression analysis, for which the Origin Pro 8 program was used. These parameters were used to evaluate the kinetics of in vitro gas production according to the methodology described by Machado et al. [36].

2.6. Fermentation Parameters

The pH and the population of protozoa in rumen fluid were determined at 24, 48, and 72 h of incubation, while in vitro DMD and DOM were evaluated at 24, 48, and 72 h of incubation. The pH was determined with a multiparametric pH edge[®] meter (Products HANNA[®] instruments, Mexico city, Mexico). For protozoa count, the technique proposed by Ogimoto and Imai [37] was used; 2 mL of the inoculum obtained after 24, 48 and 72 h of incubation was mixed with 2 mL of methyl-green formalin saline (MFS); 1 mL of the solution was centrifuged at 2500 rpm for 15 min; the supernatant was removed and 1 mL of distilled water was added to the precipitate; the mixture was shaken and a drop was placed in each compartment of the Neubauer chamber (Fuchs-Rosenthal; manufactured by Hausser Scientific, Horsham, PA, USA). Each sample of each replicate (n = 4) was counted in triplicate (a total of 12 counts per treatment) using an optical microscope (Olympus CX21, Shinjuku-ku, Tokyo, Japan) with a 40× objective lens. To estimate the degradability of DM and OM, the formula of Choi et al. [14] was used; after each incubation period, the substrate contained in each amber vial was filtered in nylon bags (made of Dacron[®] with pore size of 45 µm) and washed three times with water and; subsequently the bags were dried in an oven at 60 °C for 48 h to obtain the dry weight of the residual sample. To determine the degradability of OM, the remaining samples of the nylon bags were incinerated in a muffle at 600 °C for 8 h.

2.7. Chemical Analysis

Samples were analyzed according to the procedures described in AOAC [38]; for dry matter (DM) method 934.01 was used, for crude protein (CP) method 954.01, and for ash (AS) method 942.05. Neutral detergent fiber (aNDF: assayed with a heat stable amylase and expressed inclusive of residual ash) and acid detergent fiber (ADF: expressed inclusive of residual ash), and lignin (sa: determined by solubilization of cellulose with sulphuric acid) were determined by sequential procedures according to Van Soest et al. [39] using the ANKOM 2000 fiber Analyzer (ANKOM Technology Corp., Macedon, NY, USA).

2.8. Statistical Analysis

Data were analyzed by one-way analysis of variance (ANOVA) with the PROC GLM procedure of SAS Version 8 [40] for a completely randomized design. In vitro gas production kinetics were analyzed using the Gompertz model [35]. Tukey's test was used for comparisons of means between treatments. The results were considered statistically significant at a value of $p < 0.05$.

3. Results

3.1. Chemical Composition

Differences were observed in the chemical composition of the diets due to the effect of *sargassum* inclusion, where the concentration of DOM (90.09 to 83.93%), NDF (52.04 to 32.35%) and ADF (31.94 to 19.02%) decreased as the level of *sargassum* inclusion increased and vice versa with lignin content (2.06 to 2.49%); however, as expected, no effect on CP and DM content was observed. The decrease in fiber in the substrate was not solely due to the level of *sargassum* inclusion; the high concentration of NDF (69.31%) and ADF (45.35%) in CT-115 grass compared to *sargassum* also had an influence. It can be observed that the inclusion level of the grass decreased from 64.20 to 15.46% as the *sargassum* level increased from 0 to 30% (Table 1).

3.2. Total Gas Production and Characteristics of In Vitro Fermentation

The IVGP increased linearly ($p < 0.0001$) at 24 (from 255.27 to 311.10 mL g⁻¹ DM), 48 (from 342.00 to 389.92 mL g⁻¹ DM) and 72 h (from 416.33 to 493.42 mL g⁻¹ DM) of incubation with *sargassum* inclusion level and the lowest gas generation was found in ICD at all evaluation times (Figure 1). Consequently, DDM and DOM increased linearly ($p < 0.0001$) at 0, 24, 48, and 72 h of incubation (Table 2).

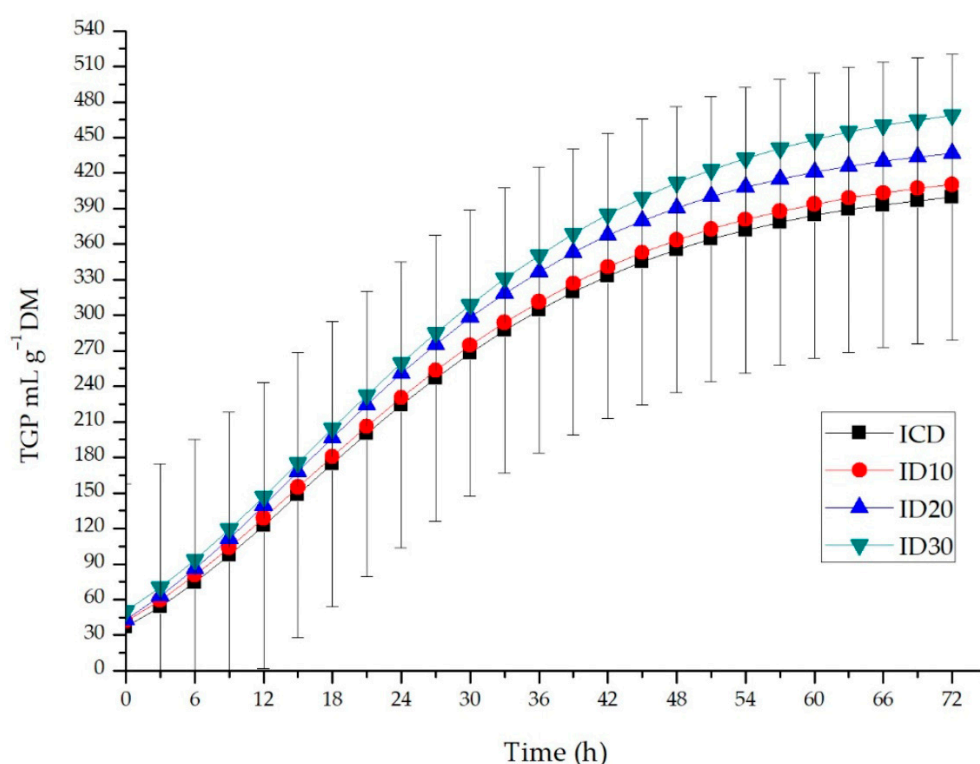


Figure 1. Effect of dosage of *sargassum* on in vitro gas production (IVGP) during 72 h of incubation. TGP: total gas production; ICD: integral diet control; ID10: integral diet with 10% *sargassum*; ID20: integral diet with 20% *sargassum*; ID30: integral diet with 30% *sargassum*.

Table 2. Effect of *Sargassum* inclusion level on in vitro total gas production, dry matter degradability, and organic matter degradability.

Time (h)	Treatments (Inclusion Level of <i>Sargassum</i>)				SEM	<i>p</i> Value
	ICD	ID10	ID20	ID30		
Total gas production (mL g ⁻¹ DM)						
24	255.27 ^c	278.19 ^b	288.81 ^b	311.10 ^a	4.844	<0.0001
48	342.00 ^b	345.21 ^b	375.96 ^a	389.92 ^a	6.382	0.0004
72	416.33 ^c	428.83 ^{bc}	453.62 ^b	493.42 ^a	5.985	<0.0001
DM degradability (g kg ⁻¹)						
0	375.68 ^b	385.96 ^b	471.29 ^a	484.26 ^a	6.282	<0.0001
24	493.39 ^c	519.75 ^b	544.44 ^b	592.57 ^a	6.066	<0.0001
48	509.07 ^c	559.83 ^b	587.54 ^{ab}	613.92 ^a	7.544	<0.0001
72	523.34 ^c	571.97 ^b	594.25 ^b	636.23 ^a	7.484	<0.0001
OM degradability (g kg ⁻¹)						
0	349.29 ^b	344.90 ^b	428.17 ^a	425.86 ^a	6.074	<0.0001
24	468.04 ^c	492.43 ^{bc}	511.82 ^b	553.68 ^a	6.419	<0.0001
48	486.77 ^c	536.07 ^b	556.98 ^{ab}	581.03 ^a	8.390	<0.0001
72	505.55 ^c	551.96 ^b	568.30 ^b	604.97 ^a	7.659	<0.0001

Different letters in the same row indicate significant differences ($p < 0.05$). SEM: standard error of the means; $n = 4$. Treatments: ICD = Control (integral diet); ID10 (integral diet with 10% *sargassum*); ID20 (integral diet with 20% *sargassum*); ID30 (integral diet with 30% *sargassum*).

The pH of the rumen fluid decreased ($p < 0.0010$) linearly at 48 h with *sargassum* inclusion level, and the lowest value was found in ID30 (6.69); while at 24 and 72 h of incubation, there were no differences ($p > 0.05$). Despite the differences in pH values reported in the present study (Table 3), they are within the optimal range (5.66–7.47).

Table 3. Effect of *sargassum* inclusion level on pH and total concentration of protozoa in rumen fluid.

Time (h)	Treatments (Inclusion Level of <i>Sargassum</i>)				SEM	<i>p</i> Value
	ICD	ID10	ID20	ID30		
pH						
24	6.83	6.88	6.93	6.93	0.027	0.0532
48	6.83 ^a	6.89 ^a	6.82 ^a	6.69 ^b	0.026	0.0010
72	6.77	6.83	6.87	6.80	0.033	0.2142
Total protozoa (×10 ⁵ cel/mL) log ₁₀						
24	7.32 ^a	7.03 ^{ab}	6.99 ^{ab}	6.75 ^b	0.109	0.0247
48	6.71	6.59	6.83	6.64	0.120	0.5401
72	6.39	6.24	6.03	5.99	0.135	0.1789
Total Entodinium (×10 ⁵ cel/mL) log ₁₀						
24	4.23	4.14	4.02	3.93	0.076	0.0834
48	3.74	3.69	3.81	3.70	0.086	0.7334
72	3.57	3.42	3.21	3.09	0.144	0.1462
Total Holotrichs (×10 ⁵ cel/mL) log ₁₀						
24	3.09	2.89	2.97	2.82	0.076	0.1289
48	2.97	2.89	3.01	2.94	0.102	0.8630
72	2.82	2.82	2.82	2.89	0.037	0.4262

Different letters in the same row indicate significant differences ($p < 0.05$). SEM: standard error of the means; $n = 4$. Treatments: ICD = Control (integral diet); ID10 (integral diet with 10% *sargassum*); ID20 (integral diet with 20% *sargassum*); ID30 (integral diet with 30% *sargassum*).

3.3. Protozoan Population

The total protozoan population was different ($p < 0.05$) at 24 h of incubation, and the lowest concentration was observed in ID30; while the genera *Entodinium* and *Holotrich* were similar ($p > 0.05$) at all inclusion levels and evaluation times (Table 3).

4. Discussion

4.1. Total Gas Production and Characteristics of In Vitro Fermentation

Total gas production is related to substrate degradability, volatile fatty acid production, and microbial growth in the rumen [41–43]. Despite the differences in pH values reported in the present study, where the lowest pH was observed at 48 h in ID30 (Table 3), these were within the optimal range (5.66–7.47) for optimal microbial growth [44]. This can be attributed to the physicochemical properties of the *Sargassum* genus of algae. The results of chemical analyses of *Sargassum* revealed that it has a low NDF and ADF content. Soluble fiber is fermented rapidly in the rumen, and thus, a large number of organic acids are produced during the decomposition process. In addition, pelagic *sargassum* contains other types of polysaccharides that are highly bioavailable to rumen microorganisms. However, after 72 h, no effects were observed between treatments. This is because ruminal microorganisms need a period of adaptation to the substrates, and once they adapt and increase their population, the rate of digestibility and fermentation efficiency in ruminants increases. These results agree with Canul-Ku et al. [25], who observed a linear decrease in rumen fluid pH at 48 h when evaluating increasing levels of pelagic *sargassum* from 10 to 30% added to Stargrass hay. In contradiction, the addition of *Sargassum fusiforme*, *Sargassum fulvellum*, and *Undaria pinnatifida* at a level of 10% to a Timothy grass hay did not cause changes in rumen fluid pH [14,45,46]. These three species of brown algae, when added at a level of 5% as ethanolic extracts in Timothy grass hay, modified the pH of the rumen liquid at 24 and 72 h of incubation and the highest value was found with *S. fusiforme* [18]; however, when they were included to a better-quality mixture (Timothy grass and corn grain mixture in a 60:40 ratio), no effects were observed although pH values at 48 h were below 6.0 [19], which does not agree with the results of the present study.

PGIV, DMD, and DOM increased linearly ($p < 0.0001$) with *sargassum* inclusion level in all incubation periods (Table 2 and Figure 1). The increase in total gas emissions could be responsible for the ruminal fermentation characteristics, which are largely affected by the pelagic *sargassum* inclusion. The present study revealed an improvement in the DMD and DOM in all fermentation periods, which could be related to the ability of non-fibrous carbohydrates and minerals present in *sargassum* to improve energy availability for rumen microorganisms. These results are partially consistent with those obtained by Canul-Ku et al. [25] when evaluating pelagic *sargassum*, who indicate that despite a slight decrease in IVGP, they observed higher DMD and DOM. Similarly, Maia et al. [20], when including 25% *Saccharina latissima* to a total mixed ration, reported no change in IVGP; however, DDM and DOM increased. Other studies carried out with brown algae indicate that despite the changes that occur in IVGP in some incubation periods, the DDM potential is not affected [18,19,45,46]. On the other hand, Widiawati and Hikmawan [47] observed a linear increase in IVGP, DMD, and DOM with increasing dose of the red alga *Eucheuma cottonii* added to an Elephant grass (*Pennisetum purpureum*). Contrary to all the studies mentioned above, Rjiba-Ktita et al. [27] report a linear decrease in IVGP and DOM when the dose of green algae increased from 10 to 40% in a substrate based on concentrated feed. Therefore, the effect of algae on fermentative parameters depends on the species of algae and the quality of the substrate. However, there is also an interaction effect between the species of algae and the basal substrate [48].

Algae cause changes in rumen microbiome, which modifies digestion and fermentation characteristics [20,21,36,46,48,49], towards a more cellulolytic profile [14,18]. This is due to its bioactive compounds (phlorotannins, polysaccharides such as: fucoidan, alginate, laminarin and mannitol), which improve DDM with an increase in fibrolytic bacterial populations an increase in fibrolytic bacterial populations such as *Fibrobacter succinogenes* and *Ruminococcus flavefaciens*. According to Huang et al. [50], phlorotannins promote the

growth of the genera *Ruminococcus*, *Lachnospiraceae*, and *Fibrobacter*, which correlates with higher DDM. As with other fermentative parameters, nutrient degradability is influenced by the species of algae [42].

The brown algae that make up pelagic sargassum are characterized by high levels of ash, low organic matter content, and high concentrations of neutral detergent insoluble protein (NDIP), which reduce DMD and CP degradability [27,51]. In contrast, red algae contain high concentrations of CP, low ash levels, and high digestibility. With regard to amino acid degradability, brown algae are low in comparison to red algae. This is due to their phlorotannin content and chemical structure of their cell walls; however, the physical properties (anatomy and morphology) and amino acid composition among different species of algae also play an important role [42]. An advantage of the latter is that it increases the supply of amino acids in the small intestine of ruminants [52]. Studies report that the OM content of pelagic *sargassum* represents between 30 and 50% of DM [20,21]; this value is lower than that reported in the present study (58.89%) and probably could influence DOM (Table 1). Other limiting factors for the inclusion of sargassum in diets for ruminants are its concentration of heavy metals such as As, which, according to reports, can contain from 100 to 500 mg kg⁻¹ DM [23,25]. Although the concentration of secondary metabolites was not determined in pelagic sargassum in this study, research on the bioactive compound content in algae of the genus *Sargassum* indicates that they have a strong influence on DDM and DOM [25,53–56]. The complex polysaccharides contained in brown algae may initially limit the availability of nutrients to rumen microorganisms, requiring the establishment of a new microbial consortium or the adaptation of an existing one to produce specific enzymes that can efficiently degrade these polysaccharides [57]. According to Saldarriaga-Hernandez et al. [53], the collection season influences the composition of carbohydrates, proteins, and phenolic compounds of *Sargassum*. For this reason, it is necessary to conduct studies on the speciation of As, as well as the determination of the concentration of bioactive compounds of pelagic *sargassum* according to the time of the year.

4.2. Protozoa Population

Rumen protozoa contribute approximately 30% of methane emissions through their symbiotic relationship with methanogens, which involves the transfer of hydrogen between them [58]. In the present study, the total protozoa population decreased at 24 h (Table 3). These results are partially consistent with Canul-Ku et al. [25], who observed a decrease in the total protozoa concentration and in population of Entodiniomorphs at 48 y 72 h of incubation; this contrast is probably due to the difference in the quality of the basal diet used and the type of diet provided to the rumen fluid donor animals; however, in the two studies the degradability of DM and OM were not negatively affected. Therefore, the reduction in protozoa population by the effect of brown algae supplied in ruminants is associated with a decrease in methanogen populations [22] and, therefore, it is related to a lower CH₄ production [14,59].

Bioactive compounds contained in macroalgae have antimicrobial properties [15,60–62], modify the abundances of cellulolytic bacteria, methanogenic archaea, and methanogens associated with ciliate protozoa in the rumen [18]. Red algae such as *Asparagopsis taxiformis* are characterized by high concentrations of bromoform, a halogenated compound that acts by inhibiting cobamide-dependent methyltransferase in the terminal stage of the methanogenic pathway [13,63,64], while brown algae contain phlorotannins, compounds that favor the increase in fibrolytic populations [18,19,50]. The antibacterial mechanism of action of these phenolic compounds is related to their ability to modify cell wall permeability and inhibit bacterial cell replication [65].

The inclusion of the red seaweed *Eucheuma cottonii* in high-fiber diets resulted in a linear reduction in the protozoa population and CH₄ concentration in the ruminal fluid as the dose increased [46]. Similar results were reported by Roque et al. [21], who indicated reductions in the abundance of methanogens in the rumen fluid accompanied by a CH₄ reduction of 95% with the addition of 5% *Asparagopsis taxiformis* to a good-quality substrate. Contrary to these studies, Molina-Alcaide et al. [42], when evaluating several species of red algae (*Mastocarpus stellatus*, *Palmaria palmata*, and *Porphyra* sp.), reported no effects on the microbial population. The differences in the effects observed in studies with red algae are related to the concentration of bromoform, which varies depending on the species of algae and the season of collection [66,67]. With brown algae, differential effects have also been observed in their antimicrobial properties [12,19]. These differential effects are due to differences in the concentration [22], chemical structure, and molecular weight of phlorotannins in brown algae, which could determine the potency of their effect [68,69]. In this same context, the characterization of the polysaccharides present in pelagic *sargassum* based on their bioactive properties in ruminants would be another topic of great interest since it has been little studied. According to Cheong et al. [69], polysaccharides such as alginate, laminarin, and fucoidan promote the growth of bacteria associated with CH₄ reduction and the suppression of methanogenic archaeal populations. In this regard, the addition of brown algae extracts to a substrate with 60% grass hay and 40% corn grain increases the abundance of cellulolytic bacteria [18]. The antimicrobial property of polysaccharides is related to the presence of sulfate groups or uronic acid [70]. The mechanism of action of these polysaccharides is related to their ability to disrupt microbial cell walls and membranes, causing cell lysis [69,71,72]. Based on our findings and supported by studies conducted with brown algae in ruminants, we can confirm that our hypothesis was proven correct.

5. Conclusions

Pelagic *sargassum* can be a potential ingredient in ruminant feed due to its bioactive compounds. The results of the study indicate that pelagic *sargassum* can be included up to 30% under in vitro conditions in integrals diets, improving fermentative parameters. It is recommended to determine the chemical composition and the concentration of secondary metabolites of *sargassum* according to the time of the year, and to carry out animal feeding studies to determine its possible effects on the rumen microbiome and productive parameters.

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