

## The marine algae *Sargassum* spp. (Sargassaceae) as feed for sheep in tropical and subtropical regions

Alejandro Marín<sup>1</sup>, Margarita Casas-Valdez<sup>1</sup>, Silvia Carrillo<sup>2</sup>, Hugo Hernández<sup>3</sup>, Alberto Monroy<sup>3</sup>, Leonor Sanginés<sup>2</sup> & Fernando Pérez-Gil<sup>2</sup>

1. Laboratorio de Macroalgas. Centro Interdisciplinario de Ciencias Marinas, Ave. Instituto Politécnico Nacional s/n, Col. Playa Palo de Santa Rita, La Paz, Baja California Sur, México, phone: (612) 12 2 53 44, ext. 82411; fax: (612) 12 2 53 22. Apdo. Postal 592. La Paz, B. C. S., México, C.P. 23090; mcasav@hotmail.com, amarinal@ipn.mx
2. Departamento de Nutrición Animal. Instituto Nacional de Ciencias Médicas y Nutrición Salvador Zubirán. Vasco de Quiroga No. 15, México, D. F., C.P. 14000; silvecarrillo3@hotmail.com; leosangines@hotmail.com; rosyo@prodigy.net.mx
3. Departamento de Zootecnia. Universidad Autónoma de Baja California Sur. Km 5 Carr. al Sur. La Paz, B. C. S., México, C.P. 23050; amonroy@uabcs.mx; hehi@uabcs.mx

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**Abstract:** The objective of this study was to evaluate *Sargassum* meal as feed for sheep through the measures of *in vivo* digestibility, dry matter degradability, pH, ammonia and volatile fatty acids in rumen. The *Sargassum* algae used in this experiment were collected at the end of spring, when they are more abundant, bigger, and have completed their reproductive cycle. Four tons (wet weight) were collected manually from the intertidal zone of La Paz bay, Baja California Sur, Mexico. These algae were sun-dried and ground in a hammer mill to obtain the *Sargassum* meal. Four fistulated Pelibuey sheep, were fed daily with diets containing the marine algae (MA) at different levels (0, 10, 20 and 30 %), using a 4 x 4 Latin-square design experiment. Feed intake was not affected ( $p > 0.05$ ). Water consumption and urine excretion increased with MA ( $p < 0.05$ ;  $r^2 = 0.54$  and  $r^2 = 0.74$ , respectively). In all treatments dry matter digestibility was of 74%-79%, and crude protein digestibility was of 85%-88%. Acid detergent fiber (59%-65%) and neutral detergent fiber (55%-66%) digestibility were greater in all treatments with MA. Ruminal pH was greater in all groups fed with MA ( $p < 0.05$ ). Ammonium concentration was not influenced ( $p > 0.05$ ) by MA. Ruminal volatile fatty acids decreased in all MA groups ( $p < 0.05$ ). The marine algae *Sargassum* spp. can be used as a feed supplement for sheep, especially in tropical and subtropical regions where these marine algae are available. Rev. Biol. Trop. 57 (4): 1271-1281. Epub 2009 December 01.

**Key words:** marine algae, sheep, *Sargassum*, digestibility, ruminal fermentation, volatile fatty acids.

In Mexico as in other countries around the world, there are arid regions where sheep and goats are severely affected during the dry season because naturally-grown feed is scarce (Yahaya *et al.* 1999, Mora *et al.* 2009). The marine algae, *Sargassum* spp. represents an important alternative as food.

*Sargassum* forms large beds in tropical and subtropical waters around the world, growing along beaches with a rocky substrate, rolling stones and pebbles (Ganzon-Fortes *et al.* 1993).

This algae is especially abundant along the Gulf of California coast, where a harvestable amount of 183 000 t has been estimated (Casas-Valdez *et al.* 2006a). This genus is a good source of minerals, carbohydrates and some essential amino-acids like arginine, tryptophane and phenylalanine. It is also rich in beta-carotene and vitamins. Furthermore, no antinutritious elements have been detected in it (Carrillo *et al.* 2002, Casas-Valdez *et al.* 2006a). Some studies have shown a seaweed digestibility ranging

from 28% to 67% (dry matter), and a protein degradability of 95% (Gojón *et al.* 1998).

Experimental works conducted with sheep and seaweeds are scarce. Arieli *et al.* (1993) mention that *Ulva lactuca* (Linnaeus) 1753 could be categorized as a low-energy high-nitrogen foodstuff, and that these algae is a useful supplement for sheep. Hansen *et al.* (2003) concluded that *Laminaria digitata* (Hudson) J.V. Lamouroux 1813 and *L. hyperborea* (Gunnerus) Foslie 1884 can be used as an alternative food for north Ronaldsoy sheep. The effects of *Sargassum* spp. on digestibility and ruminal parameters in ruminants are unknown.

The objective of this study was to evaluate *Sargassum* meal as feed for sheep through the measures of *in vivo* digestibility, dry matter degradability, pH, ammonia and volatile fatty acids in rumen.

## MATERIALS AND METHODS

**Algae and chemical analysis *Sargassum* spp.:** Specimens were collected at the San Juan de La Costa beach, which is located in La Paz

bay, Baja California Sur, Mexico (24° 27'-24° 06' N and 110° 18'-110° 40' W). Four tons (wet weight) were collected manually from the intertidal zone at depths between 60cm and 1.20m. Plants were spread on a cement surface and sun-dried for two days. Then the marine algae (MA) was ground in a hammer mill (JERZA, model L) and afterwards in a knife mill using a 1mm mesh. A sample of 1kg was taking by quartering, to grind it finely and carry out chemical analysis by triplicate (Table 1).

The following analyses were carried out on the *Sargassum* meal and diets using standard methods (AOAC 2000): moisture content (over-drying at 60°C to constant weight, AOAC 2000 method 976.05), ash (ignition at 550°C in an electric furnace, AOAC 2000 method 923.03), nitrogen content was determined using the micro-Kjeldahl method (AOAC 2000 method 976.05), and a conversion factor of 6.25 was used to calculate protein content (CP), and ether extract (EE) (Soxhlet apparatus, AOAC 2000 method 920.39). Cell wall fractions: neutral detergent fibre (NDF), acid detergent fibre (ADF), cellulose, hemicellulose and lignin were

TABLE 1  
*Ingredients and chemical composition (g/100g DM) of diets used*

Ingredients	0% MA	10% MA	20% MA	30% MA
Dehydrated alfalfa (12% CP)	50.40	48.00	42.20	28.00
Corn (8 % CP)	32.50	23.50	17.50	20.80
Marine algae <sup>a</sup>	0.00	10.00	20.00	30.00
Soybean (45 % CP)	14.80	15.70	17.50	18.80
Urea	0.30	0.30	0.20	0.30
Tallow	2.00	2.50	2.60	2.10
Total	100.00	100.00	100.00	100.00
<b>Chemical composition</b>				
Organic matter	94.09±1.77	91.29±1.93	88.70±2.16	86.66±1.88
Crude protein (g/100g)	11.31±1.28	10.69±1.65	11.25±1.58	11.07±1.71
Neutral Detergent Fibre (%)	19.88±3.41	19.85±3.65	26.51±5.45	26.53±4.23
Acid Detergent Fibre (%)	15.04±2.64	19.41±3.18	21.33±4.12	24.69±3.18
Metabolizable Energy (Mj/kg)	10.37±1.15	10.24±1.03	10.19±1.6	10.32±1.71

<sup>a</sup> Composition of *Sargassum* spp.: Moisture 7.7±0.06 %, CP 6.3±0.04 %, Ether extract 0.45±0.03 %, CF 6.4±0.08 %, Ash 33.3±0.11 %, GE 2.13±0.02 kcal/g, NDF 47.1±0.42 %, ADF 44.5±0.35 %, Cellulose 6.2±0.37 %, Mg 7.54±0.07 mg/g, K 15.9±0.06 mg/g, Na 28.7±0.16 mg/g, Ca 6.41±0.094 mg/g, P 2.7±0.07 mg/g, Fe 263±9.65 ppm, Zn 119±5.41 ppm, Cu 14±0.29 ppm, Pb 18±0.02 ppm, Se 85±0.13 ppb, Hg 181±0.26 ppb, Tannic acid 22±0.53 mg/g, Saponines ++, Cyanogenic glucosides and Alkaloids not detected. NDF = Neutral detergent fiber; ADF = Acid detergent fiber.

analyzed according to the Van Soest method described by Tejada (1985), and gross energy was determined using a calorimetric Parr Bomb (Parr Instrument Bomb, Mod. 6300).

To determine Ca, Mg, Na, K and Fe samples of MA meal were subjected to acid digestion and analyzed by atomic absorption spectrophotometry (Brand GBC, Mod. Avanta PM) following the procedures described by AOAC (2000) method 968.08 (Table 1). Zn and Cu by AOAC (2000) method 999.11. P was obtained by colorimetric (Clesceri *et al.* 1989, method 4500). Pb, Hg y Se (NOM-117-SSA1-1994).

Metabolizable Energy (ME), Digestible Energy (DE) and Total Digestible Nutrients (TDN), were calculated: ME=DE\*0.82; DE=TDN\*4409 kcal; TDN= crude fiber(0.5)+crude protein(0.75)+nitrogen free extract (0.75)+(etheral extract (0.90 \* 2.25)).

**Experimental designs:** The diets utilized were: control diet (0% MA), diet supplemented with 10%, 20% and 30% of *Sargassum* spp. meal (10% MA, 20% MA and 30% MA). The four diets met the sheep's nutritional requirements (12.8% of crude protein and 10.19Mj/kg) (NRC 1996) (Table 1).

The study was conducted at La Paz City, Baja California Sur, Mexico. The weather in this region is typically warm and semi-arid, with an annual precipitation of 220 mm, a 50% average humidity and a 28°C average temperature.

Four Pelibuey sheep (mean BW=24.5kg, 18 months old) fitted with ruminal cannulae (Plastisol 1"; Bar Diamond) were randomly distributed in a replicated 4 x 4 Latin-square design, and were kept in metabolism cages with restricted access to feeding. All animals were treated with anthelmintics. The experimental protocol was approved by the Scientific Committee of the Universidad Autónoma de Baja California Sur.

Feed (0.600 kg of diet) was provided twice a day at 06:00 and 18:00 hours. Water intake was provided *ad libitum*. Each one of the four experimental periods comprised 15 days; 10

days for diet-adaptation period, and 5 days for total feces and urine collection. feed intake (the difference between feed offered and feed refusal) and water intake were recorded daily. Total feces and urine were collected daily. In addition, dry matter (DM), CP, ADF and NDF digestibility (total fecal collection) were determined.

The DM digestibility coefficient of each diet was estimated using the following formula:

$$DC = \frac{FI - FO}{FI} \times 100$$

Where: DC=digestibility coefficient (%); FI=feed intake (DM basis) and FO=fecal output (DM basis).

Rumen degradability of dry matter for each diet was determined *in situ* using a nylon bag technique. The nylon bags (224 of 10 x 5 cm; pore size=60 µm) were filled with 3g of each diet and suspended in the rumen of each sheep. The bags were incubated for 3, 6, 9, 12, 24, 30, 36, 48 and 72 hours (zero time disappearance was obtained by suspending the nylon bags in a NaCl 0.15 N solution, at 37°C for 5min). Upon removal from the rumen, bags were washed five times during one min in a mechanical agitator until obtaining a clear and transparent rinsing liquid and afterwards, dried at 60°C and weighed (Harmon & Richards 1997, Stern *et al.* 1997) before determining DM disappearance which was calculated as follows:

$$DM (\%) = \frac{((WB + WBI) - (WB + WAI)) \times 100}{SW}$$

Where: DM (%)= dry matter disappearance; WB= bag weight; WBI= sample weight prior to incubation; WAI= sample weight after incubation; SW= sample weight.

Result of the *in situ* digestibility of DM were fitted to the non-linear equation: P= a+b(1-e<sup>-ct</sup>) (Orskov & McDonald 1979) using the Neway Excel program, where: P= disappeared at time t; (a+b)= potential degradability;

c= rate of degradation. The effective degradation was estimated at different degradation rates (fraction  $h^{-1}$ ): 0.02, 0.05 and 0.08.

Ruminal content samples of 150 ml were collected at 03:00, 06:00, 09:00, 12:00, 18:00 and 24:00 hr through the ruminal cannulae, for five days. One portion of the ruminal content was filtered by squeezing out eight layers of gauze and the pH of the filtrate was recorded immediately using a potentiometer (Orion Mod. 701A) with a Corning electrode (AOAC 2000). Ammoniacal nitrogen was determined by the distillation method (Clesceri *et al.* 1989, method 4500). To determine volatile fatty acids (VFA's), 5ml of filtrate were mixed with 1ml of a 3:1 (25 %) metaphosphoric acid/formic acid solution followed by centrifugation at 2 300g for 30min, then the supernatant was frozen and stored until analyzed. VFA's content were determined using a Varian Star 3400 CX chromatograph (AOAC 2000, method 996.06). Data were analyzed by triplicate.

**Statistical analysis:** Data of digestibility *in vivo*, kinetic of digestibility *in situ* of dry matter, feed and water intake, and urine excreted were examined using analysis of variance with a Latin square design (4 x 4) (Kuehl 1999) and Tukey's test for a multiple comparisons of means, with a significance level of  $p < 0.05$ .

Correlation analysis was made to know if there was a relationship between the concentration of *Sargassum* in the diet and: a) water intake, b) urine excretion, as well as between water intake and urine excretion. Ruminal pH, ruminal ammonia, and VFA data were analyzed as repeated measures using the Statistical Analysis System program (SAS 1985).

## RESULTS

**Digestibility:** No significant difference was found in feed intake among all experimental diets ( $p > 0.05$ ) (Table 2). There was significant difference in water intake and urine excretion between the control diet and the diets with MA ( $p < 0.05$ ). A progressive increase in water intake ( $r^2 = 0.54$ ) and urine excretion ( $r^2 = 0.74$ ) (Table 2) was observed as the concentration of MA added in the diets increased; a positive correlation ( $r^2 = 0.80$ ) was found between water intake and urine excretion. Feces output did not present significant difference among all experimental diets ( $p > 0.05$ ); however, the feces output of the animals fed with the diets 20% and 30% MA were more humid.

The *in vivo* digestibility coefficient of DM and CP showed no significant differences among control diet and the diets with MA ( $p > 0.05$ ) (Table 2). The digestibility of ADF of the

TABLE 2

*Feed intake, water consumption, feces output and urine excreted, dry matter (DM), crude protein (CP), acid detergent fiber (ADF) and neutral detergent fiber (NDF) digestibility in ewes fed diets supplemented with Sargassum spp.*

	Treatments				SEM
	0% MA	10% MA	20% MA	30% MA	
Feed intake-DM (kg/d)	1.15±0.09 <sup>a</sup>	1.15±0.10 <sup>a</sup>	1.20±0.07 <sup>a</sup>	1.11±0.08 <sup>a</sup>	0.18
Water consumption (l/d)	3.45±0.74 <sup>a</sup>	4.26±0.88 <sup>b</sup>	4.84±0.70 <sup>c</sup>	4.87±0.48 <sup>bc</sup>	0.38
Urine excreted (l/d)	0.59±0.29 <sup>a</sup>	0.99±0.36 <sup>b</sup>	1.14±0.28 <sup>b</sup>	1.57±0.44 <sup>c</sup>	0.20
Feces output-DM (kg/d)	0.26±0.07 <sup>a</sup>	0.24±0.03 <sup>a</sup>	0.27±0.04 <sup>a</sup>	0.28±0.07 <sup>a</sup>	0.03
DM coefficient of digestibility	0.77±0.02 <sup>a</sup>	0.79±0.02 <sup>a</sup>	0.77±0.04 <sup>a</sup>	0.74±0.03 <sup>a</sup>	0.02
CP coefficient of digestibility	0.87±0.01 <sup>a</sup>	0.88±0.01 <sup>a</sup>	0.86±0.02 <sup>a</sup>	0.85±0.01 <sup>a</sup>	0.01
ADF coefficient of digestibility	0.3±0.04 <sup>a</sup>	0.59±0.07 <sup>b</sup>	0.64±0.04 <sup>b</sup>	0.65±0.04 <sup>b</sup>	0.02
NDF coefficient of digestibility	0.44±0.07 <sup>b</sup>	0.55±0.08 <sup>ab</sup>	0.67±0.05 <sup>a</sup>	0.66±0.07 <sup>a</sup>	0.03

<sup>a,b,c</sup> Different letters in each row indicate statistically significant differences ( $p < 0.05$ ).

control diet was smaller in regard to all other treatments ( $p < 0.05$ ). Moreover, the digestibility of NDF was greater in the 20% and 30% MA compared to the control diet ( $p < 0.05$ ).

**Dry matter disappearance:** During the ruminal incubation of diets, no significant differences were found in the DM disappearance among treatments in the first 24 hours ( $p > 0.05$ ), but from 48 to 72 hr the disappearance of the control diet was higher (Fig. 1). Differences were observed in each treatments as time passed by ( $p < 0.05$ ), DM disappearance increased with time of incubation.

**Kinetics of the *in situ* digestibility of DM:** The soluble fraction (a) exhibited significant difference between the control diet and the diet with 30% MA which is higher ( $p < 0.05$ ). The potentially digestible fraction (b) was higher in control diet than 10% MA ( $p < 0.05$ ), but there was not significant difference in relation to 20% and 30% MA. Not any significant difference was found between the diets in the

potential degradability ( $p > 0.05$ ). No significant difference was found among the diets in degradability rate (Rate constant (fraction/hr)) ( $p > 0.05$ ). The effective degradation was higher ( $p < 0.05$ ) at the lowest degradation rate (0.02/hr) in all treatments (Table 3).

**pH, ammonia and volatile fatty acids in the rumen:** The lowest pH value was recorded on the control diet ( $p < 0.05$ ), compared to the diets with MA, therefore *Sargassum* spp. meal favoring a neutral pH (Table 4). Patterns of pH variation were similar among treatments.

No differences were observed in ammonia concentration among treatments ( $p > 0.05$ ) (Table 4). Ruminal  $\text{NH}_3$  concentration (mg/l) was of 215 in the control group, and varied between 221 and 265 in the treatments with MA.

Acetic acid declined in all treatments 12 hours after the first meal (Table 5). Significant differences ( $p < 0.05$ ) were observed among 20% and 30% MA whose concentration was smaller than the control group. Propionic acid also displayed a similar pattern (Table 5), with

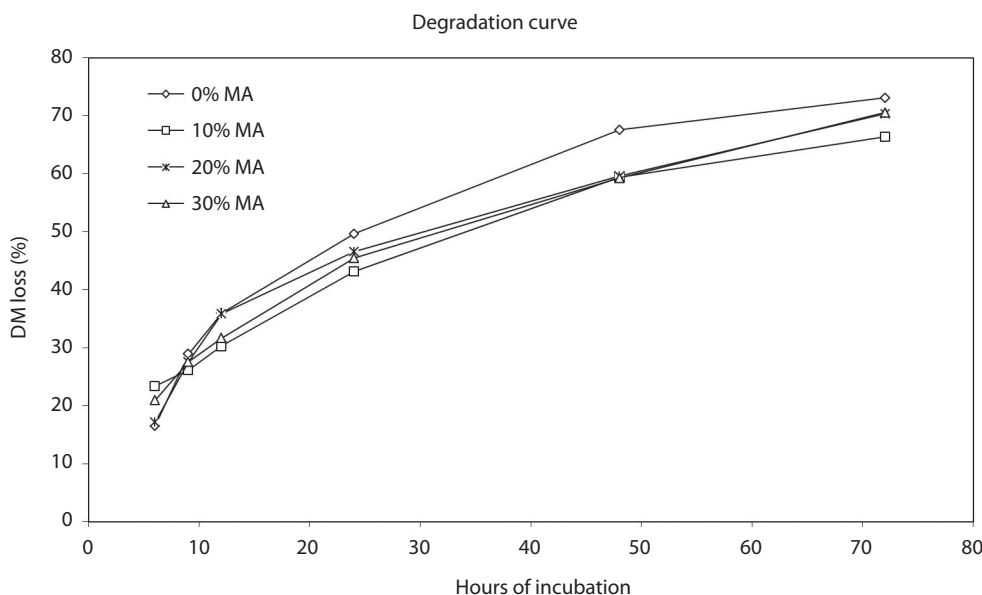


Fig. 1. Ruminal dry matter disappearance of: control diet (0% MA), diet supplemented with 10% of *Sargassum* spp. (10% MA), diet supplemented with 20% of *Sargassum* spp. (20% MA) and, diet supplemented with 30% of *Sargassum* spp. (30% MA).

TABLE 3  
Kinetics of the *in situ* disappearance of dry matter from experimental diets

Treatments	0% MA	10% MA	20% MA	30% MA
Soluble fraction (a) (%)	7.42±0.94 <sup>b</sup>	10.27±2.06 <sup>ab</sup>	11.03±1.69 <sup>ab</sup>	12.54±1.71 <sup>a</sup>
Fraction (b) (%)	72.87±2.72 <sup>a</sup>	59.57±4.51 <sup>b</sup>	62.35±5.32 <sup>ab</sup>	64.55±10.31 <sup>ab</sup>
Potential degradability (%)	80.27±2.45 <sup>a</sup>	69.80±6.38 <sup>a</sup>	73.40±6.10 <sup>a</sup>	77.20±11.13 <sup>a</sup>
Rate constant (fraction/hr)	0.036±0.01 <sup>a</sup>	0.036±0.005 <sup>a</sup>	0.033±0.005 <sup>a</sup>	0.029±0.005 <sup>a</sup>
Effective degradability (%) at different disappearance rates Kd (hr <sup>-1</sup> ):				
0.02	53.82±3.87 <sup>aA</sup>	48.35±2.79 <sup>bA</sup>	50.025±3.48 <sup>abA</sup>	50.30±5.24 <sup>abA</sup>
0.05	37.70±4.06 <sup>abB</sup>	35.05±1.69 <sup>abB</sup>	36.07±2.30 <sup>abB</sup>	35.92±3.33 <sup>abB</sup>
0.08	29.92±3.47 <sup>abB</sup>	28.87±1.91 <sup>abB</sup>	29.47±1.73 <sup>abB</sup>	29.52±2.70 <sup>abB</sup>

<sup>a,b</sup>Different letters within each row indicate significant differences (p<0.05).

<sup>A,B</sup>Different letters within each column indicate significant differences (p<0.05).

TABLE 4  
Patterns of variation of ruminal pH and ruminal ammonia concentration (mg/l) over a 24 hr period in sheep supplemented with *Sargassum spp.*

pH					
Hour	0% MA	10% MA	20% MA	30% MA	SEM
0300	6.61±0.52	6.80±0.49	6.95±0.39	6.74±0.58	0.07
0600	6.53±0.42	6.65±0.55	6.82±0.45	6.82±0.51	0.07
0900	6.32±0.57	6.55±0.67	6.64±0.62	6.66±0.60	0.08
1200	6.51±0.48	6.69±0.52	6.69±0.69	6.79±0.58	0.06
1800	6.86±0.26	7.03±0.41	7.11±0.43	7.14±0.27	0.06
2400	7.15±0.18	7.18±0.36	7.38±0.22	7.24±0.14	0.05
Mean	6.61±0.14 <sup>a</sup>	6.81±0.12 <sup>b</sup>	6.93±0.16 <sup>b</sup>	6.89±0.17 <sup>b</sup>	
Ammonia, mg/l					
Hour	0% MA	10% MA	20% MA	30% MA	SEM
0300	235.1±54.8	378.1±218.9	316.9±177.8	368.2±128.6	0.41
0600	234.1±116.0	190.6±122.0	218.0±157.6	353.6±203.7	0.45
0900	177.0±142.3	235.8±196.6	337.3±186.3	328.1±140.3	0.48
1200	319.8±249.4	133.9±90.1	390.3±154.6	237.2±109.1	0.69
1800	136.1±66.2	232.2±179.2	148.5±103.2	113.2±77.9	0.32
2400	186.0±71.7	156.0±73.3	178.4±137.0	124.4±83.8	0.17
Mean	214.7±94.0 <sup>a</sup>	221.1±89.1 <sup>a</sup>	264.9±105 <sup>a</sup>	254.1±107 <sup>a</sup>	

<sup>a,b,c</sup>Different letters in columns indicate significant differences among treatments (p<0.05).



TABLE 5  
Variation of volatile fatty acids (mM/l)

Acetic acid					
Hour	0% MA	10% MA	20% MA	30% MA	SEM
0600	73.29±14.36	73.77±8.64	63.65±8.88	56.54±3.05	4.14
1200	78.16±18.40	79.15±10.58	73.02±3.93	61.69±4.58	4.00
1800	72.45±11.41	67.60±10.82	58.85±8.04	56.67±12.20	3.70
2400	53.71±17.28	48.04±14.75	46.47±11.10	43.01±7.87	2.23
Mean	69.4±5.38 <sup>a</sup>	67.1±6.78 <sup>ab</sup>	60.5±5.52 <sup>b</sup>	54.5±4.00 <sup>c</sup>	
Propionic acid					
0600	25.11±9.80	21.40±5.96	19.32±5.28	24.75±2.06	1.39
1200	30.02±11.11	23.47±6.28	22.08±4.88	17.20±4.07	2.64
1800	25.50±7.64	19.32±3.84	16.49±5.25	14.26±7.00	2.43
2400	15.44±7.18	12.25±5.67	12.73±5.69	9.58±3.89	1.20
Mean	24.0±6.14 <sup>a</sup>	19.1±4.88 <sup>abc</sup>	17.7±4.00 <sup>bc</sup>	16.4±6.36 <sup>c</sup>	
Butyric acid					
0600	10.80±5.43	6.1±2.313	7.57±4.65	8.13±1.21	0.97
1200	12.81±7.52	8.14±3.99	9.11±4.68	10.04±3.29	1.01
1800	13.18±6.94	8.33±4.08	8.28±3.74	9.87±3.19	1.15
2400	8.73±5.01	5.65±4.26	6.03±3.83	7.12±4.20	0.69
Mean	11.4±2.05 <sup>a</sup>	7.1±1.37 <sup>b</sup>	7.7±1.31 <sup>b</sup>	8.8±1.41 <sup>b</sup>	

<sup>a,b,c</sup>Different letters in columns indicate significant differences among treatments ( $p < 0.05$ ).

levels decreasing at 18:00 (12 hours after the first meal). The 20% and 30% MA treatments presented significant differences ( $p < 0.05$ ) with respect to 0% MA. Butyric acid increased from 06:00 to 18:00 hr in 0% MA and 10% MA and of 06:00 to 12:00 in 20% MA and 30% MA (Table 5). Levels of butyric acid were always lower in diets containing *Sargassum* spp. these showed significant differences ( $p < 0.05$ ) with 0% MA.

## DISCUSSION

The chemical composition of the algae varies seasonally and annually in relation to environmental factors, geographic distribution and physiological variations (Jimenez & Goñi 1999, Aguilera *et al.* 2005). Pérez (1999) reports variations in the chemical composition of *Sargassum* spp., collected in February, March, April and May in La Paz bay, in 1995. Their main components varied in a range of:

protein 3.5 to 4.7%, ash 26.5 to 31.7%, crude fiber 10.2 to 11.4%, and ether extract 0.1 to 0.4. As *Sargassum* presents their maximum abundance, bigger size and completed their reproductive and life cycle at the end of spring, this seaweed is commonly collected then to do different experiments, as in the present research. Results about the chemical composition of *Sargassum*, collected at the end of spring, show the following values, protein 6.1 to 7.7%, ash 31 to 38%, crude fiber 6.5 to 9.3%, and ether extract 0.34 to 1.8% (Carrillo *et al.* 2002, Marín *et al.* 2003, Casas-Valdez *et al.* 2006a, 2006b). The results obtained in this study (protein 7.7%, ash 33.3%, crude fiber 6.4% and ether extract 0.45%) are similar to the ones previously mentioned. There are others components, like minerals analyzed in this study that also have seasonally and annually variations (Aguilera *et al.* 2005).

It seems that the palatability and taste of the different diets were not influenced by the

inclusion of *Sargassum* meal as a feed ingredient, since there were not significant differences in feed intake. Water intake increased, as a result of the concentration of *Sargassum* included in the diet, particularly due to the large amount of Na and K in the algae. Consequently, the amount of urine excreted per day increased, being more abundant when sheep were fed with diets containing algae, as a result of the rise in water intake (Forbes 1995). Mora *et al.* (2009) also observed a progressive increase in the water intake and the urine excreted as the concentration of the alga *Macrocystis pyrifera* was higher (from 10 % to 30 %) on diets given to goats. It is likely that fecal matter excreted by sheep was more humid in the 20% MA and 30% MA groups, because of the water intake increase.

The digestibility of the diets including *Sargassum* meal, showed an adequate utilization of algal nutrients (79% to 74%). Our results suggest that values obtained for dry matter digestibility with diets supplemented with *Sargassum* spp. are similar to commonly used forages, like bean and soybean with 71.8 % and 69.4 %, respectively (Valdez *et al.* 2002).

The concentration of seaweeds included in the diets, did not affect the digestibility of CP. The digestibility of cellular walls (FDN and FDA) increased, as a result of the concentration of *Sargassum* in the diet. This could be due to the presence of alginates, in the cellular walls of the algae.

The *in situ* digestibility allows determine whether degradation characteristics of vegetative species could be used to predict its nutritional value (Shem *et al.* 1995). The dry matter disappearance obtained in this work at 72 hr varied from 65% to 68% as the concentration of *Sargassum* meal was increased. These values are in the range of those reported by Ramírez *et al.* (2000) for different shrubs from Baja California Sur, with a mean of 0.68, and are closer to sorghum values (0.68) reported by Martínez *et al.* (1995). Mora *et al.* (2009) found a similar behavior in the dry matter disappearance when including the alga *Macrocystis pyrifera* on diets for goats, which

rose from 0.73 to 0.80 as the concentration of the algae were increased from 10% to 30%. This result is considered to be due to the fact that *Macrocystis* has a higher concentration of soluble carbohydrates (Rodríguez-Montesinos & Hernández-Carmona 1991) than *Sargassum* (Casas-Valdez *et al.* 2006a), which is degraded faster in the rumen. Gojón *et al.* (1998) reported a 54.8% disappearance of *Sargassum* dry matter in an experiment conducted with bovines. This difference might be attributed to the fact that they evaluated the algae alone.

It is important to evaluate the kinetics of the digestion since it allows the proportion of nutrients of dry matter consumed that can be absorbed, and assimilated by the animal (Mertens 1993). The soluble fraction (a) was higher at all diets that included *Sargassum* with respect to the control diet. This is related to the high content of soluble ashes in the algae. According to Gojón *et al.* (1998) the minerals of *Sargassum* disappear from the nylon bag up to 78% at time 0 by diffusion.

The values of potentially digestible fraction of dry matter (b) obtained in the diets that included the alga (59% to 64%), are higher than those found by Ramírez *et al.* (2000) in nine shrubs from Northwest Mexico supplied to sheep (15.5% to 54.7%), and similar to those reported by Galina *et al.* (2004) for diets that included maize, fish flour and urea (59 %). As well as those obtained by Mora *et al.* (2009) when supplying diets that included 10%, 20% and 30% of the alga *M. pyrifera* to goats (61 to 64%).

There was not any significant difference on the degradable fraction among the diets used in this experiment, observing values from 70% to 80%, what exhibits a low percentage of the indigestible fraction within each diet. These values were lower to those reported by Mora *et al.* (2009) (84% to 87%) when included *M. pyrifera* at concentrations from 10% to 30% on diets for goats. This could be related to the high content of ashes and soluble carbohydrates in *Macrocystis* (Mora *et al.* 2009).

The highest values of effective degradation were obtained at a disappearance or passage



rate (fraction/hr) of 0.02/hr in comparison to the rates of 0.05/hr and 0.08/hr; similar to the results obtained by Mora *et al.* (2009) with a diet containing *M. pyrifera* supplied to goats. Because when the flow is slower, it allows the feed to have a longer retention time in the rumen, obtaining a better degradation, as Forbes (1995) mentions that digestibility is the product of the retention time.

The rise in ruminal pH in sheep fed with marine algae could be as a consequence of the greater water consumption as mention Casas *et al.* (2003) and Mora *et al.* (2009). Furthermore, the addition of feedstuff with large amounts of starch or soluble carbohydrates will result in lower pH values (Fimbres *et al.* 2002, Giri *et al.* 2004) compared to diets with a prevalence of cellulose or other carbohydrates that are metabolized slowly. The proportion of *Sargassum* spp. in the diet affected positively ruminal pH, because it favors a neutral pH that helps cellulolytic bacteria replication, which are responsible for the degradation of the cellulose in the feed (Weimer 1996).

Obara *et al.* (1991) suggested that lower levels of ruminal  $\text{NH}_3\text{N}$  are associated to increased rumen fermentation rates by stimulating an increased  $\text{NH}_3\text{N}$  uptake for microbial protein synthesis. In this study, ruminal  $\text{NH}_3\text{N}$  levels for sheep fed with *Sargassum* spp. were higher than the critical level (20mg/l) suggested by Satter & Slyter (1974) for microbial protein synthesis and digestibility.

The total VFA's concentration varies due to several factors. This may decline to a minimum 30mM or rise above 200mM, but it is normally between 70 and 130mM. The values obtained in this study fell within this range, except at 24:00 hr when VFA's concentration was below 70mM in treatments with *Sargassum* meal. Volatile fatty acids concentration in the rumen at any given time reflects the balance between production and loss rates. Immediately after feed intake, VFA's production exceeds the loss and the concentration increases, but subsequently this situation is reversed and VFA's concentration falls (France & Siddons 1993), as was observed in this work. However,

the acetate:propionate:butyrate molar proportions in VFA's obtained with diets containing *Sargassum* spp. were always lower than those in the control group. This can be explained because VFA's are produced in the rumen as end-products of microbial fermentation. Therefore, it is likely that *Sargassum* polysaccharides, namely mannuronic and guluronic acids from alginate, the fucose and xylose (soluble polysaccharide), were not fully degraded by microbial populations in the rumen.

It is concluded that the marine algae *Sargassum* spp. meal can be used as a feed supplement for sheep, especially in tropical and subtropical regions where this marine algae is available.

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## RESUMEN

El objetivo de este estudio fue evaluar la harina del alga marina *Sargassum* como alimento para ovejas, midiendo la digestibilidad *in vivo*, la degradabilidad de la materia seca, así como el pH y los ácidos grasos volátiles en rumen. El alga *Sargassum* utilizada en este experimento, fue recolectada a finales de la primavera, cuando esta alga es más abundante, alcanza su mayor talla y ha completado su ciclo reproductivo. Se recolectaron manualmente, cuatro toneladas (peso húmedo) de la zona intermareal en la Bahía de La Paz, Baja California Sur, México. Estas algas fueron secadas directamente al sol y molidas en un molino de martillos, para obtener la harina. Se utilizaron cuatro borregos Pelibuey fistulados, distribuidos en un arreglo factorial de 4 x 4. Los animales fueron alimentados diariamente con dietas que contenían el alga marina (AM) *Sargassum* a diferentes niveles (0, 10, 20 y 30%). El consumo de alimento no se vio afectado con la inclusión del alga ( $p > 0.05$ ). El consumo de agua y la excreción de orina se incrementaron conforme aumentó la concentración de AM en las dietas ( $p < 0.05$ ;  $r^2 = 0.54$  and  $r^2 = 0.74$ , respectivamente). En todos los tratamientos la digestibilidad de la materia seca fue de

74% a 79%, la digestibilidad de la proteína cruda fue de 85% a 88%. La digestibilidad de la fibra ácido detergente (59%-65%) y de la neutro detergente (55%-66%) fue mayor en todos los tratamientos con AM, lo mismo ocurrió con el pH en rumen ( $p < 0.05$ ). La concentración de amonio en rumen no se vio afectada por AM ( $p > 0.05$ ). La concentración de ácidos grasos volátiles se redujo en todos los tratamientos con AM ( $p < 0.05$ ). El alga marina *Sargassum* spp. puede ser usada como complemento alimenticio para ovejas, especialmente en las regiones tropicales y subtropicales donde está disponible.

**Key words:** alga marina, *Sargassum*, ovinos, digestibilidad, fermentación ruminal, ácidos grasos volátiles.

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