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Chemical and biological evaluation of the nutritive value of Algerian green seaweed *Ulva lactuca* using *in vitro* gas production technique for ruminant animals

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Abstract

This study aimed to determine the nutritive value of seaweed *Ulva lactuca* collected from the Algerian coast by estimation of its chemical composition and fermentation characteristics, comparatively to vetch-oat hay (control), using *in vitro* gas production technique. Seaweed and control were incubated with rumen liquor taken from fistulated and non lactating cows. Gas production was recorded at: 2, 4, 6, 8, 12, 24, 48 and 72h. The *in vitro* rumen fermentation parameters were measured after 24h and 72h of fermentation. Proteins degradability was investigated by the enzymatic technique using a protease extracted from *Streptomyces griseus*. The results showed a wide variation between the chemical components of seaweed and conventional fodder used as control. The main constituents in *Ulva lactuca* were ashes (39.1% DM), fibers (22.8 % DM) and proteins (15.3% DM). The gas production profile of seaweed during 72h of incubation showed a slow and low gas production. Cumulative gas production obtained after 24h for seaweed (42 ml/g OM) was significantly lower than control (128.4 ml/g OM) (P<0.001). pH, ammonia, volatile fatty acids (VFAs), apparent digestibility of dry matter (DDM), organic matter digestibility (OMD) and metabolizable energy (ME) were significantly different between seaweed and vetch-oat hay (6.86, 290.1 mg/ml, 1.25 mmol/g DM, 38.3%, 29.2%, 3.81MJ/Kg DM respectively for seaweed). Protein degradability measurement indicates the weak hydrolysis of seaweed crude protein which is beneficial for ruminants. This study showed that the use of *Ulva lactuca* as non conventional feed in ruminant nutrition can be considered under certain conditions.

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Introduction

Algeria is a country with a littoral stretching over 1200 Km. This ecosystem is not well known, despite that it constitutes a reservoir of rich biodiversity. High quantities of seaweeds are available and very little valued; some species are washed-up and become a source of bad smell and pollution after decomposition.

Over the past fifty years, the use of seaweeds has increased considerably, with the consequent increase in applied research in various related fields (Jiménez-Escrig et al., 2000). As known, seaweeds are used as sources of food for human nutrition in many countries because these natural resources are rich in soluble dietary fibers, proteins, minerals, vitamins, antioxidants and polyunsaturated fatty acids, with a low calorific value (Mohamed et al., 2012). They are also exploited in industry for agar, alginate and carrageenan productions. Their use as fertilizer, as fuel and cosmetics products has been also pointed (McHugh, 2003).

Seaweeds could be a potentially valuable resource for ruminants feeding but not yet valued by the Algerian scientific community, although their utilization as feed supplements for livestock is not new. Arieli et al (1993) showed that seaweed *Ulva lactuca* is an interesting feed supplement for sheep, but not for poultry. Ventura and Castanon (1998) pointed out that this species represents medium quality forage for goats, with high protein content. Hansen et al (2003) concluded that seaweeds *Laminaria digitata* and *Laminaria hyperborea* have the potential to be used as an alternative feed source for small ruminants under some conditions. Mora castro et al (2009) suggested that marine algae *Macrocystis pyrifera* represents a good unconventional feeding as a nutritional supplement for goats. Rjiba ktita et al (2010) concluded that seaweeds *Ruppia maritima* and *Chaetomorpha linum* could be used as alternative feed resources for growing lambs during drought periods. Therefore, the recourse for seaweeds as local alternative feed resources is encouraged to overcome the forage deficit for feeding livestock in Algeria. In this context, we have explored the nutritive value of the local green seaweed *Ulva lactuca* by the analysis of the chemical composition and prediction of protein degradability in the rumen using enzymatic technique. *In vitro* gas production technique was used to assess biological values of the substrate and by estimating the fermentation end-products: ammonia (N-NH₃) and volatile fatty acids (VFAs). Energy estimation was also considered by determination of apparent dry matter digestibility (DMD), organic matter digestibility (OMD) and metabolizable energy (ME). *Ulva lactuca* species known by the common name as sea lettuce is a green seaweed belonging to Chlorophyta division.

Material and Methods

Sample collection and preparation

Samples of *Ulva lactuca* were collected in winter period from Annaba situated in the North-East of Algeria (36°55'2" latitude, 7°46'6" longitude). Fresh samples were collected manually in their natural habitat, up to 0.50 m deep. They were successively rinsed with seawater, tap water and distilled water to eliminate sand, shells and epiphytes. The vetch-oat hay forage used as control was collected in spring period from the Technical Institute of Great Crops (ITGC, Constantine, Algeria). Samples were air-dried and subsequently ground to pass through a 1-mm screen using a Cyclotec (1093, Sample Mill, FOSS Electric A/S, Hilleroed, Denmark), prior to chemical analyses and *in vitro* gas production measurements.

Chemical analyses

The following analyses were carried out on the seaweed and control samples according to (AOAC, 1990). All measurements were done in triplicate. The dry matter (DM) was determined by oven-drying at 105°C to constant weight and ash by incinerating samples at 550°C for 8h. Nitrogen content was measured by the Kjeldahl method and a conversion factor of 6.25 was used to calculate protein content (CP). Ether extract (EE) was determined using Soxhlet apparatus. Neutral detergent fibers (NDF), acid detergent fibers (ADF) and acid detergent lignin (ADL) were analyzed as described by Van Soest et al (1991), using the automated fiber analyzer (ANKOM²⁰⁰ Fiber Analyzer) equipped with ANKOM filter bags. Gross energy was determined using an adiabatic oxygen bomb calorimeter (1241 Adiabatic Calorimeter, PARR Instrument CO., Illinois, USA).

In vitro gas production measurements

The rumen fluid for the *in vitro* incubations was collected just before morning feeding from two rumen non-lactating Holstein cows (BW= ± 750Kg) fitted with a permanent ruminal cannula and housed in the study center of animal production (C.E.P.A) according to the standards. The cows were fed twice daily and have free access to water. The total mixed ration of the cows was composed of 8 kg of meadow hay (0.69 UFL/kg DM) and 3 kg of concentrate mixture (Bovi Brio 20, SCAM, Belgium) and was calculated to satisfy the maintenance requirements of the animals. Equal volumes of the rumen fluid, strained through a mesh screen, was collected from each cow and combined together. All laboratory handling of rumen fluid was carried out under a continuous flow of carbon dioxide to minimize changes in microbial populations and to avoid oxygen contamination.

In vitro gas production was determined as described by Menke and Steingass (1988). *In vitro* ruminal batch cultures were conducted using the ANKOM^{RF} gas production system (ANKOM Technology). Approximately (0.2 g) of sample was weighed into the 250-ml flasks. 20 ml of buffer solution and 10 ml of ruminal fluid were added to each flask, whereas blanks were prepared by adding the buffer and ruminal fluid to vessels without substrate. Samples were incubated in triplicate. Temperature was maintained at 39°C and incubation vessels were stirred continuously. Gas pressure was recorded automatically every 5 min. A wireless gas pressure monitoring system measure the changes in pressure inside the flask relative to atmospheric pressure, as a consequence of the gas produced during fermentation. The modules communicate information to a computer using radio frequency transmission. From the computer interface, the operator can control numerous variables such as the release of pressure through internal valves.

Fermentation parameters

After 24 h of incubation, the pH values of the *in vitro* cultures were recorded using a pH meter (Model 3110 SET 2, Germany). For ammonia determination, 10 ml of rumen fluid was acidified with 10 ml of hydrochloric acid (HCl, 0.2 N) and stored at -20°C until further processing. The concentration of ammonia in the fermentation cultures were measured by a calorimetric method Weatherburn (1967) using UV-visible spectrophotometer (UV-1650 PC, Shimadzu Corporation, Japan). At the end of the fermentation (72h), volatile fatty acids were determined by a High-Performance Liquid Chromatography (WATERS ALLIANCE 2690 HPLC System) equipped with UV Detector (WATERS 286) and a column (AMINEX HPX-87H, 300×7.8 mm, BIO-RAD). The apparent dry matter digestibility (DMD) was estimated according to the procedure described by Mellenberger et al (1970). The contents of the flasks were centrifuged at 1800g for 15mn. The supernatant was discarded and the pellet was suspended in 35ml H₂O and centrifuged 1800 x g for 15mn. The residue was dried at 100°C for 48h.

In vitro degradability of seaweed proteins

Prediction of degradability of seaweed proteins was done according to the method described by Aufrère and Cartailier (1988). Protein hydrolysis was recorded after 1h (DE₁) and 24h (DE₂₄) in presence of a protease, extracted from *Streptomyces griseus*, and borate-phosphate buffer solution (12.2 g/l NaH₂PO₄×2H₂O; 8.91g/l Na₂B₄×10H₂O, pH= 8), 0.5g of substrate sample were added to 50 ml of enzymatic solution and incubated at 40°C. Then, the solution was centrifuged at 3000 rpm for 5 mn. After filtration, nitrogen was measured in the supernatant using Kjeldhal method. The DE is the ratio between the quantities of nitrogen solubilised and the initial nitrogen content of the sample. Each series was adjusted with the control.

Statistical analysis and calculations

The data collected from the current experiment were analyzed with the software SAS (Statistical Analysis System, 2006). The procedure Proc GLM of SAS was used for the variance analysis. The Fisher test was used to determine the significance of the treatment effect. Means were compared two by two by the Student's t test.

- In order to estimate the fermentation kinetics variables, gas production data were fitted to the model proposed by Groot et al (1996) as follows:

$$G = A / (1 + (B/t)^C)$$

Where, G (ml g⁻¹DM) denotes the amount of gas produced per gram of dry matter, A (ml g⁻¹ DM) represents the asymptotic gas production, B (h) represents the time after incubation at which half of the asymptotic amount of gas has been formed, C is a constant determining the shape of the curve, R_{max} (ml g⁻¹ DM h⁻¹) is the maximum rate of gas production and T_{max} (h) is the time at which R_{max} is reached.

- Metabolizable energy (ME) and organic matter digestibility (OMD) were calculated using the equations proposed by Menke and Steingass (1988):

$$\text{ME (MJ/kg DM)} = 2.2 + 0.136 \text{ GP} + 0.057 \text{ CP}$$

Where, GP is the 24 h net gas production (ml /200 mg DM) and CP is the crude protein (%).

$$\text{OMD \%} = 14.88 + 0.889 \text{ GP} + 0.45 \text{ CP} + 0.0651 \text{ XA}$$

Where, GP is the 24 h net gas production (ml/ 200 mg DM); CP is the crude protein from the seaweed sample (%) and XA is the ash content from the seaweed sample (%).

Results and discussion

Chemical composition of *Ulva lactuca*

The chemical composition of *Ulva lactuca* and vetch-oat hay is given in **Table1**. There was a wide variation between the chemical components of the control and seaweed. The main constituents in *Ulva lactuca* were ashes (39.1% DM), fibers (22.8% DM) and proteins (15.3% DM). The high concentration of *Ulva lactuca* in minerals is not surprising and can be explained by its habitat rich in salt and the diversity of minerals present in this ecosystem (MacArtain et al., 2007). Previous studies have reported that ash content of seaweed varies between 8 and 40% (Mabeau and Fleurence, 1993). Our result (39.1%) is in perfect accordance with the maximum value of this interval, but it was considerably higher than that reported for this seaweed species by other authors who noted (19.6% DM) and (11.0 % DM) for Yaich et al (2011) and Ortiz et al (2006), respectively. These differences can be explained by the fact that the mineral contents of seaweeds vary according to factors such: species, geographical origin, seasons, environmental and physiological variations and the mineralization procedure (Mabeau and Fleurence, 1993; Kaehler and Kennish, 1996; Sanchez-Machado, 2004; Siddique et al., 2013).

Regarding crude protein content, it was relatively high (15.3% DM) and within the range of (10-21% DM) reported by Fleurence (1999) for the *Ulva lactuca* species. However, this quantity was higher than that reported by Wong et al (2000) and lower than that noted by Vantura and Castanon (1998). Protein content varies greatly among seaweeds species, depending on the season and environmental growth conditions (Dawczynski et al., 2007; De Oliveira et al., 2009).

Lipid content (1% DM) recorded for *Ulva lactuca* was low but in accordance with the results reported by Wong et al (2000, 1.6%) and Ortiz et al (2006, 0.3%). However, Yaich et al (2011) noted a concentration seven times superior to our result (7.9%). It is reported that seaweeds are not a conventional source of energy with low total lipid content, but their polyunsaturated fatty acids content can be high as those of terrestrial vegetables (Darcy Vrillon, 1993). At same, lipid content varies among species, geographical location, season, temperature, salinity and light intensity as well as interactions among these factors and also the extraction method (Myachita et al., 2013; Yaich et al., 2011).

Seaweeds are known to have a high polysaccharide content which could imply a high level of soluble and insoluble dietary fibers (Lahaye, 1991). In their study, Ortiz et al (1991) showed that *Ulva lactuca* has soluble and insoluble dietary fiber contents higher than that determined in fruits and vegetables. In this study, analysis of insoluble dietary fibers (cellulose, hemicelluloses and lignin) contents showed that hemicelluloses were the most abundant fraction (15.2%). This result was in agreement with the result reported by Yaich et al (2011) for *Ulva lactuca* species collected in Tunisia. Lignin was the less abundant fraction (1.9 %). This result was not far from the result obtained by Ventura and Castanon (1998) who reported a value of 2.7%. The cellulose content (5.71%) was lower to that determined by Yaich et al (2011) and Ventura and Castanon (1998).

In this study, the NFC calculated was 21.7% and it was lower than that reported by Ventura and Castanon (1998, 38.1%). Gross energy was (9.4 MJ kg⁻¹ DM). This result was not in agreement with the result of Ventura and Catanon (1998) who reported a value of (14.1 MJ kg⁻¹DM). However, Marsham et al (2005) obtained in their study a value of (15.7 MJ kg⁻¹ DM).

***In vitro* gas production and kinetics parameters**

Cumulative gas production recorded after 72h of fermentation of *Ulva lactuca* and control in batch systems and the model parameters are shown in **Table2**, **Figure1** and **Figure2**.

The gas production profile of seaweed during 72h of incubation showed a slow and low gas production. The asymptote of the curve had been reached after 36h. Wide variations were observed in the cumulative gas production at different incubation times between seaweed and vetch-oat hay. The results indicated that the gas volume at all incubation times was significantly different ($p < 0.05$). Gas production obtained after 24h for *Ulva lactuca* (42 ml/g OM) was significantly lower than control (128.4 ml/g OM) ($P < 0.001$). This is probably due to the discordances between their chemical components; effectively vetch-oat hay is rich in fibers which contribute highly to gas production. However, the *Ulva lactuca* is characterized by its high contents in minerals and crude protein which contribute enormously to biomass production rather than gas production. Menke and Steingass (1988) reported that gas volume at 24 h after incubation has relationship with metabolisable energy in feedstuffs. The value observed for theoretical maximum gas production (A) and rate of gas production (Rmax) were higher for vetch-oat hay than that of seaweed. As mentioned by Getachew et al (2004), the extent of gas production is correlated to chemical components of feeds such as starch, soluble sugars, NFC, NDF and CP. Then, the result obtained for *Ulva lactuca* can be explained by the nature of their polysaccharides which are different to terrestrial plants. Effectively, seaweeds contain mainly alginate, carrageenans, agar, fucoidans, xylans, ulvans, laminarin (β -1, 3-glucan) and florideans starch (Burtin, 2003). These fractions may limit the availability of nutrients to rumen microbiota and/or needs specific enzymes for their degradation.

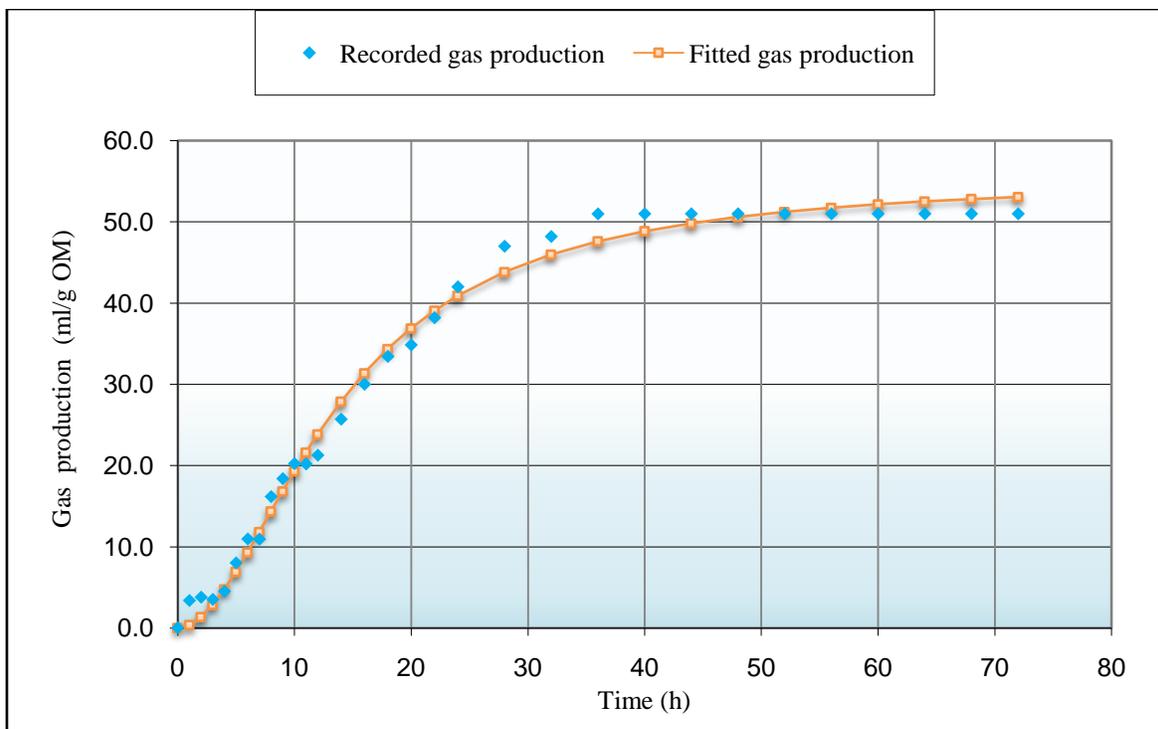


Figure 1: *In vitro* gas production volume of *Ulva lactuca* at different incubation times

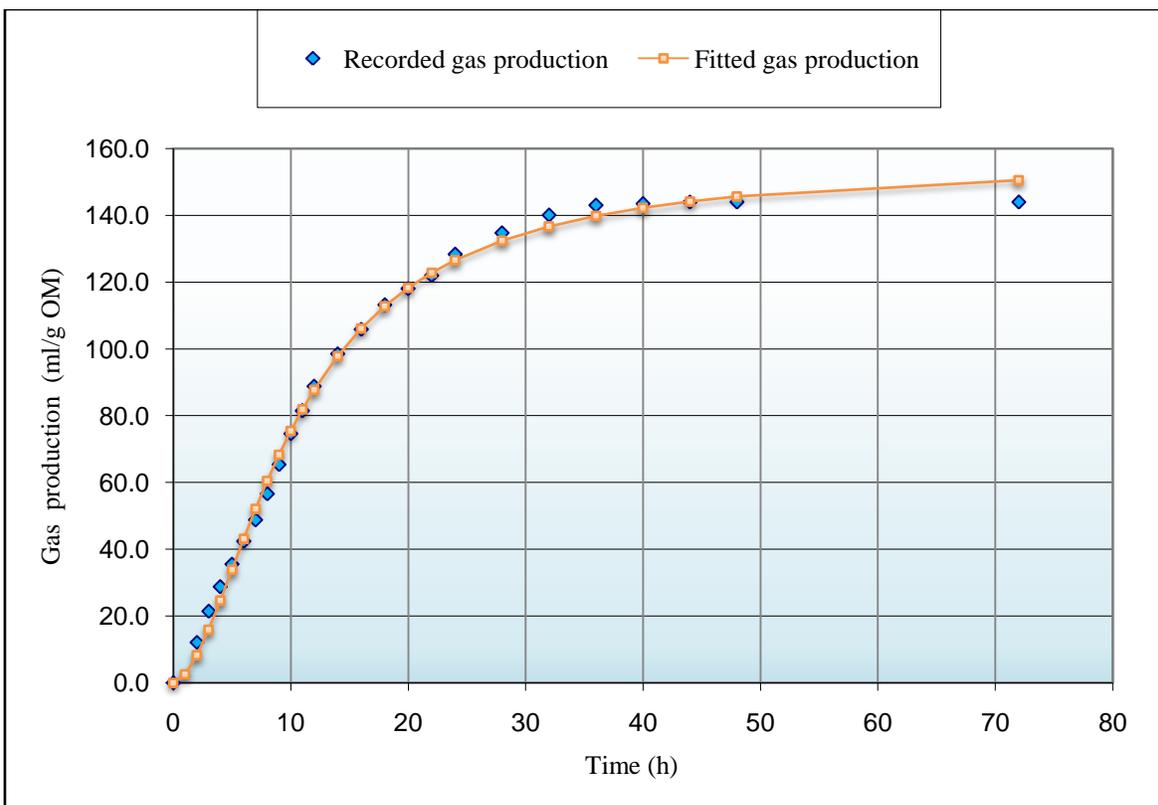


Figure 2: *In vitro* gas production volume of control at different incubation times

Table 1. Dry matter content (g kg⁻¹), chemical composition (g kg⁻¹ DM) and energy content (MJ Kg⁻¹DM) of seaweed (*Ulva lactuca*) and control diet (Vetch-oat hay)

Components	<i>Ulva lactuca</i>	Vetch-oat hay
DM	836.4	911.5
Ash	391.4	77.4
CP	153.2	80.5
EE	10.2	25.8
NDF	228.4	547.8
ADF	76.0	356.7
ADL	18.9	93.0
Cellulose	57.1	263.8
Hemicelluloses	152.2	191.1
NFC	216.9	269.3
GE	9.4	17.8

DM= Dry matter, CP= Crude protein, EE= Ether extract, NDF=Neutral detergent fiber, ADF= Acid detergent fiber, ADL=Acid detergent lignin, GE= Gross energy, NFC= Non fibrous carbohydrates: 100 – (% NDF + % CP + % EE + % Ash) NRC (2001)

Table 2. Cumulative gas production and fermentations kinetics parameters Groot et al (1996)

	Cumulative gas production (ml/ g OM)							
	2h	4h	6h	8h	12h	24h	48h	72h
Seaweed	3.8	4.5	10.9	16.2	21.3	42.0	51.0	51.0
Control	12.2	28.8	42.4	56.6	88.8	128.4	144.0	144.0
S.E.M	1.80	1.55	1.22	1.10	2.18	3.35	5.99	5.98
P	*	***	***	***	***	***	***	***

	Kinetics parameters				
	A (ml g ⁻¹ OM)	B (h)	C	R _{max} (ml g ⁻¹ OM h ⁻¹)	T _{max} (h)
Seaweed	56.1	14.1	1.9	2.5	7.6
Control	155.5	10.3	1.7	9.3	4.9
S.E.M	7.04	0.43	0.08	0.14	0.45
P	***	***	NS	***	*

NS: (P>0.05); *: (P<0.05); **: (P<0.01); *** (P <0.001); S.E.M: Standard error of the mean; P:Probability; A (ml g⁻¹ OM) is the asymptotic gas production; B (h) represents the time after incubation at which half of the asymptotic amount of gas has been formed; C is a constant determining the shape of the curve; R_{max} (ml g⁻¹ OM h⁻¹) is the maximum rate of gas production; T_{max} (h) is the time at which R_{max} is reached.

Table 3. *In vitro* fermentation parameters of *Ulva lactuca* and control (pH, ammonia (N-NH₃), volatile fatty acids, apparent dry matter and organic matter digestibility, and metabolizable energy)

Items	Seaweed	Control	S.E.M	P
pH	6.86	6.75	0.02	**
Ammonia(mg/l)	290.1	151.5	0.22	***
Total VFAs (mmol/gDM)	1.25	5.16	0.18	***
Molar proportion of VFAs (mol/100mol)				
Acetate (C2)	64.2	67.4	0.53	**
Propionate (C3)	25.8	21.1	0.15	***
Butyrate (C4)	10.0	11.6	0.41	**
A/P Ratio	2.48	3.20	0.04	***
DDM (%), 72h	38.3	79.1	0.54	***
OMD (%), 24h	29.2	40.0	0.51	***
ME (MJ/Kg DM)	3.81	5.87	0.08	***

** (P < 0.05); ***: (P < 0.001); S.E.M: Standard error of the mean; P:Probability ,VFAs: Volatile fatty acids; DDM: Dry matter digestibility; OMD: Organic matter digestibility; ME: Metabolizable energy

Table 4. Enzymatic degradation (%) of *Ulva lactuca* and vetch-oat hay proteins

Time of reaction	Seaweed	Control
Soluble nitrogen content (mg/ml) after 1h	0.32 (mg/ml)	0.35 (mg/ml)
Soluble nitrogen content (mg/ml) after 24h	0.43 (mg/ml)	0.39 (mg/ml)
Enzymatic degradation (%) after 1h	26 %	50 %
Enzymatic degradation (%) after 24h	38 %	61 %

Ruminal fermentation parameters

The results obtained after fermentation for pH, ammonia (mg/l), total volatile fatty acids (mmol/g), apparent dry matter and organic matter digestibility and metabolizable energy are illustrated in **Table 3**. The pH value (6.86) recorded for *Ulva lactuca* was higher ($p < 0.01$) than that of control. This result is corroborated with some works conducted *in vivo* and *in vitro*. Indeed, Marin et al (2009), in their study *in vivo*, have reported that the proportion of *Sargassum spp.* affected positively ruminal pH and favors neutral pH. According to Mora et al (2009), who also noted an increase in pH values with the augmentation of algae proportion in the diet, this increase in pH values can be due to the greater water consumption which increase with the concentration of mineral salts supplied by the algae in the diets. However, Arhab et al (2013) explain the stability of pH in neutral zone in batch cultures by the excessive liberation of ions carbonates from the buffer solution for neutralization acidity resultant from volatile fatty acids production and/or high ammonia production.

The higher ammoniacal nitrogen concentration was recorded for *Ulva lactuca* (290 mg/l) and the lowest was observed for control (152 mg/l) ($p < 0.001$). This concentration was five times superior to level (50 mg/l) needed for suitable microbial protein synthesis and digestibility (Satter and Slyter, 1974).

Volatile fatty acids (VFAs) are produced in the rumen as end products of microbial fermentation and represent the major source of energy for ruminants. In this study, their concentration was significantly different between seaweed and control diet ($p < 0.001$). The highest value was recorded for vetch-oat hay (5.16 mmol/g DM) and the lowest was observed for *Ulva lactuca* (1.25 mmol/g DM). According to Getachew et al (2004) who observed strong correlation between 24h gas production, VFA production and non fibrous carbohydrate (NFC). The weak VFA production observed for seaweed can be partially attributed to their low NFC content comparatively to vetch-oat hay. Other authors have observed that CP content was highly correlated to valerate and isovalerate proportions (Getachew et al., 2002; Blümmel et al., 1999).

Molar proportion of VFAs indicated a predominance of acetate production. The values observed for *Ulva lactuca* and control were 64 and 67%, respectively. Furthermore, the highest concentration of butyrate was noted for control (11.6 %) and the lowest was observed for seaweed (10 %). However, propionate rate was higher for seaweed (26%) than vetch-oat hay (21%). According to Mc Donald et al (1995), the fermentation of feed rich in starch tends to produce propionate while fibrous substrates give high concentration in acetate. These observations were reinforced by the high A/P ratio noted for control. This parameter is an indication of proportionally higher digestible NDF in the feeds (Getachew et al., 2004).

Apparent dry matter (DDM) and organic matter digestibility (OMD) are also illustrated in **Table 3**. In this case also the highest values were determined for control (79.14 and 40%). The apparent DMD and OMD of *Ulva lactuca* measured in this study were low than that determined in previous studies performing *in sacco* or *in vivo* tests or in the same conditions. Ventura and Castanon (1998) have reported that OMD of *Ulva lactuca* species was 62.1%. Hansen et al (2003) have mentioned in their paper a high *in sacco* DM degradability (71.7% at 48h). Similarly, Mora et al (2009) noted a high *in situ* digestibility after inclusion of 30% of *Macrocystis pyrifera* (82.2%). In their experiment Marin et al (2009) mentioned an adequate utilization of algal nutrients and suggested that values obtained for dry matter digestibility of *Sargassum* meal are similar to commonly used forages like bean and soybean.

The predicted metabolizable energy is also presented in **Table 3**. It was significantly different between seaweed and control diet ($P < 0.001$). The value of ME calculated in this study (3.81 MJ/ KgDM) is not in agreement with the findings of Ventura and Castanon (1998). These authors have obtained a value of 10.2 MJ/Kg DM for the same species. This value was similar to that reported for alfalfa hay with medium quality (NRC, 1989). These discordances can be attributed in part to the equation used by authors for ME calculation (NRC, 1989).

Proteins Degradability

The results of proteins degradability of seaweed and control are illustrated in **Table 4**. It indicates a difference in proteins degradation between the two substrates after 1h and 24h of incubation. The *Ulva lactuca* proteins were weakly (38%) degraded by proteases after 24h than that of control (61%). Moreover, the ratio DE1/DE24 for *Ulva lactuca* (68 %) lesser than that of control (82 %) demonstrated that the seaweed's proteins were degraded to a slower rate in the rumen. This situation let us supposing that *U. lactuca* can play an important role for supplying animals by nitrogen after its gut degradation. As recommended by Fleurence (1999) seaweeds, especially those that are part of Chlorophyceae and Rhodophyceae, could be a complementary source of proteins for human and animal nutrition. This nutritional value of proteins depends on the nature, composition and digestibility of their amino acids. The proteins of *U. lactuca* contained a high level of amino acids, especially essential amino acids, like: valine, leucine, lysine and threonine (Yaich et al., 2011). These compounds can play an important role for improving meat and milk quality when they are valorized in ruminant gut.

Conclusion

This study showed that the energy potential of *Ulva lactuca* is limited by its high mineral content. The proteins content is interesting, but further analysis is needed to confirm the nature and the intestinal digestibility of these proteins. Based on the results of *in vitro* measurements, the use of *Ulva lactuca* can be considered as supplement to cover animal maintenance needs. Mixtures including studied seaweed with conventional forage in Algeria could be an interesting future investigation.

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