

## Effect of dietary seaweed (*Ulva lactuca*) supplementation on growth performance of sheep and on in vitro gas production kinetics

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**Abstract:** This study was carried out to determine the effect of dietary seaweed (*Ulva lactuca*) supplementation on growth performance of sheep, in vitro gas production, estimated energy, and microbial protein synthesis. A total of 18 Naimey male sheep with average live weight of  $22.78 \pm 0.24$  kg were randomly allocated to 3 groups. Sheep in group 1 were fed a diet containing commercial feed without seaweed as a control diet, sheep in group 2 were fed the control diet with 3% seaweed, and sheep in group 3 were fed the control diet with 5% seaweed. The supplementation of seaweed had no effect ( $P > 0.05$ ) on the average daily gain, feed intake, and feed conversion efficiency. The extent of gas production at 72 h was similar among the 3 groups ( $P > 0.05$ ). There were no significant differences ( $P > 0.05$ ) in the potential degradability, gas production rate, metabolizable energy, net energy, organic matter digestibility, and microbial protein synthesis among the experimental diets. This study concluded that dietary supplementation of seaweed (*Ulva lactuca*) has no effect on growth of sheep, in vitro gas production, potential degradability, estimated energy, organic matter digestibility, and microbial protein synthesis.

**Key words:** Seaweed, *Ulva lactuca*, growth, gas production, energy, microbial protein synthesis

### 1. Introduction

Seaweeds are accumulating in all world coast zones and are considered as an environmental hazard. Their collection and use as a nonconventional source for animal feeding may contribute in solving the environmental problem (1,2). Use of seaweed as a feed supplement for animals has been known by farmers for centuries, and thus the recent attempts to use it as a source of forage for livestock are not new (3–5). Seaweeds have higher protein, minerals, and vitamin levels and lower fat contents compared to some vegetables (6–8), and they are known as a useful feed supplement for sheep (3). Green seaweed (*Ulva lactuca*) has been described as a medium-quality forage for goats through in sacco and in vitro trials (5). Moreover, seaweeds have also shown positive effects on semen quality and fertility traits in ruminants (9,10) and nonruminant animals such as rabbits (11,12) under summer conditions. However, digestibility studies on seaweeds, and particularly *Ulva lactuca*, as a feed supplement to animals are scarce (3,13). More studies are needed to evaluate seaweed as a feed supplement in the diets of ruminants; therefore, the current study was conducted to evaluate the effects of feeding a diet supplemented with different amounts of seaweed (*Ulva lactuca*) on the growth performance

of Naimey sheep and in vitro gas production, estimated energy, and microbial protein synthesis.

### 2. Materials and methods

#### 2.1. Seaweed collection and preparation

Seaweed (*Ulva lactuca*) was collected from the sea and washed with fresh water. Thereafter, it was sun-dried and further dried at 60 °C for 72 h. Dried seaweed was grounded through a 1-mm stainless-steel screen using a Wiley mill grinder and was chemically analyzed. Finally, seaweed was incorporated into the experimental diets at 0%, 3%, and 5% DM. The experimental diets were then mixed through a feed mill and processed as pelleting diets.

#### 2.2. Animals and diets

A total of 18 young lambs were weighed (average live weight of  $22.78 \pm 0.24$  kg) and randomly allocated to 3 groups, each group consisting of 3 separately housed replicates of 2 lambs. Sheep were fed 3% of their body weight of commercial feed with and without different levels of seaweed (*Ulva lactuca*). The lambs in each group were fed as follows (Table 1): Sheep in group 1 were fed a diet containing commercial feed without seaweed as a control diet (CG), sheep in group 2 were fed the control diet supplemented with 3% seaweed (SW3), and sheep in

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**Table 1.** The experimental diets' composition.

Ingredients	Experimental diets (% of DM) <sup>2</sup>		
	CG	SW3	SW5
Alfalfa hay	40	37	35
Seaweed <sup>1</sup>	0	3	5
Barley	25	25	25
Corn	25	25	25
Soybean meal	7	7	7
NaCl	1	0.8	0.7
Lime stone	1	1	1
NaHCO <sub>3</sub>	0.8	1	1.1
Vitamins	0.2	0.2	0.2
Total	100	100	100

<sup>1</sup>Green algae (*Ulva lactuca*).

<sup>2</sup>CG, control diet without seaweed; SW3, control diet with 3% seaweed; SW5, control diet with 5% seaweed.

group 3 were fed the control diet supplemented with 5% seaweed (SW5).

### 2.3. Growth trial

The experiment lasted for 60 days. Feed intake for each group was recorded weekly and daily feed intake was calculated. The animal's weights were recorded every week before the morning meal. The daily weight gains and feed conversion ratio were calculated.

### 2.4. In vitro trial

#### 2.4.1. Gas production rate and potential degradability

The in vitro gas production technique was performed as described by Menke and Steingass (14). Rumen liquor was obtained from slaughtered sheep. Buffer solution was prepared according to Onodera and Handerson (15) and placed in a shaker water bath at 39 °C under continuous flushing with CO<sub>2</sub>. Approximately 200 mg of air-dried of experimental diets was placed into syringes. Thirty-six syringes were divided into 3 treatment groups; each group consisted of 6 replicates with 2 syringes each. Thirty milliliters of rumen fluid and buffer mixture (1:2 v/v) was placed into each syringe containing the samples (16). The incubation procedure was repeated 3 times. The gas production was recorded after 3, 6, 9, 12, 24, 48, and 72 h of incubation. Cumulative gas production values were fitted to the potential equation according to the model of Ørskov and McDonald (17): Gas (Y) = a + b (1 - exp<sup>-ct</sup>), where a = gas production from the immediately soluble fraction, b = gas production from the insoluble fraction, a + b = potential degradability, c = gas production rate constant for insoluble fraction b, and t = incubation time.

#### 2.4.2. Estimation of energy, organic matter digestibility, and microbial protein synthesis

The energy values of experimental diets were calculated from the amount of gas produced at 24 h of incubation with supplementary analysis of crude protein, ash, and ether extract (14) as follows:

$$\text{ME (MJ/kg DM)} = 1.06 + 0.157 \text{ Gp} + 0.084 \text{ CP} + 0.22 \text{ CF} - 0.081 \text{ CA},$$

$\text{OMD (\%)} = 14.88 + 0.889 \text{ Gp} + 0.45 \text{ CP} + 0.0651 \text{ CA}$ , where ME is the metabolizable energy, OMD is the organic matter digestibility, Gp is 24-h net gas production (m/200 mg DM), CP is crude protein, and CA is ash.

$$\text{NE (Mcal/lb)} = (2.2 + (0.0272 \text{ Gp}) + (0.057 \text{ CP}) + (0.149 \text{ EE})) / 14.64,$$

where NE is net energy, Gp is 24-h net gas production (mL/g DM), CP is crude protein, EE is ether extract, and the NE unit is then converted to MJ/kg DM.

Microbial protein (MP) was calculated as follows, according to Czerkawski (18):

$$\text{MP (g/kg OMD)} = \text{OMD} \times 19.3 \times 6.25,$$

where OMD is organic matter digestibility for 24 h.

### 2.5. Chemical analysis

Feeds were dried at 70 °C for 24 h and then ground through a 1-mm screen. Samples of feed were analyzed for dry matter (DM), crude protein (CP), ether extract (EE), and ash according to the AOAC (19). Neutral detergent fiber (NDF) and acid detergent fiber (ADF) were determined according to Van Soest et al. (20).

### 2.6. Statistical analysis

The data were statistically analyzed by ANOVA using the general linear models procedure of SAS (21). Means showing significant differences in ANOVA were tested using the PDIF option. The probability value, which denotes statistical significance, was  $P < 0.05$ .

## 3. Results

The proximate analysis of the diets fed to the experimental animals is shown in Table 2.

### 3.1. Growth trial

The results of the growth performance trial are presented in Table 3. There were no significant differences ( $P > 0.05$ ) among the 3 groups in the final weight of animals after 60 days. Dietary seaweed supplementation for lambs had no effect on the average daily gain (ADG) and feed intake (Table 3). The control group (CG) fed the diet without seaweed showed the numerical best feed conversion efficiency compared to the other groups (SW3 and SW5), although the difference was not significant ( $P > 0.05$ ). Moreover, dry matter intake did not differ significantly among the experimental groups ( $P > 0.05$ ).

**Table 2.** The proximate analysis of the 3 experimental diets.

Items <sup>2</sup>	Proximate analysis (% of DM) <sup>1</sup>					
	DM %	CP	EE	Ash	NDF	ADF
CG	93.38	15.87	2.52	8.74	27.59	16.62
SW3	93.18	15.75	2.47	9.72	27.16	16.38
SW5	93.47	16.07	2.43	10.30	26.46	16.14

<sup>1</sup>DM, dry matter; CP, crude protein; EE, ether extract; NDF, neutral detergent fiber; ADF, acid detergent fiber.

<sup>2</sup>CG, control diet without seaweed; SW3, control diet with 3% seaweed; SW5, control diet with 5% seaweed.

**Table 3.** Effect of seaweed (*Ulva lactuca*) additives on growth, feed intake, average daily gain and feed conversion (mean  $\pm$  SE).

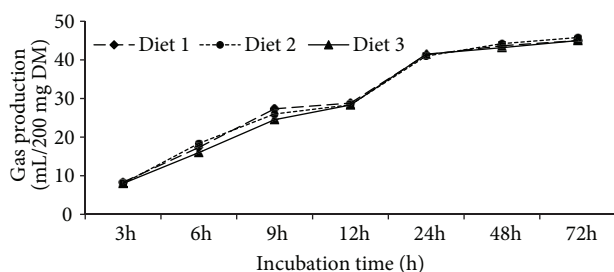
Parameters	Experimental diets <sup>1</sup>			P-value
	CG	SW3	SW5	
Period (days)	60	60	60	
Initial weight (kg)	22.99 $\pm$ 0.521	22.55 $\pm$ 0.583	22.78 $\pm$ 0.483	0.88
Final weight (kg)	38.25 $\pm$ 0.593	36.08 $\pm$ 1.383	36.75 $\pm$ 0.882	0.39
Gain weight (kg)	15.26 $\pm$ 0.170	13.53 $\pm$ 0.981	13.97 $\pm$ 0.453	0.19
Average daily gain (kg)	0.25 $\pm$ 0.003	0.23 $\pm$ 0.022	0.23 $\pm$ 0.013	0.19
Feed intake (kg)	1.26 $\pm$ 0.012	1.25 $\pm$ 0.014	1.26 $\pm$ 0.004	0.08
Feed conversion	4.97 $\pm$ 0.060	5.65 $\pm$ 0.341	5.43 $\pm$ 0.171	0.13

<sup>1</sup>CG, control diet without seaweed; SW3, control diet with 3% seaweed; SW5, control diet with 5% seaweed.

### 3.2. In vitro trial

#### 3.2.1. Gas production rate and potential degradability

Cumulative gas production produced from the experimental diets during 72 h of incubation is shown in the Figure. There were no significant differences ( $P > 0.05$ ) among the 3 diets on gas production; however, dietary seaweed supplementation at 2 levels (3% and 5%) had no effect on gas production during the incubation times.

**Figure.** Cumulative gas production produced from the experimental diets during 72 h of incubation.

There were no significant differences ( $P > 0.05$ ) among the experimental diets in potential degradability (a + b) (Table 4). The gas production rate (c) is shown in Table 4. There was also no significant difference ( $P > 0.05$ ) among the experimental diets concerning gas production and potential degradability.

#### 3.2.2. Estimation of energy, organic matter digestibility, and microbial protein synthesis

Predicted values of ME, NE, OMD, and MP are shown in Table 5. There were no significant differences ( $P > 0.05$ ) among the experimental diets in ME, NE, OMD, and MP.

### 4. Discussion

#### 4.1. Growth trial

Dietary seaweed supplementation for lambs had no effects ( $P > 0.05$ ) on the final weight, ADG, feed intake, and feed conversion efficiency (Table 3). There were no differences ( $P > 0.05$ ) in dry matter intake among the 3 groups; however, the control group had the best feed conversion

**Table 4.** Parameters of gas production produced from mixed diets contained seaweed during 72 h of incubation (mean  $\pm$  SD).

Parameters	Experimental diets <sup>1</sup>			P-value
	CG	SW3	SW5	
a + b (mL)	44.80 $\pm$ 0.923	45.47 $\pm$ 0.664	44.85 $\pm$ 0.207	0.50
c (mL/h)	0.10 $\pm$ 0.013	0.10 $\pm$ 0.006	0.10 $\pm$ 0.004	0.25

<sup>1</sup>CG, control diet without seaweed; SW3, control diet with 3% seaweed; SW5, control diet with 5% seaweed.

Cumulative gas production data were fitted to the model of Ørskov and McDonald (17): Gas (Y) = a + b (1 - exp<sup>-ct</sup>), where a = gas production from the immediately soluble fraction, b = gas production from the insoluble fraction, a + b = potential degradability, c = gas production rate constant for insoluble fraction b, t = incubation time.

**Table 5.** Predicted of metabolizable energy (ME), net energy (NE), organic matter digestibility (OMD), and microbial protein (MP) in vitro from mixed diets contained seaweed during 24 h of incubation (mean  $\pm$  SD).

Parameters	Experimental diets <sup>1</sup>			P-value
	CG	SW3	SW5	
ME (MJ/kg DM)	7.26 $\pm$ 0.709	7.92 $\pm$ 0.550	7.75 $\pm$ 0.557	0.24
NE (MJ/kg DM)	5.44 $\pm$ 0.464	5.27 $\pm$ 0.422	5.18 $\pm$ 0.403	0.58
OMD (%)	55.51 $\pm$ 4.443	54.45 $\pm$ 4.034	53.09 $\pm$ 3.934	0.60
MP (g/kg OMD <sup>2</sup> )	66.96 $\pm$ 5.359	65.68 $\pm$ 4.866	64.04 $\pm$ 4.748	0.60

<sup>1</sup>CG, control diet without seaweed; SW3, control diet with 3% seaweed; SW5, control diet with 5% seaweed.

<sup>2</sup>MP (g/kg OMD) = OMD  $\times$  19.3  $\times$  6.25 according to Czerkawski (18), where OMD is organic matter digestibility for 24 h.

efficiency compared to the other groups (SW3 and SW5), which might be due to the higher ADG values in control group. The current study showed that the control group had the best weight gain, ADG, and feed conversion efficiency as compared to the other groups (SW3 and SW5), in agreement with the results reported by Allen et al. (22), who showed that brown seaweed extract supplementation did not affect the body weight of steers. Further, Al-Shorepy et al. (23) reported that supplementation of 1% seaweed meal had no significant influence on relative growth of body components of lambs. Seaweed extract supplementation did not increase the concentration of volatile fatty acids in the rumen of goats (22). However, a decrease in the proportion of propionate and increases in the proportions of butyrate and isobutyrate in the rumen of cattle (24) might be attributed to the observed numerical decrease in weight gain and ADG in the current study.

In general, the use of seaweeds in agriculture research resulted in inconsistent findings. The discrepancies might be due to the variance in seaweed genera (*Palmaria*, *Ulva*, *Ascophyllum*), species (red algae, green algae, and brown algae), differences in harvesting and processing procedures (collecting, washing, and drying), or the levels used (25).

## 4.2. In vitro trial

### 4.2.1. Gas production rate and potential degradability

The supplementation of seaweed in mixed diet had no effect on gas production during the incubation times or the potential degradability (P = 0.50). Ventura and Castañón (5) reported that the potential degradability of seaweed using an in situ technique was 57.4%, which was higher than the potential degradability in the present study (44.80 to 45.47 mL). This discrepancy might be due to the difference of methods used, animal species, levels of seaweed used, or microbial utilization (16).

#### 4.2.2. Estimation of energy, organic matter digestibility, and microbial protein synthesis

The supplementation of seaweed had no effect ( $P > 0.05$ ) on ME and NE. Ventura and Castaño (5) estimated the digestible energy of the seaweed as 10.2 MJ/kg DM, which is equivalent to 8.26 MJ/kg DM for ME. This value is quite similar to the ME in the present study. There were no significant differences ( $P > 0.05$ ) among the experimental diets in OMD and MP. The values of OMD reported in the current study were lower than that reported for brown algae mixture (*L. digitata* and *L. hyperborean*) in vitro (78.3%) by Hasen et al. (26) and for *U. lactuca* (62.1%) by Ventura and Castaño (5), and also less than that reported for *U. lactuca* (60%) in vivo using sheep (3). The results of MP were also lower than that reported by Hasen et al. (26), Ventura and Castaño (5), and Arieli et al. (3), who found values of 94.45, 74.91, and 72.38 g/kg OMD, respectively. As mentioned before, the

differences between the various studies may be due to the different species of seaweed, harvesting time, species of experimental animals, processing procedures of seaweed, or the type of feeding.

The present study concluded that diets containing seaweed (*Ulva lactuca*) did not improve the ADG, feed conversion, gas production, potential degradability, estimated energy, organic matter digestibility, or microbial protein synthesis. This might be due to the lower levels used in the present study. Therefore, more studies using higher levels of seaweed supplementation to the diets of ruminants are needed.

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