Evaluate adding green seaweed to different rations by *In vitro* gas production technique

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Abstract: *In vitro* gas production technique used to evolution supplemented green seaweed (*Ulva lactuca*) with different levels on two rations containing rice straw and concentrate and effects on some rumen parameters, digestibility, degradability (DM, OM, NDF, ADF, hemicellulose and cellulose) and gas production values. Three rations were used to *In vitro* techniques and the rations were: R1(standard ration):40% clover hay + 60%concentrate, R2: 40% rice straw + 60%concentrate, R3: 50% rice straw + 50% concentrate. Supplemented green seaweed (*Ulva lactuca*) with different levels were (0,0.8,1.6,2.4 and 3.2% from DM ration)**.** The results indicated that the rate of fermentation gas production was high in all rations adding green seaweed. Ruminal pH and rate gas production per hour (Rate GP / h) was not affected during fermentation processes, Short chain fatty acid (SCFA) and metabolic energy (ME) cleared no significant differences among all different adding and control groups in the experiment. Ammonia concentration recorded the lowest value of ammonia in R2(1.6%) and R3 (0.8%). Microbial protein (MP) and efficiency microbial protein (EMP) recorded high values in all different addition of green seaweed compared control (0%). The highest significant value of DMD ($p<0.05$) was found in R2 adding seaweed 0.8 % and 3.2% (63.21 and 63.54 %). OMD in R2 recorded the highest values in all supplementation especially level of 3.2% compared 0% and R1, but in R3 the level of 0.8%adding seaweed was the highest value only. It was noticed, that the values of hemicellulose digestibility increased with increase in the level of green seaweed compared 0 %. Cellulose digestibility (Cellul. D) values were recorded the highest value in all adding green seaweed especially level 3.2 in R2 and level 1.6 in R3 (76.58 and 48.92 %, respectively) compared with R1 (28.84%). Degradability of DM, OM, NDF, ADF, Hemicell and Cellu. were increasing with adding green seaweed with different levels supplementation in R2 especially added 3.2% was the highest value compared with 0% added. In R3 the highest value was adding 0.8% green seaweed only but any adding were low values compared 0% added. It concluded that supplementation seaweed *(Ulvalactuca*) with different levels due to improving digestibility, kinetics of gas production, growth of microbial protein biomass, efficiency of microbial protein and degradability especially R2 containing 40% rice straw but R3 containing 50% rice straw in one level adding 0.8% DM and equaled values standard ration (R1) containing clover hay.

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1. Introduction

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 (**Arieli *et al.* (1993)** and **Ventura and Castañoń (1998)**). Seaweeds have higher protein, minerals, and vitamin levels and lower fat contents compared to some vegetables (**Beatrice, 1993 and Wong and Cheung, 2000)**, and they are known as a useful feed supplement for sheep (**Arieli *et al.* (1993)**). Green seaweed (*Ulvalactuca*) has been described as a medium-quality forage for goats through in sacco and *In vitro* trials (**Ventura and Castañoń, 1998)**. Moreover, seaweeds have also shown positive effects on semen quality and fertility traits in ruminants (**Kellogg *et al.*, 2006 and Yates *et al.*, 2010)**) and non-ruminant animals such as rabbits (**Okab *et al.*,2008 and Okab *et al.*, 2013)**) under summer conditions. However, digestibility studies on seaweeds, and particularly *Ulvalactuca*, as a feed supplement to animals are scarce (**Arieli *et al.*, 1993** and **Ventura *et al.*, 1994)**). **EL-waziry *et al.*, 2015)** found that the supplementation of seaweed in mixed diet had no effect on gas production during the incubation times or the potential degradability. **Ventura and Castañoń (1998)** reported that the potential degradability of seaweed using an in situ technique was 57.4%. This discrepancy might be due to the difference of methods used, animal species (microbial in rumen) or levels of seaweed used **(Blümmel and Orskov 1993)**. **EL-waziry*et al.* (2015)** recorded that the supplementation of seaweed had no effect ($P > 0.05$) on ME and NE. **Ventura and Castañoń (1998)** estimated the digestible energy of the seaweed as 10.2 MJ/kg DM, which is equivalent to 8.26 MJ/kg DM for ME. 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 thanthat reported for brown algae mixture (*L. digitata* and *L. hyperborean*) *In vitro* (78.3%) by **Hasen *et al.* (2003)** and for *U. lactuca* (62.1%) by **Ventura and Castañoń (1998)**. **Hasen *et al.* (2003),** who found values of 94.45, 74.91, and72.38 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. **EL-waziry *et al.* (2015)** concluded that diets containing seaweed (*Ulvalactuca*) did not improve the gas production, potential degradability, estimated energy, organic matter digestibility, or microbial protein synthesis which it might be due to the lower levels used.

The aim of the current study using *Invitro* gas production technique to evaluate supplemented green seaweed (*Ulvalactuca*) with different levels on two rations containing rice straw and concentrate and effects on some rumen parameters, digestibility, degradability and gas production values.

2. Materials and Methods

In vitro gas productiontechnique

Two days before beginning of the experiment, 400 ± 4 mg of sample for each level (contained clover hay as a roughage and concentrate ratio of 50:50%) was weighed into 125 mL glass bottles. These bottles have a total volume of 125±2 mL. A buffer solution was prepared before addition of rumen fluid as described by **Szumacher-Strabel *et al.* (2002)** and flushed continuously with CO_2 at 39◦C during sample inoculation. Rumen fluid was obtained from slaughter house and it was collected from buffalo. The collected rumen fluid was mixed into a bottle (1L) with an O2-free headspace and immediately transported to laboratory at 39°C. Upon arrival at the laboratory, the rumen fluid was filtered through four layers of cheesecloth to eliminate large feed particles. The buffer solution was added to rumen fluid at ratio 4:1. forty mL of this inoculum was added to each bottle, then the headspace of each bottle was flushed with CO2, and closed. The initial pH of the inoculums was from 6.8-6.9. Triplicates of each sample were used in two separate runs.

Digestibility

After 24 hours incubation Dry matter digestibility (%DMD) was calculated as the (Substrate dry matter incubated – (residue dry matter – blank dry matter) / Substrate dry matter incubated * 100). NDF and ADF of the residuals after fermentation were analyzed with the same methods used for feed ingredient analysis. Digestibility of NDF, ADF, cellulose and hemicellulose were calculated as the dry matter digestibility calculated.

Degradability

After 24 hours gas production substrate and degradability was calculated as:

GPDM (ml/g DM) = total gas production (ml) / substrate DM (g). GPOM, GPNDF, GPADF, GPcell. and GPhemi. calculated as the same equation in GPDM.

GPdDM (ml/g degraded DM) = total gas production (ml) / degraded DM (g). GPdOM, GPdNDF, GPdADF, GPdcell. and GPdhemi. calculated as the same equation in GPdDM.

Total gas production

After 24 hours of samples incubation, the total gas production was estimated by the displacement of syringe piston, which was connected to the serum flasks. The gas produced due to fermentation of substrate was calculated by subtracting gas produced in blank vessels (without substrate) from total gas produced in the vessels containing buffered rumen fluid and substrate.

Calculation

Metabolizable energy (ME, Mcal/kg DM), *Invitro* organic matter digestibility (OMD, g/kg OM) were estimated according to **(Menke and Steingass, 1988)**, (SCFA) Short Chain Fatty Acid concentrations were calculated according to **Getachew *et al.* (2002)**. Microbial Biomass Production (MCP) and Efficiency of Microbial Biomass Production (EMP) were calculated according to **Blummel *et al.* (1997)** as:

- ME (mJ/kg DM) = 2.20 + 0.136 GP + 0.057 CP (%),
- OMD = 14.88 + 0.889 GP+ 4.5 CP (%) + 0.0651 ash (%),
- SCFA (mmol/200 mg DM) = -0.00425 + 0.0222 * GP
- MCP (mg/g DM) = mg dDM- GP*2.2
- EMP = (mg dDM- GP*2.2))/ mg DMD

where

GP is net GP in mL from 200 mg of dry sample after 24 h of incubation, 2.2 mg/ mL is a stoichiometric factor that expresses mg of C, H, and O required for the SCFA gas associated with production of 1 mL of gas.

After 24 hours of incubation, the filtrated rumen liquor for each sample was subjected for further investigation. The pH of rumen fluid was measured (pH meter) and quantitative analysis of ammonia concentration was carried out by Nesler method modified by **Szumacher-Strabel *et al.* (2002).** total volatile fatty acids (TVFA's) **(Barnett and Reid, 1956).**

Proximate analysis and Cell wall constituents analysis

The proximate analysis of concentrate, green

seaweed, rice straw and clover hay were determined according to **A.O.A.C. (1997)**. The proximate analyses were used to determine dry matter (DM), crude protein (CP), crude fiber (CF), Ether Extract (EE) and ash. The nitrogen free extract (NFE) was obtained by the difference. Concentrate, rice straw and clover hay were analyzed according to **Van Soest*et al.* (1991**) to determine neutral detergent fiber (NDF), Acid detergent fiber (ADF) and acid detergent lignin (ADL). Hemicellulose, cellulose and lignin were determined by difference.

Seaweed collection and preparation

Green Seaweed (*Ulvalactuca*) 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, green seaweed was supplementation five levels into the experimental diets at 0%, 0.8%, 1.6%, 2.4% and 3.2% DM ration. The experimental diets were then mixed through a feed mill diets.

Standard rations

Three rationsused to *Invitro* techniques andthe rations were: R1(standard ration): contents 40% clover hay + 60%concentrate, R2: contents 40% rice straw + 60%concentrate, R3: contents 50% rice straw + 50% concentrate. Chemical composition and cell wall constituents of clover hay, rice straw, green seaweed, concentrate and rations showed in Table (1).

Statistical analysis:

The data of *Invitro*digestibility, *Invitro*degradability, energy, some rumen parameters and microbial protein synthesis were statistically analyzed according to statistical analysis system User's Guide, **(S.A.S., 1998).** Separation among means was carried out by using Duncan Multiple test, **(Duncan, 1955).** The following model was used:

$Y_{ij} = \mu + S_i + Bk + \alpha_{ij}k$

Where:

Y ij = the observation of the model.μ = General mean common element to all observation.

Si = the effect of the treatment (i = 1... 3). B_K= the effect of the levels of treatment (K = 1….5). α ij = The effect of error.

3. Results and Discussion

Gas value and Ruminal parameters

Potential gas production of R1standard ration (40% clover hay + 60% concentrate), R2 (40% rice straw + 60% concentrate) and R3 (50% rice straw + 50% concentrate) showed in Table 2. Potential gas production was significantly ($P < 0.05$) affected by adding green seaweed (*Ulvalactuca*) to R 2 and R3, after 24 hours the rate of fermentation varied among difference additives from 0.8, 1.6 and 2.4 had relatively high rate of fermentation in adding 2.4 in R2 and 1.6 in R3compared 0%. Ruminal pH and rate gas production per hour (Rate GP / h) was not affected during fermentation processes.Several studies have suggested that pH relatively more stable and meet the needs of rumen microbes to perform its activity, whichduring fermentation increasing pH and used by rumen microbes due to more stable pH **(Elghandour *et al.,* 2014).** Ammonia concentration recorded the same values in added green seaweed compared R1but added green seaweed on R2 (1.6) and on R3 (0.8) recorded the lowest value of ammonia. The result may be due to added green seaweed affect on bacteria activity which increased growth and activity ruminal bacteria and causes increase of bacteria to digestion of protein. Increasing protein content of the ration caused an increased gas production R1 in ration standard. However, fermentability of protein produces relatively small gas production compared to carbohydrate fermentation **(Makkar *et al.,* 1995).** The gas production, form any substrate, depends mainly on nutrient availability for rumen microorganisms **(Elghandour *et al.,* 2014).** Total volatile fatty acids (TVFA's) concentration recorded the highest value in added seaweed in R3 especially 2.4% compared R1 and R2 in Table 2. The result may be due to added seaweed affect on bacteria activity which increased growth and activity ruminal bacteria and increase of cellulolytic bacteria to digestion of cellulose. The result cleared that the adding seaweed to R2 due to fermentation of fiber approximately the same trained in R1 (standard ration) while in R3 fermentation increased higher than anther rations. Supplementation of seaweed (2.4 %) improved gas value, ammonia and TVFA's than the anther dose. The nature of substrate, and the Invitro procedure are responsible about the varied response with a different level of seaweed with different rations. In case of rumen modulator such as yeast supplementation at different rates, yeast could change the fermentation rate and cause different substrate depletion, resulting in different responses **(Mao *et al.,* 2013).**

Fermentation of dietary carbohydrates to acetate, propionate and butyrate produces gases in the rumen. So, it is well clear that the increased TVFAs, MP and EMP were a result of increased adding of greenseaweed to rations. It is well known that microorganisms has the ability to increase ammonia production in the rumen **(Hristov *et al.,* 2013)** by increased protein degradation. Microbial protein (M P) and efficiency microbial protein (EM P) recorded high values in all different added of green seaweed compared control (0%). Short chain fatty acid (SCFA) and metabolic energy (ME) cleared no significant differences among all different adding of green seaweed.

In these study, the high dose of green seaweed used (2.4 and 3.2%) improvedkinetics of fermentation, NH3 and MP than the low dose of green seaweed. The highly activity reflected on higher microbial protein synthesis, and higher digestibility. This can be generalized for the effect of green seaweed addition on the fermentation activity. They returned their results to the high activities of microbes in the rumen as a result of produced growth factors for microbial growth and activity in the rumen, and to the ability of green seaweed to provide conducive anaerobic conditions to microbial growth. **Siegel (1991)** suggested that gas production from cereal straws and in different classes of feeds incubated *Invitro* in buffered rumen fluid was closely related to the production of SCFA which was based on carbohydrate fermentation. **Bakker *et al.* (1995)** reported a close association between SCFA and gas production *Invitro*, suggests a potential to make energy available to the ruminants.

Digestibility

Data in Table (3) showed that after 24 hours the highest significant value of DMD increase ($p<0.05$) was found in R2 adding seaweed 0.8 % and 3.2% *(*63.21 and 63.54 %), but in R3 all addition levels were higher than 0%control and R1. OMD after 24 hours in R2 recorded the highest values in all supplementation especially level of 3.2% compared 0% control and R1, but in R3 the level of 0.8%adding seaweed was the highest value only. Results indicate that decrease values in DM and OM digestibility in R3with increase Ash percentage in composition of R3 compared R1 and R2.

In these studies showed that after 24 hours, the highest value of hemicellulose digestibility (Hemi. D) found inR2 adding green seaweed 3.2% (29.98 %) compared control 0%(26.87%). It was noticed, that the values of hemicellulose digestibility increased with increase level of green seaweed (*Ulva)* compared control 0 % ration. Cellulose digestibility (Cellul. D) values were recorded the highest value in all adding green seaweed in experimental rations especially level 3.2 in R2 and level 0.8 in R 3 (76.58 and 49.45 %, respectively) compared R 1 (28.84%). These results may be due to R2 and R3 containing high values of cellulose (in rice straw) and adding green seaweed high affect on cellulose digestibility which increase growth of cellulolytic bacteria and increase fermentation of cellulose but standard ration containing high value of hemicellulose (in clover hay) which high digestibility. **Colombatto *et al.*, (2007)** stated that fibrolytic enzymes enhanced the fermentation of cellulose and xylan by a combination of pre- and post- incubation effects. Exogenous fibrolytic enzymes might enhance attachment and improve access to the wall matrix by ruminal microorganisms and by doing so, accelerate the rate of digestion **(Nsereko *et al.*, 2000)**.

Kinetics of gas production value and Degradability

Gas production fermentation (ml /1gm substrate in rations) of DM, OM, NDF, ADF, Hemicellulose and Cellulose after 24 hours incubation showed in Table 4. After 24 hours incubation gas production of fermentation of DM, OM, NDF, ADF, Hemicell and Cellu. were increasing with adding green seaweed with different level supplementation in R2 especially added 3.2% was the highest value compared control 0% added while in R3 the highest value was adding 0.8% green seaweed only.

Gas production degradableof DM, OM, NDF, ADF, Hemicellulose and Cellulose after 24 incubation showed in Table 5. After 24 hours incubation gas production degradability of DM, NDF, ADF, Hemicellulose and Cellulose of control standard (R1) without adding was the lowest value compared any adding with R2 and R3.

Degradability of DM, OM, NDF, ADF, Hemicell and Cellu. were increasing with adding green seaweed with different level supplementation in R2 especially added 3.2% was the highest value compared control 0% added. In R3 the highest value was adding 0.8% green seaweed only. The results showed that adding green seaweed to rations implying that increase growth of cellulolytic bacteria, may be increase fermentation of cellulose and improve degradability of experimental rations. The higher extent of gas production and rate of degradation of *M. oleifera* suggests that rumen microbes were able to utilize the feed better probably due to a higher content of fermentable nutrients. A higher potential gas production can contribute significantly to energy supply via short chain fatty acid production **(Remesy *et al.*, 1995).**

For gas volume and *Invitro* gas production characteristics, **Lina *et al.* (2009)** suggested that gas volume at 24h after incubation is an indirect relationship with metabolisable energy in feedstuffs. Gas production can be regarded as an indicator of carbohydrates degradation, **(Rajendran, 2013)** suggested that gas volume is a good parameter from which to predict digestibility, fermentation end product and microbial protein synthesis of the substrate by rumen microbes in the *Invitro* system. Gas production is basically the result of fermentation of carbohydrates to acetate, propionate and butyrate and substantial changes in carbohydrates fractions were reflected by total gas produced **(Te-Hsing *et al.*, 2007).** Mathematical descriptions of gas production profiles allow analysis of data evaluation of substrates and media related differences and fermentability of soluble and slowly fermentable components of feeds **(Newman *et al.*, 2009)**. Although gas production is a

nutritionally wasteful products **(Ingale and Chaudhari, 2013)**. but provides useful basis from which ME, OMD and SCFA may be predicted **(Yang and Sun, 2006)**. There was a positive correlation between metabolisable energy calculated from *Invitro*gas production together with CP and fat content with metabolisable energy value of conventional feeds measured *Invivo*, **(Kaiser *et al.*, 2014)**. **Iravani *et al.* (2014)** found a high precision in prediction of *Invivo* OMD. This group further used a correlative approach to predict the ME content of feed by *Invitro*gas volume measurement and chemical constituents and concluded that the prediction of ME is more accurate when based on gas and chemical constituents only. Other workers **Rajendran *et al.* (2013)** have also reported significant correlation between *Invitro* gas measurement and *Invivo* digestibility. **Akinfemi *et al.* (2009)** the *Invitro* gas production techniques can be used to assess the nutritive value of tropical agricultural wastes and to differentiate between their potential digestibility and metabolisable energy contents. Chemical composition and *Invitro* digestibility are very useful in estimation of OMD, SCFA and ME.

Vinoj Kumar and Kaladharan (2007) reported that the nutritional value of six tropical seaweeds (*Sargassum wightii*, *Ulva lactuca*, *Kappaphycus alvarezii*, *Hypnea musciformis*, *Acanthophora spicifera* and *Gracilaria corticata*) as complementary source of dietaryproteins for human and animal nutrition based on amino acid profile was evaluated. All these species showedsimilar non-essential amino acid patterns in which aspartic and glutamic acids constituted together a large partof the amino acid fraction (25.2% to 29.5%). Among these, *Hypnea musciformis* possessed higher amino acid content and better amino acid profile and all of them were generally rich in phenylalanine, tyrosine, threonine and tryptophan and deficient in methionine, cysteine, leucine and lysine. Except *U. lactuca* all others showeda balanced amino acid profile comparable to **FAO (1981**) reference pattern. Seaweeds being rich in minerals, vitamins, polyunsaturated fatty acids as well as phycocolloids, partial substitution of costly protein sources inanimal feeds with seaweed protein may improve feed quality while reducing the cost. It can be concluded that all the six species of seaweeds are generally rich in aromatic amino acids, threonine and tryptophan and deficient in sulphur containing amino acids, leucine and lysine. Except *U. lactuca* all the five seaweeds showed a balanced amino acid profile comparable to thatof **FAO (1981).** Also the use of seaweed proteins with good aminoacid profile in feed for fish and cattle seems to be apromising way for the utilization of this marine resource that remains under-exploited in our coasts.

Most algae species have high protein content and favorable amino acid composition relative to WHO/FAO standards and have a relatively low content of potentially troublesome non-protein nitrogen (**Lavens and Sorgeloos, 1996; Becker, 2007).** Carbohydrate utilization by animals depends on their digestion system. The cellulose content of algal cell walls (~10% of dry weight) will affect the digestibility by non-ruminant animals. In land plants, cellulose may account for 20% to 50% (w/w) of the biomass (**Becker, 2007**). Microbial fermentation, however, enables ruminants to utilize cellulose efficiently and algal feed supplements have been used successfully to increase growth rates of calves (**Chowdhury *et al.*, 1994**) and improve milk composition in dairy ewes (**Papadopoulos *et al.*, 2002**). Algal cell walls vary among taxa (**Domozych, 2011**). Algae feeding tests performed so far indicate that their overall digestibility is high **(Becker, 2004).** Algal lipids (DHA, and other Omega 3s) have a positive impact as an animal feed on healthy fat marbling in cattle **(Stamey *et al.*, 2012).**

Table 1: Chemical composition and cell wall constituents of experimental rations

Item	Clover hay	Rice straw	Concentrate	Green seaweed	R 1	R 2	R3
DM	92.40	91.39	87.05	92.10	89.19	88.79	89.22
OM	86.79	82.24	95.73	54.18	92.15	90.33	88.98
CP	17.41	1.66	16.19	18.68	16.68	10.38	8.93
EE	3.98	2.21	4.77	0.28	4.45	3.75	3.49
CF	40.94	42.13	20.67	6.55	28.78	29.25	31.40
NFE	24.46	36.24	54.10	28.67	42.24	46.95	45.17
Ash	13.21	17.76	4.27	45.82	7.85	9.66	11.02
Cell wall constitutes							
NDF	40.94	68.74	20.66	-	28.77	39.89	44.70
ADF	26.88	50.83	53.94	-	43.12	52.69	52.38
ADL	5.80	8.37	1.41	-	3.16	4.19	4.89
Hemicell.	14.66	17.91	15.24	-	15.01	16.31	16.57
Cellulose	12.53	42.40	3.98	-	7.40	19.35	23.19

Where: concentrate: 55.9 %Corn grain, 22 %Soybean meal,, 20 %Wheat bran,0.8 % salt (Na cl),1% lime stone
R1: 40% clover hay + 60%concentrate (standard); R2: 40% rice straw + 60%concentrate; R3: 50% rice straw + 50%concentrate

Table 2: Gas value (kinetics of gas production in ration ml/ 500 mg DM)

item	R1	R2					R3					SE
Level of Algae	0	0	0.8	1.6	2.4	3.2	0	0.8	1.6	2.4	3.2	±
Gas production												
Total GP	74.66 ^{b}	72.33 ^{b}	75.66 ^{a}	77.00 ^{a}	78.33 ^{a}	74.66 ^{b}	70.00 ^{c}	74.66 ^{b}	76.66 ^{a}	75.30 ^{a}	73.30 ^{b}	1.37
Rate GP / h	3.11	3.01	3.15	3.21	3.26	3.11	2.92	3.11	3.19	3.14	3.05	0.93
Rumen parameters												
pH	6.84	6.85	6.80	6.77	6.81	6.84	6.82	6.81	6.81	6.78	6.80	0.01
NH3	7.57	6.51	7.73	5.77	7.61	7.57	7.32	5.31	7.53	7.26	7.52	0.77
TVFA's	6.11 ^{d}	7.38 ^{c}	5.73 ^{e}	7.28 ^{c}	7.16 ^{c}	6.11 ^{d}	6.77 ^{d}	7.60 ^{c}	7.17 ^{c}	11.85 ^{a}	10.97 ^{b}	0.68
MP	276.87 ^{b}	241.79 ^{e}	274.29 ^{b}	274.69 ^{b}	276.87 ^{b}	376.00 ^{a}	248.73 ^{e}	268.73 ^{b}	234.95 ^{d}	250.93 ^{c}	226.20 ^{e}	20.75
EMP	43.54 ^{b}	40.21 ^{c}	43.38 ^{b}	43.65 ^{b}	43.88 ^{b}	45.42 ^{a}	40.55 ^{c}	42.61 ^{b}	40.30 ^{c}	41.98 ^{c}	39.55 ^{c}	3.11
SCFA	3.62	3.61	3.75	3.85	3.95	4.56	3.60	3.78	3.51	3.51	3.49	0.10
M E	3.87	3.87	3.87	3.86	3.87	4.15	3.89	4.29	3.94	3.84	3.83	0.05

a, b, c, d & e means within the same row with different superscripts differ significantly ($P < 0.05$)

Where:

R1: 40% clover hay + 60%concentrate (standard) R2: 40% rice straw + 60%concentrate

R3: 50% rice straw + 50%concentrate MP: microbial protein (mg/100 ml rumen liqour)

EMP: efficiency of microbial protein SCFA: short chin fatty acid (µm) M E: metabolic energy (MJ/kg DM)

Table 3: Digestibility of DM, OM, NDF, ADF, Cellulose and Hemicellulose after 24 hours on DM basic

item	R1	R2					R3					SE
Level of Algae	0	0	0.8	1.6	2.4	3.2	0	0.8	1.6	2.4	3.2	±
DMD,%	63.54 ^{a}	60.00 ^{c}	63.21 ^{a}	62.01 ^{b}	62.85 ^{b}	63.54 ^{a}	61.29 ^{b}	56.09 ^{e}	58.29 ^{d}	59.83 ^{c}	57.19 ^{d}	1.32
OMD,%	45.18 ^{c}	45.07 ^{c}	45.91 ^{c}	46.88 ^{c}	47.18 ^{b}	52.78 ^{a}	45.59 ^{c}	47.42 ^{b}	44.31 ^{d}	44.29 ^{d}	44.23 ^{d}	0.83
NDFD,%	40.67 ^{c}	39.43 ^{d}	46.26 ^{b}	46.38 ^{b}	46.67 ^{b}	61.61 ^{a}	41.78 ^{c}	42.85 ^{c}	41.80 ^{c}	39.94 ^{d}	35.78 ^{e}	3.61
ADFD,%	41.50 ^{b}	39.89 ^{c}	39.93 ^{c}	39.68 ^{c}	41.50 ^{b}	45.82 ^{a}	43.64 ^{b}	37.81 ^{d}	37.59 ^{d}	45.19 ^{a}	40.39 ^{c}	3.48
Hemi.D,%	39.47 ^{a}	26.87 ^{f}	26.92 ^{e}	26.62 ^{f}	28.84 ^{e}	29.98 ^{a}	31.76 ^{d}	32.35 ^{d}	34.44 ^{c}	33.65 ^{c}	37.85 ^{b}	6.66
Cellul.D,%	28.84 ^{f}	38.77 ^{d}	55.40 ^{b}	55.97 ^{b}	39.47 ^{d}	76.58 ^{a}	38.64 ^{d}	49.15 ^{c}	48.92 ^{c}	35.02 ^{e}	37.96 ^{d}	4.30

a, b, c, d, e & f means within the same row with different superscripts differ significantly ($P < 0.05$)

Where:

R1: 40% clover hay + 60%concentrate (standard) R2: 40% rice straw + 60%concentrate

R3: 50% rice straw + 50%concentrate

Table 4: Gas value (kinetics of gas production in ration ml / 1g substrate content) after 24 incubation based DM

item	R1	R2					R3					SE
Level of Algae	0	0	0.8	1.6	2.4	3.2	0	0.8	1.6	2.4	3.2	±
GPDM	162.98 ^{c}	162.83 ^{c}	164.66 ^{c}	167.55 ^{b}	175.98 ^{b}	205.27 ^{a}	165.56 ^{b}	170.25 ^{a}	158.16 ^{d}	157.91 ^{d}	157.17 ^{d}	4.70
GPOM	163.51 ^{c}	161.07 ^{d}	163.51 ^{c}	166.91 ^{c}	170.51 ^{b}	199.99 ^{a}	166.93 ^{c}	170.68 ^{b}	160.47 ^{d}	160.83 ^{d}	160.57 ^{d}	4.43
GPNDF	374.27 ^{b}	363.06 ^{b}	364.96 ^{b}	366.01 ^{b}	374.27 ^{b}	635.95 ^{a}	330.54 ^{d}	363.35 ^{b}	321.67 ^{e}	322.65 ^{e}	324.07 ^{e}	14.71
GPADF	632.98 ^{c}	615.26 ^{c}	617.71 ^{c}	620.07 ^{c}	632.98 ^{c}	1307.29 ^{a}	525.68 ^{d}	599.11 ^{b}	512.14 ^{e}	512.78 ^{e}	515.31 ^{e}	29.21
GPHemi	915.75 ^{b}	855.72 ^{e}	891.98 ^{c}	893.29 ^{c}	915.75 ^{b}	1238.39 ^{a}	890.43 ^{c}	929.16 ^{b}	864.91 ^{d}	870.18 ^{d}	873.23 ^{d}	27.13
GPCell	769.99 ^{b}	748.55 ^{c}	751.45 ^{c}	754.39 ^{c}	769.99 ^{b}	1689.83 ^{a}	636.41 ^{d}	772.42 ^{b}	620.07 ^{e}	620.76 ^{e}	623.84 ^{d}	36.08

a, b, c, d, e & f means within the same row with different superscripts differ significantly ($P < 0.05$)

Where:

R1: 40% clover hay + 60%concentrate (standard) R2: 40% rice straw + 60%concentrate

R3: 50% rice straw + 50%concentrate

Table 5: Degradability of DM, OM, NDF, ADF, Hemicellulose and Cellulose (ml /g degraded substrate content) after 24 incubation based DM

item	R1	R2					R3					SE
Level of Algae	0	0	0.8	1.6	2.4	3.2	0	0.8	1.6	2.4	3.2	±
GPdDM	256.62 ^{e}	271.74 ^{d}	275.35 ^{c}	280.64 ^{b}	286.62 ^{a}	284.09 ^{a}	270.20 ^{d}	309.39 ^{a}	271.35 ^{d}	236.72 ^{e}	274.79 ^{c}	4.14
GPdOM	361.88 ^{b}	357.26 ^{c}	358.48 ^{d}	358.54 ^{d}	361.88 ^{b}	378.86 ^{a}	362.04 ^{b}	367.04 ^{a}	362.12 ^{b}	362.83 ^{b}	363.39 ^{b}	3.01
GPdNDF	926.32 ^{e}	790.15 ^{f}	805.17 ^{e}	943.15 ^{d}	989.32 ^{c}	1032.54 ^{b}	794.14 ^{f}	1590.23 ^{a}	773.01 ^{f}	809.51 ^{e}	938.73 ^{d}	15.15
GPdADF	1528.64 ^{e}	1546.53 ^{e}	1598.95 ^{e}	1685.86 ^{d}	1757.44 ^{c}	1876.87 ^{b}	1210.45 ^{f}	2806.72 ^{a}	1362.65 ^{f}	1136.18 ^{f}	1279.89 ^{f}	60.24
GPdHemi	2473.42 ^{e}	2540.22 ^{d}	2611.01 ^{c}	2754.54 ^{b}	2773.42 ^{b}	2816.81 ^{a}	2413.73 ^{e}	2847.44 ^{a}	2806.76 ^{a}	2852.89 ^{a}	2528.43 ^{d}	64.13
GPdCell	2690.80 ^{c}	2804.9 ^{b}	2817.24 ^{b}	2864.44 ^{b}	2890.80 ^{b}	3147.49 ^{a}	2227.47 ^{d}	2698.86 ^{c}	1638.57 ^{f}	1857.37 ^{e}	2266.08 ^{d}	120.42

a, b, c, d, e & f means within the same row with different superscripts differ significantly ($P < 0.05$)

Where:

R1: 40% clover hay + 60%concentrate (standard) R2: 40% rice straw + 60%concentrate

R3: 50% rice straw + 50%concentrate

Conclusion

Degradability of DM, OM, NDF, ADF, Hemicell and Cellu. were increasing with adding green seaweed with different levels supplementation in R2 especially 3.2% was the highest value compared 0% added. In R3the highest value was adding 0.8% green seaweed only but any adding were low values compared 0% added. It concluded that supplementation seaweed *(Ulva lactuca*) with different levels due to improving digestibility, kinetics of gas production, growth of microbial protein biomass, efficiency of microbial protein and degradability especially R2 containing 40% rice straw but R3 containing 50% rice straw in one level adding 0.8% DM and equaled values standard ration (R1) containing clover hay. More studies are needed to evaluate seaweed as a feed supplement in the diets of ruminants.

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