RESEARCH ARTICLE

Effect of supplementation of *Kappaphycus alvarezii* based seaweed product on rumen fermentation parameters under in vitro conditions

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Abstract: In vitro trials were conducted to study the effect of fortification of different levels of *K. alvarezii* based seaweed product (*K. Alvarezii* powder: *Gracilaria salicornia* powder: *K. alvarezii* sap powder in 1: 1: 1 ratio) to basal substrate on digestibility and rumen fermentation parameters. The results showed that supplementation of different levels (0, 0.5, 1.0, 1.5, 2.0, 2.5 and 3.0%) of *K. alvarezii* based seaweed product to the basal substrate consisting of sugargraze fodder (sorghum × sorgho × sudan grass hybrid) and concentrate mixture in 60: 40 ratio) did not affect *in vitro* true DM and OM digestibility, total gas production, methane production, ammonia-N, microbial biomass production, individual volatile fatty acid production and metabolisable energy values to any significant extent.

Keywords: In vitro, *Kappaphycus alvarezii*, Fermentation, Methane, *Gracilaria salicornia*, Seaweed product, Volatile fatty acids

Introduction

India possesses 434 species of red seaweeds (Rhodophyta), 194 species of brown seaweeds (Phaeophyta) and 216 species of

green (Chlorophyta) seaweeds (Modayil, 2004). *Kappaphycus alvarezii* and *Gracilaria salicornia* are two important species of cultivated red seaweed targeted for carrageenan production which is mostly utilized as thickening agent and stabilizer in the food industry (Pang et al. 2010, Mondal et al. 2015). Seaweeds have been used as livestock feed for many years. The by-products after extraction of carrageenan can be used as animal feed.

Evaluation of seaweeds or their byproducts using in vivo trials is time consuming, laborious and costly and in vitro evaluation is preferred in such investigations. Some literature exist on utilization of brown and green algae as supplements in pure form or in combination with two or three species (Bozic et al. 2009; Machado et al. 2014; El-Waziry et al. 2015), however, recently attention has been focused on utilization of red seaweeds for their nutritional benefits in ruminants which includes reduction in methane production (Rajauria, 2015). Thus, the present study was planned to investigate the effect of *K. alvarezii* based seaweed product on rumen fermentation parameters under in vitro conditions

Materials and Methods

Substrate for In vitro fermentation

Concentrate mixture and green fodder (sugargraze) were collected from Livestock Research Centre of ICAR-National Dairy Research Institute, Karnal, Haryana. The samples were dried in hot air oven at 65°C for 2 days until constant weight was attained. The dried samples were ground through 1 mm sieve using electrically operated Willey mill. The basal substrate was prepared using concentrate mixture and sugargraze fodder (sorghum × sorgho × sudan grass hybrid) fodder in 40:60 ratio on DM basis. Basal substrate was fortified with varying levels of commercially available *Kappaphycus alvarezii* based seaweed product (SWP; M/s AQUAAGRI Processing Pvt. Ltd., Manamadurai, Tamilnadu) consisted of *K. alvarezii* powder, *Gracilaria salicornia* powder and *K. alvarezii* sap powder in 1: 1: 1 ratio. Substrate components and SWP were analysed for proximate principles (AOAC, 2005) and cell wall constituents (Van Soest et al. 1991).

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In vitro rumen fermentation studies

In vitro rumen fermentation of total mixed substrate with varying levels of SWP such as; 0, 0.5, 1.0, 1.5, 2.0, 2.5 and 3.0%. was carried out using 6 replicates each. Rumen liquor was collected from 3 male adult crossbred cattle maintained to meet the nutrient requirement (ICAR, 2013) prior to feeding and watering, strained using muslin cloth into a pre-warmed thermos flask and brought immediately to the laboratory. The incubations were carried out in 100 mL calibrated glass syringes (Menke and Steingass, 1988). The proportion of medium mixture solution to rumen liquor was 2: 1. Just after mixing the medium and rumen liquor, 30 mL of incubation medium was injected to the syringes using auto dispenser. The syringes were shaken gently and residual air or air bubbles, if any, was removed and outlet was closed. At the end of the incubation, 5 μ L of gas was withdrawn using Hamilton gas tight syringe and analyzed for its methane level with the help of Gas Chromatograph (Nucon 5700, India) fitted with stainless steel column packed with Porapak-N and Flame Ionization Detector (FID). The standard gas used for methane estimation (Spantech House, Surrey, England) composed of 50% methane and 50% CO₂. Subsequently, substrate and inoculum was recovered. Substrate was analysed for true DM and OM digestibility (TDMD and TOMD) according to Van Soest et al. (1991).

For analysis of individual fatty acids (IVFA), the rumen fermentation was arrested by chilling at 4°C and the syringe contents were then centrifuged at 3000 rpm for 10 min. A portion of 5 mL of supernatant was added to 1 mL of 25% metaphosphoric acid and kept overnight at 4°C (Patra et al. 2006). The mixture was centrifuged at 3000 rpm for 15 min. and 2 mL of supernatant was taken and stored at -20°C for VFA analysis. The individual VFA in the samples were determined using Gas Chromatograph (Nucon 5700, Nucon Engineers, New Delhi) equipped with flame ionization detector and stainless steel column packed with chromosorb 101 mesh 80-100 (length 1.5 m; o.d 3.175 mm; i.d. 2 mm). Analytical conditions for fractionation of VFA were as follows: Injection port temperature 210°C, column temperature 180°C and detector temperature 230°C. The flow rate of the carrier gas N₂ was 40 mL/min). Individual volatile fatty acids (Acetate, propionate and butyrate) in the samples were determined on the basis of retention time and their concentration was calculated by comparing the retention time as well as the peak area of the standard after blank correction.

For estimation of NH₃-N, 5 mL of acidified supernatant was mixed with 10 mL of NaOH (1 N) and immediately steam distilled using KEL PLUS® - N analyzer (Pelican, India). The NH₃ evolved was collected in boric acid solution (20% w/v) having mixed indicator and titrated against N/100 H₂SO₄.

Metabolizable energy (ME) was calculated using the prediction equation of Menke and Steingass (1988). Microbial biomass production (MBP) was calculated using data of TDOM and net gas volume (Blummel et al. 1997; Blummel and Lebzien, 2001).

Statistical analysis

The data were analyzed by two-way ANOVA and difference between the means was compared by Turkey's-B multiple range test as per Snedecor and Cochran (1994) using software package SPSS version 20.0 (2012).

Results and Discussion

Chemical composition of feeds/sea weed product

The concentrate mixture contained 20.50% CP and 4.33% EE, 30.26% NDF and 14.20% ADF on DM basis. Sugargraze fodder had 8.78, 1.73, 53.43 and 30.42% of CP, EE, NDF and ADF, respectively. The total ash content in sea weed product was 72.55% and it possessed 5.58% CP and 1.98% EE (Table 1). Thus, SWP contained very high level of total ash while CP level was lower in comparison to concentrate mixture and sugargraze fodder. *K. alvarezii* collected from Malaysia contained 66.66, 23.25, 5.35, 4.50, 0.23% CHO, total ash, protein, fibre and lipid, respectively (Ahmad et al. 2012) indicating that type of seaweed or formulated product affect the chemical composition.

In vitro evaluation of SWP

TDMD and TOMD were 81.05±0.45 and 82.31±2.78%, respectively. The total gas production was 4.50±0.28 mL/200 mg substrate or 27.50±1.44 mL/g substrate. Methane production was 1.37±0.02 mL/200 mg DM and this represented 25.05±0.38% of total gas production. The values of TDMD and TOMD of SWP as found in the present study were higher as compared to other studies. The values of OMD reported by El-Waziry et al. (2015) were lower than that 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 Castanon (1998) and also less (60%) than that reported for *U. lactuca* (Arieli et al. 1993). The *in vivo* energy digestibility of *Ulva lactuca* in young rams has been reported as 60% (Arieli et al. 1993). The in vitro OM digestibility of brown and red seaweeds when measured with rumen fluid obtained from seaweed-fed sheep was found to be higher for brown algae *Laminaria digitata* (94%), *Saccharina latissima* (97%) and *Alaria esculenta* (81%) and red algae *Palmaria palmata* (81%). However, it was lower for other brown seaweeds such as *Ascophyllum nodosum* (33%), *Fucus serratus* (15%) and *Fucus vesiculosus* (26%) (Greenwood et al. 1983). In comparison to brown algae, *Macrocystis pyrifera* and *Sargassum sp.*, the *in situ* DM degradability of *Macrocystis pyrifera* was found to be lower (50%) but higher than that of *Sargassum* (29%) due to a better composition of the former. Degradability values for the green algae, *Ulva lactuca* were also relatively lower (54 and 41% for OM and protein, respectively) as reported by Arieli et al. (1993). The ME value of SWP in the present study was 2.86 MJ/kg DM which was 3.3 times lesser than estimate of Ventura and Castanon (1998) in seaweed.. The high mineral content of

seaweeds limits their gross, digestible, metabolisable and net energy values (Arieli et al. 1993).

Supplementary effects of SWP on rumen fermentation variables

The total gas production ranged from 34.50 to 38.25 mL/200 mg substrate and methane production from 7.51 to 9.16 mL/200 mg substrate in treatments supplemented with 0-3% SWP in the substrate (Table 2). Methane production varied from 21.01 to 25.81% of total gas production. Statistical analysis showed that supplementation of SWP @ 0-3% of the substrate did not affect total gas and methane production to any significant level.

The TDMD values ranged from 68.25 to 72.35% while TOMD values varied from 71.60 to 75.10% across the treatments (Table

3). ME values ranged from 6.88 to 8.39 MJ/kg DM and the MBP values ranged from 40.54 to 46.76 mg/tube. The $\text{NH}_3\text{-N}$ concentration ranged from 10.03 to 11.42 mg/dL. TDMD, TOMD, ME, MBP and $\text{NH}_3\text{-N}$ were comparable between control and treatments. The proportion of acetate, propionate and butyrate ranged from 66.44 to 68.33%, from 24.61 to 27.85% and from 5.01 to 8.50%, respectively (Table 4). Acetate: propionate ratio was 2.40 to 2.48. Our results are in conformity with those of (El-Waziry et al. 2015) who reported no significant differences in the potential degradability, gas production rate, ME, net energy, OMD and MBP synthesis in diets supplemented with 3 and 5% of *Ulva lactuca*. On the other hand, there was reduction in enteric CH_4 production due to macroalgae supplementation (Wang et al. 2008; Bozic et al. 2009). Addition of sea seaweeds showed inhibitory effect on rumen fermentation resulting in decreased total gas

Table 1 Chemical composition of feed stuffs used for in vitro studies

Parameter	Concentrate mixture	Sugargraze	Seaweed product
Dry matter	89.9	22.25	94.6
Organic matter	92.72	90.28	27.45
Crude protein	20.5	8.78	5.58
Total ash	7.28	9.72	72.55
Ether extract	4.33	1.73	1.98
Neutral detergent fibre	30.26	53.43	15.03
Acid detergent fibre	14.2	30.42	9.92

Table 2 In vitro gas and methane production from TMR supplemented with graded levels of sea weed product

Level of SWP supplementation (%)	Total gas production (mL/200 mg substrate)	CH_4 production (mL/200 mg DM)	CH_4 production (%)
0	34.50±0.64	8.10±0.09	23.49±0.28
0.5	37.00±1.08	8.62±0.33	23.29±0.87
1	35.75±2.59	7.51±0.20	21.01±0.56
1.5	38.25±1.25	9.02±0.96	23.58±2.51
2	35.50±0.65	8.31±0.10	23.43±0.30
2.5	35.50±0.96	9.16±0.13	25.81±0.37
3	35.00±1.96	8.41±0.10	24.04±0.31

Table 3 Effect of supplementation of graded levels of sea weed product on digestibility, microbial biomass and energy value under in vitro conditions

Level of SWP supplementation (%)	TDMD (%)	TOMD (%)	ME (MJ/kg)	MBP (mg)	$\text{NH}_3\text{-N}$ (mg/100 mL)
0	71.61±1.15	72.67±0.57	6.88±0.13	46.76±0.29	10.03±0.37
0.5	72.08±0.84	74.67±0.22	8.22±0.27	41.00±6.18	10.11±0.51
1	68.90±1.14	71.60±2.50	8.05±0.16	42.67±0.99	10.40±0.32
1.5	72.35±0.67	75.10±0.75	8.39±0.10	41.75±3.07	11.21±0.75
2	68.25±1.09	71.60±1.80	8.02±0.19	40.54±6.97	11.10±0.35
2.5	70.10±2.12	73.45±2.1	8.02±0.28	40.85±4.15	11.33±0.32
3	72.32±0.16	74.97±0.12	7.95±0.17	46.67±2.74	11.42±0.55

TDMD: true dry matter digestibility, TOMD: true organic matter digestibility, ME: metabolisable energy, MBP: microbial biomass production

Table 4 Individual VFA's as affected by addition of seaweed product at graded levels

Level of SWP supplementation (%)	Acetate	Propionate	Butyrate	Acetate: propionate ratio
Control	67.37±0.78	26.62±1.69	6.03±0.2	2.56±0.18
0.5	66.88±1.24	24.77±0.78	8.50±1.57	2.70±0.10
1	67.34±1.00	27.63±0.22	5.01±0.89	2.43±0.05
1.5	68.33±2.08	24.61±0.88	7.04±2.47	2.78±0.11
2	66.66±1.35	27.95±1.33	5.38±0.63	2.40±0.17
2.5	66.44±1.09	25.30±0.70	8.24±0.46	2.63±0.12
3	66.46±0.86	26.88±0.43	6.64±1.01	2.47±0.04

production, methane production and volatile fatty acids production under *in vitro* conditions (Machado et al. 2014). There were no significant changes in digestibility of nutrients in Sahiwal cows when mineral mixture (3%) in the concentrate was replaced by 20% *Sargassum wightii* (Singh et al. 2015).

Conclusions

The supplementation of *K. Alvearzi* based seaweed product at 0, 0.5, 1.0, 1.5, 2.0, 2.5 and 3.0% level of substrate containing sugargraze and concentrate mixture in 60: 40 ratio did not affect *in vitro* DM and OM digestibility, rumen fermentation parameters (IVGP, IVFA, NH₃-N and microbial biomass production) and ME values

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