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Screening of macroalgae on *in vitro* methane production potential by *in vitro* gas production technique

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Abstract
On screening the twenty species of marine macroalgae, collected from Gulf of Mannar, South east coast of India. This experiment was dealt to identify the promising marine macroalgae responsible to decrease methane emission among the collected twenty species of marine macroalgae by *in vitro* gas production technique. Further, the total gas production, methane production, per cent methane on total gas production, *in vitro* true dry matter digestibility and methane per 100 mg truly digested substrate were estimated by *IVGPT*. In the process of screening 20 marine macroalgae on methane production potential by *IVGPT*, *Acanthophora spicifera*, *Hypnea musciformis* and *Valoniopsis pachynema* were promising macroalgae species, significantly (P<0.01) reduced the total gas production, methane production, per cent methane on total gas production and methane production per 100mg of truly digested substrate.

Keywords: Seaweed, Methane emissons, Ruminant Production

Introduction
B Greenhouse gas emission from the ruminant production systems is of particular importance because of their consequence on global climate. Methane (CH_4) is one of such potent GHG emitted by ruminants and it has 28 times more global warming potential than that of carbon dioxide (IPCC 2014). Methane emission from agriculture and waste management account for 62 % of global anthrapogenic emission (Kirschke *et al*. 2013) [2], while enteric fermentation responsible for 58% of agricultural contribution (Olivier *et al*. 2005) [3]. Enteric CH_4 is a consequence of anaerobic fermentation of feed organic matter (OM) by a microbial consortium that produces substrate CO_2 and hydrogen in a reduction pathway used by methanogens (Morgavi *et al.,* 2010) [4]. A part from environmental issues, the methane emission also accounts for 5 % loss of gross energy of feeds (Hristov *et al.,* 2013) [5] results in lower performance of cattle. Hence, there several feeding strategies were developed to mitigate methane emission form livestock, increase production performance and decrease the livestock contribution to global warming. These strategy focuses on using the nutritional and biochemical properties of feeds, including secondary metabolites, to manipulate ruminal microbial populations and metabolism to reduce the production of enteric CH4, enhance the efficiency of energy use, and consequently the productivity of livestock

Material and Methods
The *in vitro* gas production studies were carried out by using Hohenheim gas production technique as per the procedure of Menke and Steingass (1988) [6]. The rumen liquor was collected from three cows maintained on grazing and the contents were pooled in to a thermo cud transport container under constant flushing of CO_2 and this composite sample was brought to the laboratory. The rumen contents were strained using four layered muslin cloth to an Erlenmeyer flask under continuous flushing with CO_2 and it was maintained at the temperature of 39 ºC. The rumen fluid was mixed with media solution prepared as described by Menke and Steingass (1988) [6]. Two hundred mg of macroalgae was weighed and taken in 100 ml calibrated syringes.

To all the glass syringes, 30 ml of rumen liquor containing inoculum was anaerobically transferred and it was incubated in a shaking water bath at 39 ºC for 24 hours period. At the end of incubation period, the total gas produced was measured and the gas samples were collected in vaccutainer for estimation of methane and fermented liquids were collected for *in vitro* true dry matter degradability.

Total gas production (TGP)

The total gas production was measured by noting down the raise in the piston due to gas production after 24 hours of incubation in a shaking water bath (Menke and Steingass, 1988) [6].

Methane production

Methane concentration was estimated by Gas chromatography as per the method of Sitaula *et al*. (1992) [7]. The gas chromatography (Perkin Elmer, Clarus 500 model) was fitted with Flame Ionization Detector (FID) and capillary column (30 meter length and 250 micrometer diameter). Nitrogen gas was used as carrier gas with oven, injector and detector at 60, 100 and 110 ºC, respectively. The gas collected in the vaccutainers were injected in to the column and detected in FID. Methane concentration in samples (%) and methane emission was calculated using the following formulas.

$$\text{Methane concentration (\%)} = \frac{\text{Peak area of sample gas}}{\text{Peak area of standard gas}} \times \text{Methane concentration in standard gas}$$

$$\text{Methane emission (ml)} = \frac{\text{Methane concentration (\%)}}{100} \times \text{Net gas production (ml)}$$

The percentages of methane in the samples were calculated by comparing with the standard methane gas mixture containing 21.86 per cent of methane.

Percentage of methane on total gas production

Per cent methane in total gas produced was calculated using the following formulae

$$\text{Methane (\%)} = \frac{\text{Methane emission (ml)}}{\text{Total gas (ml)}} \times 100$$

Methane production per 100 mg of truly digested substrate

For estimating methane production per 100 mg truly digested substrate, the *in vitro* true dry matter degradability was evaluated. The *in vitro* true dry matter degradability of the fermented feed was estimated by centrifuging the fluid. Then the residue was transferred in to sintered glass crucible and fitted in Fibretec (Model No. 1020, Tecator, Sweden). To each crucible, 100 ml of neutral detergent solution (NDS) was added and it was refluxed for one hour after which the residue was recovered. The true dry matter degradability was calculated as the weight of basal diet incubated minus the weight of the residue after NDS treatment (Van Soest and Robertson, 1985) [8]. Methane production per 100 mg truly digested substrate was calculated using the following formula

$$\text{Methane production per 100 mg truly digested substrate} = \frac{\text{Methane emission (ml)}}{\text{Degradability (\%)}} \times 0.1\text{gm}$$

Results & Discussion

In vitro gas production study

The effect of *in vitro* gas production study parameters of twenty marine macroalgae species was presented in below Table.

Total gas production

The total gas production of marine macroalgae is presented in below Table. The effect of macroalgae on total gas production was significantly (P<0.01) affected among the species and ranged from 3.00 to 18.00 ml. *Hypnea musciformis, Acanthophora specifera, Valoniopsis pachynema, Chaetomorpha linum, Halimeda macroloba, Halimeda opuntia, Ulva lactuca, Champia compressa, Gracilaria edulis, Kappaphycus alavaerezii, Dictyopteris australis, Sargassum swatzii and Stoechospermum marginatum* are found lower total gas production among macroalgae species. Similarly, Machado *et al*. (2014) [9] analysed twenty species of marine macroalgae and reported total gas production was significantly (P<0.01) lower among all the macroalgae species and also present results were linear to Kinley *et al.* (2016) and Molina Alcaide *et al.* (2017) [10, 11].

Methane production

The methane production potential of twenty species of marine macroalgae is presented in Table 5. Methane production potential is significantly (P<0.01) varied among the macroalgae species and observed methane production range of 0.223 to 2.417 ml/200mg. *Hypnea musciformis, Acanthophora specifera* and *Valoniopsis pachynema* are shown lower methane production compared to other macroalgae species. *Halymenia dilatata* shown higher methane production compared to other species of macroalgae. Similarly, Machado *et al*. (2014) [1] analysed twenty species of marine macroalgae and reported methane production was significantly (P<0.01) lower among all the species and observed range of 0.2 to 16.3 ml/1.2g.

Percentage of methane on total gas production

The percent methane production on total gas production is presented in Table.

The effect of macroalgae on percent methane on total gas production was significantly (P<0.01) affected among the macroalgae species and found range of 7.40 to 15.62 %. *Acanthophora specifera, Gracilaria corticata, Hypnea musciformis,* and *Caulerpa racemosa* found lower percent methane on total gas production compared to other macroalgae species. Similarly, Dubois *et al*, (2013) [12] evaluated fifteen species of macroalgae and reported a highly significant (P<0.01) difference in percent methane on total gas production among species.

Percentage of *in vitro* dry matter digestibility

The percent *in vitro* dry matter digestibility of marine macroalgae is presented in Table. The *in vitro* dry matter digestibility was significantly (P<0.01) affected among the macroalgae species and shown range of 59.65 to 83.65 %. Similarly, Greenwood *et al*., (1983) [13] analysed six macroalgae species and reported *in vitro* dry matter digestibility in the range of 15.00 to 81.00 %.

Methane production per 100 mg truly digested substrate

The effect of methane producient per 100 mg truly digested substrate was significantly (P<0.01) affected among the macroalgae species and shown range of 0.125 to 1.507 ml. *Acanthophora specifera, Hypnea musciformis* and *Valoniopsis pachynema* are the promising marine macroalgae to reduce methane production per 100 mg truly digested substrate. Similarly, Dubois *et al*, (2013) [12] evaluated fifteen species of macroalgae and reported *Cystoseria* sp., is having highly significant (P<0.01) decrease in methane ml when compared to other species of macroalgae.

Table 1: The effect of 20 marine macroalgae on *in vitro* total gas production (ml), methane production (ml), percentage of methane on total gas production and methane per 100 mg of truly digested substrate (ml) at 24 hr of incubation (*Mean ± SE)

Marine Macroalgae species	Total Gas production, *ml*	Methane production, *ml*	% of CH_4 on total gas production	% IVDMD	CH_4 production per 100mg of truly digested substrate, *ml*
Red macroalgae					
Acanthophora spicifera	3.25^{a} ± 0.06	0.311^{ab} ± 0.01	9.56^{ab} ± 0.43	68.25^{e} ± 1.31	0.227^{ab} ± 0.01
Champia compressa	3.75^{ab} ± 0.07	0.511^{bcde} ± 0.01	13.62^{f} ± 0.56	62.94^{bc} ± 0.87	0.405^{bcd} ± 0.01
Gracilaria corticate	5.50^{bc} ± 0.13	0.499^{bcde} ± 0.01	9.07^{ab} ± 0.66	62.05^{b} ± 1.11	0.402^{bcd} ± 0.01
Gracilaria edulis	4.00^{ab} ± 0.09	0.587^{cde} ± 0.01	14.67^{g} ± 0.62	76.45^{gh} ± 1.21	0.383^{i} ± 0.01
Halymenia dilatate	18.00^{f} ± 1.43	2.417^{i} ± 0.09	13.42^{f} ± 0.51	80.14^{i} ± 1.46	1.507^{h} ± 0.05
Hypnea musciformis	3.00^{a} ± 0.04	0.223^{a} ± 0.01	7.40^{a} ± 0.45	70.25^{f} ± 0.92	0.125^{a} ± 0.01
Kappaphycus alaverezii	4.00^{ab} ± 0.08	0.488^{bcd} ± 0.01	12.20^{e} ± 0.54	64.32^{cd} ± 1.54	0.314^{bc} ± 0.01
Porteria hornemannii	11.25^{e} ± 0.87	1.205^{h} ± 0.06	10.71^{cd} ± 0.23	69.05^{ef} ± 1.39	0.872^{g} ± 0.01
Brown macroalgae					
Dictyopteris australis	3.75^{ab} ± 0.08	0.406^{abcd} ± 0.01	10.82^{cd} ± 0.62	63.36^{bc} ± 1.43	0.320^{bc} ± 0.01
Padina boryano	8.00^{d} ± 0.62	0.989^{g} ± 0.06	12.36^{e} ± 0.57	68.15^{e} ± 1.09	0.725^{fg} ± 0.01
Padina tetrastomatica	8.31^{d} ± 0.66	0.875^{fg} ± 0.07	10.52^{bc} ± 0.53	66.16^{d} ± 1.21	0.713^{fg} ± 0.01
Sargassum swatzii	4.25^{ab} ± 0.11	0.594^{de} ± 0.01	13.97^{fg} ± 0.57	64.35^{cd} ± 1.16	0.461^{cd} ± 0.01
Stoechospermum marginatum	4.50^{ab} ± 0.15	0.530^{cde} ± 0.01	11.77^{d} ± 0.54	77.65^{h} ± 1.21	0.341^{bc} ± 0.01
Turbinaria conoides	7.00^{cd} ± 0.56	0.989^{g} ± 0.06	14.12^{g} ± 0.52	76.70^{gh} ± 1.32	0.644^{ef} ± 0.01
Green macroalgae					
Caulerpa racemosa	5.50^{bc} ± 0.23	0.433^{bcd} ± 0.01	7.87^{a} ± 0.71	59.65^{a} ± 1.13	0.362^{bcd} ± 0.01
Chaetomorpha linum	4.00^{ab} ± 0.24	0.484^{bcd} ± 0.01	12.10^{e} ± 0.53	80.34^{i} ± 1.26	0.301^{bc} ± 0.01
Halimeda macroloba	4.75^{ab} ± 0.27	0.602^{de} ± 0.01	12.67^{ef} ± 0.62	83.65^{j} ± 1.43	0.359^{bcd} ± 0.01
Halimeda opuntia	3.50^{ab} ± 0.14	0.501^{bcde} ± 0.01	14.31^{g} ± 0.43	75.65^{g} ± 1.32	0.331^{bcd} ± 0.01
Ulva Lactuca	4.50^{ab} ± 0.36	0.703^{ef} ± 0.01	15.62^{h} ± 0.56	65.32^{d} ± 1.12	0.537^{de} ± 0.01
Valaniopsis pachynema	3.75^{ab} ± 0.13	0.384^{abc} ± 0.01	10.24^{bc} ± 0.59	68.95^{ef} ± 1.21	0.278^{ab} ± 0.01

*Mean of six observations; Means bearing different superscripts a, b and c in the same column differ significantly (P<0.01)

Conclusions

In the process of screening 20 marine macroalgae on methane production potential by *IVGPT*, *Acanthophora spicifera*, *Hypnea musciformis* and *Valoniopsis pachynema* were promising macroalgae species, significantly (P<0.01) reduced the total gas production, methane production, per cent methane on total gas production and methane production per 100mg of truly digested substrate.

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