Indian J. Anim. Nutr. 2020. 37 (3): 296-298
doi: 10.5958/2231-6744.2020.00048.1

Indian Journal of Animal Nutrition

SHORT COMMUNICATION

Influence of Seaweed (*Sargassum johnstonii*) Supplementation on *in vitro* Digestibility and Methane Production

Sarishti Katwal[1*], M.M. Trivedi[2], P.R. Pandya[3], M.Y. Bhavsar[3] and D.T. Prajapati
Department of Livestock Production and Management,
College of Veterinary Sciences and Animal Husbandry Anand,
Anand Agricultural University, Anand-388001, Gujarat, India

ABSTRACT

The study was conducted to evaluate the effect of seaweed (*Sargassum johnstonii*) supplementation to substrate simulating total mixed rations (TMRs) on *in vitro* digestibility and methane production. Different substrates were formulated using concentrates and wheat straw in ratio of 60:40 and used as basal diet. Seaweed was incorporated at 0, 2, 4, 6, 8 and 10% level and incubated for 24 h. There was no significant difference in *in vitro* methane production and DM digestibility among groups. Therefore, inclusion of macroalgae *(S. johnstonii)* upto 8% level in TMR had no adverse effect on *in vitro* DM digestibility and methane emission.

Key words: *In vitro* methane, IVDMD, Seaweed, Mixed substrtaes

Marine macroalgae or seaweeds are found both in intertidal and deep water of coastal region where 0.01% photosynthetic light is available and belongs to three division: Brown algae (Phaeophyta), Green algae (Chlorophyta) and Red algae (Rhodophyta). The anti-methanogenic properties of macroalgae and macroalgae-based functional products have been demonstrated by *in vitro* studies (Wang *et al.,* 2008; Kinley and Fredeen, 2015), but there is much heterogeneity in the anti-methanogenic potency between types and species of macroalgae. So, aim of the present study was to investigate effect of seaweed (*Sargassum johnstoni*) based mixed substrtaes on *in vitro* digestibility and methane production.

Mixed substrtae was prepared by mixing concentrates and wheat straw (60:40) and used as basal diet for *in vitro* studies. The seaweeds was incorporated to mixed substrtae at 0, 2, 4, 6, 8 and 10% in treatments S0, S1, S2, S3, S4 and S5, respectively. Ingredient composition of TMR with different levels of seaweed is given in Table 1.

Rumen liquor was collected from two crossbred cows maintained on maintenance ration (1.5 kg compound concentrate mixture, 10 kg cereal green fodder and *ad lib.* jowar straw), after two hours of feeding in the morning through a stomach tube against negative pressure created by a suction pump. The rumen liquor was strained through four-layer muslin cloth and was mixed with artificial saliva prior to incubation. About 500 mg and 200 mg samples of substrates (Table 1) were grinded using 1.0 mm screen and taken into 100 ml glass syringes (3 replicates) for *in vitro* digestibility and methane production, respectively (Menke *et al.,* 1979). Three syringes were kept as blank. The required volume of strained rumen liquor, water, micro and macro minerals solution, buffer and resazurin were mixed in flask and CO2 was flushed continuously in the medium. For methane estimation, 30 ml and for *in vitro* digestibility, 40 ml of inoculums was dispensed into the syringe and kept in shaker water bath at 39°C for 24 h of incubation. For *in vitro* digestibility, the contents of each syringe were filtered through pre-weighed sintered glass crucible, dried and weighed. The crucibles containing undigested residues were oven dried at 70°C for 24 h, cooled in the desiccators and weighed to reach at IVDMD values. To determine *in vitro* methane, gas samples from each syringe were directly injected into Gas Chromatography (Thermofisher ceres 800) and methane concentration was determined against standard methane gas. All

[*]Corresponding email :srishtikatwal@gmail.com; [1]Department of Livestock Production Management; [2]Department of Animal Sciences, AAU Anand; [3]Department of Animal Nutrition, AAU, Anand

Table 1. Ingredient composition (%) of mixed substrtaes

Ingredient	Mixed substrates with different levels of seaweed					
	S0	S1	S2	S3	S4	S5
Wheat straw (*Triticum aestivum*)	40	40.0	40.0	40.0	40.0	40.0
Compound concentrate mixture	46.0	46.0	46.0	46.0	46.0	44.0
Seaweeds (*Sargassum johnstonii*)	0.0	2.0	4.0	6.0	8.0	10.0
Deoiled rice bran	8.0	6.0	4.0	2.0	0.0	0.0
Molasses	5.0	5.0	5.0	5.0	5.0	5.0
Mineral mixture	1.0	1.0	1.0	1.0	1.0	1.0

samples were analysed in triplicate using GC instrument, fitted with a 4 ft long by 3.2 mm internal diameter stainless steel column packed with Porapack N (80 to 100 mesh), equipped with a flame ionization detector (FID). Column temperature was maintained at 50ºC and nitrogen was used as a carrier gas with flow rate of 30 ml/min. Data were analysed by one way analysis of variance using statistical package SPSS9.00 software as per Snedecor and Cochran (1994).

The results of *in vitro* DM digestibility (IVDMD) and methane production of mixed substrate with or without seaweeds are presented in Table 2. The IVDMD was observed at par in all the groups without any significant difference. *In vitro* CH4 production was found statistically similar among the groups.

In vitro DM digestibility of mixed substrates with different levels of seaweeds (*S. johnstonii*) was in the range of 51.55 to 55.33% with non-significant differences (Anon., 2020). The authors reported no adverse effect of seaweeds on *in vitro* DM digestibility of TMR. They also studied *in vitro* methane production of TMR with seaweeds (*S. johnstonii*) at 0 to 7% level. The results revealed significant reduction by 36% in methane production in TMR with 5% level of sea weeds (1.32 ml/100 mg DM) compared to TMR without seaweeds (2.07 ml /100 mg DM). Choi *et al.* (2020) observed non-significant difference for methane emission *in vitro* trial with *Sargassum fulvellum* after 12, 24, 48, and 72 h with inclusion at concentrations of 1, 3, 5 and 10% of total ration. The DM digestibility ranged from 44-47% for all groups. Maia *et al.* (2016), Molina-Alcaide *et al.* (2017) and De la Moneda *et al.* (2019) evaluated different species of seaweeds for *in vitro* methane emission and they observed both lower and higher methane production potential of different seaweed species. They also observed that substrates used with seaweeds and season of collection have pronounced effect on methane production and *in vitro* digestibility. *Asparagopsis taxiformis* and *Oedogonium sp*. were efficient in methane reduction as dose

Table 2. *In vitro* DM digestibility and methane production

Group	IVDMD (%)	CH4(ml/100 mg DM)	CH4(ml/100 mg DDM)
S0	54.53±1.47	3.00±0.05	2.52±0.04
S1	54.20±3.10	2.78±0.58	2.37±0.49
S2	52.41±2.10	3.41±0.09	3.10±0.08
S3	55.46±1.55	2.91±0.12	2.36±0.10
S4	54.86±0.99	2.71±0.14	2.25±0.11
S5	56.3±0.75	3.27±0.08	2.58±0.06
SEM	1.72	0.254	0.208
C.D.	NS	NS	NS
CV %	5.407	14.075	14.373

increased, but digestibility shows inverse relationship with increased doses of seaweed (Machado *et al.,* 2016). The supplementation of *Kappaphycus alvearzii* 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 digestibility and methane production (Sharma *et al.,* 2019).

Therefore, inclusion of macroalgae *(S. johnstonii)* upto 8% level in TMR had no adverse effect on *in vitro* DM digestibility and methane emission.

ACKNOWLEDGEMENT

The financial assistance from ICAR under Outreach Project on Methane and necessary facilities provided by the Anand Agricultural University, Anand, Gujarat are gratefully acknowledged to carry out this study.

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Received on 20-08-2020 and accepted on 27-09-2020