VETERINARSKI ARHIV 93 (5), 513-524, 2023 DOI: 10.24099/vet.arhiv.1858

The effect of seaweed formulations on *in vitro* fermentation, nutrient utilization and growth performance in crossbred calves

Bornalee Handique*, Putan Singh, Ashok Kumar Verma and Rojita Yengkhom

Animal Nutrition Division, ICAR-Indian Veterinary Research Institute, Izatnagar, Bareilly, Uttar Pradesh, India

HANDIQUE, B., P. SINGH, A. KUMAR VERMA, R. YENGKHOM: The effect of seaweed formulations on *in vitro* fermentation, nutrient utilization and growth performance in crossbred calves. Vet. arhiv 93, 513-524 2023.

ABSTRACT

The aim of this research was to study the effect of seaweed formulations on *in vitro* rumen fermentation, nutrient utilization and growth performance of crossbred calves. The study included 18 male crossbred calves of 130±7.56 kg body weight, divided into 3 groups of 6 each following a completely randomized design. Calves in the control (T_0) were fed a standard diet, while treatment groups T_1 and T_2 were supplemented with AF-KWP or AFRD-5 at the rate of 4% of concentrate mixture, with 1% calcium carbonate and 1% dicalcium phosphate replacing the mineral mixture, and common salt offered in T_0. The experiment was conducted for a period of 120 days. At the end of the experiment, a metabolism trial was conducted to study the nutrient utilization. *In vitro* total gas and methane production was significantly ($P<0.05$) lower in the seaweed supplemented groups. A significant difference ($P<0.05$) was also observed in the retention of calcium and phosphorus between the different groups. A non-significant difference was observed in nutrient digestibility, dry matter intake, total gain in body weight, average daily gain and feed conversion ratio between the three treatment groups**.** It might be concluded that seaweed formulations significantly reduced *in vitro* total gas and methane production without affecting true dry matter digestibility and true organic matter digestibility. Moreover, seaweed formulation supplementation did not affect the growth performance and nutrient utilization in the crossbred calves.

Key words: seaweed formulations; crossbred calves; nutrient utilization; growth performance; *in vitro* rumen fermentation

Introduction

The total livestock population in our country is 535.78 million, which contributes 28.36% of the total agriculture GDP in India. There is scope to improve the livestock sector from low production and poor productivity using alternative feed resources to bridge the gap in feed resources between requirement and availability. The deficit in feed resources amounted to 44% concentrate feeds, 35.6% green fodder and 10.95% dry crop residues (IGFRI, 2013). There are many potentially important feed resources with significant nutritional value which are available inexpensively in large quantities. Aquatic plant-based non-conventional feed resources are one of these. Marine macro algae, popularly known as seaweeds, are renewable natural resources that grow in large quantities

*Corresponding author:
Bornalee Handique, PhD scholar, Animal Nutrition Division, ICAR-Indian Veterinary Research Institute, Izatnagar, Bareilly, Uttar Pradesh-243122, India, phone: +91 6000925483, e-mail: mail: bornalee16@gmail.com

ISSN 0372-5480
Printed in Croatia

along the coasts of India, and the estimated length of the coastline in India is 7516.6 km. In European countries and the USA, there were numerous reports of occasional or systematic use of seaweeds to feed livestock in the 19th and *early* 20th centuries (HANSEN et al., 2003; MAKKAR et al., 2016). There are about 10,000 species of seaweed (GUIRY, 2014) but only a few of them are used for animal feeding. There are 3 different groups of seaweed on the basis of thallus color, brown, red and green seaweed. They also differ in terms of many ultra-structural and bio-chemical features, including photosynthetic pigments, storage compounds, composition of cell walls and the presence or absence of flagella.

Seaweeds are markedly rich in organic minerals, complex carbohydrates, proteins and low molecular weight nitrogenous compounds, lipids, vitamins, volatile compounds and pigments (MAKKAR et al., 2016). They have been used as an additive to livestock feeds because they are the richest source of minerals (ITO and HORI, 1989). Seaweeds contain 10–20 times more minerals than land plants, and thus are potential sources of minerals (MAKKAR et al., 2016). However, they also contain heavy metals and some minerals are in toxic concentrations that may interfere with the availability of other minerals (CABRITA et al., 2017). Seaweeds are a rich source of sodium, potassium, magnesium, chlorine, sulfur, phosphorus, iodine, iron, zinc, copper, selenium and molybdenum (OKAB et al., 2013). In contrast to conventional mineral supplements, seaweeds are unique in being of plant origin, containing a wide range of naturally balanced chelated minerals, trace elements, amino acids and vitamins. The use of seaweeds in livestock feed increased growth rate and feed conversion efficiency in ruminants (CHOWDHURY et al., 1995) and reduced enteric CH_4 production (BOZIC et al., 2009). Many species of macro-algae have anti-bacterial, anti-viral, anti-oxidant and anti-inflammatory properties that improve animal health and function (BACH et al., 2008).

There is limited information on feeding with seaweed and its effect on feed intake, nutrient utilization and growth performance in crossbred calves. Thus, the present experiment was conducted to evaluate the effect of seaweed formulations on the *in vitro* fermentation, nutrient utilization and growth performance of crossbred calves.

Materials and methods

The study was conducted at the Experimental Animal Shed of the Animal Nutrition Division, ICAR-IVRI, Izatnagar in Uttar Pradesh, India. The care and management of calves and the biological sampling procedures were approved by the Institute's Animal Ethics Committee under the supervision of the Committee for the Purpose of Control and Supervision of Experiments on Animals.

In vitro estimation

Housing and feeding of fistulated animals. Four rumen fistulated animals were housed individually in well ventilated sheds with provision for individual feeding, and were fed a standard diet to meet their nutrient requirements (ICAR, 2013). All animals were provided *ad lib* fresh and clean drinking water twice daily.

Rumen liquor sampling. Rumen liquor was collected before feeding in the morning. After collection, the rumen liquor samples were screened using 4 layers of muslin cloth in a preheated thermos at 39^0C, and transported to the laboratory for inoculation of substrates. *In vitro* gas production was studied as per the procedure of MENKE and STEINGASS (1988). The substrate (200 mg per syringe) used comprised the concentrate with a mineral mixture and salt, wheat straw and maize fodder 50:30:20 in the control (T_0), and the concentrate mixture with seaweed formulations in the treatment groups (T_1 and T_2).

Estimation of total gas and methane. Gas production (ml/g DM) after 6, 12, 24, 48, 72 and 96 h of incubation was recorded by piston displacement. Net gas produced by feed fermentation was calculated by subtracting the gas produced in the blank from the total gas produced. For methane estimation, 100 µl of gas sampled from the headspace (after 24h of incubation) of the syringe was injected into a Nucon-5765 gas chromatograph equipped with a Porapak Q column and flame ionization detector. A mixture of 50% carbon dioxide and 50% methane (Spancan; Spantech Products Ltd, Godstone, UK) was used as the standard.

True Dry Matter (TDM) and True Organic Matter (TOM) digestibility. After recording total gas volume, the entire syringe content was drained through the spike into 500 ml spoutless beakers. The syringe was washed three times with 70 ml NDS using a dispenser to dispense NDS through the spike of the syringe. The beakers were kept on the heater and refluxing was done one hour after the boiling started. After that, the contents were filtered through a sintered glass crucible (G-1) and kept in a hot air oven at 100^0C for drying. The true DM digestibility was calculated as the difference between the weight of the DM incubated and the DM residue left. The residue in each crucible was kept in a Muffle Furnace at 550^0C for 2h to determine the organic matter content. The DM digestibility was calculated as the weight of DM incubated minus the weight of DM residue. The ratio of truly digestible substrate (mg) to the gas volume (ml) was termed as the partitioning factor.

In vivo studies

Experimental design. The experiment was conducted on 18 male crossbred calves of 8-10 months (130 ±7.56 kg live weight) of age. The calves were divided into 3 groups of 6 each using a completely randomized design. The calves were kept in a well-ventilated shed with provision for individual feeding under hygienic and uniform management conditions. The concentrate mixtures for the calves in groups T_1 and T_2 were supplemented with AF-KWP and AFRD-5 at 4% with 1% calcium carbonate and 1% dicalcium phosphate, respectively, replacing the mineral mixture and common salt. AF-KWP contains *Kapaphycus alvarezi* (thrashed weed), *Gracilaria salicornia* (washed weed) and *Kapaphycus* water extract at a ratio of 1:1:1 while AFRD-5 comprises *Kapaphycus alvarezi* (thrashed weed), *Gracilaria salicornia* (washed weed) and *Turbinaria conoides* at a ratio of 2:2:1.

Feeds and feeding. The experimental calves were offered concentrate mixture, wheat straw and green fodder to meet their nutrient requirements as per ICAR (2013) feeding standard recommendations for a body weight gain of 500 g/day. All the animals were provided fresh and clean drinking water twice per day *ad lib*. The feeding trial was conducted for 120 days. The concentrate mixture was formulated with crushed maize, de-oiled soybean meal, wheat bran, mineral mixture and common salt for feeding the calves in the control (T_0) group. Two different concentrate mixtures were formulated with crushed maize, de-oiled soybean meal, wheat bran, calcium carbonate, dicalcium phosphate, seaweed based formulations for feeding the T_1 and T_2 groups. Treatment groups T_1 and T_2 were supplemented with AF-KWP or AFRD-5 at 4%, with 1% calcium carbonate and 1% dicalcium phosphate replacing the mineral mixture and common salt (Table 1). The roughage to concentrate ratio in the present experiment was 50:50. Available green fodder was given to the experimental calves to meet the Vitamin A or carotene requirements.

Table 1. Ingredient compositions of concentrate ration

Ingredients	T_0	T_1	T_2
Crushed maize	40	`40	40
Deoiled-soybean	22	22	22
Wheat bran	35	32	32
Mineral mixture	02	-	-
AF-KWP	-	04	-
AFRD-5	-	-	04
Calcium carbonate	-	01	01
Dicalcium phosphate	-	01	01
Common salt	01	-	-
Total	100	100	100

Feed offered and residues left were measured daily, and representative samples were pooled and stored after drying (100±2°C) in a hot air oven, for further analysis. A record of feed intake and live weight was maintained to assess the nutrition and growth performance of the calves during the entire experimental period.

Metabolism trial. A metabolism trial of nine days was conducted at the end of the experiment, including three days of adaptation to the metabolic cages and six days of collection of feed, feces and urine. Sample aliquots of feces and urine voided in 24 hours by individual animals were collected and processed for storage and analysis. The feed and feces samples were dried at 100 ± 2°C in a hot air oven for dry matter estimation, and then pooled and ground to pass through a 1 mm sieve and stored in airtight polypropylene containers. A separate aliquot of fecal samples was preserved with dilute (25% v/v) sulfuric acid for nitrogen (N) estimation. Similarly, urine excreted by the animals over 24 hours was collected under acidic (dilute sulfuric acid) conditions and sampled for estimation of nitrogen.

Analysis of feed, seaweed, feces and urine. Representative sub-samples of the feeds offered and feces voided were oven dried (100°C, 24 hr), ground (1mm screen) and then analyzed following the methods of the Association of Official Analytical Chemists (2012) to determine DM by the oven drying method, and organic matter by muffle furnace incineration. Feeds, feces and seaweed samples were also analyzed for the proximate principles (AOAC, 2012) and fiber fractions, viz. neutral detergent fiber (NDF) and acid detergent fiber (ADF) (VAN SOEST et al., 1991).

Statistical analysis. The statistical analyses were performed as per the standard methods using the SPSS computer package (SPSS version 20.0, SPSS Inc., Chicago, USA). The data were statistically analyzed using ANOVA with Duncan's post hoc testing to compare the experimental groups. For all statistical analyses, probability values less than 0.05 were considered as significant.

Results

The chemical composition of the concentrate, green maize and wheat straw fed to the crossbred calves are presented in Table 2. The crude protein (CP) content of T_0, T_1 and T_2 was similar in all groups. The CP content of wheat straw and maize fodder was 3.65 and 9.85% respectively. Total ash content of T_2 (7.18%) was slightly lower than groups T_0 (7.21%) and T_1 (7.24%). Seaweed formulations (AF-KWP and AFRD-5) contained a higher amount of AIA, and as a result AIA content was higher in CM_1 and CM_2. The calcium (Ca) and phosphorus (P) content of the seaweed formulation supplemented groups was higher than in the unsupplemented group. Wheat straw on average contained 57.13, 85.20%, 0.25%, and 0.28% of ADF, NDF, Ca and P, respectively. Maize fodder on average contained 37.91, 58.42, 1.30 and 0.50% of ADF, NDF, Ca and P, respectively.

Table 2. Chemical composition (%) of seaweed formulations, concentrate mixture and roughages

Attributes	AF-KWP	AFRD-5	T_0	T_1	T_2	Wheat straw	Maize fodder
Dry matter	93.10	91.33	88.74	88.76	88.77	93.55	19.00
Organic matter	37.90	44.60	92.86	92.82	92.76	93.47	89.80
Crude protein	`4.87	7.26	18.73	18.65	18.62	3.65	9.85
Ether extract	0.90	1.20	2.30	2.16	2.15	1.79	1.07
Total ash	62.10	55.40	7.21	7.24	7.18	6.53	10.20
Acid insoluble ash	7.67	8.35	1.36	1.44	1.46	2.20	9.00
Neutral detergent fibre	10.17	20.52	24.10	22.43	22.66	85.20	58.42
Acid detergent fibre	9.04	19.11	7.23	7.18	7.20	57.13	37.91
Acid detergent lignin	1.40	3.52	1.38	1.35	1.31	7.53	6.96

Table 2. Chemical composition (%) of seaweed formulations, concentrate mixture and roughages (continued)

Attributes	AF-KWP	AFRD-5	T_0	T_1	T_2	Wheat straw	Maize fodder
Hemicellulose	1.13	1.41	16.87	15.25	15.33	28.07	20.51
Cellulose	7.64	15.59	5.85	5.83	6.02	48.77	30.95
Calcium	2.70	1.95	0.73	1.31	1.18	0.25	1.30
Phosphorus	0.078	0.090	0.82	0.78	0.79	0.28	0.50

The data pertaining to *in vitro* fermentation parameters are presented in Table 3. The *in vitro* total gas production was significantly (P<0.05) lower in the seaweed formulation supplemented groups. Likewise, the *in vitro* methane production was also significantly (P<0.05) lower in the seaweed formulation powder supplemented groups. However, true dry matter digestibility (TDMD) and true organic matter digestibility (TOMD) was found comparable in all groups.

Table 3. Mean *in vitro* total gas production, methane production, TDMD and TOMD

Particulars	Dietary treatments			SEM	P value
	T_0	T_1	T_2		
Total gas (ml/g DM)	148.33^{c}	131.67^{b}	129.17^{a}	3.15	0.015
Methane (% total gas)	24.57^{c}	21.80^{b}	19.45^{a}	0.59	0.047
TDMD (%)	64.18	66.63	67.31	1.63	0.194
TOMD (%)	65.68	67.61	68.15	0.65	0.276

[abc]Mean values bearing different superscripts in a row differ significantly (P<0.05)
DM: Dry matter
TDMD: True dry matter digestibility
TOMD: True organic matter digestibility

The nutrient intake (g/d) and digestibility (%) by crossbred calves fed on the experimental diets, assessed at the end of feeding through the metabolism trial, are presented in Table 4. The intake and digestibility of dry matter, organic matter, crude protein, ether extract (EE), total carbohydrate, ADF and NDF were comparable in all the three groups, and with no significant differences (P>0.05) between the groups.

The average daily intake and retention (% intake) of Ca, P and N are presented in Table 5. The results show that all the experimental calves were in positive Ca, P and N balance. The calcium intake (g/d) was significantly (P<0.001) higher in the seaweed supplemented groups T_1 (48.82) and T_2 (45.54), than T_0 (35.59). Calcium retention (% intake) was significantly (P<0.05) lower in T_0 (44.39) as compared to groups T_1 (48.91) and T_2 (49.60). However, phosphorus intake (g/d) was significantly lower (P<0.001) in the seaweed supplemented groups T_1 (28.21) and T_2 (28.26) than T_0 (29.35). Phosphorus retention as a percentage of intake was significantly (P<0.05) higher in both the seaweed formulation powder treated groups (T_1 and T_2) as compared to the control group (T_0). Significant differences were also observed between groups. The nitrogen intake and retention was non - significant in all three groups.

Table 4. Mean intake and digestibility of nutrients in different experimental groups

Attributes	Dietary treatments			SEM	P- value
	T_0	T_1	T_2		
Nutrient intake(g/d)					
DM	5103.36	5070.35	5041.31	13.56	0.178
OM	4714.77	4682.32	4656.76	12.59	0.172
Crude protein	628.58	621.82	619.11	1.76	0.168
Ether extract	93.55	91.39	91.74	0.32	0.105
Total carbohydrate	3855.68	3837.68	3813.20	10.23	0.247
ADF	1535.96	1527.46	396.30	4.49	0.522
NDF	2622.46	2570.31	2561.97	9.47	0.209
Digestibility (%)					
DM	62.79	64.24	63.75	3.41	0.777
OM	64.98	66.64	66.45	0.82	0.918
Crude protein	65.66	69.44	67.08	1.76	0.699
Ether extract	70.92	71.49	71.50	1.71	0.315
Total carbohydrate	56.62	57.33	57.25	0.55	0.860
ADF	45.42	42.12	47.37	1.41	0.324
NDF	57.86	53.41	54.21	1.53	0.476

DM: Dry matter
OM: Organic matter
ADF: Acid detergent fiber
NDF: Neutral detergent fiber

Table 5. Mean intake (g/d) and retention (%intake) of calcium, phosphorus and nitrogen in different experimental groups

Attributes	Treatment			SEM	P value
	T_0	T_1	T_2		
Calcium (Ca)					
Ca intake (g/d)	35.59^a	48.82^b	45.54^b	0.48	0.012
Ca retention (% intake)	44.39^a	48.91^b	49.60^c	1.00	0.029
Phosphorus (P)					
P intake (g/d)	29.35^b	28.21^a	28.26^a	0.43	0.000
P retention (% intake)	47.73^a	50.66^b	52.65^c	0.24	0.001

Table 5. Mean intake (g/d) and retention (%intake) of calcium, phosphorus and nitrogen in different experimental groups (continue)

Attributes	Treatment			SEM	P value
	T_0	T_1	T_2		
Nitrogen (N)					
N intake (g/d)	98.69	97.58	97.17	0.73	0.141
N retention (% intake)	48.68	49.25	50.90	3.56	0.654

[abc]Mean values bearing different superscripts in a row differ significantly (P<0.05)
Ca: Calcium
P: Phosphorus
N: Nitrogen

Data pertaining to growth performance are presented in Table 6. The initial body weight of the experimental calves was similar in all the groups, and the final body weight was comparable between the T_0, T_1 and T_2 groups. Total dry matter intake (DMI) did not differ significantly (P>0.05) among the three treatment groups. A non-significant (P>0.05) difference was also observed in the total gain in body weight, average daily gain (ADG) and feed conversion ratio (FCR) between the three treatment groups.

Table 6. Mean changes in body weight, gain in body weight, dry matter intake and feed conversion ratio of crossbred calves

Attributes	Treatment			SEM	P value
	T_0	T_1	T_2		
Growth performance					
Initial BW (Kg)	130.05	130.13	130.83	7.56	0.999
Final BW (Kg)	196.82	198.17	200.35	9.48	0.987
Total BW gain (Kg)	66.77	68.04	69.52	1.35	0.928
ADG (g)	556.42	567	576.67	10.48	0.932
Total DMI (kg)	570.22	553.85	546.43	13.56	0.978
FCR	8.54	8.14	7.86	0.33	0.620

BW: Body weight
ADG: Average daily gain
DMI: dry matter intake
FCR: Feed conversion ratio

Discussion

The total ash, calcium and phosphorus content of the concentrate mixture containing calcium and phosphorus seaweed formulation (AF-KWP and AFRD-5) powder, were higher than in the control with the mineral mixture, due to higher content of OM (more than 50%). The CP, CF, EE, NDF and ADF values was similar to that of values reported by MUNDE (2018) in crossbred calves. The CP,

ADF and NDF values were lower in the seaweed supplemented groups. Our results are similar to those of ABDOUN et al. (2013), who reported that NDF and ADF were lower in seaweed (*Ulva lactuca*) supplemented groups than the control. The chemical composition of maize fodder and wheat straw was within the normal range reported by RANJHAN (1988).

There was a significant ($P<0.05$) decrease in the *in vitro* total gas and methane production in the seaweed formulation supplemented groups. Our results are in agreement with many studies that have reported decreased *in vitro* gas production and methane production (WANG et al., 2008; DUBOIS et al., 2013; MAIA et al., 2016). Moreover, MUNDE (2018) reported that there was a decrease in the *in vitro* gas production and methane when *Gracilaria salicornia was* included at more than 1% and *Kappaphycus alvarezii* was included at more than 2%. Likewise, KINLEY et al. (2016) reported that inclusion of *Asparagopsis* in the substrate reduced *in vitro* gas production. Our results are in similar with ZITOUNI et al. (2014) who reported that gas production with seaweed (42 ml/g OM) was significantly lower than in the control (128.4 ml/g OM) after 24 h. The seaweed formulations used in the present experiment are rich sources of halogenated low molecular weight compounds, mainly brominated and chlorinated haloforms (PAUL et al., 2006) which inhibit methyl transfer reactions for methanogenesis (LIU et al., 2011). Additionally, they are rich sources of plant secondary metabolites (WANG et al., 2008; KINLEY and FREDEEN, 2015). This might be the reason for the reduction in *in vitro* gas and methane production in the present experiment. The differences in *in vitro* dry matter and organic matter digestibility were non-significant among the groups. However, significant dry matter digestibility was reported by HANSEN et al. (2003) for brown algae mixture (*Laminaria digitata* and *Laminaria hyperborean*) which was 78.30% and by VENTURA and CASTANON (1998) for *Ulva lactuca* (62.10%).

There was a non-significant effect on DMI and OMI from feeding of calcium and phosphorus added seaweed formulation (AF-KWP and AFRD-5) powders in crossbred calves. Our findings are similar to other studies that have reported the non-significant influence of seaweed supplementation on DMI (CVETKOVIC et al., 2004; ABDOUN et al., 2013; SINGH et al., 2017). Likewise LEE et al. (2004) reported that supplementation of brown seaweed *Undaria pinnatifida* made a non-significant difference to DMI. VENKETESWARAN (2018) also reported a non-significant effect on DMI and organic matter intake (OMI) from feeding brown seaweed (*Turbinaria conoides*) powder. In the present experiment, a numerically lower feed intake was observed in the seaweed formulation supplemented groups. This could be due to the presence of secondary metabolites or high mineral content (CABRITA et al., 2017). CABRITA et al. (2017) also reported that DMI was reduced by 24 and 25% when alfalfa hay was supplemented with *Gracilaria vermiculophylla* and *Ulva rigida* at 25% of the diet on a dry matter basis.

The difference in the digestibility of OM, DM, CP, EE, total carbohydrate, NDF and ADF was non-significant among the groups. Therefore it is evident that supplementation of seaweed formulations has no adverse impact on the overall rumen microbial and digestive efficiency of the animals. Our results corroborate the study of ANTAYA et al. (2001) who reported that there was no treatment effect of kelp meal supplementation on the digestibility of OM, DM, ADF and NDF. Our findings are contradictory with FIKE et al. (2005) who reported that feeding of *Ascophyllum nodosum*at 1% in lambs improved both N and OM digestibility in comparison with the control. The calcium intake and retention showed a significant difference ($P<0.05$) between the different groups. Similar results were also shown by SINGH et al. (2016), who reported a significant difference in calcium balance from feeding 20% *Sargassum wightii* to dairy cows. The phosphorus intake and retention showed a significant difference between the different groups. The phosphorus retention (% intake) was significantly ($P<0.05$) higher in the seaweed supplemented groups than the unsupplemented group. Our results are in agreement with KESHAVARZ (2000), who reported that the efficiency of phosphorus retention increases with the reduction in its dietary concentration.

VENKETESWARAN (2018) also found significant differences in calcium and phosphorus balance in crossbred calves by feeding 2% and 4% brown seaweed *Turbinaria conoides*. In contrast to our results, MUNDE (2018) did not find any significant difference in P balance by feeding red seaweed to crossbred calves. Nitrogen intake and retention were non-significant between the different treatment groups. Similarly, MUNDE (2018) reported that retention of N did not differ significantly among the groups after feeding red seaweed *Kappaphycus* and *Gracilaria*. VENKETESWARAN (2018) also reported that the average daily intake and retention of nitrogen were statistically similar in all the three groups after feeding 2% and 4% brown seaweed *Turbinaria conoides*.

The body weights of experimental calves at different fortnights were comparable among the T_0, T_1 and T_2 group. The differences in total gain in body weight, ADG, total DMI and FCR were observed to be non-significant among the groups. Our results are in agreement with SINGH et al. (2016) who reported that feeding of 20% brown seaweed *Sargassum wightii* had no effect on body weight changes and growth rate. In the present study, the results were similar to the findings of many other workers. ANDERSON et al. (2006) did not observe any influence of *Ascophyllum nodosum* supplementation on the growth performance of crossbred cattle. Likewise, inclusion of brown seaweed by-products did not affect daily gain and feed efficiency (HONG et al., 2015). Our results are also in agreement with ABDOUN et al. (2013) who reported that seaweed supplementation did not affect body weight gain and feed conversion efficiency. Moreover, dietary supplementation with brown seaweed in goats did not show any significant effect on weight gain (YATES et al., 2010). Our results are in contrast with many other studies that found that seaweed improved body weight gain (AL-SHOREPY et al., 2001; TURNER et al., 2002).

Inclusion of seaweed formulations did not affect the nutrient utilization and growth performance of the experimental calves. However, it decreased the *in vitro* total gas and methane production. Therefore, on the basis of the results obtained from the present study, it might be concluded that seaweed formulations significantly reduced *in vitro* total gas and methane production without affecting the true dry matter digestibility and true organic matter digestibility. Moreover, dietary supplementation of seaweed formulations did not affect the nutrient utilization and growth performance in crossbred calves.

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Received: 19 January 2022
Accepted: 13 June 2022

HANDIQUE, B., P. SINGH, A. KUMAR VERMA, R. YENGKHOM: Učinak hranidbe s dodatkom algi na *in vitro* fermentaciju, iskorištavanje hranjivih tvari i svojstva rasta u teladi križanaca različitih pasmina. Vet. arhiv 93, 513-524 2023.

SAŽETAK

Cilj je ovoga rada bio istražiti učinak hranidbe s dodatkom algi na *in vitro* fermentaciju, iskorištavanje nutrijenata i svojstva rasta u teladi. Istraživanje je uključilo 18 teladi muškog spola, križanaca različitih pasmina, tjelesne mase 130±7,56 kg, koja su metodom slučajnog izbora podijeljena u tri skupine po šest jedinki. Telad u kontrolnoj skupini (T_0) hranjena je uobičajenom prehranom, dok je u prehranu pokusnih skupina T_1 i T_2 dodane mješavina algi AF-KWP odnosno mješavina algi AFRD-5 u količini od 4% koncentrirane mješavine s 1% kalcijeva karbonata i 1% dikalcijeva fosfata. Navedene mješavine dodane su kao zamjena za mineralnu mješavinu i običnu sol koju su dobivale jedinke iz kontrolne skupine T_0. Pokus je trajao 120 dana nakon čega je istražen metabolizam teladi kako bi se proučilo iskorištavanje hranjivih tvari. Ukupna *in vitro* proizvodnja plina i metana bila je znakovito niža ($P<0,05$) u skupinama kojima su u prehranu dodane mješavine s algama. Znakovita je razlika između skupina teladi ($P<0,05$) uočena i u zadržavanju kalcija i fosfora. U probavljanju hranjivih tvari, unosu suhe tvari, ukupnom prirastu tjelesne mase, prosječnom dnevnom prirastu i omjeru konverzije hrane među trima skupinama nije uočena znakovita razlika. Može se zaključiti da je dodatak mješavina s algama u teladi pokusnih skupina znatno smanjio ukupnu *in vitro* proizvodnju plina i metana, ne utječući pritom na probavljanje suhe i organske tvari. Štoviše, dodatak morskih algi prehrani nije utjecao ni na rast ni na iskorištavanje hranjivih tvari u teladi križanaca različitih pasmina.

Ključne riječi: hranidba s dodatkom algi; telad križanci; iskorištavanje hranjivih tvari; svojstva rasta; *in vitro* fermentacija