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Dietary effect of combination of nitrate, sulphate and saponin on growth rate and methane mitigation on crossbred calves

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Abstract
The present study was conducted to evaluate the effect of dietary Nitrate, Sulphate and Saponin supplementation on enteric methane emission and growth performance in crossbred calves. Twenty four (24) crossbred calves divided in four groups (8-10 months of age) were selected from the Livestock Research Complex of ICAR- National Dairy Research Institute, Karnal. Control, T1, T2 and T3. Control group was fed without any supplementation, T1 group was fed with Nitrate and Sulphate, T2 group with Nitrate and Saponin and T3 group with Nitrate, Sulphate and Saponin, each was supplemented @1.5% of dietary concentrate mixture. The roughage to concentrate ratio was 65:35. Total feeding trial was conducted for four months. . Enteric CH4 emissions were measured for a total of 5 days, using the SF6 tracer gas technique. Non- significant(p>0.05) effect was observed on fortnightly body weight, feed intake, ADG, FCR, digestibility of nutrients and hematological parameters however There was significant (p<0.05) reduction in methane emission (11.84 in T1 – 26.78% in T3) in crossbred calves in treatment groups (38% reduction in T3) than control. On the basis of above results it can be concluded that supplementation of combination of Nitrate, Sulphate and Saponin affects the enteric methane emissions without adversely affecting growth and ruminal fermentation parameters.

Keywords: Methane, Nitrate, Saponin, mitigation, emission, ADG, FCR

Introduction

The present scenario of animal husbandry is changing day by day due increasing human population and increase in demands for livestock products. The contribution by livestock is about $1.4 trillion to the global asset (Thronton, 2010) [23], equal to 50% of the total economy of the agricultural sector (Herrero *et al*., 2016) [4]. Globally, it has been observed that the per capita consumption of livestock products has become doubled in the past few decades (Herrero *et al*., 2016) [4]. The supply and demands of milk has been changed drastically in last few decades and it is estimated that will rise by 25% in the coming years. Livestock especially ruminants produces significant amounts of methane as part of their normal digestive processes. It is estimated that they contribute 12–18% to the global anthropogenic greenhouse gases (GHG) in carbon dioxide equivalents (CO2-eq) (Westhoek *et al*., 2011) [28]. Enteric methane emission is the largest source of GHG from agriculture. This contribution will likely continue to increase over the next few decades due to growing demands for meat and milk primarily driven by human population growth and improved standard of living in developing countries (Patra, 2014) [20]. Many types of methane inhibitors have been repeatedly tried, in this study a combination of Nitrate, Sulphate and Saponin has been used to reduce the methane emission from livestock. In the previous study a combination of nitrate and saponin was shown to reduce methane production dramatically (by 32% at 5 mM nitrate and 0.6 g/L saponin; and by 58% at 10 mM nitrate and 1.2 g/L saponin) using an *in vitro* model of rumen cultures (Patra and Yu, 2013) [21]. In the nitrate–saponin combination, three modes of action were shown to function additively in reducing methane production: (1) saponin functioning as an inhibitor to rumen protozoa, decreasing hydrogen production by protozoa and protozoa-associated methanogens, (2) nitrate acting as an electron sink and competing with CO_2 for electrons, and (3) nitrite, the first intermediate of nitrate reduction, exerting toxicity to methanogens (Bozic *et al*., 2009; Zhou *et al*., 2011) [2, 29]. Dissimilarly sulfate reduction by sulfate reducing bacteria (SRB) is thermodynamically more favorable ($\Delta G0$ = -42.2 kJ/mol H2) than hydrogenotrophic methanogenesis ($\Delta G0$= -33.9 kJ/mol H2) (Ungerfeld and Kohn, 2006) [24]. Indeed, previous studies have shown that sulfate outcompeted CO2 as an electron acceptor in anaerobic habitats. One recent study has shown that sulfate can suppress methane production in sheep (van Zijderveld *et al*., 2010) [26]. Thus it is hypothesized that combinations of nitrate, sulfate, and saponin may further reduce methane production by rumen microbial communities.

Material and Methods

Twenty four (24) crossbred calves (8-10 months of age) were selected from the Livestock Research Complex of ICAR-National Dairy Research Institute, Karnal. The study was conducted in the experimental shed no. 6 of ICAR National Dairy Research Institute, Karnal, India. The institute is located at 29° 42" N and 79° 54" E at an altitude of 834 feet above the sea level. The maximum ambient temperature in summer goes up to 45°C and minimum temperature in winter comes down to about 4°C with a diurnal variation to the order of 15-20°C. Average annual rainfall is 700 mm, most of which is received during early July to mid-September. The experiment was conducted from December 1st, 2019 to April 30th, 2020. The cross-bred calves were housed in experimental sheds and were maintained on similar basal ration consisting of concentrate, wheat straw and green fodder. Prior to onset of experimental feeding, feed intake data was collected for about 10 days to have an idea about the intake and accordingly their ration was formulated. During this period animals also got acclimatized to the changed environment, after this adaptation animals were weighed consecutively for two days and these were blocked in four groups. Supplementation combinations of Nitrate, Sulphate, Saponin were fed for feeding experiment on cross bred calves. Treatment dose was provided over and above the concentrate and each was given @1.5% of concentrate mixture (diet composition: Conc. 35% +Green +straw), Control- fed with according ICAR 2013, T1- Control +Nitrate+ Sulphate, T2- Control + Nitrate +Saponin and T3- Control + Nitrate+ Sulphate+ Saponin. The feed samples were collected from NDRI, Karnal. The samples were dried in hot air oven 60°C for a day till a constant weight was attained. The dried samples were ground through 1mm sieve using electrically operated Willey mill. The ground samples were stored in sample bottles of 200 mL capacity, labeled properly and kept for further analysis. Pooled samples were analyzed for chemical composition according to standard methods of AOAC (2005) [5]. Fiber fractions were assayed using by procedures of Van Soest *et al*. (1991). Acid detergent lignin was recovered from ADF by solubilizing cellulose with 72% (w/w) sulphuric acid. Fortnightly body weight, Daily DMI was recorded and blood for hematological parameter was taken monthly. Hematological parameters were carried out by Hematology analyzer MS4Se made in France. A metabolic trial of 5 days of adaptation and 7 days for collection period was also carried for nutrient digestibility. All analyses were done in triplicates. Enteric CH4 emissions were measured for a total of 5 days, using the SF6 tracer gas technique as described by Johnson *et al*. (2007) [7]. A permeation tube containing SF6, an inert gas tracer, was placed into the rumen of each animal approximately 2 days before CH4 measurements commenced.

Data were analyzed using the general linear models (GLM) procedure of SPSS 16.0 computer package.

Results and Discussion

Chemical composition of feedstuffs fed to the experimental animals

The proximate composition and cell wall constituents of feed ingredients *viz*., concentrate mixture, oat fodder and wheat straw has been presented in table 1. The DM of concentrate mixture, oat fodder and wheat straw was 91.42, 24.23 and 91.46% respectively. The CP and TDN content of feed ingredients varied from 2.98 to 19.22, and 46.22 to 75.25 respectively. These values for chemical composition NDF, ADF, NDICP and ADICP of feed ingredients were found in accordance with previous reports.

Table 1: Chemical composition of feedstuffs fed during experiment DMB (%)

Parameter	Oat fodder	Wheat straw	Concentrate mixture
DM	24.23	91.46	91.42
OM	90.09	89.64	87.89
CP	8.02	2.98	19.22
TA	9.91	10.36	12.11
EE	2.77	0.83	4.81
NDF	57.39	81.26	26.93
ADF	44.75	59.97	15.68
NDICP	2.82	1.80	2.74
ADICP	1.34	0.87	0.89
TDN	60.79	46.22	75.25

Body weight, DMI, ADG and FCR of the experimental animals during trial

The data regarding body weight, DMI, ADG and FCR has been presented in table 2. In *in vivo* feedings of nitrate, Sulphate and Saponin resulted invariable effects on growth and production performance of animals. Several studies have been conducted using nitrate supplementation to reduce the methane emission in livestock. A similar study was conducted by Velazco *et al*. (2014) [27] on beef cattle using calcium nitrate @ 2.6% of body weight observed non-significant ($p>0.05$) change in DMI and body weight of the experimental animals. Zijderveld *et al*. (2011) [23] also conducted an experiment supplemented with calcium nitrate @1% of DMI to the dairy cattle and observed non-significant changes in DMI and body weight gain by the animals. Similar results were also found in the study by Lee *et al*., (2015), the experiments was conducted on beef cattle using calcium nitrate @2.5 of DMI. However Klop *et al*. (2016) [8] found decrease in DMI fed with Nitrate @ 21g/kg DMI in lactating dairy cows. Kumar at al. (2017) [11] conducted an experiment on goat kits using saponin @2.6 of DMI observed non-significant change in DMI, ADG and FCR. Similar results were also observed by Li and Powers, (2012) they used saponin @1.5 of DMI in Holstein steers.

Table 2: Overall performance of crossbred calves during trial

Attributes	Control	T1	T2	T3	p- value
Initial Body weight(kg)	152.35±4.04	148.12±3.99	149.78±4.99	153.48±4.58	0.357
Final Body weight(kg)	205.86±4.8	207.46±4.4	203.48±5.04	209.02±4.56	0.428
DMI(kg/d)	4.97±0.9	5.15±1.08	4.93±0.97	5.10±0.92	0.730
ADG(g/d)	528.18±4.8	538.69±20.0	525.87±5.48	535.08±5.53	0.518
FCR	9.34±0.29	9.33±0.41	9.16±0.33	8.98±0.27	0.584

The apparent digestibility and nutritive value of various nutrients during trial

The data pertaining apparent digestibility and nutritive value has been presented in table No.3. We observed non-significant difference in digestibility of DM, OM, CP, NDF, and ADF among different treatments groups during metabolic trial of different treatments groups. Nasri *et al*. 2011[15] and Aazami *et al* (2013) [1] found similar results to our findings on digestibility with feeding saponin containing diet in ruminants. Some studies shows that feeding of Nitrate may reduce organoleptic properties of animals which results in decrease in feed intake. However, in this study Nitrate had no influence on nutrient intake or digestibility, which could likely be due to the unchanged organoleptic issues. Meanwhile, in other studies, supplementation of sheep diet with 4% Potassium Nitrate (Nolan *et al*., 2010) [17] and 2% of concentrate mixture (Pal *et al*., 2015) [19] also did not affect the dietary DMI, nutrient intake and digestibility.

Table 3: Apparent digestibility and nutritive value during trial

Apparent digestibility (%)					
Parameter	**Control**	**T1**	**T2**	**T3**	**p- Value**
DM	60.16±1.40	58.29±0.94	59.94±1.44	61.24±1.10	0.641
OM	61.08±1.56	63.60±0.79	62.54±0.11	62.81±1.36	0.542
CP	64.61±0.99	63.05±1.14	64.42±1.08	67.89±2.00	0.581
EE	74.93±0.78	75.20±0.87	73.64±1.29	72.28±0.77	0.672
NDF	51.82±1.09	48.70±1.08	53.26±1.92	53.19±1.28	0.832
ADF	38.93±1.98	36.44±1.40	41.77±2.26	40.53±1.70	0.326
Nutritive Value (%)					
CP	13.08±0.005	12.91±0.40	13.02±0.003	13.21±0.005	0.219
DCP	8.61±0.13	8.70±0.14	8.35±0.12	8.16±0.23	0.421
TDN	66.71±5.43	64.26±3.06	65.34±3.11	65.38±4.50	0.532

Hematological parameters of different groups during trial

The values related predicting hematological value are shown in table no. 4 The hematological parameters were similar in different treatment groups which indicated that supplementation had no adverse effect on these parameters, similar findings were also reported in studies by Pal *et al*. (2015) [19]; Zijderveld *et al*. (2011) [23] and Kumar *et al*. (2017) [11]. The similar hemoglobin values indicated that supplementation had no adverse effect on these parameters, similar findings were also reported in studies by Pal *et al*. (2015) [19]; Zijderveld *et al*.(2011) [25]; Nasri *et al*.(2011) [15] and Kumar *et al*. (2017) [11]. So these findings indicated that these supplements are safe for ruminants feeding. The normal haematocrit value depicts that supplementation had no adverse effect on the experimental animals as also observed in findings of Nasri *et al*. (2011) [15] and Kumar *et al*. (2017) [11].

Table 4: Hematological parameters of different groups during trial**.**

Attributes	Control	T1	T2	T3	p- value
RBC counts (m/mm3)	5.77±0.12	5.13±0.13	5.17±0.15	5.57±0.25	0.542
Hb (mg/dL)	11.85±0.25	11.96±0.24	12.38±0.24	12.03±0.37	0.415
Hematocrit values (%)	36.68±0.42	36.87±0.35	36.57±0.35	37.14±0.36	0.241

Effect of Nitrate, Sulphate and Saponin supplementation on enteric methane (CH4) emission in different treatment groups.

The enteric methane emission parameters have been presented in table 5. The methane emission in Control, T1, T2 and T3 group was 112.13±6.48, 98.85±5.78, and 96.46±5.51and 82.30±4.66 g/d respectively. The present study showed a significant (*p*<0.05) reduction in methane emission in treatment groups in comparison to control. The similar results have been observed in previous studies also (Nolan *et al*., 2010 Hulshof *et al*., 2012 van Zijderveld *et al*., 2011, Newbold *et al*., 2014, Li *et al*. 2013, Lee *et al*., 2015 and Granja-Salcedo., 2019) [17, 6, 25, 14, 23] when nitrate was fed (0 to 4%/ kg DMI) as a supplement to their experimental animals.

Table 5: Effect of Nitrate, Sulphate and Saponin supplementation on enteric methane (CH_4) emission of different treatment groups.

Attribute	Control	T1	T2	T3	p-value
CH4 (gm/d)	112.13^a ±6.48	98.85^b±5.78	96.46^b ±5.51	82.30^c±4.66	0.021
CH4 (g / kg B.wt)	54.67^a ±4.29	47.40^b ±5.15	46.83^b±6.24	35.09^c ±4.60	0.034
CH4 (g / kg DMI)	21.84^a ±3.25	19.36^b ±2.98	17.65 ^{b}±2.03	13.87^c ±2.70	0.002
CH4 (g / kg CPI)	169.69 ^{a} ±10.25	143.33^b ±9.89	146.48 ^{b}±9.76	192.57^c±11.83	0.042
CH4 (g / kg EEI)	386.37^a±12.5	356.12^b ±13.45	362.35^b ±17.56	295.34^c±12.67	0.041
CH4 (g / kg NDFI)	48.54^a ±4.36	38.6^b±3.54	42.30^b±4.17	35.38^c±3.93	0.026
CH4 (g / kg ADFI)	57.60^a ±6.87	56.24^b±5.59	60.23^b±6.14	41.48^c±4.19	0.025
CH4 (g / kg TDN)	76.83^a ±4.17	58.48^b ±4.31	63.88^b±4.79	49.28^c±4.45	0.015
CH4 Energy (MJ/d)	12.47±1.12	11.92±1.08	11.86±1.15	11.74±1.38	0.071
CH4 Energy loss (%GEI)	4.78±1.23	4.63±1.19	4.58±1.64	4.34±1.58	0.095
CH4 Energy loss (%MEI)	9.88±1.76	9.46±1.22	9.52±1.53	9.38±1.48	0.074

a, b,c Means having different superscripts in the same row differ significantly,(p<0.05).

Conclusions

Although there are several methods for reduction of methane emission from livestock but none of them is long term effective, in our study which was carried out for four months shows a significant (p<0.05) reduction in methane emission (11.84 – 26.78%) in T1, T2 & T3 group supplemented with Nitrate, Sulphate and Saponin combinations. On the basis of above results it can be concluded that supplementation of Nitrate, Sulphate and Saponin at 1.5% of concentrate mixture had decreased the enteric methane emissions without adversely affecting growth and ruminal fermentation parameters.

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