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EFFECT OF SUPPLEMENTARY SODIUM NITRATE AND SULPHUR ON METHANE PRODUCTION AND GROWTH RATES IN SHEEP AND GOATS FED FORAGE BASED DIET LOW IN TRUE PROTEIN

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ABSTRACT

Ruminants are major contributorsof methane (CH_4) production. Serious global efforts are required to reduce CH_4 produced through their digestive tract, which isgenerally termed as enteric methane production (e.m.p). Feeding diets containing nitrates and/or sulphates could be used to reduce e.m.p. The study was conducted to evaluate the influence of varying levels of sodium nitrate (SN) with or without sulphur (S) on the growth performance and e.m.p in *Lohi* sheep and *Teddy* goats in a 2×3×2 factorial arrangement under Randomized Complete Block Design. In the experiment, 48 male animals (24 *Lohi* sheep and 24 *Teddy* goats of approximately nine months of age), were randomly divided into 12 groups, 4 animals in each group. Six *iso-nitogenous* and *iso-caloric* diets were formulated. The control diet SN0-S0 contained neither SN nor S. Whereas SN0-S4, SN2.5-S0, SN2.5-S4, SN5-S0 and SN5-S4 diets had 0% SN and 0.4% S, 2.5% SN and 0% S, 2.5% SN and 0.4% S, 5% SN and 0% S and 5% SN and 0.4% S, respectively. Nonprotein nitrogen was same across all diets. The Gases were measured by using infra-red biogas analyzer. The nutrient intake and digestibility in both sheep and goats were similar ($P> 0.05$) across all diets. The animals fed diet containing 5% SN with 0.4% S showed better nitrogen balance. The enteric CH_4 was 19.6% reduced ($P<0.05$) in sheep and 18.2% in goats fed diet containing 5% SN and 0.4% S compared to those fed control diet. Daily live weight gain of both sheep and goats fed diet containing 5% SN and 0.4% S was 143 and 59 g/day, respectively. The best feed conversion values ($P<0.05$) were observed in sheep fed both SN2.5-S4 and SN5-S4 diets. However, best feed conversion ratio was observed in goats fed SN5-S0 and SN5-S4 diets. Both in sheep and goats non-significant differences ($P> 0.05$) were observed in blood metabolites including blood urea nitrogen, glucose and Creatinine. Similar trend ($P> 0.05$) was observed in hematology. None of the animal showed any kind of abnormal behaviour or signs of illness during the whole experimental period. It can be concluded that supplementation of forage-based diet with SN in combination with sulphur is not only effective to reduce the e.m.p but also improves the weight gain in fattening sheep and goats.

Keywords: Lohi, Methane, Nitrate, Sulphate, Teddy.

INTRODUCTION

Among various challenges of twenty first century, continuous enteric CH_4 emission from ruminants is a serious threat to climate change (Alemu *et al*, 2011). Forster *et al*. (2007) pointed out that CH_4 has 25 times more global warming potential than CO_2. It is predicted that growing demand of livestock will boost up e.m.p resulting in its 30% growth by year 2020 (O'Mara, 2011). O'Mara (2011) highlighted the importance to develop new means to abate enteric CH_4 in ruminants.

Agro-industrial by-products are primarily fed to ruminants in developing countries. Supplementing urea to such diets improves rumen microbial growth thus improving productive performance of animals. In ruminants, the excess hydrogen which is generated as a result of rumen fermentation is normally required to be removed for efficient and continuous microbial growth (Beauchemin *et al*., 2008). This hydrogen is removed by reducing CO_2 into CH_4 which is eventually emitted by eructation (Ungerfeld, 2015). Methane emission is also an energy loss (Broucek, 2014). This CH_4 emission can be reduced by substituting the CO_2 with another electron acceptor (McAllister and Newbold, 2008).

Nitrates as replacement for urea can replace CO_2 as an electron acceptor. They are reduced to nitrite and then to NH_3. One mol of nitrate reduces CH_4 in equivalent amount which in turn produces one mol of NH_3 (Leng, 2008). So, the supplementation of nitrates in diet may reduce the CH_4 emission by ruminants. However, in past their use was limited because of possible risk of nitrate/nitrite toxicity. While, Leng (2008) proposed that addition of nitrates to foarage based diet may significantly reduce e.m.p without causing nitrate toxicity, in slowly acclimatized animals. Furthermore, he also stated that supplementation of

sulphur in nitrate containing diets may also be effective in reducing nitrite accumulation in rumen. He urged the need of more *in-vivo* work to evaluate the efficiency of nitrates and sulphates to abate CH_4 production in various ruminant animals.

It is very complex to measure CH_4 production from individual animals because of its gaseous properties. One of the important considerations while attempting these experiments is the precision and accuracy of CH_4 measuring methods or techniques. Various methods used to determine CH_4 production from animals (Makkar and Vercoe, 2007) include respiration chambers (Frankenfield, 2010), CH_4 estimations from the VFA production (Hegarty and Nolan, 2007), ventilated hood techniques (Odongo *et al*., 2007), isotopic (Hegarty et al., 2007), non-isotopic tracer techniques (Johnson *et al*., 1994) and tunnel technique (Murray *et al*., 2007). Recently, a new method for estimating enteric CH_4 production from ruminants has been developed. It is based on the use of naturally emitted CO_2 as a tracer gas instead of SF_6 (Storm *et al*., 2012; Madsen *et al*., 2010). This technique is easy, fast, cost effective and less labour intensive. By this technique, data from more number of animals can be collected in limited period of time. Also the results from animals are in their natural environment. However, this technique is yet to be tested against standard respiration calorimetry chamber technique. In conclusion, the respiration chamber technique is the best however, technique based on the use of naturally emitted CO_2 as a tracer gas is more feasible in developing countries.

The scientific evidence regarding the use of nitrate with or without S to mitigate *in-vivo* CH_4 production is limited. Therefore, the present study was planed to determine the effect of varying levels of SN with or without S on growth performance and e.m.p in fattening *Lohi* sheep and *Teddy* goats fed forage based diet. The hypothesis was that nitrate and S will mitigate the enteric CH_4 production in both sheep and goats. Both nitrate and S will give an additive effect on the CH_4 reduction.

MATERIALS AND METHODS

Experimental site: The study was conducted at University College of Agriculture, University of Sargodha, Pakistan.

Experimental animals and feeding: In this experiment, 48 male animals (24 *Lohi* sheep (initial weight 38.7 + 0.17 Kg) and 24 *Teddy* goats (initial weight 24.1+ 0.15 Kg) of approximately nine months of age), were randomly divided into 12 groups, 4 animals in each group. Guidelines of ethical use of animals (Dua, 2004) were followed. Each group was maintained in a separate pen measuring 10' x 10'. Animals were fed separately. Six *iso-nitrogenous* (crude protein 18%) and *iso-caloric* (metabolizable energy 2.0 Mcal/Kg) diets using ~ 0, 2.5 and 5% SN with or without 0.4% S (anhydrous $MgSO_4$) were formulated. In this experiment sodium nitrate and urea was used as fermentable nitrogen sources. Nonprotein nitrogen was same across all diets. Moreover, all treatments contained same basal diet. The control diet (SN0-S0) contained neither SN nor S. Whereas SN0-S4, SN2.5-S0, SN2.5-S4, SN5-S0 and SN5-S4 diets had 0% SN and 0.4% S, 1.5% SN and 0% S, 1.5% SN and 0.4% S, 3% SN and 0% S and 3% SN and 0.4% S, respectively (Table 1). The total mixed rations were offered twice a day and fresh clean water was made available round the clock during experimental period. The animals were weighed fortnightly. This experiment lasted for three months (Octber-November-December).

The daily feed intake was recorded and representative samples were taken and analyzed for DM and CP using the procedures described by AOAC (1990). Neutral detergent fiber (NDF) and acid detergent fiber (ADF) were determined by the methods described by Van Soest *et al*. (1991).

Digestibility and nitrogen balance trials were conducted during the last week of experiment. For digestibility and nitrogen balance trials, all animals were shifted to metabolic crates for 7 days to ensure complete collection of feces and urine. Locally made metabolic collection crates, each measuring 5'x4'x5', were used to collect the urine and feces of individual animal and then urine and feces were mixed by animal. Metabolic collection crates consisted of a collection tray and two plastic urine collection bowls. Removable trays were fitted on floor of metabolic collection crates and animals stood on these trays. Removable urine collection bowls were set beneath the floor of metabolic collection crates. During total collection method, urine was collected in urine collection bowls through the small hole at the bottom of the collection tray. These bowls had measured amount of solution acidified with 50% H_2SO_4 to avoid N losses during collection (Nisa *et al*., 2004). Feces and urine were collected, weighed and representative samples were stored at -20^{o}C for further analysis. At the end of collection period, urine and feces samples from individual pens were thawed, composited and homogenized. Composite samples were dried at 55^{o}C and ground through 1-mm screen. Feed and fecal samples were analyzed for NDF and ADF by the methods described by Van Soest *et al*. (1991) and DM and CP were determined by the methods described by AOAC (1990). At the end of experiment, one hour after morning feed, blood samples (10 mL/animal) were collected from jugular vein punctured into vacutainer tube containing 81 µL of 15% EDTA solution and analyzed in local pathological laboratory for blood urea nitrogen, glucose, creatinine and methemoglobin (MetHb; Evelyn and Malloy, 1938).

Enteric CH_4 was analyzed at the end of the experiment using method described by Madsen *et al*. (2010) in which naturally emitted CO_2 was used as tracer gas (Storm *et al.*, 2012). Data of e.m.p was recorded one hour after each feeding. Each animal was kept in an especially designed closed enclosure (each measuring 5'x4'x5') for 15 minutes and e.m.p was measured during the last 5 minutes with the help of infra red CH_4 analyzer (Gasmet Dx-4030, Finland). For Zero point calibration nitrogen gas was used. In order to minimize stress factor all animals were made accustomed to enclosure 15 days prior to data collection.

The CO_2 and CH_4 in background air were measured at the same time. Measuring the CH_4 to CO_2 ratio combined with the measuring of total CO_2 produced, the amount of CH_4 was calculated as follows.

$CH_4:CO_2 = (a-b)/(c-d)$

Where "a" is CH_4 concentration in mixed eructed gas plus air, "c" is CO_2 concentration in mixed eructed gas plus air, "b" is CH_4 in background air and "d" the CO_2 in background air.

As feed intake did not differ among the treatments it was assumed that CO_2 production was also similar and could be used as internal marker as described by Madsen *et al*. (2010).

Methane reduction was derived from the equation proposed by Leng and Preston (2010) in which it was assumed that:

If the CH_4 production rate is "A", the CO_2 entry rate "B" is same on all diets, and the ratio of CH_4 to CO_2 is "R", then the following equations apply:

For urea-fed animal……A_U (Urea) = $B * R_1$

For the nitrate-fed animal……A_N (nitrate) = $B * R_2$

The $\%CH_4$ reduction rate is then…$B (R_1-R_2) / BR_1*100$

Statistical analysis: The data collected were analyzed using 2×3×2 factorial arrangement under RCBD. The data were analyzed by methods described by Steel *et al*. (1996). Treatment means were compared by Tukey's test using statistical software Statistix 8.1.

RESULTS

The nutrient intake and digestibility in both sheep and goats were similar (P> 0.05) across all diets (Table 2). Both in sheep and goats non-significant differences (P> 0.05) were observed in blood metabolites including blood urea nitrogen, glucose and Creatinine (Table 3). Similar trend (P> 0.05) was observed in hematology (Table 4). None of the animal showed any kind of abnormal behaviour or signs of illness during the whole experimental period. The animals fed diet containing 5% SN with 0.4% S showed better nitrogen balance (Table 5). Daily live weight gain was 138, 139, 142, 143, 141 and 143 g/day in *Lohi* sheep and was 55, 54, 57, 57, 59 and 59 g/d in *Teddy* goats fed SN0-S0, SN0-S4, SN2.5-S0, SN2.5-S4, SN5-S0 and SN5-S4 diets, respectively (Table 6). The FCR in sheep fed SN0-S0, SN0-S4, SN2.5-S0, SN2.5-S4, SN5-S0 and SN5-S4 diets were 12.7, 12.5, 12.3, 12.2, 12.3 and 12.2, respectively. Moreover, the FCR were 13.9, 14.1, 13.5, 13.5, 13.0 and 13.0 in goats fed SN0-S0, SN0-S4, SN2.5-S0, SN2.5-S4, SN5-S0 and SN5-S4 diets, respectively (Table 6). The best feed conversion values (P<0.05) were observed in sheep fed both SN2.5-S4 and SN5-S4 diets. However, best FCR was observed in goats fed SN5-S0 and SN5-S4 diets. The difference in enteric CH_4 reduction was 0, 2.20, 3.09, 8.06, 12.46 and 19.62% in *Lohi* sheep and 0, 3.12, 3.94, 7.66, 11.55 and 18.27% in *Teddy* goats fed SN0-S0, SN0-S4, SN2.5-S0, SN2.5-S4, SN5-S0 and SN5-S4 diets, respectively (Table 7).

DISCUSSION

Nutrient Intake and digestibility: In present study, the DMI remained unaltered across all diets. These observations are similar to those reported by Sangkhom *et al*. (2012) who observed similar DMI in cattle fed nitrates or urea as NPN source in their diets. Zijderveld *et al*. (2010) and Sophea and Preston (2011) also reported unaltered DMI in response to nitrates supplementation in sheep and goat, respectively. It is considered that nitrate feeding may cause DMI depression and the extent to which nitrates can cause DMI depression depends upon their dose and the rate of their conversion into nitrites in rumen. Dry matter intake decreases with increasing accumulation of nitrites in the rumen. In present study, no adverse effect of nitrates on DMI might be attributed to less accumulation of nitrites in rumen due to slower conversion of nitrates into nitrites. Phuong *et al*. (2012) also reported no effect of nitrates on DMI in cattle but they observed decreased feed intake in cattle fed sulphate-supplemented diet. This might be related to bitterness in taste of the diets due to sulphur addition at higher doses. However, in our study sulphate was included at a rate of 0.4% S which was lower as compared to that of used by Phuong *et al*. (2012) or Silivong *et al*. (2011) in their studies on cattle or goats, respectively. Other findings (Hulshof *et al*., 2010; Bruning-Fann and Kaneene, 1993) did not agree with our present results who reported that nitrate supplemented diets negatively affect DMI. Nitrite accumulation depresses the colonies of some bacterial species growth, which could lower the feed digestibility. It negatively affects volatile fatty acid production, reduces microbial biomass thus lowering intake by the animal. Results by Thanh *et al*. (2012) supported our findings on nutrient digestibility. They reported similar digestibility with nitrate or urea supplemented diets. Our results were also supported by the findings of Nolan *et al*. (2010) who used nitrates as feed additive in diets. Feed intake can be categorized as important factors among many others

which influence digestibility. In our study feed consumption remained unchanged which might have resulted in similar digestibility.

Blood Chemistry: All values of blood metabolites and haematology were within the normal physiological range, which is a sign of good health. None of the animals had nitrate or nitrite toxicity throughout the study. During the whole study animal remained healthy without any abnormal behaviour. Present study data made it possible to predict that either slow nitrate acclimation of animal or balance between nitrate to sulphur ratios tended to maintain the activity of both nitrate reducing bacteria and sulphur reducing bacteria (Leng, 2008). The present results close by related to the observations of Ngoc Huyen Le Thi (2010) who reported no evidence of nitrate or nitrite toxicity even at higher levels of inclusion in the diet. Minor incidence of nitrate toxicity was reported by Zijderveld *et al*. (2010). Methemoglobinemia condition develops only when MetHb level is 30 to 40% of Hb (Bruning-Fann and Kaneene, 1993).

Growth performance: Better live weight gain and FCR observed in animals fed nitrate and sulphate supplemented diets were supported by the findings of Sangkhom *et al*. (2012) and Trinh phucHao *et al*. (2009) who observed that nitrate supplementation results in better weight gain and FCR in cattle and goat, respectively. However, Thanh *et al*. (2012) observed no effect of nitrates supplementation on weight gain in sheep. Likewise, Trinh phucHao *et al*. (2009) also found similar weight gain in goat fed diet supplemented with or without nitrates. According to Hulshof *et al*. (2010) nitrate might be responsible for reduced growth rates. Methane mitigation improves nutritive value of feed, which in turn resulted into better animal performance (Beauchemin *et al*., 2008).

These findings suggest that, if nitrite accumulation did not occur in the rumen, it might result in NH_3 production at a rate equal to assimilatory microbial growth rate. During this process the electron transfer of NH_3 preserves more energy in ruminal end products as compared to CH_4 emission. This conserved energy might result in increased growth efficiency of ruminal microbes. Thus improved the efficiency of nitrate supplemented diets (Leng, 2008).

Abatement of enteric methane: In our study we observed significant enteric CH_4 reductions with nitrate supplemented diets. Various studies (Phuong *et al*., 2011: Sangkhom *et al*., 2011; Guo *et al*., 2009) supported our findings. Our observations are also concordant with reports of Leng (2008) who inferred that 1% inclusion of potassium nitrate could reduce e.m.p up to 10% in ruminants. Findings of Leng and Preston (2010) also supported our observations who reported nitrates as better option to abate e.m.p rather than urea. Generally dissimilatory nitrate reduction to NH_3 and assimilatory nitrate reduction to NH_3 are two pathways considered responsible for nitrate reduction in anaerobic system. Nitrate reduction in ruminal fluid encourages the growth of nitrate reducing bacteria. Thermodynamically nitrate conversion to NH_3 is more favourable than CH_4 formation, if ruminants are fed nitrate containing diets (Morgavi *et al*., 2010). According to Leng (2008) one mol of nitrate can produce one mol of NH_3 by reducing CH_4 in equivalent amounts. As reported by Ungerfeld and Kohn (2006) thermodynamic conversion of sulphate to hydrogen sulphide gas is more favourable than methanogenesis. We therefore concluded in our study that both nitrate and sulphate supplementation in diets gave an additive effect resulting in better e.m.p. Nitrate adaptation in our study improved nitrite-reducing capacity of ruminal microbes. This is evident from our present study findings that we did not observe even a single incidence of nitrite toxicity, even at higher levels of nitrate inclusion. Inconsistent results in CH_4 abatement with dietary nitrates were reported by many researchers. In sheep trial, Zijderveld *et al*. (2010) observed 32% enteric CH_4 reduction. Contrarily Nolan *et al*. (2010) observed 23% CH_4 reduction in the same specie fed nitrate containing diets as compared to those fed urea as NPN. In cattle fed diets with nitrate as feed additive Hulshof *et al*. (2010) observed 32% CH_4 reduction. Maximum 60% CH_4 reduction were observed in goats by Sophea and Preston (2011) while minimum 16% CH_4 reduction were observed in dairy cows fed nitrate supplemented diets. The possible reason for variable results might be attributed to CH_4 measuring technique used in animals. Calorimetric chambers (Zijderveld *et al*., 2011) and SF_6 technique (Hulshof *et al*., 2010) is mostly applied techniques for CH_4 estimation from animals. Enteric CH_4 production can also be estimated via volatile fatty acids (Nolan *et al*., 2010). Sophal *et al*. (2013) and Sangkhom *et al*. (2012) estimated e.m.p using technique proposed by Madsen *et al*. (2010) in which CO_2 was used as tracer gas. Diet composition may also affect rumen nitrite accumulation. This is evident from the work of some researchers (Hulshof *et al*., 2010; leng, 2008). Hulshof *et al*. (2010) observed that sugarcane based favoured nitrite accumulation in the ruminal fluid. Other researchers (Johnson *et al*., 1994; Grainger *et al*., 2007) found variable results of CH_4 estimation from SF_6 method as compared to chamber measurements.

Table 1. Ingredient and chemical composition of experimental diets.

Ingredients (%)	Diets[1]					
	SN0		SN2.5		SN5	
	S0	S4	S0	S4	S0	S4
Wheat Straw	35.0	35.0	35.0	35.0	35.0	35.0
Cotton seed meal (CP 47%)	10.00	10.00	10.00	10.00	10.00	10.00
Rice bran	10.00	10.00	10.00	10.00	10.00	10.00
Corn gluten 30%	5.00	5.00	5.00	5.00	5.00	5.00
Hay (Lucerne)	21.00	21.00	21.00	21.00	21.00	21.00
Enzose	14.00	14.00	14.00	14.00	14.00	14.00
Anhydrous Mag. Sulphate	0.00	1.50	0.00	1.50	0.00	1.50
Sodium Chloride	1.00	1.00	1.00	1.00	1.00	1.00
Di-Calcium Phosphate	2.00	2.00	2.00	2.00	2.00	2.00
Sodium Nitrate	0.00	0.00	2.45	2.45	4.90	4.90
Urea	1.70	1.70	0.85	0.85	0.00	0.00
Chemical composition						
Dry Matter	88.08	89.58	89.68	91.18	91.28	92.78
Crude Protein	18.0	18.0	18.0	18.0	18.0	18.0
Neutral Detergent Fiber	44.36	44.36	44.36	44.36	44.36	44.36
Acid Detergent Fiber	31.38	31.38	31.38	31.38	31.38	31.38
Metabolizable Energy (Mcal/kg)	2.06	2.06	2.06	2.06	2.06	2.06

[1]SN0, SN2.5 and SN5 stand for sodium nitrate at ~ 0, 2.5 and 5%, respectively. S0 and S4 stand for 0 and 0.4% sulphur, respectively.

Table 2 Effect of different levels of sodium nitrate with or without sulphur on nutrient intake and their digestibility in *Lohi* sheep and *Teddy* goat.

			Nutrients intake, g/d				Nutrient digestibility, %			
			DM	CP	NDF	ADF	DM	CP	NDF	ADF
Sheep	Diets[1] SN0	S0	1740[a]	313[a]	772[a]	546[a]	58.5[abc]	63.5[c]	52.0[ab]	43.5
		S4	1738[a]	313[a]	771[a]	545[a]	59.0[ab]	62.5[cd]	51.0[ab]	41.8
	SN2.5	S0	1736[a]	313[a]	771[a]	546[a]	57.0[abcde]	62.5[cd]	51.5[ab]	42.8
		S4	1736[a]	312[a]	770[a]	545[a]	58.0[abcde]	62.5[cd]	52.3[a]	44.0
	SN5	S0	1735[a]	312[a]	770[a]	545[a]	58.3[abcd]	62.5[cd]	50.5[ab]	44.8
		S4	1738[a]	313[a]	771[a]	546[a]	59.5[a]	61.0[d]	51.8[ab]	43.8
Goats	SN0	S0	766[b]	138[b]	340[b]	241[b]	54.0[de]	70.5[a]	49.5[ab]	41.8
		S4	768[b]	138[b]	341[b]	241[b]	55.0[bcde]	70.5[a]	48.8[ab]	42.0
	SN2.5	S0	767[b]	138[b]	340[b]	241[b]	53.8[e]	70.0[a]	49.0[ab]	41.0
		S4	766[b]	138[b]	340[b]	240[b]	54.5[cde]	70.3[a]	51.0[ab]	43.8
	SN5	S0	765[b]	138[b]	339[b]	240[b]	54.3[cde]	69.3[ab]	48.0[b]	41.5
		S4	769[b]	138[b]	341[b]	241[b]	55.8[abcde]	68.0[b]	50.3[ab]	42.3
SEM[2]			2.85	0.57	1.32	0.89	0.63	0.31	0.85	0.92
Significance[3]	A		NS	NS	NS	NS	NS	*	NS	NS
	B		NS	NS	NS	NS	NS	*	NS	NS
	AB		NS	NS	NS	NS	NS	*	NS	NS
	ABC		*	*	*	*	*	*	*	NS

[1] SN0, SN2.5 and SN5 stand for sodium nitrate at ~ 0, 2.5 and 5%, respectively. S0 and S4 stand for 0 and 0.4% sulphur, respectively. [2]SEM stand for Standard error mean. [3]A, B, C and ABC stand for Nitrate levels, Sulfur levels, Species and their Interaction, respectively while AB stand for Interaction of nitrate levels with sulphur levels. NS stand for Non significant (P>0.05) and * stand for Significant (P<0.05).[a,b,c] Means in a row with different superscripts differ significantly (p<0.05). DM, CP, NDF and ADF stand for dry matter, crude protein, neutral detergent fibre and acid detergent fibre, respectively.

Table 3. Effect of different levels of sodium nitrate with or without sulphur on blood metabolites in *Lohi* sheep and *Teddy* goat

				Items (mg/dL)		
				Blood Urea Nitrogen	Blood Glucose	Creatinine
Sheep	Diets[1]	SN0	S0	18.3 [a]	74.5 [a]	2.4 [a]
			S4	18.5 [a]	73.3 [a]	2.5 [a]
		SN2.5	S0	19.3 [a]	73.0 [a]	2.4 [a]
			S4	19.0 [a]	72.3 [a]	2.4 [a]
		SN5	S0	18.5 [a]	73.5 [a]	2.5 [a]
			S4	19.0 [a]	73.8 [a]	2.4 [a]
Goats		SN0	S0	26.5 [b]	96.0 [b]	1.6 [b]
			S4	26.5 [b]	95.5 [b]	1.5 [b]
		SN2.5	S0	26.5 [b]	94.0 [b]	1.6 [b]
			S4	26.0 [b]	96.5 [b]	1.5 [b]
		SN5	S0	26.5 [b]	96.5 [b]	1.6 [b]
			S4	26.5 [b]	96.3 [b]	1.7 [b]
SEM[2]				0.57	0.86	0.09
Significance[3]			**A**	NS	NS	NS
			B	NS	NS	NS
			AB	NS	NS	NS
			ABC	*	*	*

[1] SN0, SN2.5 and SN5 stand for sodium nitrate at ~ 0, 2.5 and 5%, respectively. S0 and S4 stand for 0 and 0.4% sulphur, respectively. [2]SEM stand for Standard error mean. [3]A, B, C and ABC stand for Nitrate levels, Sulfur levels, Species and their Interaction, respectively while AB stand for Interaction of nitrate levels with sulphur levels. NS stand for Non significant (P>0.05) and * stand for Significant (P<0.05).[a,b,c] Means in a row with different superscripts differ significantly (p<0.05).

Table 4. Effect of different levels of sodium nitrate with or without sulphur on hematology in *Lohi* sheep and *Teddy* goat

				Hb	Met	Neut	Lymp	Mono	Eosin	Baso	Plat
				g/dL	%	%	%	%	%	%	k/µL
Sheep	Diets[1]	SN0	S0	14.0[a]	2.0	45.0 [a]	46.8 [a]	2.8	4.8	0.8	561.3
			S4	13.5[abc]	2.0	44.5 [a]	46.5 [a]	2.8	4.8	1.5	577.5
		SN2.5	S0	14.0 [a]	1.7	45.3 [a]	43.3 [a]	3.8	6.5	1.3	576.3
			S4	13.8[ab]	2.2	44.0[abc]	44.5 [a]	3.8	6.3	1.5	563.8
		SN5	S0	13.5[abc]	1.8	43.8[abcd]	47.5 [a]	3.3	4.3	1.3	558.8
			S4	14.3 [a]	2.0	44.3[ab]	46.3 [a]	3.5	4.5	1.5	567.5
Goats		SN0	S0	11.0 [d]	1.7	39.3[cde]	54.0 [b]	2.5	3.8	0.5	541.3
			S4	11.0 [d]	2.0	37.5 [e]	56.3 [b]	2.3	3.8	0.3	551.3
		SN2.5	S0	11.3 [cd]	2.2	38.5 [e]	54.0 [b]	2.8	4.3	0.5	547.5
			S4	11.0 [d]	1.8	39.5[bcde]	53.0 [b]	3.0	4.0	0.5	561.3
		SN5	S0	11.5[bcd]	2.0	37.5 [e]	56.3 [b]	2.0	4.0	0.3	548.8
			S4	11.3 [cd]	2.0	39.0 [de]	53.5 [b]	2.5	4.5	0.5	558.8
SEM[2]				0.48	0.14	0.96	0.92	0.50	0.82	0.38	10.58
Significance[3]			**A**	NS	NS	NS	*	NS	NS	NS	NS
			B	NS	NS	NS	NS	NS	NS	NS	NS
			AB	NS	NS	NS	*	NS	NS	NS	NS
			ABC	*	NS	*	*	NS	NS	NS	NS

[1] SN0, SN2.5 and SN5 stand for sodium nitrate at ~ 0, 2.5 and 5%, respectively. S0 and S4 stand for 0 and 0.4% sulphur, respectively. [2]SEM stand for Standard error mean. [3]A, B, C and ABC stand for Nitrate levels, Sulfur levels, Species and their Interaction, respectively while AB stand for Interaction of nitrate levels with sulphur levels. NS stand for Non significant (P>0.05) and * stand for Significant (P<0.05).[a,b,c] Means in a row with different superscripts differ significantly (p<0.05). Hb, Met, Neut, Lymp, Mono, Eosin, Baso and Plat stand for haemoglobin, methemoglobin, neutrophils**,** lymphocytes**,** monocytes**,**eosinophils,basophils and platelets, respectively.

Table 5. Effect of different levels of sodium nitrate with or without sulphur on nitrogen balance in *Lohi* sheep and *Teddy* goat

				Items (g/day)			
				NI	FN	UN	NBal
Sheep	Diets[1]	SN0	S0	50.1[a]	18.1[b]	24.7[a]	7.3[c]
			S4	50.1[a]	18.6[b]	24.1[ab]	7.4[ac]
		SN2.5	S0	50.0[a]	18.6[b]	23.9[b]	7.5[ab]
			S4	50.0[a]	18.6[b]	23.8[b]	7.6[a]
		SN5	S0	49.9[a]	18.6[b]	23.9[b]	7.4[ac]
			S4	50.1[a]	19.3[a]	23.1[c]	7.7[a]
Goats		SN0	S0	22.0[b]	6.6[c]	12.5[de]	2.9[e]
			S4	22.1[b]	6.6[c]	12.6[d]	2.9[e]
		SN2.5	S0	22.0[b]	6.7[c]	12.3[de]	3[de]
			S4	22.0[b]	6.6[c]	12.4[de]	3[de]
		SN5	S0	22.0[b]	6.9[c]	12.1[de]	3[de]
			S4	22.1[b]	7.1[c]	11.9[e]	3.1[d]
	SEM[2]			0.08	0.12	0.14	0.04
Significance[3]			A	NS	*	*	*
			B	NS	*	*	NS
			AB	NS	*	*	*
			ABC	*	*	*	*

[1] SN0, SN2.5 and SN5 stand for sodium nitrate at ~ 0, 2.5 and 5% respectively. S0 and S4 stand for 0 and 0.4% sulphur, respectively. [2]SEM stand for Standard error mean. [3]A, B, C and ABC stand for Nitrate levels, Sulfur levels, Species and their Interaction, respectively while AB stand for Interaction of nitrate levels with sulphur levels. NS stand for Non significant (P>0.05) and * stand for Significant (P<0.05).[a,b,c] Means in a row with different superscripts differ significantly (p<0.05). NI, FN, UN and NBal stand for nitrogen intake, faecal nitrogen, urinary nitrogen and nitrogen balance, respectively.

Table 6. Effect of different levels of sodium nitrate with or without sulphur on growth performance in *Lohi* sheep and *Teddy* goat

				Items		
				weight gain (g/d)	Feed consumed (g/d)	FCR
Sheep	Diets[1]	SN0	S0	138[c]	1740[a]	12.7[de]
			S4	139[bc]	1738[a]	12.5[de]
		SN2.5	S0	142[a]	1736[a]	12.3[e]
			S4	143[a]	1736[a]	12.2[e]
		SN5	S0	141[ab]	1735[a]	12.3[e]
			S4	143[a]	1738[a]	12.2[e]
Goats		SN0	S0	55[e]	766[b]	13.9[ab]
			S4	54[e]	768[b]	14.1[a]
		SN2.5	S0	57[de]	767[b]	13.5[bc]
			S4	57[de]	766[b]	13.5[bc]
		SN5	S0	59[d]	765[b]	13.0[cd]
			S4	59[d]	769[b]	13.0[cd]
	SEM[2]			0.62	2.85	0.11
Significance[3]			A	*	NS	*
			B	NS	NS	NS
			AB	*	NS	*
			ABC	*	*	*

[1] SN0, SN2.5 and SN5 stand for sodium nitrate at ~ 0, 2.5 and 5% respectively. S0 and S4 stand for 0 and 0.4% sulphur, respectively. [2]SEM stand for Standard error mean. [3]A, B, C and ABC stand for Nitrate levels, Sulfur levels, Species and their Interaction, respectively while AB stand for Interaction of nitrate levels with sulphur levels. NS stand for Non significant (P>0.05) and * stand for Significant (P<0.05). a,b,c Means in a row with different superscripts differ significantly (p<0.05). FCR stand for feed conversion ratio.

Table 7 Effect of different levels of sodium nitrate with or without sulphur on enteric methane production in *Lohi* sheep and *Teddy* goat

			Items (ppm)			
			Animal (CH_4)	Animal (CO_2)	CH_4 (Animal - Air) : CO_2 (Animal - Air)	CH_4 Reduction (%)
Sheep	Diets[1] SN0	S0	65.2[bc]	1134.8[a]	0.0870[d]	0
		S4	63.8[c]	1134.8[a]	0.0850[de]	2.22
	SN2.5	S0	63.4[cd]	1136.0[a]	0.0847[de]	3.09
		S4	60.2[de]	1136.0[a]	0.0803[ef]	8.06
	SN5	S0	57.3[e]	1134.8[a]	0.0763[f]	12.46
		S4	53.1[f]	1138.5[a]	0.0703[g]	19.62
Goats	SN0	S0	70.2[a]	1094.8[b]	0.0998[a]	0
		S4	68.1[ab]	1094.8[b]	0.0965[ab]	3.12
	SN2.5	S0	67.6[ab]	1096.0[b]	0.0955[ab]	3.94
		S4	65.2[bc]	1097.3[b]	0.0920[bc]	7.66
	SN5	S0	62.3[cd]	1094.8[b]	0.0880[cd]	11.55
		S4	58.0[e]	1098.8[b]	0.0813[e]	18.27
SEM[2]			0.64	2.93	0.0010	
Significance[3]		**A**	*	NS	*	
		B	*	NS	*	
		AB	*	NS	*	
		ABC	*	*	*	

Air (Methane gas in ppm) = 1.98; Air (carbon dioxide gas in ppm) = 410: [1] SN0, SN2.5 and SN5 stand for sodium nitrate at ~ 0, 2.5 and 5% respectively. S0 and S4 stand for 0 and 0.4% sulphur, respectively. [2]SEM stand for Standard error mean. [3]A, B, C and ABC stand for Nitrate levels, Sulfur levels, Species and their Interaction, respectively while AB stand for Interaction of nitrate levels with sulphur levels. NS stand for Non significant (P>0.05) and * stand for Significant (P<0.05).[a,b,c] Means in a row with different superscripts differ significantly (p<0.05).

Conclusion: In conclusion, animals fed diets containing nitrate in combination with sulphur not only gained more weight but e.m.p was also reduced in these animals compared to those fed control diet. Enteric CH_4 reductions were better in *Lohi* sheep as compared to *Teddy* goats fed diet containing nitrate in combination with sulphur.

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