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IN VITRO METHANE PRODUCTION OF ERAGROSTIS HAY TREATED WITH GRADED LEVELS OF UREA OR NITRATE

F. A. Adejoro and A. Hassen*

Department of Animal and Wildlife Sciences, University of Pretoria, Private bag X20, Hatfield 0028, Pretoria. South Africa

*Corresponding author E-mail address: abubeker.hassen@up.ac.za.

ABSTRACT

Urea treatment of protein deficient feeds serve as a source of ammonia nitrogen supply for rumen fermentation. This study was undertaken with the objective of determining the effect of treating *Eragrostis curvula* hay with varying levels of urea or nitrate on digestibility and *in vitro* fermentation. Grass hay was sprayed with urea solution at 0.5%, 1.0%, and 1.5% DM and calcium nitrate was used as a replacement of urea on an iso-nitrogenous basis. This was followed by 30 days anaerobic storage in airtight bottles with each treatment having three replicates. Following anaerobic treatment, hay samples were dried, milled and evaluated for their chemical composition, *in vitro* organic matter digestibility, and *in vitro* fermentation and methane production. Feed treatment with both urea and nitrate reduced ADF content of hay, while crude protein content was increased. *In vitro* organic matter digestibility of treated hays increased with inclusion levels although urea recorded higher values than nitrate. Nitrate treatment significantly reduced *in vitro* methane by 14-33% while there was no significant methane reduction in the urea treated diets. Total volatile fatty acid, ammonia N and pH across treatments were statistically not different (P>0.05). While urea treatment seems to improve digestibility better, it did not confer additional benefits when compared to nitrate treatment that provided the additional benefit of methane reduction with an acceptable level of improvement in feed digestion and fermentation. Nitrate can thus be incorporated into feed treatment to improve the nutritional value of poor quality hays.

Key words: ammoniation, digestibility, Eragrostis hay, nitrate, methane emission.

INTRODUCTION

In much of tropical and sub-tropical areas of Africa and Asia, small ruminants contribute a significant proportion of the income of farmers, who exploit their ability to convert roughage feeds to edible meat or milk (Ben Salem and Smith, 2008). With changing climatic patterns, and decline in rangeland resources, there remains a shortfall in total feed resources available to these class of animals. They therefore often rely on hays, straws and other crop residues to meet their dietary requirements especially during the dry seasons (Ben Salem and Smith, 2008).

Various chemical treatment methods have been developed to improve the feeding value of these poor quality roughage feeds, such as alkali treatment, use of aqueous ammonia or by urea treatment (ammoniation) under anaerobic condition (Wanapat *et al.* 2009; Mapato *et al*. 2010). Urea treatment involves spraying with aqueous urea followed by anaerobic storage for up to 3 weeks prior to feeding. It not only improves ammonia nitrogen supply in the rumen but also the ammoniation process helps to soften fibre structure of feed for greater microbial attachment thus leading to improved digestibility (Vadiveloo, 2003). Urea hydrolyses to ammonia during feed treatment, incorporates NPN to the treated material and is also an effective preservative or fungicide (Oji *et al.* 2007). Non-protein nitrogen (NPN) in chemical treatment of feed is particularly valuable with feedstuffs high in fermentable carbohydrates but low in crude protein (Do *et al.* 2011). There is usually an increase in feed intake and nutrient digestibility in ruminants feeding on low quality roughages with supplemental proteins or non-protein nitrogen. This is because digestibility of these materials is dependent on adequate colonization of cellulolytic bacteria, which needs adequate supply of ammonia nitrogen for their own microbial synthesis (Mahr-un-Nisa *et al.* 2004).

Replacing urea with nitrate salts have recently received wide consideration because of its potential benefits in mitigating enteric methane production (Sophal *et al.* 2013). Nitrate is able to recycle hydrogen ions which is easily converted to methane by the rumen archaea (Thanh *et al.* 2012). Nitrate is able to favourably compete with these methanogenic archaea, for the available hydrogen ions in the rumen thus capable of reducing methane by up to 80% (Leng, 2008). The method of nitrate application is capable of determining its dose and toxicity level in ruminant animals (Zijderveld *et al.* 2010). The focus of this study therefore is to know the effect of Eragrostis hay treated with graded levels of urea and calcium nitrate on *in vitro* digestibility, gas production and methane emission.

MATERIALS AND METHODS

This study was conducted at University of Pretoria's Experimental Farm after the approval of the trial protocol by the Animal Ethics Committee of University of Pretoria (No. EC061-14).

Feed treatment and experimental design: *Eragrostis curvula* hay was collected from the feedstock in the University of Pretoria experimental farm, chopped to about 5cm and used as experimental diet. Feed treatment was carried out following the procedure described by Tesfayohannes *et al*. (2013). Approximately 3 kg of hay was mixed with urea or nitrate at iso-nitrogenous level. Both urea and calcium nitrate were feed-grade fertilizers obtained from Introlab Chemicals (Pty.) Ltd., Pretoria, South Africa. The experimental treatments were arranged to include two nitrogen sources (urea or nitrate) at three levels of inclusion (2.33g, 4.66g and 6.99g nitrogen kg^{-1} hay DM) in a 2 x 3 factorial design plus a control treatment that contains no additive. Urea and calcium nitrate were solubilized in water to form a concentrated solution and the entire solution was uniformly sprayed on the hay. The final mix was done to achieve a total moisture content of approximately 40%. Grass hay was thoroughly hand mixed with additives inside a big plastic container, filled into glass bottles which served as mini silos, compressed, and subsequently sealed anaerobically in a vacuum with three replicates per treatment group. A control diet was mixed with corresponding amount of distilled water but had no additive. Each bottle was stored at room temperature (25°C) for 30 days. After the 30 d period, the glass bottles were opened, the upper 5cm discarded, and the contents emptied into a big plastic container, then hand mixed and sub-sampled as urea or nitrate treated diets. All experimental diets were freeze-dried and milled to pass through a 1 mm screen (Wiley mill) before analysis.

***In vitro* fermentation:** Rumen buffer, macro-mineral and micro-mineral solutions were prepared as described by Goering and Van Soest (1970) with the modifications of Mould *et al.* (2005). The *in vitro* fermentation procedure of Menke *et al.* (1979) was followed. The prepared buffer solution was kept inside water bath at 40°C and continuously purged with CO_2 until the solution turned colorless. Rumen fluid was collected from two rumen-cannulated merino wethers fed Lucerne hay (*Medicago sativa*) *ad libitum*. Detailed procedures have been previously described by Hassen *et al.* (2015). Gas pressure was taken at 2, 4, 8, 12, 24, and 48 h after commencement of incubation while gas samples were taken inside Hamilton syringes for the analysis of methane concentration. At the end of incubations, fermentation was terminated by removing the serum bottles and immersing them in ice to impede microbial activity. Rumen fluid pH was measured after incubation using a pH meter (Metler Toledo 230 pH meter) while supernatant was collected and stored at -20°C for ammonia-N (Broderick and Kang, 1980) and VFA analysis.

Chemical composition analysis: Urea and calcium nitrate treated hay samples were analysed for dry matter, ash and crude protein by the Leco/Dumas method all according to AOAC, (2000) as indicated in ID 934.01, ID 942.05 and ID 968.06, respectively. Neutral detergent fibre (NDF) inclusive of residual ash was determined according to Robertson and Van Soest (1981) without the use of heat stable amylase while acid detergent fibre (ADF) and acid detergent lignin (ADL) were also determined according to Robertson and Van Soest (1981).

***In vitro* organic matter digestibility:** The *in vitro* organic matter digestibility (IVOMD) of diets was determined using the two-phase digestion method of Tilley and Terry (1963) as modified by Engels and Van Der Merwe (1967). During the first stage, 200 mg of feed samples were incubated in four replicates of each diet with rumen liquor for 48h at 39°C under anaerobic conditions. Blanks and a standard feed were included in each batch of incubation. This was followed by an acid-pepsin digestion phase for 48 h. After digestion, the residual material was oven dried at 105°C for 18 h, weighed, and subsequently ashed in a muffle furnace at 550°C for 3h. *In vitro* organic matter digested was estimated from the weights of starting material and residuals.

Methane production measurement: Gas samples from the *in vitro* incubations was taken using a Hamilton syringe on duplicate incubation bottles at 2, 12, 24 and 48 h incubation time (Gemeda and Hassen 2015). Methane concentration was analysed with a gas chromatography (8610C BTU Gas Analyser GC System; SRI Instruments GmbH, Bad Honnef, Germany). The GC was pre-equipped with a solenoid column, packed with silica gel and a flame ionisation detector (FID). Methane concentration values were related to the total gas production in order to estimate its concentration (Tavendale *et al.* 2005).

Calculations and statistical analysis: The concentrations of acetate, propionate, butyrate, valerate, and iso-butyrate were evaluated as molar proportions (mmol100 mol^{-1}) while total volatile fatty acid (TVFA) concentration was expressed in mmol L^{-1} and ammonia-N concentration in mg dL^{-1}. Total gas and net methane was expressed in mL and in mass values per unit of IVOMD (g kg^{-1} IVOMD) and TVFA unit methane (mmol $mmol^{-1}$). The ratio of non-glucogenic to glucogenic volatile fatty acid is expressed as acetate/propionate molar ratio. The chemical composition of samples was analysed as a one-way analysis of variance while *in vitro* data were

analysed as a 2 X 3 factorial plus control, using the GLM procedure of SAS (Statistical Analysis System, version 9.3) with single degree of freedom contrasts to compare means between i) control vs NPN-treated hay ii) urea treated vs nitrate treated hay iii) the linear effect of urea levels iv) the quadratic effect of urea levels v) the linear effect of nitrate levels vi) quadratic effect of nitrate levels.

RESULTS

The chemical composition of untreated, urea treated and nitrate treated Eragrostis hay is shown in Table 1. The anaerobic treatment increased ($p \leq 0.05$) crude protein (CP) content of Eragrostis hay by between 8-25% in the urea diets and between 15-33% in the nitrate diets. However, nitrate was more effective in increasing the nitrogen content of the treated hay than urea at the same level of inclusion. There was a decrease in cell wall content (NDF and ADF) of treated hay as inclusion levels of urea increased ($p \leq 0.05$). However, there was no reduction ($p \geq 0.05$) in the NDF content of treated hay with increasing inclusion levels of nitrate but the ADF content decreased ($p \leq 0.05$).

The result of the *in vitro* gas production and digestibility of Eragrostis hay, untreated or treated with urea or calcium nitrate is shown in Table 2. Simple effects of nitrogen source showed that total gas production at 48 h was not affected generally by the average effects of nitrogen treatment when compared to the untreated hay (105 vs 98.9 mL) but within the treated group, gas production was on average, higher ($p \leq 0.05$) in urea treated hay compared to nitrate treated hay (105 vs 84.5 mL). Increasing levels of inclusion did not affect gas production in both urea and nitrate treated hays ($p \geq 0.05$). Generally, urea treated hays showed higher methane production ($p \leq 0.05$) compared to nitrate treated hays although increasing inclusion levels of both urea and nitrate did not show any linear or quadratic response ($p \geq 0.05$). As high as 32% reduction in methane production was recorded in nitrate treated hay at 6.99 g nitrogen kg^{1} DM of calcium nitrate inclusion level compared to the untreated hay.

Both nitrogen sources improved *in vitro* organic matter digestibility (IVOMD) of treated Eragrostis hay compared to control. There was both linear and quadratic increase in IVOMD with increasing inclusion levels in both urea and nitrate treated hays. Values ranged from 455 g kg^{-1} in untreated hay to 502 g kg^{-1} in urea treated hay, with urea treated hays consistently resulting in better improvement in digestibility compared to nitrate treated hays across all three levels of inclusions (463 vs 458; 476 vs 460; 501 vs 485 g kg^{-1}) respectively. The ratio of methane to total gas produced showed that nitrate treated hay recorded lower methane ($p \leq 0.05$) as a proportion of the total gas when compared to the urea treated hays. Similarly, there was a reduction ($p \leq 0.05$) in the ratio of methane produced per unit of organic matter digested in the nitrate treated hay compared to urea treated hay. However, methane produced per unit of organic matter digested did not produce any linear or quadratic response across the inclusion levels in the nitrate treatment. In contrast, there was a linear increase in methane per unit of organic matter digested with increasing levels of urea inclusion. The ratio of TVFA to methane showed that nitrate treated hays recorded higher TVFA per unit of methane when compared with urea treated hays ($p = 0.07$) however, there was no linear or quadratic response with increasing inclusion levels.

In vitro fermentation characteristics of urea and nitrate treated Eragrostis hay is shown in Table 3. Total VFA production was not influenced by nitrogen source or levels of inclusion across the diets. Nitrate treated hays recorded higher acetate concentration compared to urea treated hay ($p \leq 0.05$). Increasing inclusion levels showed a linear response in the nitrate treated hays. Butyrate, and valerate concentrations did not show any differences between the nitrogen sources ($p \geq 0.05$) while propionate and iso-butyrate concentrations were lower in nitrate treated hays compared to urea treated hays ($p \leq 0.05$). This reduction in propionate also led to an increase in the ratio of non-glucogenic to glucogenic volatile fatty acids as shown by the A/P molar ratio where the average effect of nitrate treated hays showed higher A/P molar ratio compared to the average effect of urea treated hays ($p \leq 0.05$). There was no difference in rumen ammonia nitrogen concentrations between urea and nitrate diets ($p \geq 0.05$) while the pH of the rumen fluid, after 48 h incubation was also not affected by the nitrogen source or levels of inclusion.

Table 1. Chemical Composition (Mean ± SE) of untreated, urea treated and nitrate treated Eragrostis hay.

Parameters[1]	~~Untreated~~ Control	Urea-treated hay[2]			Nitrate-treated hay[2]			*P*-value
		I	II	III	I	II	III	
DM (g kg^{-1} DM)	912.5±1.8	882.5±0.6	863.2±0.8	850.7±1.4	836.7±1.1	875.4±3.2	880.5±2.5	0.325
CP (g kg^{-1} DM)	53.8^{e}±0.16	58.3^{d}±0.38	62.6^{c}±0.15	67.0^{b}±0.11	61.9^{c}±0.15	65.0^{b}±0.14	71.7^{a}±0.26	0.002
Ash (g kg^{-1} DM)	30.4±0.4	29.7±0.1	29.5±0.3	30.0±0.2	29.6±0.3	28.8±0.4	29.4±0.6	0.152
NDF (g kg^{-1} DM)	746.6^{a}±3.2	732.8^{b}±1.7	712.5^{c}±1.4	709.8^{c}±2.8	736.3^{b}±1.1	734.0^{b}±2.0	731.0^{b}±1.5	0.0002
ADF (gkg^{-1} DM)	449.4^{a}±0.9	442.8^{b}±2.1	433.5^{c}±2.5	414.1^{d}±1.2	452.1^{a}±2.6	443.6^{b}±1.8	428.3^{c}±2.5	≤0.0001
ADL (gkg^{-1} DM)	79.1±1.5	79.0±0.6	78.8±1.1	78.6±0.8	78.6±1.2	79.1±3.2	78.7±2.4	0.275

[1]DM, dry matter; CP, crude protein; NDF, neutral detergent fibre; ADF, acid detergent fibre; ADL, acid detergent lignin. [2]I, II, III; 2.33, 4.66 and 7.0g N kg^{-1} hay & equivalent to 5, 10 and 15g urea and 13.6, 27.3 and 40.9g calcium nitrate kg^{-1} hay respectively. SE, standard error. Means in the same row with different superscripts are significantly different ($P \leq 0.05$).

Table 2. *In vitro* gas production, digestibility and fermentation efficiency in *Eragrostis curvula* hay ammoniated with different levels of urea and nitrate.

Parameter[1]	Control	Urea[2]			Nitrate[2]			SEM	Contrast *P*-Values[3]					
		I	II	III	I	II	III		C vs N	U vs N	U_L	U_Q	N_L	N_Q
48H Gas (mL)	98.9	106	103	106	88.7	93.8	82.4	3.63	0.78	0.02	0.21	0.94	0.61	0.78
48H Methane (mL)	23.2	26.3	27.6	26.6	19.7	18.7	15.8	1.37	0.79	0.002	0.07	0.96	0.40	0.48
IVOMD (g kg^{-1} DM)	455.1	463.0	475.8	501.9	457.6	459.8	486.1	3.64	≤.0.001	0.001	≤.0.001	0.001	0.003	0.001
Methane/Total Gas	0.24	0.25	0.26	0.25	0.22	0.20	0.18	0.01	0.59	0.001	0.01	0.82	0.45	0.29
Methane/IVOMD (g kg^{-1} IVOMD)	91.0	101.8	103.9	94.7	76.6	72.7	54.5	2.02	0.57	0.003	0.04	0.87	0.79	0.38
TVFA/Methane	6.85	5.82	6.70	7.29	8.82	8.93	9.34	0.03	0.56	0.07	0.16	0.87	0.76	0.62

[1]IVOMD, *in vitro* organic matter digestibility; TVFA, total volatile fatty acid.[2]I, II, III; 2.33g, 4.66g and 6.99g N kg^{-1} hay DM respectively & equivalent to 5 g, 10 g, & 15 g urea kg^{-1} hay & 13.6 g, 27.3 g, & 40.9 g calcium nitrate kg^{-1} hay. [3]Contrast analysis across treatments: C vs. N, control vs. the average of NPN-treated diets; U vs. N, average of urea-treated diet vs. average of nitrate-treated diets; U_L, linear effect of urea levels; U_Q, quadratic effect of urea levels; N_L, linear effect of nitrate levels; N_Q, quadratic effect of nitrate levels.

Table 3. *In vitro* fermentation parameters of *Eragrostis curvula* hay as influenced by ammoniation with different levels of urea and nitrate.

Parameter[1]	Control	Urea[2]			Nitrate[2]			SEM	[3]Contrast *P*-values					
		I	II	III	I	II	III		C vs N	U vs N	U_L	U_Q	N_L	N_Q
TVFA (mmol L^{-1})	157	132	166	173	162	173	153	12.0	0.87	0.97	0.98	0.69	0.67	0.85
Acetate (mmol 100ml^{-1})	64.6	60.2	64.1	62.0	61.9	64.5	68.7	2.89	0.35	0.003	0.41	0.50	0.04	0.25
Propionate (mmol 100mol^{-1})	24.9	25.9	25.0	25.4	24.3	24.1	21.1	1.90	0.37	0.002	0.001	0.04	0.75	0.61
Butyrate (mmol 100mol^{-1})	5.93	7.88	6.64	8.09	8.65	7.11	7.37	0.50	0.003	0.64	0.18	0.01	0.02	0.58
Isobutyrate (mmol 100mol^{-1})	2.49	3.28	2.01	2.25	2.30	1.93	1.33	0.33	0.11	0.001	≤.0.001	0.03	0.09	0.28
Valerate (mmol 100mol^{-1})	2.12	2.81	2.31	2.23	2.80	2.37	2.36	0.24	0.08	0.69	0.73	0.07	0.84	0.05
A/P Molar ratio	2.62	2.32	2.57	2.44	2.55	2.67	3.25	0.14	0.66	0.01	0.12	0.25	0.60	0.04
NH_3-N (mg dL^{-1})	11.9	12.2	13.5	15.8	12.7	14.7	14.8	2.1	0.84	0.54	0.55	0.97	0.63	0.55
pH	6.78	6.77	6.78	6.78	6.87	6.87	6.87	0.02	0.57	0.12	0.18	0.85	0.96	0.95

[1]TVFA, total volatile fatty acid; A/P Molar ratio, acetate to propionate molar ratio; NH_3-N, ammonia nitrogen. [2]I, II, III; 2.33 g, 4.66 g and 6.99 g N kg^{-1} hay DM respectively & equivalent to 5 g, 10 g, & 15 g urea kg^{-1} hay & 13.6 g, 27.3 g, & 40.9 g calcium nitrate kg^{-1} hay.[3]Contrast analysis across treatments: C vs. N, control vs. the average of NPN-treated diets; U vs. N, average of urea-treated diet vs. average of nitrate-treated diets; U_L, linear effect of urea levels; U_Q, quadratic effect of urea levels; N_L, linear effect of nitrate levels; N_Q, quadratic effect of nitrate levels.

DISCUSSION

Treating Eragrostis hay with urea or calcium nitrate up to 1.5% of DM increased its nitrogen content. A similar trend of increased nitrogen content after anaerobic treatment of roughage feeds has been observed in the urea treatment of hays and crop residues as reported in literature (Fadel Elseed *et al.* 2003; Oji *et al.* 2007). The solubilisation of NPN (urea or calcium nitrate) during the anaerobic treatment was more pronounced in the urea treated diet as judged by the lower residual nitrogen content of urea treated hay compared to the nitrate treated hay which recorded higher residual nitrogen content. For the urea treatment, the breakdown of urea can be related to the optimal conditions that favour the activity of urease enzyme which enables the hydrolysis of urea to ammonia. According to Oji *et al.* (2007), moisture content of treated forage above 375g kg^{-1} favours the urea hydrolysis to ammonia. The dissimilatory reduction of calcium nitrate to ammonia has been noted as the major pathway for nitrate breakdown under anaerobic conditions and often in the presence of high organic matter concentrations. However, high ammonia accumulation have also been noted to impede the further breakdown of nitrate under such conditions (Simon, 2002; Leng 2008). The higher IVOMD of Eragrostis hay in urea treated hay compared with nitrate treated hay may also be an indication that the feed treatment process favours the hydrolytic reduction of urea more than the nitrate reduction process. The ability of aqueous ammonia to penetrate and soften the cell wall structure of roughage feeds for improved digestibility is an indication of an effective ammoniation treatment.

Generally, treatment with urea or nitrate decreased the cell wall content of Eragrostis hay and this was consistent with previous research. Oji *et al.* (2007) observed a trend of reduction in NDF and ADF contents of maize stalks, maize cobs and maize husks following urea and aqueous ammonia treatment and this can be related to increased digestibility. Tesfayohannes *et al.* (2013) also noted that improved IVOMD following feed treatment is attributable to a reduction in NDF and hemicellulose content of poor quality feeds and increase in the degradable portion of ADF. Solubilisation of fibre fractions due to linkage disintegration following treatment have been reported by Mason *et al.* (1988) while Zorrilla-Rios and Owens (1985) noted increased fragility of wheat straw following ammoniation. The process of ammoniation like many other hydrolytic treatment methods helps to improve feed digestibility by the disruption of cell wall structure by ammonia and increased swelling resulting in higher affinity for microbial attachment (Jung *et al.* 1993). Treatment of poor quality roughage diets with urea or aqueous ammonia have been found very effective in improving their digestibility (Uza *et al.* 2005; Adejoro and Hassen 2017). However, several factors have been found to influence the feed treatment process such as type of feedstuff, and other treatment conditions (Mason *et al.* 1988).

From the result of this study, it appears a considerable amount of nitrate was hydrolysed during feed storage to account for the differences in fibre composition and *in vitro* digestibility of treated hay with a small residual nitrate remaining to potentially act as hydrogen scavenger during rumen fermentation *in vitro*. The activities of the residual nitrate in the treated hay generally played a role in mitigating methane production when compared to the average effects of urea treated hay, but the levels of inclusion of nitrate was not significant. In contrast, a linear response was observed in terms of increased methane production associated with the inclusion level of urea in the urea treated hay. This was similar to previous experiments involving nitrate supplementation, where nitrate resulted in methane reduction both *in vitro* and *in vivo* (Nolan *et al.* 2010; Hulshof *et al.* 2012; Thanh *et al.* 2012; Sophal *et al.* 2013).

Values of ammonia nitrogen concentration did not show any differences across treatment groups. They were lower than the minimum of 15-20 mg dL^{-1} proposed by Preston and Leng (1987) for effective feed intake and fibre digestion in roughage based diets. The pH ranges observed in this study were however within the normal range of 5.5 to 7.0 for optimum rumen function (Krause and Oetzel 2006). With considerable hydrolysis of both urea and nitrate during feed treatment, higher inclusion levels of urea or nitrate may thus be required in treating Eragrostis hay to provide allowance for residual nitrate or urea to meet the ammonia-nitrogen requirements of the animals. Inclusions levels of up to 20 g kg^{-1} DM have been reported for urea treatment of rice straws (Fadel Elseed *et al.* 2003) but at the same time, considerations must be given to the total amount of nitrogen consumed by each animal per day to avoid excessively high ammonia nitrogen accumulation in the rumen. The reduction in the molar proportion of propionate in the nitrate treated hay is consistent with the findings of Nolan *et al.* (2010) for nitrate supplementation because nitrate tend to compete for hydrogen with propionate synthesis (Van Zijderveld *et al.* 2011) and this also explains the higher A/P molar ratio recorded in the nitrate treated hays. The ability of nitrate to recycle hydrogen ions into ammonia, thus competing for reducing equivalents away from propionate synthesis have been noted as capable of limiting animal productivity. However, the results of other *in vivo* trials have not shown any significant reduction in productivity of animals in terms of energy in milk, energy retention or nitrogen retention (Van Zijderveld *et al.* 2011; Sophal *et al.* 2013).

Conclusion: The treatment of Eragrostis hay with graded levels of calcium nitrate showed on average significant methane suppression than urea treated hay, with both urea and nitrate increasing *in vitro* digestibility compared to the untreated hay. However, the improvement in *in vitro* digestibility was somewhat smaller in the nitrate treated hay than with the urea treated hay. The additional benefit observed in terms of reduction in methane production implies the potential of incorporating nitrate into feed ammoniation as a possible climate smart agricultural practice.

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