Tropical Animal Health and Production (2019) 51:171–177
https://doi.org/10.1007/s11250-018-1673-9

REGULAR ARTICLES

Mineral salt intake effects on faecal-N concentration and the volume and composition of beef cattle urine

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Received: 17 May 2018 / Accepted: 17 July 2018 / Published online: 26 July 2018

Abstract
The effect of mineral salts on water ingestion and urine volume in cattle has been extensively studied. However, recently, this effect has been investigated as a potential mitigator of environmental aspects related to the nitrogen (N) cycle, such as nitrate (NO_3^-) lixiviation, ammonia (NH_3) volatilisation, and nitrous oxide (N_2O) emissions. The effect of mineral salts, particularly sodium chloride (NaCl), on urine-N concentration, urine volume, the proportion of N compounds in the urine, and faecal-N concentration has not yet been explored in field conditions with respect to environmental aspects of beef cattle production. The present study investigated the effect of dietary mineral salt rates on these parameters. A Latin square (5 × 5) experimental design was utilised with five concentrations of mineral salts in the diet: 0.0, 2.0, 4.0, 6.0, and 8.0 g based on dry matter (DM) ingestion (g/kg DM). The nitrogen concentration in the urine and urine volume increased linearly. The total N excreted (g/day) via urine did not vary with increasing mineral salt concentrations. When evaluated, the N compounds of urine (urea-N, allantoin-N, and hippuric acid-N) also reacted to the increased mineral salt concentrations, while creatinine-N did not. Urea-N, allantoin-N, and hippuric acid-N linearly increased their proportions in total N-urine. The N concentration in faeces was not affected by mineral salt concentrations. The urine volume, concentration of N, and proportion of N compounds in the urine affected N_2O emissions and NH_3 volatilisation. Therefore, mineral salt utilisation may be an option for mitigating N pollution from beef cattle, especially for grasslands in tropical countries.

Keywords Urinary N compounds · Sodium chloride · Nitrogen usage efficiency · Urea-N

Introduction

In tropical areas of South America, India, and Africa, 600 million beef cattle are reared in extensive production systems (FAO 2015). In these systems, grassland does not contain all the necessary minerals for the animals; therefore, mineral supplementation is required (Pereira et al. 2018). Mineral supplementation is offered in a mixture form; it consists of sodium chloride (NaCl) that has a diuretic effect by inducing greater drinking-water intake, increasing urination frequency, and decreasing urine nitrogen (N) concentration and urine-N deposition rate. This has the potential to decrease N leaching (Ledgard et al. 2015) and appears to be a potential mitigation option for N pollution.

Livestock influences the environment and is responsible for 15% of greenhouse gas emissions (Gerber et al. 2013). Therefore, strategies are needed to mitigate the environmental effects of beef production. An increase in the efficiency of N

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usage resulting in a reduction in the concentration of N and N compounds in the excreta, especially in the urine, might reduce the effect of N on the environment (Spek et al. 2013). Spek et al. (2012) showed that increasing concentrations of mineral salt intake linearly increased urine production and total urinary N excretion in dairy cattle. We hypothesised that the concentration of mineral salts in the diet influenced urinary volume, total N excretion via urine and faeces, and the proportion of urinary N compounds in beef cattle.

Urine excreted from grazing cattle creates spots that represent a substantial addition of N to pastures. Following urination, N undergoes hydrolysis and mineralisation, which may be nitrified or denitrified. The urine volume and concentration of N influence these mechanisms (Dijkstra et al. 2013). If the urine volume is constant, total N in the urine does not have a dominant effect on the proportion of N_2O emitted (Cardoso et al. 2017). Contrastingly, in another study, when the volume of urine was increased from 1 to 2 L (with equal amounts of N) the fraction of N_2O released was observed to decrease linearly (Cardoso et al. 2018). The concentrations of several urine compounds have been shown to influence N_2O emissions, with Van Groenigen et al. (2006) and Bertram et al. (2009) observing that hippuric acid in cattle urine acts as a natural inhibitor of N_2O emission, probably owing to the temporary inhibition of nitrification and denitrification processes. Liu and Zhou (2014) discovered that a high salt dietary treatment decreased the average emission rate of NH_3 by 48% and N_2O by 26% from soil treated with sheep urine than what was observed in the control group.

The presence of Na^+ in soils negatively influences nitrification and ammonia volatilisation rates (Anderson et al. 2017) and the addition of potassium chloride to soils had a negative effect on N_2O (Cardoso et al. 2017). Increasing urine volume via increased dietary mineral content appears to be a promising N_2O mitigation strategy, particularly in extensive livestock production systems. This is of particular significance because N_2O is a greenhouse gas that has global warming potential approximately 300 times greater than that of CO_2 (IPCC 2013). The present study aimed to investigate the influence of the dietary mineral salt concentration on the quantity of N in the urine/faeces, urine volume, and the proportion of urea-N, allantoin-N, creatinine-N, and hippuric acid-N compounds of the total N excreted via the urination of beef cattle in pastures.

Materials and methods

Experimental site

The present study was a metabolism study and occurred on palisade-grass (*Brachiaria brizantha* 'Marandu') grassland located at the Jaboticabal campus in São Paulo State, Brazil (21°15′22″ S and 48°18′08″ W, 595 m above sea level). The region's climate is characterised by tropical rainy summers and dry winters. The mean annual rainfall is 1424 mm with a mean air temperature of 22.3 °C. The soils are Rhodic Ferralsols (IUSS 2015) derived from basalt. The experimental area constituted 5 paddocks, each 625 m^2 in area with a total of 0.31 ha. The cattle grazed on the paddocks continuously during the experimental period. The chemical composition of forage material may interact with mineral salts and, thus, affect animal response. To avoid this influence, we assumed that the chemical composition did not vary during the time of the experiment and between paddocks. This assumption was proven correct, with dry matter (DM) and chemical composition of grass not differing during the period of study (Table 1).

Animals, experimental treatments, and feeding during the metabolism study

The experiment was conducted from 18 November to 22 December 2012. Five animals of the Nellore breed weighing 298 ± 23 kg were utilised during the experiment. The animals were offered five concentrations of mineral salts in a Latin square (5×5) experimental design. The rows were the mineral salt levels and the columns were the animals. The mineral salt concentrations were 0.0, 2.0, 4.0, 6.0, and 8.0 g/kg of DM ingestion, which were calculated based on the body weight of the beef cattle. The different treatment concentrations were based on the recommendations of the National Research Council (NRC 2016) with the recommended amount of NaCl in the diet being 6–8 g/kg of DM ingested. A commercial product, Fosbovi 40® (Tortuga CIA Zootécnica Agrária), was used and combined with NaCl, which constituted the mineral salt in the experiment. The mineral salt mixture was offered individually to the cattle in the morning, with ingestion stimulated by mixing with 0.8 kg of corn meal. Orts were only observed at the highest salt mix concentration of 8.0 g/kg of DM. The animals received the treatment for 6 days as an adaption period and sampling occurred on the seventh day. Water was available ad libitum.

Herbage sampling and chemical composition

Grazing heights were measured at 80 random points ('hits') per hectare (ha) to estimate average paddock height. To estimate herbage mass, 5 samples per paddock (average hits height) were collected from a 0.25-m^2 area (5 cm residual height) every 7 days. Samples were dried at 55 ± 5 °C to a constant weight to estimate DM/ha. To estimate herbage chemical composition, samples were milled in a Willey mill (Thomas Scientific, Swedesboro, NJ), and analysed for DM (method no. 934.01) and N concentrations (method no. 978.04) according to the Association of Official Analytical Chemists (AOAC 1995). To obtain the crude protein (CP) value in the forage, total N was multiplied by 6.25. The

Table 1 Variation of chemical composition (g/kg DM) of palisade-grass according to the day of sampling during the experimental period

		Day 7	Day 14	Day 21	Day 28	Day 35	SEM
DM*	kg/ha	9600	10,000	8002	9550	11,600	585
DM*	g/kg	403	400	400	405	413	2.4
CP*	g/kg DM	60.3	62.7	65.7	63.5	65.8	1.0
NDF*	g/kg DM	563	554	578	557	549	5.0
ADF*	g/kg DM	371	379	379	381	373	1.9
Ash*	g/kg DM	9.7	9.6	10.0	10.1	10.2	1.1

*Dry matter (DM), crude protein (CP), neutral detergent fibre (NDF), acid detergent fibre (ADF), and standard error of means (SEM, ±)

ANOVA probability values: DM ($P = 0.25$), % DM ($P = 0.97$), CP ($P = 0.57$), NDF ($P = 0.82$), ADF ($P = 0.93$), and ash ($P = 0.61$)

amounts of ash, neutral detergent fibre (NDF), and acid detergent fibre (ADF) were determined using methods described by Van Soest et al. (1991).

Excretion sampling and urine volume calculation

Each day at 21 h, GMT; 18 h, BRST, day, urine was sampled directly from the animals. The animals were immobilised in a cattle chute where collection of urine occurred. Sub-samples of 10 mL of urine were collected and diluted with 40 mL of a 0.018 mmol/L sulphuric acid solution, followed by storage in a freezer at −20 °C until analysis for N concentration and urine compounds. Fresh faeces were collected from the paddock and subsequently dried at 55 °C for 72 h, milled, sieved, and analysed for total N concentration as described above for the forage samples.

The urine volume (UV) was calculated according to the following equation:

$$\mathrm{UV}\ (\mathrm{L/day}) = (27 \times LW)/(c),$$

where 27 is the daily excretion of creatinine in mg/kg *LW*, as obtained by Posada et al. (2012) in Nellore cattle, *LW* is the live weight of the animal, and (*c*) is the concentration of creatinine (mg/L) quantified in the urine sample of the experimental animals.

Analytical procedures for urine

Total N content in the urine was determined following AOAC (1995) standardisation methods. The concentrations of allantoin and hippuric acid were quantified colourimetrically, according to Fujihara et al. (1987). Creatinine and urea were analysed using commercial kits (ANALISA) following Heinegård and Tiderström (1973) for creatinine and Bergmeyer (1985) for urea. Total N excreted daily (g/day) was calculated by utilising the N concentration in urine (g/L) and the daily urine volume (L). The proportions of urea-N, allantoin-N, creatinine-N, and hippuric acid-N were calculated by dividing the N (g/L) of the compound by the total urine-N (g/L).

Statistical analyses

To verify normality and homogeneity of the data, the Lilliefors and Cochran-Bartlett tests were utilised, respectively. Analysis of variance (ANOVA) was used to compare means of each variable. For the forage mass and chemical composition, the statistical model was completely randomised design represented by:

$$Yij = \mu + Ti + eij,$$

where *Yij* is the dependent variable (*i* and *j* denote the level of salt and the replication within the level of salt, respectively), μ is the general average effect, *Ti* is the effect of having salt level '*i*', and *eij* is the random error.

For the urinary and faeces composition, data were analysed as a 5 × 5 Latin square with mineral salt concentration, period, and heifer as factors. The statistical model was represented by:

$$Yijk = \mu + Ti + Pj + Sk + \mathrm{e}ijk,$$

where *Yijk* represents the observation, μ is the population mean, *Ti* equals the mineral salt rate effect ($i = 1–5$), *Pj* represents the period effect ($j = 1–5$), *Sk* equals the steer effect ($k = 1–5$), and e*ijk* equals the residual error.

When the ANOVA was significant, regressions analysis was performed to evaluate the effect of the mineral salt concentration on the variables. All statistical analyses were executed using R version 3.2.1 (R Core Team 2015) software.

Results

Herbage chemical composition

The forage was offered at a rate of 8002–11,600 kg of DM/ha, with the DM of the palisade-grass approximately 400 g/kg DM. CP ranged from 60.3 to 65.8 g/kg DM, the mean NDF was 550 g/kg DM, and the mean ADF was 370 g/kg DM. The ash content varied from 96 to 102 g/kg DM (Table 1). The

ANOVA of forage chemical composition was not significant for any of the parameters evaluated. The probability values were DM/ha ($P = 0.25$), DM ($P = 0.97$), CP ($P = 0.57$), NDF ($P = 0.82$), ADF ($P = 0.93$), and ash ($P = 0.61$), indicating that there was no variation in grass composition during the experimental period (Table 1). It was hypothesised that the forage chemical composition would not vary between paddocks and periods, with this being proven from the non-significant results of the statistical analyses.

Urine and faeces composition

N concentration in the faeces DM did not vary when the mineral salt concentration was increased in the diet ($P > 0.05$); ranging from 14.7 to 17.1 g N/kg DM of faeces (Fig. 1). The total N concentration in urine decreased linearly ($P < 0.01$) from 26.88 to 6.73 g/L (Fig. 1) as the mineral salt concentrations increased in the diet. The calculated urinary volume was significantly influenced by the variation in mineral salt concentration in the diet ($P < 0.01$), with this volume increasing linearly from 5.11 to 12.45 L/day with increasing mineral salt concentration. The total N excreted daily via urine decreased from 106.6 to 67.8 g/day, but these results were not significant ($P > 0.05$).

The main N compound in the urine-N excreted daily was urea-N, which increased linearly from 430 to 704 g N/kg urine-N ($P < 0.001$). Allantoin-N also increased linearly and ranged from 19.3 to 44.9 g N/kg urine-N ($P < 0.004$). The proportion of creatinine-N ranged from 24.4 to 49.0 g N/kg urine-N, but the means from the different concentrations were not significantly different ($P > 0.05$). The hippuric acid-N was significantly affected by the mineral salt concentration in the diet ($P < 0.018$) and increased linearly ($P < 0.001$) from 22 to 73 g N/kg urine-N (Fig. 2).

Discussion

Urine volume

Salt has been utilised in cattle diets as a regulator of feed and water ingestion and to supply cattle nutrient requirements. In a review, Spek et al. (2012) observed that the excreted urinary volume is a major determinant of the N concentration in urine, both in terms of water restriction and increased water intake. These authors calculated urinary volume between 5.1 and 12.4 L/day, which increased with rising mineral salt ingestion by beef cattle. In cattle, the mineral load that needs to be excreted largely determines the volume of urine. Van Vuuren and Smits (1997) verified the effect of low or high rumen protein surplus and two different concentrations of NaCl (0 and 0.38 kg/day) in lactating dairy cattle; they found that urine volumes varied from 23 to 42 kg/day and from 40 to 60 kg/day for low and high rumen protein surplus, respectively. Spek et al. (2012) studied dairy cow urine while increasing NaCl in diets from 0 to 1.01 kg/day; results showed that urine volume increased from 18 to 68 kg/day. In the present study with Nellore cattle, varying the mineral salt concentration in the diet had the same effect on urine volumes as that found in other studies with dairy cattle. This suggests that there is a similar response to variable salt concentrations in the diets of beef cattle as that observed in dairy cattle. Liu and Zhou (2014) found similar effects when evaluating sheep, i.e. a high NaCl diet increased total urine volume voided.

Nitrogen excretion via urine

There is a positive relationship between total N intake and total N excreted via urine (Kebreab et al. 2010). Poppi and McLennan (1995) indicated that protein loss occurred when the CP content of the diet exceeded approximately 210 g CP/

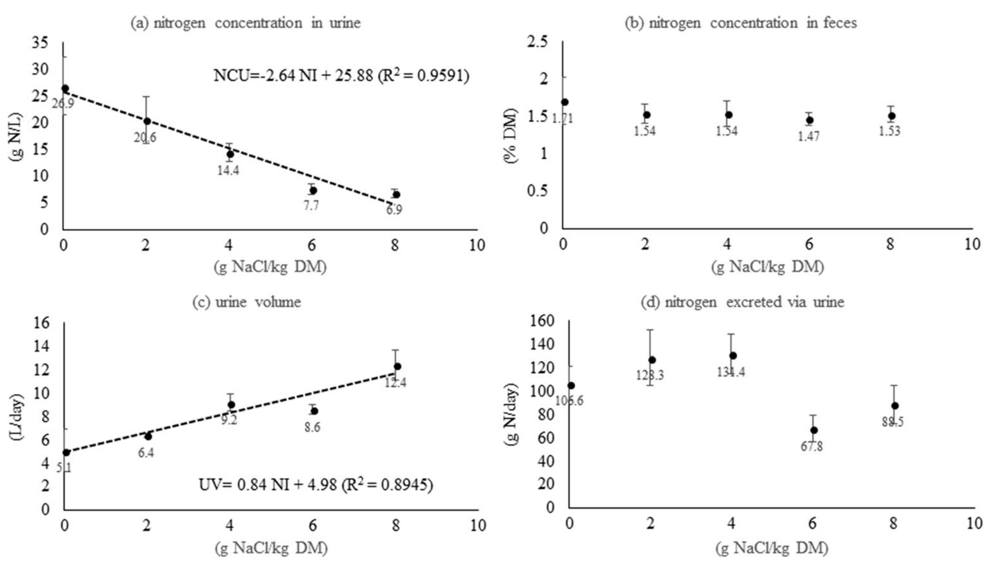

Fig. 1 Nitrogen concentration in urine (g/L), N concentration in faeces (g N/kg DM), urinary volume (L), N excreted via urine (g/day), in function of mineral salt intake concentration (g salt/kg DM). In (**a**) dashed lines are the linear equations between mineral salt intake (NI; g/kg DM intake) and NCU (g/L) and, in (**b**) with urine volume (L/day)

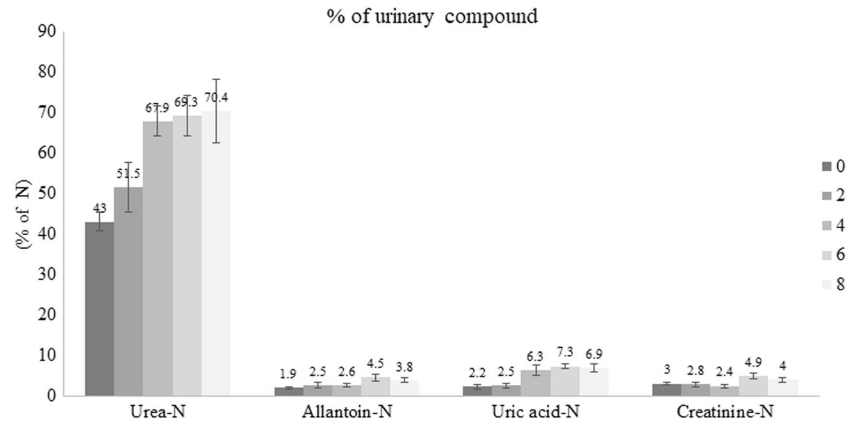

Fig. 2 Proportion of N compounds in the total urinary-N (g/kg urine-N) and effects of mineral salt intake concentration (0, 2, 4, 6 and 8 g NaCl/kg DM) on urinary N compounds. Linear equation between NaCl intake (NI, % g NaCl/kg DM intake) and urea-N (UUN, % of total urinary-N). UUN = 3.63 NI + 45.88 (R^2 = 0.8537), allantoin-N (AN, % of total urinary-N). AN = 0.28 NI + 1.92. (R^2 = 0.7435), hippuric acid-N (HN, % of total urinary-N). HN = 0.71 NI + 2.18 (R^2 = 0.8226) and creatinine-N (% of total urinary-N)

kg of digestible organic matter, with a better relationship between protein and energy occurring at approximately 160 g CP/kg digestible organic matter. This is because at the later amount there is complete net transfer of ingested protein to the intestines as microbial, undegraded, and endogenous proteins. Total N excreted daily via urine did not significantly differ ($P > 0.05$) as was expected in our study, probably because N was not altered in the experimental diet (see Table 1). The N intake was estimated based on a dataset from a study that occurred 1 km from the present study site (Oliveira 2014). The total N intake averaged 161 ± 3 g/day and the N excreted via urine calculated in the present study represented 420–790 g N/kg urine-N of total N ingested.

Urinary N compounds

Another consequence of increased salt ingestion is the dilutive effect on urinary compounds with an increase in urinary volume. NaCl is primarily responsible for the increase in urine volume, predominantly by creating a demand for greater water ingestion in animals (Pereira et al. 2018). The N concentration of cattle urine is variable and ranges from 3.0 to 20.5 g/L (Dijkstra et al. 2013). In the present study, we recorded 26.8 g/L, which is higher than what has been previously reported. The urine-N concentration in the 8.0 g/kg DM concentration of mineral salt was 6.7 g/L, which is close to the values of 6.1, 6.8, and 5.8 g/L obtained by Lantinga et al. (1987), Bristow et al. (1992), and Gonda and Lindberg (1994), respectively, and higher than the lowest concentrations of 3.9 and 3.0 g/L measured by van Vuuren and Smits (1997) and Spek et al. (2012), respectively, when feeding additional NaCl in diets. In the present study, the animals were not forced to ingest NaCl. A urine concentration of 6.7 g/L was quantified when the animals were fed 8.0 g/kg DM of mineral salt concentration mix in the diet, implying a value near to field conditions without restriction of salt and water.

Cattle urine contains diverse nitrogenous constituents and the principal form of N is urea. Urea is formed mainly in the liver as a means of detoxification of NH_3 present in the systemic circulation. It is transported in blood plasma and subsequently diffuses or is transported to other fluid pools in the body, such as milk in the udder and liquid in the rumen (Spek et al. 2013). According to Bristow et al. (1992), urea constitutes 500–900 g N/kg urine-N. Dijkstra et al. (2013) observed that the concentration of urea-N varied between 2.1 and 19.2 g/L, which represent 521–995 g N/kg urine-N. Large amounts of urea can be volatilised and NH_3 volatilisation is considered an indirect source of N_2O (de Klein et al. 2010). In the present study, the concentration of urea-N in urine (g/L) was not significantly different with an increase in the rate of dietary mineral salt concentrations ($P = 0.27$). However, the relative contribution (%) of the total urine-N was affected. When varying the NaCl content in the diet of dairy cows, van Vuuren and Smits (1997) and Spek et al. (2012) found significant differences in the quantities of total urine-N present. The former authors obtained 727–777 g N/kg urine-N in the form of urea and the later authors obtained 600–714 g N/kg urine-N. The analysed urea-N in the urine varied between 430 and 704 g N/kg urine-N in the present study (Fig. 2).

Allantoin, creatinine, and hippuric acid are other principal N compounds that occur in urine (Gardiner et al. 2018). The purine derivative allantoin is derived from rumen microbial nucleic acids. A linear effect of NaCl rates on allantoin-N was discovered ranging from 19 to 45 g N/kg urine-N of total urine-N. Bristow et al. (1992) discovered values from 22 to 118 (g N/kg) allantoin-N in total urine-N of cattle, sheep, and goats, while Gonda and Lindberg (1994), via evaluation of the urine of dairy cows, obtained values between 100 and 140 g N/kg urine-N. The proportion of creatinine-N was not significantly different ($P = 0.19$) based on the mineral salt concentrations in the present study, and, in agreement, Dijkstra et al. (2013) argue that dietary composition has a relatively minor effect on creatinine excretion. Hippuric acid is derived from plant phenolic cinnamic acids. A linear effect between mineral salt concentration in the diet and the urinary concentration of hippuric acid, which increased from 22 to 73 g N/kg urine-N, was obtained; these values were similar to those quantified in dairy cattle urine from previous studies. Studying the urinary compounds of dairy cattle, Kreula et al. (1978) obtained a variation between 2 and 107 g/kg, Bristow et al. (1992) between 34 and 80 (g/kg), and Kool et al. (2006) between 41 and 51 (g/kg) of urine-N in the form of hippuric acid. Hippuric acid has an inhibitory effect on N_2O release, via the temporal inhibition of the nitrification and denitrification processes. This is caused by the breakdown product, benzoic acid, which is a recognised antimicrobial agent. Bertram et al. (2009) have detected this inhibition previously. They showed that increasing the concentration of hippuric acid in the urine by a factor of two or three halved the emissions of N_2O.

Nitrogen concentration in faeces

Faecal N excretion increases with increased N ingestion. However, the variation in urinary N excretion is 3.5 times greater than that of faecal N excretion (e.g. Weiss et al. 2009). The response and variation in faecal N output compared with urinary N excretion are much lower (Dijkstra et al. 2013). The concentration of N in faeces did not vary according to the mineral salt concentration in the diet in the present study (Fig. 1) because the concentration of CP in the forage did not differ throughout the experimental period (Table 1). With reference to N concentration, Silva et al. (2014) quantified 10.8 g N/kg urine-N of Nellore cattle faeces and Cardoso et al. (2018) determined 19.6 g N/kg urine-N in faeces of the Girolando breed. We quantified 14.7–17.1 g N/kg urine-N in the present study.

After the mineral salt concentrations have affected the urinary N concentration, the proportions of urea-N and hippuric acid-N in the urine-N field studies, after measuring NH_3 and N_2O emissions and other N loses to the environment, are required to verify the potential for mitigating N_2O emissions, NH_3 volatilisation, and NO_3^- leaching by including mineral salts in the diet of beef cattle while grazing in the proportions recommended by the NRC (2016).

Conclusions

Varying the concentration of the mineral salt mix in the diet of beef cattle grazing on tropical palisade-grass pasture altered their urine output, the concentration of N in urine, and changed the proportion of N compounds including urea-N, allantoin-N, creatinine-N, and hippuric acid-N. The amount of N excreted in the urine per day and the concentration of N in the faeces did not differ with increased mineral salt concentrations in the diet. The effect of N concentration in the urine decreased linearly, and there was a linear increase for the urine volume, urea-N, allantoin-N, and hippuric acid-N values. Creatinine-N did not fit the curve.

Funding sources This study was funded by the Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP), under the codes 2012/06718-8 and 2011/00060-8.

Compliance with ethical standards

Ethical clearance from the study was granted by São Paulo State University Ethical Committee (protocol number 004389/13). The manuscript does not contain clinical studies or patient data.

Conflicts of interest statement We declare that there are no conflicts of interest in this project. Additionally, there is no financial or other relationship with other people or organisations that may inappropriately influence the authors' work.

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