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Carbohydrate-rich supplements can improve nitrogen use efficiency and mitigate nitrogenous gas emissions from the excreta of dairy cows grazing temperate grass

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*Temperate pasture species constitute a source of protein for dairy cattle. On the other hand, from an environmental perspective, their high N content can increase N excretion and nitrogenous gas emissions by livestock. This work explores the effect of energy supplementation on N use efficiency (**NUE**) and nitrogenous gas emissions from the excreta of dairy cows grazing a pasture of oat and ryegrass. The study was divided into two experiments: an evaluation of NUE in grazing dairy cows, and an evaluation of N-NH_3 and N-N_2O volatilizations from dairy cow excreta. In the first experiment, 12 lactating Holstein × Jersey F1 cows were allocated to a double 3 × 3 Latin square (three experimental periods of 17 days each) and subjected to three treatments: cows without supplementation (**WS**), cows supplemented at 4.2 kg DM of corn silage (**CS**) per day, and cows supplemented at 3.6 kg DM of ground corn (**GC**) per day. In the second experiment, samples of excreta were collected from the cows distributed among the treatments. Aliquots of dung and urine of each treatment plus one blank (control – no excreta) were allotted to a randomized block design to evaluate N-NH_3 and N-N_2O volatilization. Measurements were performed until day 25 for N-NH_3 and until day 94 for N-N_2O. Dietary N content in the supplemented cows was reduced by 20% ($P < 0.001$) compared with WS cows, regardless of the supplement. Corn silage cows had lower N intake ($P < 0.001$) than WS and GC cows (366 v. 426 g/day, respectively). Ground corn supplementation allowed cows to partition more N towards milk protein compared with the average milk protein of WS cows or those supplemented with corn silage (117 v. 108 g/day, respectively; $P < 0.01$). Thus, even though they were in different forms, both supplements were able to increase ($P < 0.01$) NUE from 27% in WS cows to 32% in supplemented cows. Supplementation was also effective in reducing N excretion (761 v. 694 g/kg of N_{intake}; $P < 0.001$), N-NH_3 emission (478 v. 374 g/kg of N_{milk}; $P < 0.01$) and N-N_2O emission (11 v. 8 g/kg of N_{milk}; $P < 0.001$). Corn silage and ground corn can be strategically used as feed supplements to improve NUE, and they have the potential to mitigate N-NH_3 and N-N_2O emissions from the excreta of dairy cows grazing high-protein pastures.*

perennial pasture species are particularly important when considering the characteristics of milk production systems in tropical and subtropical regions around the world. Cool-season (C3) grasses and legumes have high digestibility and constitute a source of protein for animals due to their high N levels. On the other hand, from an environmental perspective, their high N content can decrease NUE and increase N excretion of livestock. The low NUE under grazing conditions is related to the high N intake associated with a high concentration of soluble proteins, causing an imbalance between energy and N supplies in the rumen.

The adoption of feeding strategies to reduce the amount of N excreted by dairy cattle per unit of milk can be a powerful tool for mitigating the amount of N excreted into the environment (Johnson *et al.*, 2016). Therefore, the association between high-protein pastures with high-energy/low-protein supplements can reduce dietary N concentrations and increase NUE, reducing nitrogenous gas emissions (Luo *et al.*, 2008a).

Carbohydrate supplementation is an alternative for providing additional energy to dairy cows grazing high-protein pastures (Bargo *et al.*, 2004). However, the type of supplement provided can substantially influence total DM and pasture intake. Overall, supplementary forage has a drastic effect on the reduction of pasture intake compared with concentrate supplementation (Delagarde *et al.*, 2011). Therefore, responses related to N intake, production and excretion, as well as responses in nitrogenous gas emissions, may vary according to the type of supplement provided to livestock.

This study aimed to evaluate NUE and emission of $N\text{-}NH_3$ and $N\text{-}N_2O$ from the excreta of dairy cows grazing high-protein pastures with or without energy supplementation.

Material and methods

The current study was performed concomitantly with a study that aimed to assess the effects of corn silage or ground corn supplementation on enteric methane emission, milk production and total DM intake by grazing dairy cows. For details about methodological procedures related to FUNDACEP – FAPA 43) was sown in May 2016. After each experimental period, the areas were mowed to standardize pasture regrowth and fertilized with 50 kg N/ha supplied as urea. Within the experimental area, a plot of 500 m^2 was excluded from grazing for gas emission experiments. This plot was subdivided into three blocks (6×28 m in length), and each block was divided into seven sub-units (4×6 m). This area was neither grazed nor fertilized after excreta application to avoid external influences.

Experiment 1: N use efficiency

Animals, experimental design and treatments. Twelve lactating Holstein × Jersey F1 cows were separated into six homogeneous groups according to milk yield (23.3 ± 6.9 kg/day), lactation stage (101 ± 57.6 days in milk) and initial BW (492 ± 76.8 kg). Each group was considered the experimental unit, and the cows were allocated to a double 3×3 Latin square with three experimental periods lasting 17 days each (a 12-day adaptation period and a 5-day measurement period). The six experimental groups were divided into three treatments: without supplementation (**WS**) – cows fed a pasture-only diet; corn silage (**CS**) – cows fed a pasture diet and supplemented at 4.2 kg DM of corn silage per day; and ground corn (**GC**) – cows fed a pasture diet and supplemented at 3.6 kg DM of ground corn per day. The quantities of ground corn and corn silage were balanced to provide the same amount of net energy for lactation as recommended by the Institute National de la Recherche Agronomique (INRA) (2007). The chemical composition and nutritional value of the supplements are presented in Table 1. Cows without supplementation were taken to paddocks after milking. Supplemented cows had exclusive access to supplements (corn silage or ground corn) immediately after milking – from 0800 to 0900 h and

Table 1 *Chemical composition and nutritive values of the supplements (corn silage and ground corn) offered to dairy cows grazing annual ryegrass (*Lolium multiflorum *cv. Barjumbo) and oat (Avena sativa cv. FUNDACEP – FAPAR 43)*

Items	Corn silage	Ground corn

from 0430 to 1730 h. After supplements were offered, the cows were taken to paddocks where they remained until subsequent milking. All cows had *ad libitum* access to water and mineral salt. The grazing method was strip grazing, and WS cows were offered a pasture allowance of 40 kg of DM per cow per day. For CS and GC cows, pasture allowance was adjusted to maintain the same post-grazing sward height of WS treatment. This grazing management was adopted to avoid differences in sward grazed by cows from different groups, reducing losses during grazing.

Sampling. Pre- and post-grazing pasture mass was estimated using a rising plate meter (F200 model; Farmworks®, Feilding, New Zealand), which was calibrated based on DM content, taking into account the plate area (0.1 m^2) and sward height measured ('t Mannetje, 2000). During each experimental period, samples from 10 points were recorded and cut above ground level before and after grazing for calibration. The samples were dried in an oven with forced air circulation at 60°C for 72 h, and regression equations were developed for estimating pasture mass (kg DM/ha) as a function of compressed height (cm) measured with the rising plate meter. Pre-grazing extended tiller height was measured on days 14 and 16, while post-grazing extended tiller height was measured on days 16 and 18. Both measurements were taken on 100 randomly chosen tillers per paddock per period. The chemical composition of the sward was determined on days 14 and 16 per period. Twenty handfuls of randomly selected pasture were cut above ground level per paddock per period. In the laboratory, the samples were cut at the average post-grazing extended tiller height to represent the pasture selected and ingested by cows, as proposed by Delagarde *et al.* (2000). Subsequently, this subsample was dried in an oven with forced air ventilation at 60°C for 72 h and stored for further analysis. Milk yield and composition (protein and milk urea N) were recorded for each cow at each milking (0700 and 1530 h) during the last 5 days of each experimental period. Blood collection was performed by venipuncture of the jugular vein on the 13th and 17th day of each experimental period after morning milking. Chemistis (AOAC) International, 1998). Milk samples were analysed by infrared spectrophotometry (DairySpect FT; Bentley, Chaska, Minnesota, USA).

Animal measurements. Individual balances for true protein absorbable in the small intestine (**PDI**) when energy is limited for microbial synthesis in the rumen (**PDIE**) and when N is limited for microbial synthesis in the rumen (**PDIN**), as well as for the net energy for milk production (**UFL**), were calculated from the chemical compositions of pasture and supplements according to the INRA (2007). Cows' pasture intake was estimated as the difference between the total biomass between pre-grazing and post-grazing (Lantinga *et al.*, 2004), and supplement intake (corn silage or ground corn) was quantified daily as the difference between the quantity supplied and the orts on each of the last 5 days of each experimental period. Nitrogen use efficiency was calculated by dividing the total N output in milk by the total amount of ingested N. Nitrogen excretion was calculated according to the equations of the RedNex European Project (Cutullic *et al.*, 2013b), as described:

$$\mathrm{N_{dung}}(\mathrm{g/day}) = -13.7 + 6.907\,\mathrm{DMI}(\mathrm{kg/day}) + 0.1025\,\mathrm{NI}(\mathrm{g/day}) \quad (R^2 = 0.70)$$

where N_{dung} stands for faecal N excretion; DMI, DM intake; and NI, N intake.

$$\mathrm{N_{urine}}(\mathrm{g/day}) = -142.1 + 1.98\,\mathrm{Milk\ urea\ N}(\mathrm{mg/100ml}) + 0.2009\,\mathrm{NI}(\mathrm{g/day}) + 6.5\,\mathrm{NC}(\mathrm{g/kg\ of\ DM}) \quad (R^2 = 0.90)$$

where N_{urine} stands for urinary N excretion; MUN, milk urea N; NI, N intake; and NC, dietary N content.

Experiment 2: Emission of ammonia and nitrous oxide

An aliquot of each treatment sample was separated for chemical analyses. Urine was refrigerated until analysis, and dung was dried in an oven with forced air ventilation at 60°C for 72 h and stored for further analysis.

Evaluation of ammonia emissions from excreta. Aliquots of 140 g of dung and 80 ml of urine of each treatment (WS, CS and GC) plus one blank (control – no excreta) were allotted to a randomized block design to evaluate N-NH_3 volatilization (Supplementary Figure S1). Three blocks were used, and each block was equipped with seven semi-open chambers ($n = 21$). Dung and urine were applied over the soil in micro-plots delimited by circular bases (10 cm in diameter; area = 0.008 m^2). N-NH_3 volatilization was quantified on 28 October 2016, according to the methodology of Araújo *et al.* (2009) as described by Jantalia *et al.* (2012) and Lessa *et al.* (2014). The semi-open chambers were made with transparent polyethylene terephthalate bottles. The base of the chamber was inserted 2.5 cm into the soil during the whole sampling period (25 days). The N-NH_3 trapping system was composed of a polyurethane foam (0.017 g/cm^3) piece, 3 mm thick, 2.5 cm wide and 25 cm in length, suspended vertically with the aid of a 1.5-mm-diameter rigid wire inside a 50-ml plastic flask with 10 ml H_2SO_4 solution and 1 mol/dm^3 glycerine (2%, v/v). Before starting each sampling, foam was immersed in the acid solution and then compressed to absorb most of the solution. After the spreading of excreta on soil, foam pieces were set out (day 0), and they were replaced on days 2, 4, 7, 10, 13, 17, 21 and 25, totalling eight samplings. For N-NH_3 determination, 40 ml distilled water was added to the foam along with the remaining solution in each 50 ml flask of known mass (P1). After homogenization of the mixture contained in each flask, they were weighed again (P2). An aliquot of 5 ml was obtained by steam distillation and titrated for the quantification of N-NH_3, according to Alves *et al.* (1994). The difference between P2 and P1 determined the total volume of the solution. The density of the mixture was considered equal to 1 g/cm^3. The quantity of N-NH_3 (μg/cm^2) was calculated considering the amount of N recovered in the collector as a function of the interval between samplings and the area of the bases. Cumulative emissions were calculated as the

excreta application on day 0. These measurements were repeated on days 1, 3, 5, 7, 9, 13, 17, 21, 26 and 31. From day 37, measurements were performed weekly until day 94, totalling 20 samplings. Air samples were collected from closed static chambers (Mosier, 1989) made from polyurethane buckets that were 33.7 cm in diameter and 38 cm in height, totalling a volume of 33.8 l. The close static chambers were fitted to the metal bases at the beginning of each sampling at 0800 h. Air samples were collected at 0, 15 and 30 min using polypropylene syringes (20 ml) with luer-lock tips and kept in an icebox. At the end of sampling period, air samples were transferred to 12-ml sterilized evacuated vials (Exetainer; Labco, Ceredigion, UK). N_2O concentration in air samples was measured by gas chromatography at the Soil Department of the Federal University of Rio Grande do Sul (Porto Alegre, RS, Brazil) using a Shimadzu GC 2014 device with an injection temperature of 250°C, a column temperature of 70°C and N_2 as the carrier gas (30 ml/min). The device was equipped with an electron capture detector maintained at 325°C. N-N_2O flow (μg/m^2 per hour) was calculated considering a linear increase in gas concentration in the chamber as a function of time between samplings, air temperature and pressure, chamber volume and the area of bases (Gomes *et al.*, 2009). Cumulative emissions were calculated by multiplying the hourly flow by 24 h during the sampling days and by linear interpolation between the sampling days. On every day of gas sampling, soil samples were taken from the 0 to 10 cm layer and oven-dried (105°C) to determine the water content. Soil samples for bulk density calculations were also taken from the area to calculate volumetric water content and total soil porosity and hence the water-filled pore space (**WFPS**).

Calculations. According to the calibration technique for the quantification of volatilized N-NH_3 described by Araújo *et al.* (2009), the fraction of NH_3 recovered was 0.63 for a range of 100 to 1500 kg N/ha. Therefore, the values measured were multiplied by a correction factor of 1.74. N-NH_3 and N-N_2O emission factors for dung and urine were calculated according to the following equation:

$$\mathrm{N} - \mathrm{Gas}_{\mathrm{emission}}(\mathrm{g/cow\ per\ day}) = \mathrm{EF}_{\mathrm{Gas}}(\%) \times \mathrm{N}_{\mathrm{excreted}}(\mathrm{g/day})$$

where N-$\mathrm{Gas}_{emission}$ is the emission of N-N_2O or N-NH_3 from dung or urine, EF_{gas} is the emission factor of N-N_2O or N-NH_3 for dung or urine, and $N_{excreted}$ is the amount of N excreted as dung or urine.

Statistical analyses

Data were subjected to the analysis of variance using PROC MIXED performed by SAS software (version 9.3; SAS Institute, Cary, NC, USA) at a 5% significance level. The variables, averaged per group and per period ($n = 18$), were analysed using the following model:

$$Y_{ijk} = \mu + \mathrm{group}_i + \mathrm{period}_j + \mathrm{treatment}_k + e_{ijk}$$

where Y_{ijk} is analysed variable; μ is overall mean; group_i is random effect of the group; period_j is random effect of the period; $\mathrm{treatment}_k$ is fixed effect of the treatment; and e_{ijk} is residual error.

Nitrogenous emissions factor data were subjected to the analysis of variance using PROC GLM performed by SAS software (version 9.3) at a 5% significance level. The variables were analysed using the following model:

$$Y_{ij} = \mu + \mathrm{treatment}_i + \mathrm{block}_j + e_{ij}$$

where Y_{ij} is analysed variable; μ is overall mean; $\mathrm{treatment}_i$ is fixed effect of the treatment; block_j is fixed effect of the block; and e_{ij} is residual error.

Results

DM intake, grazing behaviour and enteric methane emissions

For more details of DM intake, grazing behaviour and enteric methane emissions, see Dall-Orsoletta *et al.* (2019).

N use efficiency

In this study, supplementation reduced the levels of protein truly digested in the intestine (INRA, 2007) both as PDIN and PDIE (Table 2). The reduction in PDI content was more marked in the diet of cows supplemented with corn silage compared to cows receiving ground corn. PDIN intake did not change in cows supplemented with ground corn, but PDIE and UFL intakes increased ($P < 0.01$ and $P < 0.001$, respectively) as a result of the variation in feeding patterns and nutritional values of the diets. On the other hand, PDIE and UFL intakes did not change in cows supplemented with corn silage, but PDIN intake was lower ($P < 0.01$) compared with WS cows. Milk yield ($P < 0.05$) and milk CP content ($P < 0.01$) were

Table 2 *Diet nutritive values, feed intake and milk production of dairy cows grazing annual ryegrass (*Lolium multiflorum *cv. Barjumbo) and oat (*Avena sativa *cv. FUNDACEP – FAPAR 43) without (WS) and with corn silage (CS) or ground corn (GC) supplementation*

Items ($n = 18$)	Treatments WS	CS	GC	SEM	*P*-values
Diet nutritive values					
PDIN[1] (g/kg of DM)	141a	108c	121b	2.1	<0.001
PDIE[2] (g/kg of DM)	107a	90c	102b	1.1	<0.001
UFL[3] (UFL/kg of DM)	0.98a	0.87b	1.00a	0.012	<0.001
(PDIN–PDIE)/UFL	34.5a	20.2b	18.7b	1.13	<0.001
Feed intake					
DM (kg/day)					

Table 3 *N balance of dairy cows grazing annual ryegrass (*Lolium multiflorum *cv. Barjumbo) and oat (*Avena sativa *cv. FUNDACEP – FAPAR 43) without (WS) and with corn silage (CS) or ground corn (GC) supplementation*

	Treatments				
Items ($n = 18$)	WS	CS	GC	SEM	*P*-values
Diet N content (g/kg of DM)	35a	27b	29b	0.6	<0.001
N intake (g/day)					
Pasture	418a	335c	384b	15.4	0.0163
Corn silage	–	31	–	–	–
Ground corn	–	–	49	–	–
Total	418a	366b	433a	15.7	0.039
N milk yield (g/day)	108b	107b	117a	1.8	0.006
NUE[1]	0.27b	0.32a	0.32a	0.009	0.004
Milk urea N (mg/dl)	18.1a	15.2b	15.4b	0.45	0.003
N excretion (g/day)					
Dung	111b	116b	133a	3.6	0.006
Urine	205a	139c	165b	7.1	<0.001
Total	316a	255b	298a	10.3	<0.001
N excretion (g/kg of N_{intake})					
Total	761a	698b	690b	5.6	<0.001
N balance	−6b	3ab	18a	5.1	0.026

[1]NUE = N use efficiency.
[a,b,c] Values within a row with different superscripts differ significantly at $P < 0.05$.

Table 4 *Excreta composition and N-NH_3 and N-N_2O emission factors from dung and urine of dairy cows grazing annual ryegrass (*Lolium multiflorum *cv. Barjumbo) and oat (*Avena sativa *cv. FUNDACEP – FAPAR 43) without (WS) and with corn silage (CS) or ground corn (GC) supplementation.*

	Treatments				
Item ($n = 21$)	WS	CS	GC	SEM	*P*-values
Dung					
DM content (g/kg)	101	91	157	–	–
N content (g/kg of DM)	28	24	28	–	–
EF[1] N-NH_3 (% of $N_{applied}$)	5.77a	2.96b	0.94c	0.145	0.005
EF[1] N-N_2O (% of $N_{applied}$)	0.72	0.632	0.377	0.146	0.636
Urine					
N content (g/kg)	12	8	10	–	–
EF[1] N-NH_3 (% of $N_{applied}$)	18.53	23.21	26.92	4.020	0.715
EF[1] N-N_2O (% of $N_{applied}$)	0.16	0.18	0.12	0.040	0.803

[1]EF = emission factor.
[a,b,c] Values within a row with different superscripts differ significantly at $P < 0.05$.

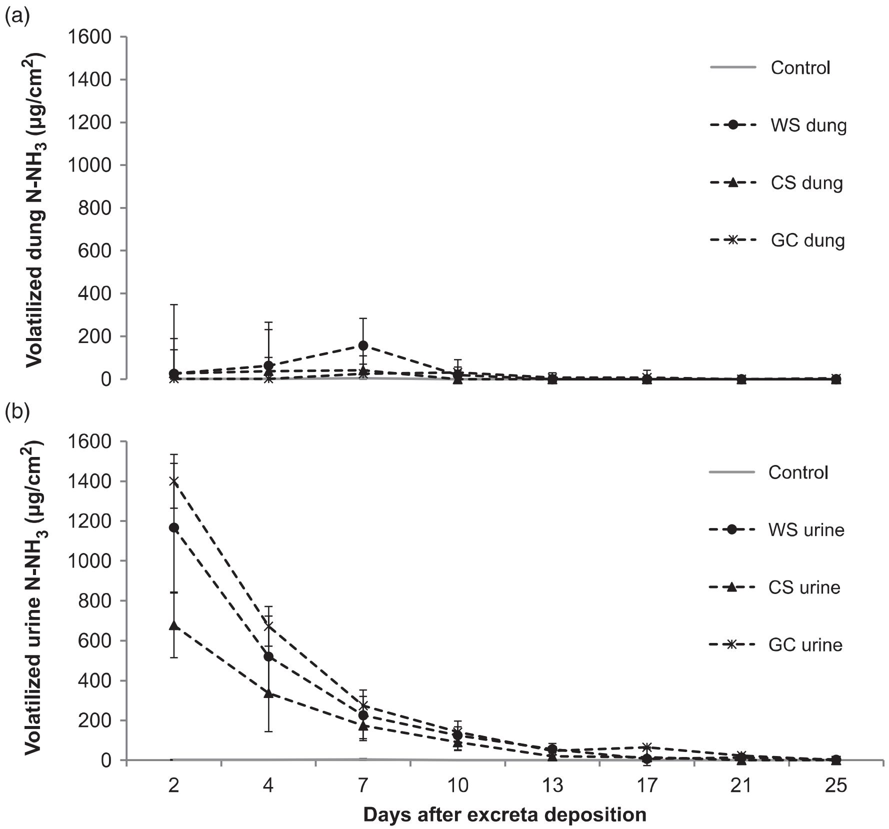


Figure 1 Ammonia volatilization from cattle dung (a) and urine (b) patches artificially deposited on an annual ryegrass and oat pasture from southern Brazil. Bars are SEM. WS = cows without supplementation; CS = cows supplemented with corn silage; GC = cows supplemented with ground corn.

$N\text{-}NH_3$ volatilization from dung micro-plots was always close to the control (without excreta) and near 0 on most days (Figure 1). On the other hand, urine micro-plots showed greater volatilization rates even in the first measurement, reaching values up to 1400 µg/cm^2.

Three days after excreta application, $N\text{-}N_2O$ fluxes were greater in all treatments compared with the control (without excreta) (Figure 2). The first $N\text{-}N_2O$ emission peak (around the fifth day) reached approximately 1100 µg/m^2 per hour, and the second peak (around the 17th day) reached >600 µg/m^2 per hour for both urine and dung micro-plots. After the 37th day, there was no more $N\text{-}N_2O$ flow for urine micro-plots; however, dung from the micro-plots

emission from the excreta of supplemented cows was lower compared with WS cows. This reduction was evident in GC cows, both in g per cow per day and in g/kg N_{milk} ($P < 0.001$). On the other hand, $N\text{-}N_2O$ emission in g/kg of N_{intake} was lower only in GC cows (1.62 *v.* 2.72, $P < 0.001$).

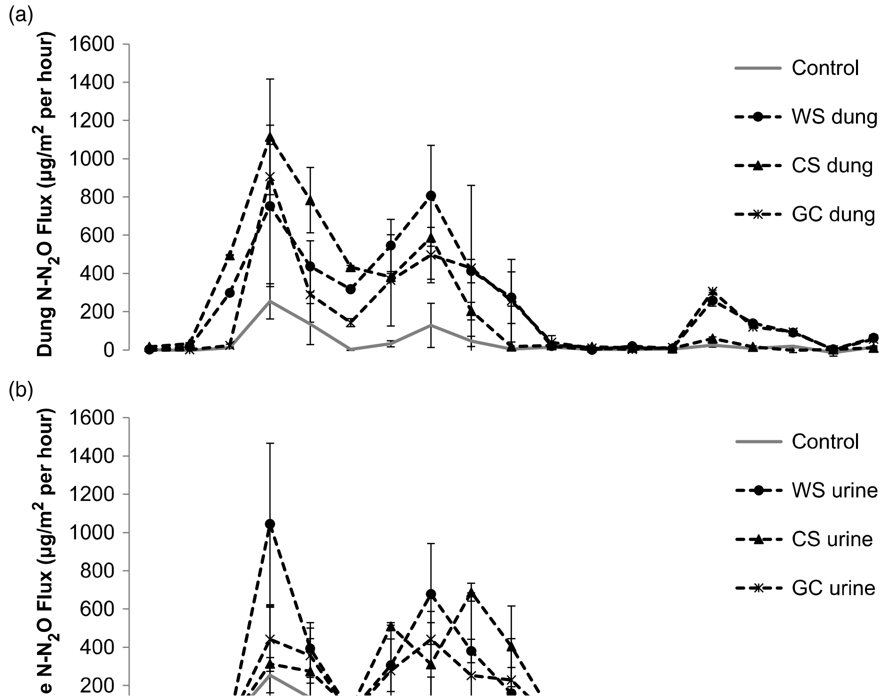
Discussion

N use efficiency. The experimental protocol principally focused on allowing cows to graze the same proportion of pasture in relation to the initial sward height, regardless

Table 5 *N-NH_3 and N-N_2O emissions from dung and urine of dairy cows grazing annual ryegrass (* Lolium multiflorum *cv. Barjumbo) and oat (*Avena sativa *cv. FUNDACEP – FAPAR 43) without (WS) and with corn silage (CS) or ground corn (GC) supplementation*

	Treatments				
Items ($n = 18$)	WS	CS	GC	SEM	*P*-values
N-NH_3 emissions					
N-NH_3 (g per cow per day)	49.74a	36.33b	45.86a	1.870	0.003
Dung	11.83a	4.11b	1.54c	0.288	<0.001
Urine	37.92b	32.22c	44.32a	1.663	0.003
N-NH_3 (g/kg of N_{intake})	119.87a	99.23c	106.13b	1.834	<0.001
N-NH_3 (g/kg of N_{milk})	478.26a	346.63b	402.27b	18.442	0.003
N-N_2O emissions					
N-N_2O (g per cow per day)	1.14a	0.99b	0.70c	0.031	<0.001
Dung	0.81a	0.74a	0.50b	0.022	<0.001
Urine	0.33a	0.26b	0.20c	0.011	<0.001
N-N_2O (g/kg of N_{intake})	2.73a	2.71a	1.62b	0.016	<0.001
N-N_2O (g/kg of N_{milk})	10.98a	9.43b	6.09c	0.332	<0.001

[a,b,c] Values within a row with different superscripts differ significantly at $P < 0.05$.



of PDIE and UFL, which were higher in GC cows than in WS and CS cows. It is known that PDIE intake is directly associated with the production and concentration of milk protein (Vérité and Delaby, 2000; Monteils *et al.*, 2002; Cantalapiedra-Hijar *et al.*, 2014). Therefore, higher energy and PDIE intakes were responsible for the higher milk yield and milk protein content in cows supplemented with ground corn.

Supplementation with corn silage or ground corn in the diets of cows grazing temperate annual pastures increased NUE by 5 percentage points. Therefore, the supply of fermentable carbohydrates may have reduced ruminal NH_3 concentration (by reducing ruminal NH_3 production or increasing microbial uptake of NH_3 in the rumen), leading to better N utilization in supplemented cows (Hristov *et al.*, 2005). The improvement of NUE in cows that received corn silage was associated with a lower N intake and maintenance of milk N yield. In contrast, the improvement of NUE in cows supplemented with ground corn may be related to the lack of variation in N intake and a higher milk N yield compared with WS cows. In both cases, NUE was higher due to a reduction of the (PDIN–PDIE)/UFL ratio and a reduction of dietary N levels (Monteils *et al.*, 2002). A higher NUE stimulates the recycling of urea for microbial synthesis (Reynolds and Kristensen, 2008), resulting in lower N excretion without affecting protein synthesis and animal performance (Cutullic *et al.*, 2013a). In our study, supplementation with corn silage or ground corn was equally effective in reducing milk urea N, corroborating other studies evaluating energy supplementation for grazing cows (Bargo *et al.*, 2002; Delahoy *et al.*, 2003) that observed reduced CP and rumen-degradable protein levels (Mutsvangwa *et al.*, 2016), agreeing with the results obtained for the (PDIN–PDIE)/UFL ratio. The relationship between milk urea N content and dietary N content on an individual cow basis was calculated and presented as supplementary material (Supplementary Figure S2).

Cows supplemented with corn silage ingested less N but produced the same amount of milk N, which resulted in lower N excretion per ingested N compared with WS cows. Cows supplemented with ground corn ingested the same amount of N as those without supplementation but produced a higher

Thus, the reduction of difference between PDIN and PDIE in relation to the dietary energy content in supplemented cows indicates that the supply of corn silage or ground corn was responsible for better N balance in supplemented cows.

Ammonia and nitrous oxide emissions factors from dung and urine patches

The average volatilization of N-NH_3 was 3.2% of N applied as dung, ranging from 0.94% to 5.77%. These results are within the range from 0% to 11.6% cited in the literature (Petersen *et al.*, 1998; Saarijärvi *et al.*, 2006; Laubach *et al.*, 2013) and are also very close to the results found in Brazil by Lessa *et al.* (2014), where N-NH_3 volatilization averaged 3.4% of N applied as dung.

The volatilization rate of N-NH_3 from urine ranged from 18.5% to 26.9% of applied N, which is also in agreement with the literature (17% to 30%; Saarijärvi *et al.*, 2006; Laubach *et al.*, 2012, 2013; Lessa *et al.*, 2014). In addition, the average of urinary N-NH_3 volatilized in this experiment was 22.9% of N applied, which is very similar to the average of 22% reported by Lessa *et al.* (2014) in a study conducted in Brazil. These results are very close to the value used by the IPCC, which considers an N-NH_3 emission factor of 20% of N deposited as excreta (Intergovernamental Panel on Climate Change, 2006).

It is well known that when excreta are allocated to the soil, N is immediately exposed to factors that favour its volatilization (Haynes and Williams, 1993). In the present study, >90% of N-NH_3 volatilization occurred during the first week, which was expected because it is in agreement with a large number of previous experiments (Cantarella *et al.*, 2003; Zaman *et al.*, 2009; Laubach *et al.*, 2012; Liu and Zhou, 2014).

In the case of N-N_2O emission, the three emission peaks (on the 5th, 17th and between the 58th and 65th days) may be explained by soil WFPS variations. Soil WFPS usually increased in response to rainfall or further anaerobic denitrification, a process directly associated with N-N_2O emission (Smith *et al.*, 2003). Hence, these results are also in agreement with what has already been observed in many other studies (De Klein *et al.*, 2003; Lessa *et al.*, 2014; Luo *et al.*, 2008b; Rochette *et al.*, 2014; Van Der

In comparison with literature values, Brazilian studies have shown N-N_2O emission factors for dung ranging from 0% to 0.4% (Lessa *et al.*, 2014; Sordi *et al.*, 2014), with the highest values found in the south of the country. In the present study, dung N-N_2O emission factors averaged 0.58%. Although slightly higher compared to the Brazilian studies, this result is still within the values described in the literature, which ranged from 0.1% to 0.7% (De Klein *et al.*, 2003, 2014; Lessa *et al.*, 2014; Luo *et al.*, 2008b; Oenema *et al.*, 1997; Rochette *et al.*, 2014; Sordi *et al.*, 2014; Van Der Weerden *et al.*, 2011; Van Groenigen *et al.*, 2005; Wachendorf *et al.*, 2008; Yamulki *et al.*, 1998) and are below the default value of 2% used by the IPCC (2006).

The relatively low urine N-N_2O emission factor (0.15%) is also in agreement with previous studies. In Brazilian grazing conditions, Lessa *et al.* (2014) found urine N-N_2O emission factors ranging from 0.01% in dryer seasons to 2.55% in wetter seasons (average = 1.3%), and Sordi *et al.* (2014) found values ranging from 0.1% to 0.45% (average = 0.26%). In the worldwide literature, urine emission factors have ranged from 0.01% to 3.8% (Oenema *et al.*, 1997; Yamulki *et al.*, 1998; De Klein *et al.*, 2003 and 2014; Van Groenigen *et al.*, 2005; Luo *et al.*, 2008b; Wachendorf *et al.*, 2008; Van Der Weerden *et al.*, 2011; Lessa *et al.*, 2014; Rochette *et al.*, 2014; Sordi *et al.*, 2014).

Finally, the combination of high soil mineral N availability and high WFPS is considered to be a trigger for the induction of soil N-N_2O fluxes (Smith *et al.*, 2003). In our study, N losses through NH_3 volatilization were high, and WFPS was frequently low (<50%). This result may explain the smaller values of N-N_2O emission factors from urine compared to the dung micro-plots.

Emission of ammonia and nitrous oxide

N-NH_3 emission from the excreta of cows grazing annual temperate pastures without supplementation was approximately 50 g per cow per day. Supplementation with ground corn did not affect N-NH_3 emission in absolute terms, whereas corn silage supplementation was able to reduce N-NH_3 emissions by 27%. The decrease in N-NH_3 emissions is related to the lower N intake and, consequently, lower excretion in cows receiving corn silage (Petersen *et al.*, 1998). emissions from the excreta of these cows and their higher milk yield compared with WS cows. Cows that received corn silage also had lower daily emissions of N-N_2O and emissions per milk N (13% and 14%, respectively), but the emission per ingested N remained similar to that of WS cows. Overall, supplementation with corn silage or ground corn was effective in mitigating the emission of N-N_2O from the excreta of dairy cows grazing temperate annual pastures, but ground corn was more effective than corn silage. These results are in agreement with Luo *et al.* (2008a), who reported a decrease in N-N_2O emissions in dairy cows grazing temperate perennial grass supplemented with corn silage. In their study, which took a systematic approach to the New Zealand dairy industry, the authors observed a reduction of 22% in N-N_2O emissions per litre of milk with the introduction of corn silage in grazing systems due to a better NUE. The present experiment reported reductions of 14% and 43% in N-N_2O emissions from the excreta of cows supplemented with corn silage or ground corn, respectively.

The absolute magnitude of N losses through volatilization should be interpreted carefully because it may vary with different conditions of temperature, soil moisture, pH and wind speed. Moreover, N excretion of dung and urine is based on empirical equations, and the measurements of emission factors are based on constant quantities of dung and urine, which may not simulate a pasture situation or capture treatment effects on excretory patterns. Despite this statement, our results are in accordance with a recent study (Voglmeier *et al.*, 2018), which showed that reductions in dietary N content induced by carbohydrate-rich supplements may reduce N emission factors.

In conclusion, both corn silage and ground corn can be strategically used as feed supplements to improve NUE and have the potential to mitigate N-NH_3 and N-N_2O emissions from the excreta of dairy cows grazing high-protein pastures.

However, further studies on indirect and direct emissions from pastures and on the production of supplements and fertilizers are required for a complete approach to this production system.

Declaration of interest

The authors declare that there is not any actual or potential conflict of interest with other people or organizations that could inappropriately influence their work.

Ethics statement

The project has the approval of the Ethics Committee on Animal Use (CETEA) from the State University of Santa Catarina – UDESC (under protocol no. 4373090816), in accordance with current legislation and ethical guidelines formulated by the Brazilian College of Animal Experimentation.

Software and data repository resources

None of the data were deposited in an official repository.

Supplementary material

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References

Alves BJR, Santos JCF, Urquiaga S and Boddey RM 1994. Métodos de determinação do nitrogênio em solo e planta. In Manual de métodos empregados em estudos de microbiologia agrícola (eds. RS Araújo and M Hungria), pp. 449–469. Embrapa-SPI, Brasília, DF, Brasil.

Araújo EDS, Marsola T, Miyazawa M, Soares LHDB, Urquiaga S, Boddey RM and Alves BJR 2009. Calibração de câmara semiaberta estática para quantificação de amônia volatilizada do solo. Pesquisa Agropecuaria Brasileira 44, 769–776.

Association of Official Analytical Chemistis (AOAC) 1998. Official methods of analysis, 16th edition. AOAC, Arlington, VA, USA.

Bargo F, Delahoy JE and Muller LD 2004. Milk production of dairy cows fed total mixed rations after a grazing period. The Professional Animal Scientist 20, 270–277.

Bargo F, Muller LD, Delahoy JE and Cassidy TW 2002. Milk response to concentrate supplementation of high producing dairy cows grazing at two pasture allowances. Journal of Dairy Science 85, 1777–1792.

Bertol I, Albuquerque JA, Leite D, Amaral AJ and Zoldan Junior WA 2004. Propriedades físicas do solo sob preparo convencional e semeadura direta em rotação e sucessão de culturas, comparadas às do campo nativo. Revista Brasileira de Ciencia do Solo 28, 155–163.

Cantalapiedra-Hijar G, Peyraud JL, Lemosquet S, Molina-Alcaide E, Boudra H, Nozière P and Ortigues-Marty I 2014. Dietary carbohydrate composition modifies the milk N efficiency in late lactation cows fed low crude protein diets.

De Klein CAM, Luo J, Woodward KB, Styles T, Wise B, Lindsey S and Cox N 2014. The effect of nitrogen concentration in synthetic cattle urine on nitrous oxide emissions. Agriculture, Ecosystems and Environment 188, 85–92.

Delagarde R, Peyraud JL, Delaby L and Faverdin P 2000. Vertical distribution of biomass, chemical composition and pepsin-cellulase digestibility in a perennial ryegrass sward: Interaction with month of year, regrowth age and time of day. Animal Feed Science and Technology 84, 49–68.

Delagarde R, Peyraud J-L, Parga J and Ribeiro Filho HMN 2001. Caractéristiques de la prairie avant et aprés un pâturage; quels indicateurs de l´ingestion chez la vache laitière? Rencontres Recherches Ruminants 8, 209–212.

Delagarde R, Valk H, Mayne CS, Rook AJ, González-Rodríguez A, Baratte C, Faverdin P and Peyraud JL 2011. GrazeIn: a model of herbage intake and milk production for grazing dairy cows. 3. Simulations and external validation of the model. Grass and Forage Science 66, 61–77.

Delahoy JE, Muller LD, Bargo F, Cassidy TW and Holden LA 2003. Supplemental carbohydrate sources for lactating dairy cows on pasture. Journal of Dairy Science 86, 906–915.

Edouard N, Hassouna M, Robin P and Faverdin P 2016. Low degradable protein supply to increase nitrogen efficiency in lactating dairy cows and reduce environmental impacts at barn level. Animal 10, 212–220.

Galloway JN, Townsend AR, Erisman JW, Bekunda M, Cai Z, Freney JR, Martinelli LA, Seitzinger SP and Sutton MA 2008. Transformation of the nitrogen cycle: recent trends, questions, and potential solutions. Science 320, 889–892.

Gomes J, Bayer C, de Souza Costa F, de Cássia Piccolo M, Zanatta JA, Vieira FCB and Six J 2009. Soil nitrous oxide emissions in long-term cover crops-based rotations under subtropical climate. Soil and Tillage Research 106, 36–44.

Haynes RJ and Williams PH 1993. Nutrient cycling and soil fertility in the grazed pasture ecosystem. Advances in Agronomy 49, 119–199.

Hristov AN, Ropp JK, Grandeen KL, Abedi S, Etter RP, Melgar A and Foley AE 2005. Effect of carbohydrate source on ammonia utilization in lactating dairy cows. Journal of Animal Science 83, 408–421.

Institute National de la Recherche Agronomique (INRA) 2007. Alimentation des bovins, ovins et caprins. Editions Quae, Versilles, France.

Intergovernamental Panel on Climate Change (IPCC) 2006. IPCC guidelines for national greenhouse gas inventories. Institute for Global Environmental Strategies, Hayama, Kanagawa, Japan.

Jantalia CP, Halvorson AD, Follett RF, Alves BJR, Polidoro JC and Urquiaga S 2012. Nitrogen source effects on ammonia volatilization as measured with semi-static chambers. Agronomy Journal 104, 1595–1603.

Johnson ACB, Reed KF and Kebreab E 2016. Short communication: evaluation of nitrogen excretion equations from cattle. Journal of Dairy Science 99, 7669–7678.

Lantinga EA, Neuteboom JH and Meijs JAC 2004. Sward methods. In Herbage intake handbook (ed. PD Penning), 2nd edition, pp. 23–52. The British Grassland Society, Maidenhead, UK.

Laubach J, Taghizadeh-Toosi A, Gibbs SJ, Sherlock RR, Kelliher FM and Grover SPP 2013. Ammonia emissions from cattle urine and dung excreted on pasture. Biogeosciences 10, 327–338.

Laubach J, Taghizadeh-Toosi A, Sherlock RR and Kelliher FM 2012. Measuring

Mosier AR 1989. Chamber and isotope techniques. In Exchange of traces gases between terrestrial ecosystems and the atmosphere: report of the Dahlem workshop (eds. MO Andreae and DS Schimel), pp. 175–187. Wiley, Berlin, Germany.

Mutsvangwa T, Davies KL, McKinnon JJ and Christensen DA 2016. Effects of dietary crude protein and rumen-degradable protein concentrations on urea recycling, nitrogen balance, omasal nutrient flow, and milk production in dairy cows. Journal of Dairy Science 99, 6298–6310.

Oenema O, Velthof GL, Yamulki S and Jarvis SC 1997. Nitrous oxide emissions from grazed grassland. Soil Use and Management 13, 288–295.

Petersen SO, Sommer SG, Aaes O and Søegaard K 1998. Ammonia losses from urine and dung of grazing cattle: effect of N intake. Atmospheric Environment 32, 295–300.

Reynolds CK and Kristensen NB 2008. Nitrogen recycling through the gut and the nitrogen economy of ruminants: an asynchronous symbiosis. Journal of Animal Science 86, 293–305.

Rochette P, Chantigny MH, Ziadi N, Angers DA, Bélanger G, Charbonneau É, Pellerin D, Liang C and Bertrand N 2014. Soil nitrous oxide emissions after deposition of dairy cow excreta in Eastern Canada. Journal of Environment Quality 43, 829.

Saarijärvi K, Mattila PK and Virkajärvi P 2006. Ammonia volatilization from artificial dung and urine patches measured by the equilibrium concentration technique (JTI method). Atmospheric Environment 40, 5137–5145.

Smith KA, Ball T, Conen F, Dobbie KE, Massheder J and Rey A 2003. Exchange of greenhouse gases between soil and atmosphere: interactions of soil physical factors and biological processes. European Journal of Soil Science 54, 779–791.

Sordi A, Dieckow J, Bayer C, Alburquerque MA, Piva JT, Zanatta JA, Tomazi M, da Rosa CM and de Moraes A 2014. Nitrous oxide emission factors for urine and dung patches in a subtropical Brazilian pastureland. Agriculture, Ecosystems and Environment 190, 94–103.

Steinfeld H, Gerber P, Wassenaar T, Castel V, Rosales M and De Haan C 2006. Livestock's long shadow: environmental issues and options. Food & Agriculture Org., Rome, Italy.

't Mannetje L 2000. Measuring biomass of grassland vegetation. In Field and laboratory methods for grassland and animal production research (eds. L 't Mannetje and RM Jones), pp. 151–177. CABI Publishing, Wallingford, UK.

Van der Weerden TJ, Luo J, de Klein CAM, Hoogendoorn CJ, Littlejohn RP and Rys GJ 2011. Disaggregating nitrous oxide emission factors for ruminant urine and dung deposited onto pastoral soils. Agriculture, Ecosystems and Environment 141, 426–436.

Van Groenigen JW, Kuikman PJ, De Groot WJM and Velthof GL 2005. Nitrous oxide emission from urine-treated soil as influenced by urine composition and soil physical conditions. Soil Biology and Biochemistry 37, 463–473.

Verite R and Delaby L 2000. Relation between nutrition, performances and nitrogen excretion in dairy cows. Annales de Zootechnie 49, 217–230.

Voglmeier K, Jocher M, Häni C and Ammann C 2018. Ammonia emission measurements of an intensively grazed pasture. Biogeosciences 15, 4593–4608.

Wachendorf C, Lampe C, Taube F and Dittert K 2008. Nitrous oxide emissions and dynamics of soil nitrogen under 15N-labeled cow urine and dung patches on a sandy grassland soil. Journal of Plant Nutrition and Soil Science 171, 171–180.

Yamulki S, Jarvis SC and Owen P 1998. Nitrous oxide emissions from excreta applied in a simulated grazing pattern. Soil Biology and Biochemistry 30, 491–500.

Zaman M, Saggar S, Blennerhassett JD and Singh J 2009. Effect of urease and nitrification inhibitors on N transformation, gaseous emissions of ammonia and nitrous oxide, pasture yield and N uptake in grazed pasture system. Soil Biology and Biochemistry 41, 1270–1280.