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Intake and digestion by wethers fed a tropical grass-based diet supplemented with increasing levels of canola meal

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ABSTRACT

Eight Polwarth × Texel wethers (31 ± 3.8 kg body weight (BW)), housed in metabolic cages and offered sudangrass (*Sorghum sudanense*) ad libitum, were used in a replicated 4 × 4 Latin Square experiment to evaluate the effects of increasing levels of canola meal supplementation on intake, digestibility, duodenal flow of N compounds and on N excretion. Four of the eight wethers were fitted with duodenal cannula. Treatments included no supplement (0) or daily supplementation with 5, 10 or 15 g/kg BW of a canola meal mixture (nine parts canola meal one part cracked corn grain). Forage dry matter (DM) intake decreased linearly and total DM, organic matter (OM), digestible OM and *N* intake increased linearly with increasing levels of supplementation. Supplementation did not affect neutral (NDF) or acid detergent fiber (ADF) digestibility whereas it tended to improve DM and OM digestibility. Fecal and urinary N excretion, as well as duodenal flow of α-amino N and non-ammonia non-microbial N increased linearly with increasing levels of supplementation. Rumen microbial protein flowing to duodenum and the efficiency of rumen microbial protein synthesis were not affected by treatments. The proportion of rumen degradable protein which was used for microbial protein synthesis decreased linearly with increasing levels of supplementation, but at a higher rate than was the reduction of the proportion of N intake which reached the small intestine as α-amino N. In conclusion, despite increasing N excretion and exerting a depressive effect on forage intake, supplementation with a high-degradable true protein source improves α-amino N and energy supply in ruminants fed tropical grass based-diets.

1. Introduction

Provision of supplementary feeds is usually required to achieve acceptable levels of production by ruminants fed tropical forage-based diets. However, although total OM intake is improved, supplementation with non-fiber carbohydrate-rich sources usually depresses forage intake and fiber digestibility in cattle (Chase and Hibberd, 1987; Delcurto et al., 1990; Sanson et al., 1990) and sheep (Kozloski et al., 2006). In other way, when N content of forage is relatively low, which could limit bacterial growth and activity, supplementation with degradable N compounds usually increases forage intake and digestibility (Moore et al., 1994; Paterson et al., 1994).

The impact of N supplementation depends on both level of supplementation and whether it is a non-protein or true protein source (Broderick and Reynal, 2009; Carro and Miller, 1999). Several in vitro studies showed rumen microbial growth and activity is stimulated by amino acids (Argyle and Baldwin, 1989; Van Kessel and Russell, 1996) or peptides (Eschenlauer et al., 2002). Moreover, although most free amino acids are fermented to ammonia and volatile fatty acids by rumen microflora (Barker, 1981), when high-soluble true protein sources are included in the diet, significant

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amounts of free amino acids and short-chain peptides, released in rumen fluid due to microbial proteolysis, may flow to the small intestine instead of be absorbed as ammonia from rumen (Volden et al., 2002). High-soluble true protein sources are widely used as supplementary feed in ruminant tropical grazing systems. However, they are usually mixed with other concentrate sources, the amount offered daily to animals is broadly variable (i.e., from approximately 1 to more than 15 g/kg BW) (Tambara, 2011), and their effect on the amount of ingested pasture is also variable and unpredictable. All these factors difficult conclusions on how much these supplements impact nutritional status of grazing animals.

The objectives of the present study were to investigate, in a controlled digestibility trial, the effects of increasing levels of a high-soluble true protein source supplementation on feed intake, energy and α-amino N supply and N utilization by wethers fed a tropical grass-based diet.

2. Materials and methods

All procedures in the study were conducted in accordance with the guidelines of the Animal Care and Ethical Committee of the Universidade Federal de Santa Maria.

2.1. Feedstuffs, animals, housing and experimental design

Eight Polwarth × Texel wethers (31 ± 3.8 kg BW), housed in metabolic cages and offered sudangrass (*Sorghum sudanense*) ad libitum, were used in a replicated 4 × 4 Latin Square experiment. Four of the eight wethers were fitted with duodenal cannula. Treatments included no supplement (0) or supplementation with 5, 10 or 15 g/kg BW/d with a mixture that included nine parts canola meal and one part of cracked corn grain. Cracked corn was included to improve palatability because animals did not ingest canola meal when it was offered as the sole supplementary feed. Wethers had free access to water and to mineral salt containing (g/kg): Ca: 100, P: 45, S: 4.12, Na: 205, Co: 0.025, Cu: 0.450, Fe: 1.5, I: 0.05, Mn: 1.0, Se: 0.009, Zn: 2.52 and F: 0.45. The chemical composition of forage and supplements are presented in Table 1.

Forage was harvested when the grass reached a height of 1.5 m. It was cut 10 cm above ground level, chopped (10–20 cm length) and stored at −20 °C until fed in each experimental period. Wethers were fed two equal meals daily at 0800 and 1700 h. Forage was offered in an amount that ensured that 100–200 g/kg of refusals remained for each wether. Supplement was fed at the same time but separately from forage and there were no supplement refusals in any of the experimental periods.

Each experimental period lasted for 15 d, with a 10 d adaptation and a 5 d measurement period. Feed offered was weighed and sampled daily from days 10 to 15 of each experimental period. Total feed refusals and feces were taken daily throughout the collection period and stored at −20 °C. At the end of each experimental period they were weighed and a sample (10% of wet weight from each sheep) was collected. All samples were oven-dried at 55 °C for at least 72 h and ground through a 1-mm screen (Willey mill, Arthur H. Thomas, Philadelphia, PA, USA) for subsequent chemical analysis. Urine was collected daily during the collection period, in buckets containing 100 mL of 7.2 N H_2SO_4. The volume of urine was measured and a sample of 10 mL/L was taken and diluted to 50 mL with distilled water and stored frozen (−20 °C) until analysis. On day 15, eight duodenal digesta samples (100 mL) were collected at 3-h intervals over a 24-h period, composited within animal and period and stored frozen (−20 °C). For analysis, these samples were thawed, an aliquot of the supernatant (10 mL) was taken and stored at −20 °C and the remaining was dried in a forced-air oven (55 °C) for 7 d and ground to pass through a 1-mm screen.

Table 1
Chemical composition of dietary components.

Item	Sudangrass	Canola meal	Corn
DM (%)	21.4	87.4	86.0
Composition (g/kg DM)			
OM	854	937	991
CP	113	444	87
NDF[a]	676	295	115
ADF[a]	455	234	23.7
Lignin	57	100	1.0
Ether extract	26	32.7	31.1
NFC[b]	84	212	766
ADIP[c] (% CP)	16.7	6.3	2.8

[a] Neutral detergent fiber (NDF) and acid detergent fiber (ADF) were not corrected for ash.
[b] Non-fiber carbohydrates (NFC)=OM−((N × 6.25))+EE+(NDF−(NDIN × 6.25)).
[c] Acid detergent insoluble protein.

2.2. Chemical analysis

Samples of feed, refusals, feces and urine were pooled on a 5 d basis within each animal and experimental period. Dry matter content was determined by drying at 105 °C for at least 8 h. Ash was determined after combustion at 550 °C for 3 h and OM by mass difference. Total N was assayed by a Kjeldahl method (Method 984.13; AOAC, 1997). The NDF analysis (assayed with heat stable α-amylase and expressed with residual ash) was based on the procedures described by Mertens (2002), except that the samples were weighed into polyester filter bags (porosity of 25 μm), and treated with neutral detergent in an autoclave at 110 °C for 40 min (Senger et al., 2008). Concentrations of ADF (expressed with residual ash) and sulphuric acid detergent lignin (ADL) were analyzed according to Method 973.18 of AOAC (AOAC, 1997). Analysis of acid detergent insoluble N (ADIN) and neutral detergent insoluble N (NDIN) were performed according to Licitra et al. (1996). Ether extract (EE) concentration was determined in a reflux system (Soxtherm 2000S 306M, Gerhardt; Königswinter, Germany) with ethyl ether at 180 °C for 2 h. The content of non-fiber carbohydrates (NFC, g/kg) was calculated as OM−[(NDF−(NDIN × 6.25))+(N × 6.25)+EE], according to Van Soest et al. (1991).

Duodenal supernatant samples were analyzed for ammonia N concentration following the procedures described by Weatherburn (1967). Dried duodenal samples were analyzed for purine bases (PB) concentration according

to Makkar and Becker (1999) as well as for α-amino N concentration. For this last analysis, a non-automated method was adapted from Palmer and Peters (1969) as follows. Approximately 0.1 g of dried duodenal samples were weighed into screw-cap tubes and treated with 2 mL of HCl 6 N at 110 °C for 24 h. After hydrolysis, 8 mL of 1.5 N NaOH was added into the tubes and the content diluted to 50 mL with distilled water. This solution was then filtered through filter paper and α-amino N concentration was analyzed colorimetrically as follows: 100 μL of filtrate was mixed into a test tube with 400 μL of distilled water, 1 mL of borate buffer (19.1 g/L of $Na_2B_4O_7 \cdot 10\,H_2O$, 0.05 M, pH 9.2) and 250 μL of 1 g/L of 2,4,6-trinitrobenzeno sulfonate (TNBS). The mixture was incubated in a water bath at 37 °C for 20 min. Thereafter, 500 μL of 1 N HCl was added to each test tube and absorbance was read at 420 nm (UV BEL Photonics 2000) against a distilled water blank. L-serine (100 mg/L) was used to make a standard curve that included test tubes containing 0.33, 0.66, 1.33 and 2.00 μg of α-amino N.

2.3. Calculations

2.3.1. Apparent digestibility of OM

Apparent digestibility of OM was calculated as follows: [OM intake (g/d) − fecal OM (g/d)]/OM intake (g/d).

2.3.2. True digestibility of OM

True digestibility of OM (OMTD) was estimated considering that neutral detergent soluble fractions of the feces are from endogenous origin and only the NDF fraction of feces is originated from feed (Van Soest, 1994) as follows: [OM intake (g/d) − fecal NDF (g/d)]/OM intake (g/d).

2.3.3. Microbial N supply

The amount of microbial nitrogen flowing to the small intestine was estimated based on the duodenal flow of PB, considering that the concentration of N in PB is 0.49, and the ratio of PB N: total N in mixed microbial mass is 0.116, according to Chen and Gomes, (1992).

2.3.4. Duodenal flow of DM and nitrogenous compounds

Duodenal flow of DM (g/d) was estimated based on lignin concentration in duodenal digesta and feces as follows: [fecal DM (g/d) × fecal ADL (g/kg of DM)]/duodenal lignin (g/kg of DM) (Porter and Singleton, 1971).

Duodenal flux of N compounds (g/d) was calculated multiplying their concentration in duodenal digesta (g/kg DM) by duodenal flux of DM (g/d). Ruminal degradability of dietary N (RDN) was calculated as 1 − [(duodenal N (g/d) − microbial N (g/d) − ammonia N (g/d))/N intake (g/d)].

2.4. Statistical analysis

Data was analyzed using the MIXED procedures (SAS, 2002, Inst. Inc., Cary, NC) following the model: $Y_{ijkl} = \mu + A_i + P_j + T_k + S_l + TS_{kl} + \varepsilon_{ijlkl}$, where Y is the dependent variable, μ is the overall mean, A is the random effect of the animal, P is the random effect of the period, T is the fixed effect of the treatment, S is the fixed effect of the Latin square, TS is the fixed effect of the treatment by Latin Square interaction and ε is the residual error. For ruminal digestibility data analysis, as only four cannulated wethers were used, the Latin Square effect was excluded from the model. Treatments effect was analyzed by linear and quadratic regression. As no quadratic responses were observed only the linear effects are indicated in tables.

3. Results

3.1. Intake and total tract digestibility

The Latin Square by treatment interaction was not significant for any variable. Forage DMI decreased ($P < 0.05$) whereas total DM, OM, NFC and digestible OM intake increased linearly ($P < 0.001$) with increasing levels of supplementation (Table 2).

Neither ADF intake nor total NDF intake was affected by treatments. However, forage NDF intake decreased linearly ($P < 0.05$) and supplement NDF intake increased linearly ($P < 0.001$) with increasing levels of supplementation. Supplementation did not affect DM, OM, NDF or ADF digestibility. The OMTD increased linearly with increasing levels of supplementation ($P < 0.05$).

Total N intake and N intake from supplement, as well as N apparent digestibility, fecal and urinary excretion of N and N retention increased linearly ($P < 0.001$) with increasing levels of supplementation (Table 3). True digestibility of N was not affected by treatments.

3.2. Ruminal digestibility and microbial protein synthesis

Duodenal flow of OM ($P < 0.05$), total N, α-amino N, ammonia N and non-ammonia non-microbial N (NANMN) ($P < 0.001$) increased linearly with increasing levels of supplementation (Table 4).

Rumen microbial N flowing to the duodenum, ruminal OM true digestibility, RDP and the efficiency of microbial protein synthesis (EMPS, g of microbial N/kg of rumen true digestible OM) were not affected by treatments ($P > 0.05$). The proportion of rumen degradable protein which was used for microbial protein synthesis linearly decreased ($P < 0.01$) with increasing levels of supplementation.

4. Discussion

4.1. Energy supply

The present experiment was designed to evaluate, in a controlled digestibility trial, what would be the nutritional status of grazing animals fed rumen degradable protein at fixed rates whereas pasture was offered ad libitum. In these situations, supplementation with concentrate usually exerts a positive effect on total feed intake despite exerting a substitutive effect on forage intake (Tamminga and Hoff, 1999). However, the effect of supplemental protein on forage intake, although variable (Figueiras et al., 2010), rarely is negative. Moore et al. (1994) based on data compiled from literature, reported that

Table 2
Effect of canola meal supplementation on intake and total tract digestibility of dry matter (DM), organic matter (OM), neutral detergent fiber (NDF), acid detergent fiber (ADF) and non-fiber carbohydrates (NFC) by wethers fed a sudangrass-based diet.

Item	Supplementation (g/kg BW)				SEM[a]	Significance[b]
	0	5	10	15		Linear
DM intake (g/d)						
Forage	531	437	413	326	52.0	*
Total	531	587	715	778	49.0	***
NDF intake (g/d)						
Forage	359	294	278	216	24.0	*
Supplement	0.00	41.7	83.6	125	3.28	***
Total	359	336	362	341	35.9	ns
Total intake (g/d)						
OM	462	527	651	717	46.0	***
ADF	239	229	248	237	13.6	ns
NFC	47.0	81.1	119	153	5.03	***
Digestible OM	344	389	502	565	38.8	***
Apparent digestibility						
DM	0.67	0.67	0.72	0.73	0.03	ns
OM	0.74	0.74	0.78	0.79	0.02	ns
ADF	0.61	0.56	0.59	0.55	0.01	ns
NDF	0.70	0.64	0.66	0.64	0.03	ns
OM true digestibility	0.76	0.77	0.81	0.83	0.02	*

[a] Standard error of means where $n=8$ per treatment.
[b] *$P<0.05$, **$P<0.01$, ***$P<0.001$, ns=not significant.

Table 3
Effect of levels of canola meal supplementation on N intake, total tract digestibility and retention by wethers fed a sudangrass-based diet.

Item	Supplementation (g/kg BW)				SEM[a]	Significance[b]
	0	5	10	15		Linear
N intake (g/d)						
Supplement	0.00	9.83	19.7	29.5	0.77	***
Total	9.72	18.2	27.5	35.8	1.47	***
N excretion (g/d)						
Fecal	2.14	3.30	4.05	4.41	0.29	***
Urinary	4.90	7.41	12.4	14.6	1.16	***
N total tract digestibility						
Apparent	0.74	0.81	0.85	0.87	0.01	***
True	0.94	0.94	0.94	0.95	0.00	ns
N retention (g/d)	3.48	7.49	11.0	16.7	1.31	***

[a] Standard error of means where $n=8$ per treatment.
[b] *$P<0.05$, **$P<0.01$, ***$P<0.001$, ns=not significant.

supplemental protein shows the potential to increase voluntary intake only of forages having digestible energy (DE):crude protein (CP) ratios (kcal/g) higher than 31 (i.e., deficit of N relative to DE). In the present study, the ratio DE:CP of sudangrass, estimating DE from digestible OM intake (i.e. digestible OM intake $\times$ 4.409) was 24. Thus, although a positive impact was not expected, there is not a clear explanation for the substitutive effect of supplement on sudangrass intake. Level of NDF intake generally has been accepted as the primary restrictive determinant of intake in forage-based diets, as it is directly related to physical distension of the reticulo-rumen (Van Soest, 1994). Once total NDF intake was similar for all treatments, rumen fill was probably the major factor affecting feed intake by wethers in the present study. Moreover, although filling effect depends on particle size and lignin content (Colluci et al., 1990; Van Soest, 1994), our results suggest that NDF from either canola meal or forage showed similar rumen filling effect.

Digestible OM intake increased 13–65% compared to the control treatment when supplement was offered at a rate of 5–15 g/kg of BW, respectively. This was a result mainly of an increase in OM intake instead of an increase in OM digestibility. Using a meta-analysis approach based on published data with lactating dairy cows fed silage based-diets, Nousiainen et al. (2009) reported a small positive effect of supplementary protein sources such as canola meal, soybean meal or fishmeal on diet

Table 4
Effect of levels of canola meal supplementation on duodenal flow of OM and N compounds, ruminal digestibility of OM and feed N and efficiency of rumen microbial protein synthesis by wethers fed a sudangrass-based diet.

Item	Supplementation (g/kg BW)				SEM[a]	Significance[b]
	0	5	10	15		Linear
Duodenal flow of OM (g/d)	152	183	205	240	18.2	*
Duodenal flow of N compounds (g/d)						
Total	5.9	9.3	12.5	15.2	0.98	***
α-Amino	3.67	6.54	8.07	9.7	0.69	***
Ammonia	0.29	0.65	1.27	1.44	0.11	***
Microbial	3.03	4.08	4.20	4.80	0.48	ns
NANMN[c]	2.30	4.57	7.02	8.95	0.25	***
Ruminal OM true digestibility	0.70	0.69	0.72	0.71	0.02	ns
Ruminal degradability of feed N	0.70	0.73	0.73	0.74	0.01	ns
EMPS[d]	13.0	15.1	11.9	11.2	1.99	ns
ERNU[e]	0.65	0.32	0.23	0.18	0.08	**

[a] Standard error of means where $n=8$ per treatment.
[b] $^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$, ns = not significant.
[c] Non-ammonia, non-microbial N.
[d] Efficiency of microbial protein synthesis, g microbial N/kg of rumen apparent digestible OM.
[e] Efficiency of ruminal N use, g of bacterial N/g of rumen degradable N.

digestibility. This response tended to be quadratic with increasing levels of supplementation, with predicted maximum digestibility at a dietary CP concentration of 220 g/kg of DM. In our study with wethers, diet CP concentration varied from 113 to 287 g/kg of DM. However, instead a quadratic effect it tended to produce a linear effect on apparent OM digestibility.

Fiber digestibility is inversely related to both lignin concentration and digesta passage rate from the rumen (Mertens, 2005; Van Soest, 1994). The ADL content of sudangrass was 4-fold lower than of concentrate (84 vs. 339 g/kg of NDF) and NDF intake from forage decreased at increasing levels of supplementation. Surprisingly, NDF digestibility was not affected by treatments in the present study. It is likely that the passage rate of particles throughout the gastrointestinal tract may have been reduced with increasing proportions of concentrate in the diet (Fachney, 2005; Fox et al., 2004). In this situation, rumen retention time of feed particles could have increased, allowing fiber degradation to increase to such an extent that it compensated for the higher ADL content in supplement.

4.2. α-Amino N supply

As expected, total N intake, as well as fecal and urinary N excretion and N retention increased with increasing levels of supplementation. However, the N apparent digestibility and retention means observed at the highest supplementation level were above the expected and seems to be unreliable. The N true digestibility was not affected by treatments and averaged 0.95, indicating that a very low proportion of fecal N was originated from feed. The difference between N true and apparent digestibility decreased from 0.20 to 0.08 at increased levels of supplementation. It is not clear why such variation occurred. Most RDP not captured by rumen bacteria is assumed to be absorbed as ammonia and partly excreted as urea in urine. The amount of N excreted in urine as proportion of RDP absorbed as ammonia (i.e. RDP which was neither captured by rumen bacteria nor reached the small intestine as ammonia) decreased from 1.40 at low N intake to 0.72 for the highest level of N intake.

These results indicate that blood urea pool and N recycling through blood and the lower gastrointestinal tract may have significantly increased at higher levels of N intake (Harmeyer and Martens, 1980; Lapierre and Lobley, 2001), increasing fecal excretion of volatile N compounds. These compounds may have volatilized during sample drying underestimating fecal N excretion.

Amino acids available for absorption in the small intestine of ruminants are derived from rumen microbial, undegraded feed and endogenous protein sources. Microbial protein reaching the duodenum is regulated by the quantity of OM fermented in rumen and by the EMPS, provided that adequate supplies of degradable N and other nutritional factors such as sulphur are not limiting (Bach et al., 2005; Owens and Isaacson, 1977; Stern and Hoover, 1979). The amount of OM fermented in the rumen increased from 285 g/d in control treatment to 411 g/d when supplement was offered at a rate of 15 g/kg BW. However, unlike expected, neither the EMPS nor the amount of microbial N flowing to duodenum were significantly modified with supplementation. The lack of statistical significance for treatment effect on these variables may have been due to its inherent high variability. For example, the coefficients of variation for microbial N flow and EMPS were 31% and 35%, respectively, whereas for apparent OM digestibility it was only 6.2%. Despite this, the EMPS was on average 13 g of microbial N/kg of fermented OM, which is within the range of values reported in literature (Owens et al., 2009; Reed et al., 2007).

Although endogenous N was not accounted for duodenal flow calculations, RDP was high and similar for all treatments (i.e., on average 0.72). Thus, as consequence of increased N intake, duodenal flow of NANMN increased linearly with increasing levels of supplement. Moreover, as shown in Fig. 1, whereas the proportion of ingested N

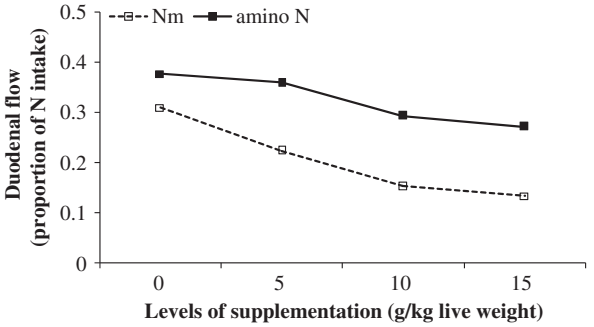

Fig. 1. Duodenal flow of microbial N (Nm) and α-amino N (amino N) as proportion of N intake in wethers fed sudangrass supplemented with levels of canola meal.

flowing to duodenum as microbial N decreased 58% (i.e., from 0.31 to 0.13) the duodenal flow as α-amino N decreased only 27% (i.e. from 0.37 to 0.27). These results indicate that at increased levels of RDP intake, the proportion of duodenal α-amino N supply from undegradable feed protein increased.

4.3. Efficiency of N utilization

The efficiency of ruminal N use, calculated as the proportion of RDP which was used for microbial protein synthesis, linearly reduced with increasing levels of supplement. Bach et al. (2005) reported that under optimal ruminal conditions (i.e. maximum N capture and maximum efficiency of energy use by microorganisms) bacteria could capture 69 g of N/100 g of available N. In our study, measured as microbial N reaching the duodenum, ruminal bacteria were able to capture 0.65 of ingested RDP in animals receiving only sudangrass and an average of 0.24 when supplement was supplied. This difference was due to the fact that while OM fermented in the rumen, which is the primary factor affecting bacterial growth, increased only 44% (i.e., 285 vs. 411 g/d), RDP intake increased 300% (i.e., 6.6 vs. 26.5 g/d) due to supplementation.

It is recognized that excessive N input and deposition on a local ecosystem may negatively affect water quality and, at a global level, contribute for greenhouse gas emissions (Steinfeld et al., 2006). Nonetheless, similar levels of N excretion and deposition may have different environmental effects depending on the ecosystem (Oltjen and Beckett, 1996). For example, oilseed cultures are the most important commodities in Brazil whereas most of its pasture soils are deficient in N availability. In these grazing production systems, increasing N input as supplemental oilseed by-products instead negative could have a positive impact on their productivity and sustainability. Systemic and long term studies needs to be carried out to test this hypothesis.

5. Conclusion

Supplementation with a high-rumen degradable true protein source shows the potential to improve both energy and α-amino N supply for ruminants fed a tropical grass based-diet, although it reduces forage intake and increases N excretion.

Conflict of interest statement

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.

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