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Nitrogen balance in Holstein steers grazing winter oats: effect of nitrogen fertilization

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Suggested short title.

Nitrogen balance in grazing steers.

Summary text for the Table of Contents.

A more detailed understanding of N balance in grazing steers is needed to improve N utilization in animals, reducing N losses to the environment and potential for environmental pollution. N fertilization of forage is a common management in Argentina, leading to modifications of the chemical composition of consumed diet, and hence, modifications in the animals. Steers grazing fertilized winter oats had greater N intake, N retention and ADG than steers grazing non-fertilized oats. Also, the steers grazing fertilized oats had greater N excretion, predominantly urinary N. These findings are part of the onset of N cycle in grazing management situations. Due to higher N excretion, it is important to study the environmental impact of animal depositions grazing fertilized oats.

Abstract. The present study evaluated the effect of nitrogen (N) fertilization of winter oats on whole-animal N balance (N intake, N excretion in urine and feces, N retention), partition of urinary N (purine-N derivatives and urea-N) and average daily gain (ADG) in grazing steers. The experimental area was divided in two plots (10 steers/plot), and samples were obtained in two periods (1 plot/period).The experimental area was divided in 2 plots, and each plot in 10 strips. Twenty Holstein steers (161.3 ± 7 kg of initial body weight) grazed, for 51 days, individual strips of fertilized (100 kg N/ha; N100) and non-fertilized (N0) winter oats during daylight (10 h/d). The daily individual grazing paddock was adjusted to offer 6 kg DM of green leaf·100 kg $BW^{-1}\cdot d^{-1}$. Chemical composition of the herbage and N diurnal variation were estimated by collecting 3 samples per paddock at 8:30, 13:30 and 18:30 h, twice on each sampling period. Forage intake and *in vivo* digestibility were estimated by the *n*-alkane technique. Individual N intake was estimated using *n*-alkane data, the ingestive behavior data and the diurnal variation of the chemical composition of the forage. N fertilization increased N content ($P<0.01$; N0=11.4% CP vs. N100=13.9% CP) and decreased the water soluble carbohydrate content (P<0.01; N0=21.1% vs. N100=16.8%) in the forage, but did not modify herbage mass or the DM content. Dry matter intake (4.72 kg DM/d), water intake (7.57 L/d) and DM digestibility (67%) were not affected by N fertilization. However, N intake and N digestibility were higher in N100 than in N0 (20 vs. 7 g N/d). While treatments had similar fecal N excretions (aerage 45.4 g N/d), there was a trend to increase urinary N excretion with N intake (P=0.08; N100=53.3 vs. N0=47.5 g N/d), a trend to increase N-allantoin excretion (P=0.11; N100=3.18 vs. N0=2.91 g/d) and an increase in urea-N excretion (P<0.01; N100=30.7 vs. N0=23.8 g/d). Increasing N intake led to greater N retention (P<0.02; N100=37.9 vs. N0=20.9 g N/d) and ADG (P<0.03; N100=860 vs. N0=698 g/d). These results suggest that fertilizing winter oats with 100 kg N/ha improves N retention and ADG in young steers under grazing conditions.

Keywords: nitrogen balance, grazing steer, nitrogen fertilization, *Avena sativa*

Introduction

Important economical and environmental reasons exist to reduce nitrogen (N) losses and improve utilization in cattle. Intensification of ruminant production has resulted in greater output of milk and meat, which is made possible, in part, by input of N fertilizers and protein-rich feeds (Dijsktra *et al*., 2011). Excess of N in the diet leads to high levels of N in urine and feces, contributing to environmental pollution, as ammonia (NH_3), nitrous oxide (N_2O) and nitric oxide (NO) lost to the atmosphere (greenhouse effects) and as nitrate (NO_3^-) in the soil and ground water. To increase our knowledge in whole-animal N metabolism under grazing conditions would allow new strategies to improve nutrient retention efficiency and animal production, and hence, minimize N excretion to the environment.

In Argentina, use of winter forages is well established in grazing systems, most of them being fertilized with N, mainly as urea. The effect of N fertilization on forages depends on the timing of fertilization and our ability to quantify it on forage sampling methodology (Wilman, 1980). Across short periods (< 20 d), increases in N content and decreases in water soluble carbohydrates (WSC) in all morphological components of the forage can be detected (Wolf and Opitz von Boberfeld, 2003). In periods of 25 or more days, N fertilization can reduce the lamina:pseudostem ratio and increase pasture height and DM production (Wilman, 1980; Delagarde *et al*., 1997; Wolf and Opitz von Boberfeld, 2003). Chemical and morphological modifications may affect DM intake, DM digestibility (DMD), and therefore, animal production. Effects of N fertilization on voluntary DM intake are not consistent; Delagarde *et al.* (1997) observed a greater DM intake in cows grazing fertilized pastures than in cows grazing non fertilized pastures. Peyraud *et al.* (1997) found no difference in DM intake between cows eating fertilized and non fertilized pastures. Demarquilly (1970) found variable results in several comparisons, while Ferri *et al.* (2004) observed a lower DM intake in animals eating winter pastures with higher levels of N fertilization.

Several authors showed a positive effect of N fertilization on animal production. In this scenario, Delagarde *et al*. (1997) observed higher milk yield in cows on fertilized pastures and Archibeque *et al*. (2001) found greater N retention in steers eating forage with high levels of N fertilization.

Although different levels of N fertilization seems not to affect DMD (Van Vuuren *et al*., 1991; Gabler and Heinrichs, 2003; Ferri *et al*., 2004; Knowlton *et al*., 2010), modifications of N and WSC concentrations could diminish the efficiency of N use of rumen microorganisms (Archibeque *et al*., 2001; Marini and Van Amburgh, 2003). This could be attributed to the relative high crude protein (CP) concentration on highly fertilized pastures and an imbalance with energy available in rumen (Hoekstra *et al*., 2007), leading to accumulation of NH_4^+ in rumen, which is absorbed through gastrointestinal tract and converted in urea in the liver. The fate of urea in peripheral blood (i.e., urinary excretion or return to gastrointestinal tract) depends partly, on diet characteristics. In diets with levels of N that exceed the animal requirements or with an energy-N rumen imbalance, the main fate of urea will be urinary excretion (Marini and Van Amburgh, 2005).

Although numerous studies on N balance have been conducted under housing conditions, to our knowledge there is no data from growing cattle where different techniques such as *n*-alkane technique, ingestive behavior, sward defoliation and total urine collection were used to estimate N balance under grazing conditions. The objective of the present study was to evaluate the effect of N fertilization of winter oats (*Avena sativa*) on a whole-animal N balance (N intake, N retention, N excretion in urine and feces), urinary N partition (DP-N and urea-N excretion in urine) and ADG in grazing steers.

Materials and methods

The study was conducted during late-winter to early-spring of 2009 (4 September to 28 October) at the facilities of Facultad de Ciencias Veterinarias de la Universidad Nacional del Centro de la Provincia de Buenos Aires (FCV – UNCPBA; 37º19´S, 59º07´W) in Tandil, Argentina. Twenty Holstein-Friesian steers (161 ± 7.0 kg of initial BW) grazed, in individual paddocks, winter oats (*Avena sativa* cv. Calén) sown on May, 2009. The study lasted for 51 d, divided into a pre-experimental period of 11 d, and an experimental period of 40 d. Procedures were conducted according to Council Directive 2010/63/EU guidelines on the protection of animals used for experimental and other scientific purposes.

Experimental design and treatments

Prior to the beginning of the experiment, the grazing area of the experimental period (180 x 100 m) was divided in 2 plots (plots A and B: 90 x 100 m), and then each plot was divided in 10 strips (90 x 10 m) using a double-wire electric fence. Thirty days before the beginning of the experiment, odd strips of plots A and B were fertilized by hand with 100 kg N/ha (230 kg urea/ha), leaving 1 m on each side without fertilizer.

Steers were stratified by initial BW and then randomly assigned to one of each treatment (N100=fertilized; N0=non-fertilized). During the pre-experimental period (d 1 to 11), steers grazed in groups according to their treatment in 2 adjacent areas (one fertilized with 100 kg N/ha) for animal adaptation to diet. On d 11, steers within treatment were randomly assigned to plots A or B and within each plot to individual strips where they remained until the end of the experiment. The experimental period was divided in two sampling periods: period I (PI; from d 22 to 30) when steers from plot A were sampled, and period II (PII; from d 31 to 39) when steers from plot B were sampled.

Steers grazed a new paddock every day from 9 to 19 h, and then were individually stalled from 19 to 9 h in a grass-free paddock with access to water (individual troughs). The daily paddock was offered in two sub-paddocks at 9 h (AM; morning sub-paddock) and 14 h (PM; afternoon sub-paddock). Herbage allowance was, approximately, 6 kg DM of green leaf·100 kg $BW^{-1}\cdot d^{-1}$. Size of the daily strips was determined according to the herbage mass of each strip and individual BW.

Herbage measurements

Herbage mass. For herbage mass, 9 randomly selected samples of herbage within each individual paddock were clipped to ground level using quadrats of 0.05 m^2 on d 1, 14, 25 and 32. Samples taken on d 25 and 32 were manually separated into lamina and pseudostem. Samples were oven-dried at 100ºC.

Chemical Composition. For chemical composition and diurnal variation, 3 randomly selected samples of 5 ungrazed tillers were collected at 8:30, 13:30 and 18:30 h on d 29 and 31 for PI and d 37 and 39 for PII, on each individual paddock cutting to ground level.

Immediately, samples were frozen in the field with liquid N and stored at -20ºC until further analysis. Prior to analysis, samples were separated in lamina and pseudostem.

Sward surface height. Sward surface height (SSH) was measured in AM and PM sub-paddocks at entry time and every 150 min (i.e., 9:00, 11:30 and 14:00 for AM and 14:00, 16:30 and 19:00 h, for PM sub-paddocks, respectively) to determine intensity of defoliation. Intensity of defoliation was determined by difference between pre and post-grazing SSH. Sward height was taken using a sward stick detailed in Nadin *et al*. (2012). Measurements were performed on d 29 and 31 for PI and d 37 and 39 for PII.

n-Alkane content of consumed forage. On d 29 (PI) and d 37 (PII), 3 herbage samples from each strip were taken and then pooled by treatment. Samples were randomly collected by hand-plucking, considering the mean post-grazing height of the paddock grazed on the previous day. Pooled samples were manually separated in lamina and pseudostem; subsamples were oven-dried at 60ºC for 48 h and then finely grounded with an electrical coffee grinder (Connoisserve CG 700, Kin Hip M & P Factory Ltd., Kowloon, Hong Kong). Subsamples were stored at room temperature until *n*-alkane analysis was performed. The remaining fresh material was dried at 100°C until constant weight, and the proportion of each component in the grazed horizon was estimated.

Animal Measurements

DM intake and fecal output. Forage DM intake and fecal output (FO) were estimated by the *n*-alkane technique. From d 22 to 30 (PI) and d 31 to 39 (PII) and before entering to a new sub-paddock, the steers were orally dosed with cellucotton stoppers (34.5 x 22 mm Carl-Roth GmbH and Co KG, Karlsruhe, Germany) containing dotriacontane (C_{32} - 120 mg/pellet) and hexatriacontane (C_{36} - 70 mg/pellet; Sigma-Aldrich, Aldrich Chemical Co, Gillingham, UK). Each dose was prepared by pippeting a controlled amount of 2 solutions of C_{32} and C_{36} in *n*-heptane, respectively, into each cellucotton stopper. During the last 4 d of *n*-alkane dose, individual daily feces were collected thrice a day from field depositions (ca. 5 g/deposition) and pooled by animal. Once sampled, feces in the field were identified by paint. Pooled samples were oven-dried at 60ºC for 48 h, grounded with an electrical coffee grinder and stored at room temperature until *n*-alkane analysis was performed.

Water intake. During the last 4 d of each sampling period, individual water intake was measured by difference between offered and refused water.

Ingestive behavior. To determine total grazing time and the temporal distribution of grazing events, the IGER behavior recorder (Rutter *et al*., 1997) was used. For this purpose, 2 recordings of 10 h for each animal were obtained during the 4 d of sampling period.

Urine collection. During the 4 d of sampling period, total urine collection was performed using harnesses containing commercial diapers with a wide-spectre antimicrobial agent as a preservative. Harnesses were changed 4 times a day at 8:30, 13:30, 18:30 and 0:00 h. Once the harnesses were removed, total urine was weighed and aliquots (~100 mL) of urine were immediately taken, acidified (pH<3) with 10% H_2SO_4 (vol/vol) and stored at -20ºC until further analyses.

Body weight. The animals were weighed on d 1 and d 51 after 48 h of fasting (steers had access to water for the first 24 h), with an electronic weighing scale (sensitivity: 50 g; Challenger SC 101; Balcoppan; Sistemas Coppan S.R.L.; Argentina).

Analyses and Calculations

For the purpose of this experiment, the N balance was calculated by difference between N intake and N excretion in urine and feces, and the N use efficiency was estimated as the retained proportion of N intake. The N content in herbage, feces and urine were performed by Kjeldahl (Nelson and Sommers, 1973) and WSC content in herbage was measured by the anthrone method (Morris, 1948).

n-Alkane analysis was carried out on morphological components of the forage, fecal samples and cellucotton stoppers. For the *n*-alkane analysis, all samples were oven-dried at 60ºC for 48 h and analyzed according to the method of Dove and Mayes (2006) with modifications (Sánchez Chopa *et al*., 2012). All calculations were performed according to the equations presented by Dove and Mayes (2006), using ratio C_{32}:C_{33} for DM intake, C_{36} for FO and their ratio for DMD estimation.

Recordings obtained from the ingestive behavior recorder were analyzed using software “Graze 8.0” (Rutter, 2000). Total grazing time was determined and temporal distribution of grazing events were divided in 4 periods of 2.5 h each, and grazing time within each period was analyzed individually.

Nitrogen intake was calculated individually according to DM intake and N content of the forage. For this purpose, N diurnal variation in the forage and moment and duration of each grazing event along the day, were taken into account. *In vivo* N digestibility was estimated individually according to N intake and N fecal excretion.

Urine was analyzed for N-allantoin (Chen *et al*., 1993) and N-urea (Marsh *et al*., 1965). Absorbed microbial purines (X, mmol/d) derived from urinary excretion of N-PD (Y, mmol/d) was estimated from the equation presented by Kahn and Nolan (2000), where Y= $0.94X + 0.385 * BW^{0.75}$. Microbial N entering duodenum (g/d) was estimated from the equation (Chen and Ørskov, 2004):

$$MN\ (g/d) = \frac{X(mmol/d) * 70}{0.116 * 0.83 * 1000}$$

where, MN is microbial nitrogen flux; 70 is the N content of purines (mg N/mmol); 0.116 is N-PD:total N ratio in ruminal microorganisms (11.6:100); and 0.83 is microbial purine average digestibility.

Statistical Analysis

All data were analyzed by ANOVA using the General Linear Model (GLM) procedure of SAS Institute (2010) according to a complete random design. Classes included in the model were treatment, period and their interaction. The general form of the model used was:

$$Y_{ij} = \mu + T_i + P_j + e_{ij}$$

Where, Y_{ij} is the observation on the ith treatment in jth Period; μ is the overall mean; T_i is the effect due to ith treatment; P_j is the effect due to jth period; e_{ij} is random error. Significant differences between mean values were tested using the Duncan´s multiple range test. Differences among means with $P < 0.05$ were accepted as representing statistically significant differences and tendencies were accepted if $0.05 < P < 0.10$.

Results

Herbage Measurements

All data are presented in Table 1. Herbage mass was not affected by N fertilization, nor forage structural characteristics. Although no differences were found for initial SSH and post-grazing SSH, the intensity of defoliation was higher for N100. The actual consumed forage was considered to be similar for both treatments and had an overall lamina:pseudostem ratio of 7:3. Nitrogen fertilization increased total N content and decreased total WSC content of *Avena sativa*, leading to a higher CP:WSC ratio for N100, not only in offered diet, but also in observed consumed forage.

(Insert Table 1 here)

Within each sampling hour and independently of the morphological component, CP was higher and WSC was lower in N100 than in N0 (Table 2), except for WSC content in N100 lamina collected at 18:30 h, which it was 17% numerically lower compared to WSC content in N0 lamina. According to N and WSC diurnal variation within each treatment, N content of N100 lamina decreased along the day. No differences were found for N content in N100 pseudostem nor N0 lamina and pseudostem, as well as for WSC content in both treatments.

(Insert Table 2 here)

Animal Measurements

Total grazing time was not affected by fertilization, and also no differences were observed in grazing time between treatments in the 4 periods in which recordings were divided (Table 3). N fertilization did not affect DM intake nor water intake. Apparent *in vivo* DMD was similar for both treatments, although it differed between sampling periods. Also, N fertilization increased apparent *in vivo* N digestibility (Table 4). To estimate N and WSC intake, mean values of N and WSC forage concentration estimated at 8:30, 13:30 and 18:30 h of each individual strip were used, since no differences in grazing time and grazing defoliation intensity –estimated by differences between initial and final SSH- between M and A were observed.

(Insert Table 3 here)

Nitrogen balance (N intake, N excretion in urine and feces and N retention) is presented in Table 4. Nitrogen intake and N retention, expressed either as g/d or g $N/BW^{0.75}$·d, were greater for N100 than N0, as well as the N use efficiency. Due to greater N intake and N digestibility in steers grazing fertilized oats, the apparent digestible N actually consumed was 33% higher in N100 than in N0. Although no differences were found for total

N excretion, urinary N excretion tended to increase with N fertilization. When N partition (urine, feces and retention) was expressed as N intake ratio, urine N remained similar (PN0=41.8%; N100=39.0%), fecal N was higher for N0 (N0=39.8%; N100=33.3%) and retained N was significantly higher for N100 (N0=18.4%; N100=27.7%).

Urinary urea-N excretion linearly increased with total urinary N excretion and represented 50 and 58% of total N excretion in urine for N0 and N100, respectively. Steers on N100 excreted more urea-N, tended to excrete more allantoin-N, while no differences were observed for non ureic-N. A trend to increase estimated microbial N flow to duodenum with N fertilization was observed (N0=47.0 g N/d; N100=51.4 g N/d). Rumen microbial N efficiency (microbial N flux to duodenum:N intake ratio) did not differ between treatments. Apparently digested N was greater for N100, not only in absolute values (N0=68.4 g N/d; N100=91.2 g N/d) but also when expressed on metabolic weight basis (g N/kg $BW^{0.75}$; N0=1.37; N100=1.79). Retention efficiency of apparently digested N also increased (N0=29%; N100=40%) with N fertilization. In agreement with the results obtained for N retention, ADG was higher in animals grazing fertilized winter oats (Table 4).

(Insert Table 4 here)

Discussion

No signs of discomfort were observed and the steers adapted well to the experimental protocol. To our knowledge, this is the first N balance performed under grazing conditions. To estimate and quantify a whole body N balance supposes reliable and precise estimates of N intake and N excretion. Research indicates that N balance under grazing conditions is affected by errors in measuring either N intake or N output, which generally results in an overestimation of retained N. Underestimation of fecal and/or urinary N artificially increases N retention, and therefore N use efficiency as seen in the present experiment.

When estimating ADG from N retention converted into CP by a coefficient of 6.25 (i.e., assuming a body protein N content of 16%), a protein gain of 130.6 g/d for N0 and 236.9 g/d for N100 is obtained and, as body protein is associated with water in an average ratio of 1:3, and that the ratio of fat:CP deposition is in average 1:1 (NRC, 2001), the calculation of a lean tissue gain of about 0.7 and 1.26 kg/d for N0 and N100, respectively. When comparing these equations with actual ADG, N100 is not consistent with the measured N balance, suggesting an overestimation of N use efficiency. Nevertheless, the N retention of N100 was higher than N0, because of the confidence placed in measured ADG.

Some possible errors of this overestimation are detailed. Fecal N could be underestimated due to volatile losses of ammonia from feces in the field (Spanghero and Kowalski, 1997) and/or during the drying of samples (Sharkey, 1970). Another source of error may be the N loss from the urine collection, due to the use of a wide-specter antibiotic as a preservative, instead of using strong acids (H_2SO_4) *in situ* so as to prevent N losses (Spanghero and Kowalski, 1997). We conclude that N balances are overestimated and that the error appears to be enhanced as the dietary N availability increases. In this experiment, applying any equation proposed by Spanghero and Kowalski (1997) to correct N balance did not modify the observed effect of N fertilization upon N balance.

Another explanation could be related to total urine excretion. There is an increase in total urine excretion in animals fed high levels of dietary N, possibly due to the need for greater urine volume to excrete the excess of N (Knowlton *et al*., 2010). Although N100 had greater N intake, total urine excretion (L/d) remained similar between treatments, in accordance with Reynald and Broderick (2005). To help to support this idea, no signs of urine loss were found each time the harnesses were changed and, within animal, the daily CV of urine excretion was between 5 and 15%, except for 2 animals of N100 (26 and 29%, respectively). What is more, the difference in N excretion in urine (12%) is not as high as observed by Knowlton et al. (2010), and could possibly explain the lack of difference in total urine excretion. Another possible explanation is that N retained may not have been transferred to lean tissue but have increased the whole-body urea pool (Archibeque *et al.*, 2001, 2002).

In the present experiment, N fertilization did not significantly affect any of the forage morphology variables studied, although it increased the N content and decreased the WSC content of lamina and pseudostem, which is in agreement with several authors (Delagarde *et al*., 1997 and Peyraud *et al*., 1997 in *Lolium perenne* L.; Mazzanti *et al*., 1997 in *Avena sativa*; Ferri *et al*., 2004 in *Secale cereale*). This chemical variation led to an increase of 58% in CP:WSC ratio of the offered forage. According to Peyraud and Astigarraga (1998), an increase in CP content of 10 g/kg DM would be related to a decrease of 8 to 10 g/kg DM in WSC content, similar to the results of the present experiment (7.5 g/kg DM of WSC). Because N fertilization did not affect the morphological components studied, and taking into account that offered forage (6 kg DM lamina/100 kg BW) did not limit DM intake, results in N balance could be attributed to effects of fertilization on forage chemical composition.

When considering the effect of N fertilization on DM intake in grazing animals, conflicting results exist. Demarquilly (1970) observed different effects of N fertilization on DM intake in 32 trials. When comparing N fertilized against non-fertilized pastures, the author reported similar, higher and lower DM intakes on fertilized pastures than non-fertilized pastures in 13, 11 and 8 trials, respectively. What is more, Delagarde *et al*. (1997) observed higher DM intake in lactating cows grazing fertilized grass (*Lolium perenne* L.; 60 kg N/ha) compared with non-fertilized grass, while Ferri *et al*. (2004) observed lower intake of fertilized rye pasture (*Secale cereale* L.) compared to non-fertilized rye pasture, in housed rams. In the present experiment, N fertilization had no effect on DM intake, in agreement with results obtained by Peyraud *et al*. (1997) and Archibeque *et al*. (2001).

Total grazing time did not differ between treatments, in agreement with Delagarde *et al*. (1997). This could be due to no effects of N fertilization in herbage mass, herbage structure nor DM content, variables related with modifications of animal ingestive behavior (Nadin *et al*., 2012). The grazing management performed in this study –access to new sub-paddock twice a day and access to forage for 10 h- could have contributed to the similar grazing times.

Nitrogen fertilization increased N content of the forage and hence N intake, but had no effects on apparent DMD , as seen by Gabler and Heinrichs (2003), Ferri *et al.* (2004) and Knowlton *et al*. (2010). On the other hand, greater N intake increased N digestibility, since fecal N excretion remained similar between treatments (Demarquilly, 1970; Delagarde *et al*., 1997; Marini and Van Amburgh, 2003, 2005; and Knowlton *et al*., 2010). Increasing N intake increased excretion of urine urea-N, as seen by several authors (Delagarde *et al*., 1997; Peyraud *et al*., 1997; Marini and Van Amburgh, 2003, 2005), while only a trend to increase the total-N excretion in urine. Urinary urea-N excretion was not a reliable indicator of N inefficiency use since the ratio of N-retained:N-intake was greater in N100 compared to N0.

N-allantoin excretion in urine tended to be greater in N100, indicating a trend towards more microbial capture and more microbial N flow to duodenum compared with N0. This observation was not expected. Considering that the apparent digestible DM intake remained similar between the treatments and that the diet consumed by N0 had a different CP:WSC ratio (N0=0.87; N100=1.34), a greater microbial N flow to duodenum was expected for N0 because of a better ruminal N to energy balance (Hoekstra *et al*., 2007; Reynolds and Kristensen, 2008). Nevertheless, the trend of more microbial N flow to the duodenum is in accordance with greater ADG for N100 than N0.

The lack of data on N balance in grazing beef cattle makes it difficult to discuss the results. A summary of results obtained by several authors in penned animals are shown in Table 5. There is no clear evidence of what to expect in N use efficiency as N intake increases. While some authors observed an increase in N use efficiency with an increase of N intake (Knaus *et al*., 1998; Archibeque *et al.*, 2002; Wickersham *et al*., 2008; Knowlton *et al*., 2010), others observed a decrease in N use efficiency (Archibeque *et al*., 2001; Marini and Van Amburgh, 2003, 2005). In this experiment, the animals of N100 had high levels of N use efficiency (27.7% of N intake), similar to the results obtained by Theurer *et al*. (2002); moreover, several authors obtained higher levels of N use efficiencies (Funaba *et al*., 1997; Archibeque *et al*., 2002; Gabler and Heinrichs, 2003; Wickersham *et al*., 2008).

In 2 studies with Holstein heifers (200-270 kg BW), Marini and Van Amburgh (2003, 2005) observed an increase in N retention with an increase in N intake, reaching a plateau in N retention when N content of diet was ~30 g N/kg DM. In the present experiment, animals consumed diets with mean N concentration of 24 and 29 g/kg DM for N0 and N100, respectively. Nevertheless, and in contrast with findings of Marini and Van Amburgh (2003), in this study higher N retention efficiency (18.4 and 27.7% for N0 and N100, respectively) was observed with greater N intake. While N intake differed by 20% between treatments, the difference in N retention was 81%. This difference in N retention could be due to greater N intake, leading to greater undegradable dietary N reaching duodenum, along with a probable increase (P=0.109) of microbial N flow to duodenum.

(Insert Table 5 here)

Conclusions

In the present study, N fertilization of winter oats increased CP:WSC ratio in diet and in consumed forage, not only by increasing the CP content of the forage but also by decreasing its WSC content. This had no effect on DM intake, apparent *in vivo* DMD, or microbial N capture efficiency. Nevertheless, increasing the amount of N offered to steers increased the N intake, N digestibility, N retention and ADG.. It is important to mention that in this study, the urinary excretion of urea-N and allantoin-N were not good indicators of N use efficiency. Since the whole-body urea pool was not measured, we cannot conclude that increasing the N intake led to higher N use efficiency.

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