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ANIMAL PRODUCTION

Nitrogen compounds balance and microbial protein synthesis in supplemented crossbred dairy cows in pasture

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ABSTRACT. The objective this work was to evaluate the supplementation of cows on *Brachiaria decumbens* pasture during the rainy-dry transition. Eight 1/2 Holstein/Zebu cows (497 ± 50 kg) were distributed in two 4 x 4 Latin squares, with 2 x 2 factorial treatments (100% mineral salt *versus* nitrogen salt containing 25:25:50% urea: corn: mineral salt, and 1.5 *versus* 3.0 kg d^{-1} concentrate per cow). The experiment lasted 56 days, divided into four periods of 14 days, the last seven days for data collection. No interaction effect ($p > 0.05$) was detected between the supplement and the different levels of concentrate for any of the evaluated variables. No effect ($p > 0.05$) of supplement or concentrate level was observed on urinary excretions of purine derivatives, microbial nitrogen compounds and microbial efficiency. Nitrogen salt increased ($p < 0.05$) nitrogen intake and increased urinary nitrogen excretion, leading to a reduced nitrogen balance, while the higher concentrate level increased ($p < 0.05$) nitrogen intake and the total amount of nitrogen excreted in milk. Therefore, it is recommended, for crossbred cows in the rainy-dry transition period, the supply of 1.5 kg d^{-1} concentrate during milking, using only mineral salt instead of nitrogen salt at will on pasture.

Keywords: concentrate; microbial growth; ruminal fermentation; supplementation.

Balanço de compostos nitrogenados e síntese de proteína microbiana em vacas mestiças leiteiras suplementadas a pasto

RESUMO. O objetivo deste trabalho foi avaliar a suplementação de vacas em pastagens de *Brachiaria decumbens* durante a transição águas-seca. Foram utilizadas oito vacas 1/2 Holandês/Zebu (497 ± 50 kg), distribuídas em dois quadrados latinos 4 x 4, sendo os tratamentos em fatorial 2 x 2 (100% de sal mineral *versus* sal nitrogenado contendo 25:25:50% de ureia:fubá e sal mineral; e 1,5 *versus* 3,0 kg d^{-1} de concentrado por vaca). O experimento teve duração de 56 dias, divididos em quatro períodos de 14 dias, sendo os sete últimos dias para coleta de dados. Não foi encontrado efeito de interação ($p > 0,05$) entre o suplemento e os diferentes níveis de concentrado para nenhuma das variáveis avaliadas. Não foi observado efeito ($p > 0,05$) de suplemento nem de nível de concentrado sobre as excreções urinárias dos derivados de purina, compostos nitrogenados microbianos e eficiência microbiana. O sal nitrogenado aumentou ($p < 0,05$) o consumo de nitrogênio e aumentou a excreção urinária de nitrogênio, levando à redução do balanço de nitrogênio, enquanto o maior nível de concentrado aumentou ($p < 0,05$) o consumo de nitrogênio e o total de nitrogênio excretado no leite. Recomenda-se, portanto, para vacas mestiças no período de transição águas-seca, o uso de 1,5 kg d^{-1} de concentrado fornecido durante as ordenhas, com uso somente de sal mineral em vez de sal nitrogenado à vontade na pastagem.

Palavras-chave: concentrado; crescimento microbiano; fermentação ruminal; suplementação.

Introduction

Dairy activity in Brazil is predominantly carried out in tropical pastures, which show seasonality in production. There is rapid vegetative growth and consequent higher supply of food with better quality in the rainy season, and lower supply of food with worse quality in the dry season of the year. Restrictions in nutrient intake (quantity and/or quality), mainly observed in the dry season, is a major factor limiting the production of grazing animals (Maggioni et al., 2009).

As a hypothesis, supplementation is expected to improve nutritional conditions and minimize the differences between periods of high and low nutrient availability. In addition to the animal response, indications for the best level of

supplementation should consider the economic factor of the benefit of supplement use and not only the fulfillment of a certain nutritional demand (Oliveira, Campos, Lana, Detmann, & Valadares Filho, 2010). There are many studies on pasture supplementation strategies, but there is little information on the optimal levels of combination of forage supply and levels of supplementation that could optimize productive and economic efficiency for milk production.

The objective of this experiment was to evaluate the effect of nitrogen supplementation and two levels of concentrate on nitrogen balance and microbial protein synthesis efficiency of crossbred cows (½ Holstein x Zebu) on *Brachiaria decumbens* pasture in the rainy-dry transition period.

Material and methods

The experiment was conducted at Boa Vista Farm, Cachoeirinha District, belonging to the Federal University of Viçosa, Viçosa, State of Minas Gerais, during the rainy-dry transition period between March and May 2013, following the rules of the Ethics Committee for Animal Experimentation of the Federal University of Viçosa.

The city of Viçosa is located in the region of Zona da Mata, in the State of Minas Gerais, at 649 m altitude, geographically defined by the coordinates 20°45'20" South latitude and 42°52'40" West longitude. The climate is Cwa, according to the classification proposed by Köppen (1948), with two defined seasons: dry, from April to September, and rainy, from October to March. The summer is hot and humid and the winter is cold and dry. The average rainfall is 1,341.2 mm yearly (Universidade Federal de Viçosa, UFV, 1997.

Eight crossbred cows (1/2 Holstein: Zebu), with initial mean body weight of 497 ± 50 kg, between the third and fourth lactation, after peak production (100 days *postpartum*) and with mean milk yield of 10 kg d^{-1} were kept in an area of *Brachiaria decumbens* pasture during the rainy-dry transition period. The experiment was evaluated according to a 4 x 4 Latin square design (four cows, four treatments and four periods), using two simultaneous squares to guarantee the adequate value of degrees of freedom for the error.

The experiment lasted 56 days, divided into four periods of 14 days, with the first seven days for adaptation and the last seven days for data collection.

The treatments consisted of a 2 x 2 factorial, two forms of supplement use (mineral salt *versus* nitrogen salt) provided at will, in a roofed trough in the pasture, and two concentrate levels (1.5 and 3.0 kg d^{-1} concentrate per cow). Concentrates had approximately 26% crude protein, based on corn meal (60%) and soybean meal (40%), divided into two meals and supplied during morning and afternoon milking. The cows were expected to eat all the concentrate during milking. The nitrogen salt contained urea:ammonium sulfate at 9:1, mineral salt and corn meal at 25:25:50%, to reach consumption of 150 g d^{-1} urea per cow or 1.2% of the total dry matter. In addition to the pasture, supplements and concentrate, water was provided at will. The chemical composition of the food and supplement is given in Table 1.

The mineral salt supplied was a commercial mineral supplement containing: calcium (15.6%); phosphorus (5.1%); sulfur (2.0%); magnesium (3.3%); sodium (9.3%); potassium (2.82%); cobalt (0.003%); copper (0.040%); chromium (0.001%); iron (0.2%); iodine (0.004%); manganese (0.135%); selenium (0.002%); fluorine (0.051%); zinc (0.170%); vitamin A (135,000.00 I.U.); vitamin D3 (68,000.00 I.U.) and vitamin E (450.00 I.U.). Phosphorus solubility of 95%.

Table 1. Chemical composition of the concentrate, nitrogen salt and *Brachiaria decumbens*.

Item	Concentrate[1]	Nitrogen salt[2]	*B. decumbens*[3]
DM[4]	85.90	86.12	32.26
OM[5]	96.92	75.29	91.50
CP[5]	26.10	66.52	7.61
NDIN[5]	26.98	4.40	35.02
EE[5]	2.66	1.29	1.16
NDFap[5]	19.02	10.15	63.21
NDFi[5]	2.03	1.42	23.10
NFC[5]	49.14	11.57	9.82

[1]Concentrate - 60% corn meal, 40% soybean meal. [2]Nitrogen salt: 50% corn, 25% urea and 25% mineral salt (corn meal; urea: ammonium sulfate 9:1; mineral supplement). [3]Mean of the samples obtained by hand simulation of grazing throughout the experimental period. [4]DM – dry matter; values in g.kg^{-1} natural matter. [5]OM – organic matter; CP – crude protein; NDIN – neutral detergent insoluble nitrogen; EE – ether extract; NDFap – neutral detergent fiber corrected for ash and protein; NDFi – indigestible neutral detergent fiber; NFC – non-fiber carbohydrates; all in g kg^{-1} DM, except for NDIN, in g kg^{-1} total nitrogen.

A pasture area of four hectares was divided in two paddocks. Each of the two paddocks was divided into seven sub-paddocks: 1-7 for the supply of nitrogen salt and 8-14 for the mineral salt. The animals and supplements were rotated in the sub-paddocks, every two days, for better pasture utilization and elimination of possible paddock effects on the treatments. At the end of each experimental period, the cows returned to the first sub-paddock. Cows were mechanically milked twice a day at 7:00h and 15:00h, with calves on foot during milking. Milk weighing was done in the morning and afternoon, on days 12 to 14 of each experimental period. Milk samples were obtained from each cow

during milking, at the afternoon hours on day 13 of each experimental period and in the morning on day 14 of each experimental period, in the division of 2/3 and 1/3, being composed by animal and by period, stored in bottles containing Bronopol®, kept at 2 to 6°C, and sent to the Laboratory of Animal Nutrition of the Federal University of Viçosa - UFV. 10 mL of milk were mixed with 5 mL 25% trichloroacetic acid for deproteinization, then filtered through filter paper to obtain the milk serum, and stored for further analysis of allantoin at the Laboratory of Department of Animal Sciences of the Federal University of Viçosa, according to Fujihara, Ørskov, Reeds, and Kyle (1987).

Estimates of intake and digestibility were measured during each period, from the 5th to the 13rd day, and the first days were used to adapt the animals to the indicators. To estimate fecal excretion, 20 grams of chromic oxide/animal/day (Cr_2O_3), packed in paper cartridges and introduced by means of an esophageal probe, were used as an external indicator in a daily portion at eight hours. To estimate the individual intake of the mineral salt and nitrogen salt supplements, titanium dioxide (TiO_2) was mixed in the supplement in the proportion of 10 grams of indicator for each kilogram of supplement. Indigestible neutral detergent fiber (NDFi) was used as an internal indicator to estimate digestibility, together with fecal excretion, making it possible to estimate dry matter intake of pasture.

From the 7th to the 14th day, nitrogen balance and microbial crude protein synthesis were evaluated. On the 14th day of the experiment, approximately four hours after feeding, blood samples of all animals were taken by puncturing the coccygeal vein using a test tube with separator gel. Soon after collection, blood samples were centrifuged at 3,000 rpm for 15 minutes and blood serum samples were collected, which were packed in properly labeled glass containers and frozen for further analysis of urea and non-esterified fatty acids.

Spot urine samples were collected on the last day of each experimental period. After homogenization and filtration, 10 mL aliquots were obtained and diluted in 40 mL of 0.036 N sulfuric acid, as described by Valadares, Gonçalves, Rodriguez, Valadares Filho, and Sampaio (1997). Samples were then conditioned in properly identified plastic containers and frozen at -20°C for further analysis of urea, total nitrogen, creatinine, uric acid and allantoin.

In blood serum and in urine, the urea concentration was evaluated using the modified diacetyl method (commercial kits) with the Biosystems A15 apparatus. The concentration of serum urea nitrogen (SUN) was obtained by the concentration of serum urea, multiplied by 0.466, corresponding to the nitrogen content in urea. The concentration of milk urea nitrogen (MUN) was calculated from the following equation, proposed by Chizzotti et al. (2007):

$$\mathrm{MUN} = 1.11 * \mathrm{SUN}.$$

Non-esterified fatty acids in blood serum were analyzed using HPLC (High Performance Liquid Chromatography) as it is the most accurate method to use. Six samples of the same animals were used in each experimental period.

In urine, the nitrogen compounds were quantified and analyses of purine derivatives (allantoin and uric acid) were performed. Uric acid was determined using the uricase method with commercial kits. The concentration of allantoin was determined by the colorimetric method, according to Fujihara et al. (1987).

The creatinine concentration in the spot sample was used to estimate the urinary volume. Quantification of the daily urinary volume of each animal was done by multiplying the respective body weight by the amount of creatinine excreted daily and dividing the product by the creatinine concentration (mg L^{-1}) in the spot sample. The mean value of 29.00 mg kg^{-1} BW obtained from the studies of Valadares, Broderick, Valadares Filho, and Clayton (1999) was used to obtain total daily excretion of creatinine. Creatinine was determined in the spot urine samples by means of the kinetic alkaline picrate method using commercial kits (Labtest) in the ARCHITECT cSystems apparatus.

Total excretion of purine derivatives was estimated by the sum of the amounts of uric acid and allantoin excreted in urine plus the amount of allantoin secreted in the milk, expressed as mmol day^{-1}.

Calculations of the absorbed microbial purines (AP, mmol d^{-1}) were performed from the excretions of the purine derivatives (Y, mmol d^{-1}) using the formula:

$$\mathrm{Y} = (\mathrm{X} - 0.385\ \mathrm{BW}^{0.75})/0.85$$

where 0.85 is the recovery of purines absorbed as urinary purine derivatives and 0.385 $BW^{0.75}$ is the endogenous purine excretion (Verbic, Chen, MacLeod, & Ørskov, 1990).

The synthesis of microbial nitrogen compounds in the rumen was determined from the absorbed purines (AP, mmol d^{-1}), with substitution of the

purine-N: total N ratio in bacteria by 0.134, according to Valadares et al. (1999):

$$\text{Nmic (g d}^{-1}) = (70 \text{ x AP})/(0.83 \text{ x } 0.134 \text{ x } 1000)$$

where 70 is the purine nitrogen content (mg N $mmol^{-1}$), 0.83 is the digestibility of microbial purines and 0.134 is the purine-N: total N in bacteria. The microbial efficiency was expressed in g microbial CP kg^{-1} of total digested organic matter (g CPmic kg^{-1} TDN).

The results were tested by analysis of variance, in a Latin square design, using the Statistical and Genetic Analysis System (SAEG). The statistical model included effects of levels of concentrate, supplement, interaction between levels of concentrate x supplement, animal and period, at the level of 5% probability, as presented below:

$$Yijklmn = \mu + Ci + Sj + C*Sij + QLk + A/QLl + P/QLm + Eijklmn$$

where:

Y_{ijklmn} = observation of the dependent variable referring to the level of concentrate i, supplement j, latin square k, animal within latin square l, period within latin square m and repetition n;

μ = mean of all observations;

C_i = effect of the i-th level of concentrate, where i = 1.5 and 3.0 kg day^{-1} per cow;

EP_j = effect of the j-th supplement, where j = 1 and 2 for mineral salt and nitrogen salt, respectively;

$C*EP_{ij}$ = interaction between level of concentrate i and level of supplement j;

QL_k = effect of latin square k, where k = 1 and 2;

A/QL_l = effect of animal within latin square, where l = 1, 2, 3 and 4;

P/QL_m = effect of period within latin square, where m = 1, 2, 3 and 4;

E_{ijklmn} = experimental error referring to the observation of level of concentrate i, supplement j, latin square k, animal within latin square l, period within latin square m and repetition n.

Results and discussion

The experiment lasted 56 days, divided into four experimental periods of 14 days each, the first seven days used for adaptation and the last seven for data collection. Farenzena, Kozloski, Gindri, and Stefanello (2017) reported the impact of dietary change on nutritional variables of sheep, including voluntary intake, digestibility and ruminal fermentation, where it was observed that the adaptation time to the new diet varied from 6 to 13 days, depending on the variable and type of diet. Lana and Russell (1996) observed that changes in ruminal microbial populations occur almost immediately, where populations of microorganisms adapted to the new diet rapidly replace those less adapted. These studies corroborate, therefore, the collection of samples from the 7th to the 14th day of each period in the present study.

No interaction effect ($p > 0.05$) was detected between the supplement and the different levels of concentrate for any of the evaluated variables.

No effect ($p > 0.05$) of supplement or concentrate level was observed on the urinary excretions of purine derivatives, microbial nitrogen compounds and microbial efficiency (Table 2). Although there has been a higher intake of protein by cows with increasing levels of concentrate, the results obtained in this study are similar to those reported by Pereira et al. (2005), who found no effect of increasing CP levels on allantoin in cows in the initial and middle thirds of lactation. Yu, Egan, Boon-Ek, and Leury (2002) concluded that allantoin and uric acid excretions may be affected by dietary protein and energy sources, intake of DM, energy and protein, body weight as well as food additives. On the other hand, Fonseca et al. (2006) also concluded that there was an increase in the amount of allantoin excreted in urine with the increase in CP content of the diet. Therefore, it can be said that the nitrogen ingested was not properly used or that the pastures were at the appropriate levels to meet the nutritional requirements.

Due to the lack of significant effect on microbial nitrogen compounds and microbial synthesis efficiency (Table 2), it can be stated that the energy supplied to the animals was not being used properly. An essential character in the yield of microbial production is the synchronism between ruminal degradation of carbohydrates and protein. For this, an evaluation should be made regarding the degradation rates of each fraction contained in the carbohydrates and proteins ingested, synchronizing the time of rumen availability of these substrates to the microorganisms, maximizing the use of degraded protein in the rumen (Alves et al., 2010). The efficiency of microbial synthesis was not influenced by treatments and presented a mean of 145.86 g CP kg^{-1} TDN. Similarly, Paixão et al. (2006), working with feedlot animals receiving increasing levels of urea, did not detect effects on the efficiency of microbial protein synthesis, with level of 113 g CP kg^{-1} TDN. Among the factors affecting the microbial protein synthesis, the availability and synchronization between energy and nitrogen compounds in the rumen have been recognized as the most important Silva et al. (2007).

Table 2. Means for urinary excretion, microbial nitrogen compounds, microbial synthesis efficiency, urea nitrogen in serum and milk, and non-esterified fatty acids in serum, according to the different treatments.

Item	Supplement		Level of concentrate, kg		SEM	P-value		
	MS	NS	1.5	3.0		Sup	LC	Sup x LC
Urinary excretion								
AL (mmol d^{-1})	160.40	179.09	159.60	179.89	15.83	0.999	0.999	0.251
UA (mmol d^{-1})	27.22	28.72	27.21	28.73	2.76	0.999	0.999	0.999
TP (mmol d^{-1})	196.32	216.11	195.29	217.14	17.05	0.999	0.999	0.999
AP (mmol d^{-1})	183.37	207.04	182.26	208.15	19.91	0.999	0.999	0.999
Nmic (g d^{-1})	112.88	127.46	112.20	128.14	12.26	0.999	0.999	0.999
MSE (g CPmic kg^{-1} TDN)	138.86	152.86	147.32	144.40	19.58	0.222	0.999	0.999
SUN (mg dL^{-1})	13.27	14.34	13.26	14.35	0.79	0.087	0.999	0.217
MUN (mg dL^{-1})	14.76	15.94	14.75	15.95	0.88	0.087	0.999	0.217
NEFA (mmol dL^{-1})	0.33	0.37	0.40	0.30	0.04	0.999	0.130	0.330

MS = mineral salt; NS = nitrogen salt; SEM = standard error of the mean; Sup = supplement; LC = level of concentrate; AL = allantoin, UA = uric acid, TP = total purines; AP = absorbed purines; Nmic = microbial nitrogen compounds; MSE = microbial synthesis efficiency; CP = crude protein; TDN = total digestible nutrients; SUN = serum urea nitrogen; MUN = milk urea nitrogen; NEFA = non-esterified fatty acids.

There was no effect ($p > 0.05$) of treatments on serum urea nitrogen (SUN) and milk urea nitrogen (MUN), as well as on non-esterified fatty acids (Table 2). According to the literature, the level of serum urea nitrogen cannot exceed 13-15 mg dL^{-1}, as it would cause loss of proteins in the form of urine urea, commonly associated with excess degradable protein or lack of fermentable carbohydrate in the rumen (Chizzotti et al., 2007; Silva et al., 2014). In the present study, values varied between 13.3 and 14.3 mg dL^{-1} (Table 2), within the range considered desirable, and without treatment effect. On the other hand, Teixeira et al. (2015) found high values of SUN and MUN in dairy cows consuming mineral supplements containing 20% urea (18 to 20 and 20 to 22 mg dL^{-1}, respectively); and Souza Júnior et al. (2016) found high NUS values in dairy cow diets with 16 versus 12% crude protein (27.9 vs 15.0 mg dL^{-1}).

Nitrogen salt promoted an increase ($p < 0.05$) in nitrogen consumption and an increase in urinary nitrogen excretion, leading to a reduced nitrogen balance, while the higher level of concentrate provided an increased ($p < 0.05$) consumption of nitrogen and total nitrogen excreted in milk (Table 3). According to Wright, Moscardini, Luimes, Susmel, and McBride (1998), the first route of nitrogen excretion would be through urine, especially when there is high protein intake or high nitrogen loss in the rumen, intestine or mammary glands. Therefore, in the present study, urea from nitrogen salt was not efficiently used in ruminal microbial protein synthesis, which caused a reduced nitrogen balance, probably due to the energy deficit of tropical pasture. Accordingly, Teixeira et al. (2015) also found high values of urinary urea nitrogen in dairy cows consuming mineral supplements containing 20% urea (621 to 764 mg dL^{-1}). On the other hand, when the concentrate was used in a higher level, the input of energy favored the synthesis of ruminal microbial protein, justifying the increase in total nitrogen excreted in milk.

There was no effect of the nitrogen salt and increase in the level of concentrate on the microbial protein synthesis (Table 2). Likewise, there was no improvement in the nitrogen balance with the use of nitrogen salt (Table 3) since the increase in nitrogen intake was much lower than the loss of urinary nitrogen. Part of these effects may be due to the lack of stimulation to ruminal microbial synthesis, which would increase the true protein supply in the small intestine. According to Wright et al. (1998), when the animal is under food restriction, the excretion of nitrogen by urine is increased, depriving the microorganisms of obtaining the specific nutrients in the rumen.

Table 3. Means for ingested nitrogen (IN), nitrogen in the feces (NF), nitrogen in the urine (NU), nitrogen in milk (NM), nitrogen balance (NB) and retained nitrogen (RN) according to the different treatments.

Item	Supplement		Level of concentrate (kg)		SEM	P-value		
	MS	NS	1.5	3.0		Sup	LC	Sup x LC
	g d^{-1}							
IN	195.2	226.03	187.52	233.71	10.80	0.008	0.001	0.999
NF	79.61	80.34	76.26	83.69	4.78	0.999	0.131	0.999
NU	74.56	144.38	98.40	120.54	20.74	0.002	0.295	0.999
NM	40.22	45.83	41.79	44.26	2.11	0.999	0.021	0.999
NB	50.46	-15.78	9.56	25.12	23.13	0.008	0.999	0.999
RN	-4.19	-44.51	-29.94	-18.76	-21.15	0.067	0.999	0.999

MS = mineral salt; NS = nitrogen salt; SEM = standard error of the mean; Sup = supplement; LC = level of concentrate.

Conclusion

Considering the results obtained for urinary excretion of purine derivatives, balance of microbial nitrogen compounds and microbial synthesis efficiency, it is recommended the lower supplementation for crossbred cows in the middle third of lactation, on pasture in the rainy-dry transition period, or using 1.5 kg d^{-1} concentrate supplied during milking, with the use of mineral salt instead of nitrogen salt at will in pastures, thus reducing supplementation costs.

References

Alves, E. M., Santos Pedreira, M., Oliveira, C. A. S., Ferreira, D. N., Moreira, B. S., & Freire, L. D. R. (2010). Importância da sincronização do complexo proteína/energia na alimentação de ruminantes. *PUBVET, 4*, 844-849.

Chizzotti, M. L., Valadares Filho, S. C., Valadares, R. F. D., Chizzotti, F. H. M., Marcondes, M. I., & Fonseca, M. A. (2007). Consumo, digestibilidade e excreção de uréia e derivados de purinas em vacas de diferentes níveis de produção de leite. *Revista Brasileira de Zootecnia, 36*(1), 138-146. doi: 10.1590/S1516-35982007000100017

Farenzena, R., Kozloski, G. V., Gindri, M., & Stefanello, S. (2017). Minimum lenght of the adaptation period in digestibility trials with sheep fed ad libitum only forage or forage plus concentrate. *Journal of Animal Physiology, 101*, 1057-1066. doi: 10.1111/JPN.12550

Fonseca, C. E. M., Valadares, R. F. D., Valadares Filho, S. C., Leão, M. I., Cecon, P. R., Rodrigues, M. T., ... Araújo, A. M. (2006). Estimativa da produção microbiana em cabras lactantes alimentadas com diferentes teores de proteína na dieta. *Revista Brasileira de Zootecnia, 35*, 1169-1177. doi: 10.1590/S1516-35982006000400031

Fujihara, T., Ørskov, E. R., Reeds, P. J., & Kyle, D. J. (1987). The effect of protein infusion on urinary excretion of purine derivatives in ruminants nourished by intragastric nutrition. *The Journal of Agricultural Science, 109*(1), 7-12. doi: 10.1017/S0021859600080916

Köppen, W. (1948). *Climatologia: con um estúdio de los climas de la tierra.* Mexico, MX: Fondo de Cultura Economica.

Lana, R. P., & Russell, J. B. (1996). Use of potassium depletion to assess adaptation of ruminal bacteria to ionophores. *Applied and Environmental Microbiology, 62*(12), 4499-4503.

Maggioni, D., Marques, J. A., Rotta, P. P., Zawadzki, F., Ito, R. H., & Prado, I. N. (2009). Ingestão de alimentos. *Semina: Ciências Agrárias, 30*(4), 963-974. doi: 10.5433/1679-0359.2009v30n4p963

Oliveira, A. S., Campos, J. M. S., Lana, R. P., Detmann, E., & Valadares Filho, S. C. (2010). Estimate of the optimal level of concentrates for dairy cows on tropical pastures by using the concept of marginal analysis. *Revista Brasileira de Zootecnia, 39*(9), 2040-2047. doi: 10.1590/S1516-35982010000900024

Paixão, M. L., Valadares Filho, S., Leão, M. I., Valadares, R. F. D., Paulino, M. F., Marcondes, M. I., ... Pina, D. (2006). Uréia em dietas para bovinos: consumo, digestibilidade dos nutrientes, ganho de peso, características de carcaça e produção microbiana. *Revista Brasileira de Zootecnia, 35*(6), 2451-2460. doi: 10.1590/S1516-35982006000800035

Pereira, M. L. A., Valadares Filho, S. C., Valadares, R. F. D., Campos, J. M. S., Leão, M. I., Pereira, C. A. R., & Mendonça, S. S. (2005). Consumo, digestibilidade aparente total, produção e composição do leite em vacas no terço inicial da lactação alimentadas com níveis crescentes de proteína bruta no concentrado. *Revista Brasileira de Zootecnia, 34*(3), 1029-1039. doi: 10.1590/S1516-35982005000300036

Silva, Á. C., Figueiredo, M. P., Bonomo, P., Pereira, M. L. A., Luz, Y. d. S., & Santos, E. d. J. (2014). Microbial protein synthesis and nitrogen metabolism in cows bred on tropical pasture and fed on cassava root and corn. *Acta Scientiarum. Animal Sciences, 36*(2), 185-192. doi: 10.4025/actascianimsci.v36i2.22161

Silva, E. A., Berchielli, T. T., Reis, R. A., Pires, A. V., Sato, K. J., Paes, J. M. V., & Lopes, A. D. (2007). Teores de proteína bruta para bovinos alimentados com feno de capim-tifton 85: parâmetros ruminais, eficiência de síntese microbiana e degradabilidade *in situ*. *Revista Brasileira de Zootecnia, 36*(1), 225-236. doi: 10.1590/S1516-35982007000100027

Souza Júnior, A. H., Lana, R. P., Teixeira, C. R. V., Gionbelli, M. P., Veloso, C. M., & Rennó, L. N. (2016). Protected fat and variable level of protein in diets of crossbreed cows in early lactation. *Acta Scientiarum. Animal Sciences, 38*(1), 107-112. doi: 10.4025/actascianimsci.v38i1.29150

Teixeira, C. R. V., Lana, R. P., Rennó, L. N., Veloso, C. M., Carvalho, J. C., & Menezes, A. A. (2015). Urea and salt as supplementary diet for crossbreed milk cows. *Acta Scientiarum. Animal Sciences, 37*(3), 281-287. doi: 10.4025/actascianimsci.v37i3.26890

Universidade Federal de Viçosa [UFV]. (1997). *Dados climáticos*. Viçosa, MG: UFV.

Valadares, R. F. D., Broderick, G. A., Valadares Filho, S. C., & Clayton, M. K. (1999). Effect of replacing alfalfa silage with high moisture corn on ruminal protein synthesis estimated from excretion of total purine derivatives. *Journal of Dairy Science, 82*(12), 2686-2696. doi: 10.3168/jds.S0022-0302(99)75525-6

Valadares, R. F. D., Gonçalves, L. C., Rodriguez, N., Valadares Filho, S. C., & Sampaio, I. (1997). Níveis de proteína em dietas de bovinos. 4. Concentrações de amônia ruminal e uréia plasmática e excreções de uréia e creatinina. *Revista Brasileira de Zootecnia, 26*(6), 1270-1278

Verbic, J., Chen, X., MacLeod, N., & Ørskov, E. (1990). Excretion of purine derivatives by ruminants. Effect of microbial nucleic acid infusion on purine derivative excretion by steers. *The Journal of Agricultural Science, 114*(03), 243-248. doi: 10.1017/S0021859600072610

Wright, T. C., Moscardini, S., Luimes, P. H., Susmel, P., & McBride, B. W. (1998). Effects of rumen-undegradable protein and feed intake on nitrogen balance and milk protein production in dairy cows. *Journal of Dairy Science, 81*(3), 784-793. doi: 10.3168/jds.S0022-0302(98)75635-8

Yu, P., Egan, A. R., Boon-Ek, L., & Leury, B. J. (2002). Purine derivative excretion and ruminal microbial yield in growing lambs fed raw and dry roasted legume seeds as protein supplements. *Animal Feed Science and Technology, 95*(1-2), 33-48. doi: 10.1016/S0377-8401(01)00314-5

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