Revista Brasileira de Zootecnia
© 2016 Sociedade Brasileira de Zootecnia
ISSN 1806-9290
www.sbz.org.br

R. Bras. Zootec., 45(9):572-580, 2016

Performance and metabolite profile of dairy cows fed tropical grasses and concentrates containing crude protein with low or high degradability

Raphael dos Santos Gomes[1], Tadeu Silva de Oliveira[1], José Carlos Pereira[2], Ricardo Augusto Mendonça Vieira[3], Douglas Sampaio Henrique[4], Alberto Magno Fernandes[3], Fernando de Paula Leonel[5]

[1] Universidade Estadual do Norte Fluminense Darcy Ribeiro, Programa de Pós-graduação em Ciência Animal, Campos dos Goytacazes, RJ, Brazil.
[2] Universidade Federal de Viçosa, Departamento de Zootecnia, Viçosa, MG, Brazil.
[3] Universidade Estadual do Norte Fluminense Darcy Ribeiro, Laboratório de Zootecnia, Campos dos Goytacazes, RJ, Brazil.
[4] Universidade Tecnológica Federal do Paraná, Dois Vizinhos, PR, Brazil.
[5] Universidade Federal de São João del Rei, Departamento de Zootecnia, São João del Rei, MG, Brazil.

ABSTRACT - Ten Holstein-Zebu crossbred cows distributed into two simultaneous Latin squares (5 × 5) as a 2 × 2 factorial arrangement formed by chopped sugarcane or elephant grass silage, both with high or low protein degradability supplements and a corn silage as a control treatment, were compared using orthogonal contrasts. The studied variables were the performance, plasma concentrations of urea-N, glucose, and creatinine, urine-N and milk urea-N, and the nychthemeral variation in NH_3-N in the rumen fluid of dairy cows. Nutrient intake, milk production, and milk composition were affected by the treatments. The total mixed ration containing elephant grass silage combined with rumen undegradable protein (RUP) provided balanced amounts of carbon and nitrogen in the rumen. This effect may explain the 18% increase in milk yield compared with the other treatments. The diurnal pattern of ruminal NH_3-N was interpreted with a sinusoid model. In general, cows fed elephant grass silage exhibited higher concentrations of blood plasma and milk urea-N than animals fed sugarcane. The cows that consumed elephant grass silage with rumen degradable protein concentrate showed a higher milk urea-N compared with animals that consumed elephant grass silage with the RUP concentrate. The use of diets based on corn silage leads to a better use of nitrogen compounds because these diets resulted in lower levels of urea-N in the plasma, urine, and milk at the same level of milk production compared with diets containing elephant grass silage or chopped sugarcane as roughages. In sugarcane-based diets, even greater nitrogen losses in the urine are observed, despite the presence of readily fermentable carbohydrates in the diet.

Key Words: intake, milk yield, nitrogen metabolites, tropical forages

Introduction

Rumen degradable protein (RDP) deficiency can limit microbial growth, especially when diets containing high concentrations of rumen undegradable protein (RUP) are provided (Nocek and Russell, 1988). This scenario leads to reduced ruminal digestion of the fibrous fraction of the feed and restrictions on feed intake due to the rumen fill effect of fiber (Mertens, 1987; Van Soest, 1994; Vieira et al., 2008a,b; Allen et al., 2009). By contrast, the use of RUP sources increases the flow of amino acids into the small intestine (Zinn and Owens, 1993). Such a strategy will be efficient every time the source of RUP shows a biological value superior to that of the microbial protein source (AFRC, 1993; NRC, 2001). Rumen ammonia, in turn, derives from dietary protein degradation, the hydrolysis of non-protein nitrogen sources, rumen recycled urea, and the lysis of microbial cells. The concentration of rumen ammonia is an indicator of protein degradation, dietary nitrogen utilization, and microbial growth (Russell et al., 1992; Russell, 2002). Similarly, the blood urea concentration is a sensitive and immediate indicator of the animal protein metabolism, whereas the milk urea-N is a better indicator than blood urea for evaluating the metabolism of N compounds in the rumen because homeostatic mechanisms do not regulate milk urea-N, a variable less affected by postprandial variations (Jonker et al., 1998). Considering the amino acid requirement of lactating animals and dietary amino acid supply, it is possible that RUP sources can promote better productive responses when combined with forages such as sugarcane or elephant grass silage. Sugarcane exhibits a pronounced imbalance of nutrients and is usually only supplemented with NPN (Fernandes et al., 2001), whereas both elephant grass and corn silages provide more protein and RDP levels (Cabral

Received October 7, 2015 and accepted June 2, 2016.
Corresponding author: ramvieira@uenf.br
http://dx.doi.org/10.1590/S1806-92902016000900010

et al., 2000; Lacerda et al., 2004). Thus, it is possible that supplementation with RUP reduces ammonia production in the rumen (Seymour et al., 1992). Therefore, the present study aimed to evaluate the performance, the concentration of urea-N, glucose, and creatinine in blood plasma, urea-N in the urine and milk, and the pattern of diurnal variation of NH_3-N in the rumen fluid of dairy cows fed chopped sugarcane or elephant grass silage, both supplemented with high and low RDP concentrates. A control diet based on corn silage was used as a reference.

Material and Methods

The experiment was conducted in the municipality of Bambuí (20°00' S, 45°59' W, and elevation 661 m above the sea level), state of Minas Gerais, Brazil. The climate is Cwa (Kottek et al., 2006), with cold and dry winters and hot and humid summers. The monthly means (last 15 years) of dry-bulb temperature, wet-bulb temperature, relative humidity, rainfall, insolation, and wind speed were 23.6±2.4 °C, 21.7±2.5 °C, 73.5±6%, 110±119 mm, 5.9±1.4 h, and 1.28±0.17 m s^{-1}, respectively.

Ten Holstein-Zebu crossbred multiparous cows with a mean initial body weight of 535±66 kg, producing on average 19.8±3.8 kg day^{-1} of milk with a fat content of 36.1±3.8 g kg^{-1} of raw milk, were used. The experimental period was 115 days, for which the 10 initial days were intended for adapting the animals.

The experiment was distributed into two simultaneous Latin squares (5 × 5). Five cows between 24 and 40 days of lactation composed the first square, and the other square comprised five cows between 41 and 60 days of lactation. The experimental period was divided into five 21-day phases (14 days for adapting to the diets and seven days for sampling). Three different forages were the roughages used in the present study: corn silage, elephant grass silage, and chopped sugarcane. The five diets were the applied treatments, as follows: corn silage (CS) and conventional concentrate; elephant grass silage (EGS) combined with concentrate with RDP (EGS/RDP) or with RUP (EGS/RUP); and chopped sugarcane (SC) combined with concentrate with RDP (SC/RDP) or with RUP (SC/RUP). The experimental diets were formulated according to the CNCPS feed library (Fox et al., 1992; Russell et al., 1992; Sniffen et al., 1992), which contains crude protein and carbohydrate analyses and digestion and passage estimates of kinetic parameters that allow the calculations of RUP and RDP fractions and amounts to achieve the expected cow production levels of the present study. The diets were formulated to have forage: concentrate ratios on a dry matter basis of 780:220, 760:240, and 600:400 for CS, EGS, and SC, respectively (Tables 1 and 2). The body weights of the cows were measured by weighing the animals individually, with no previous fasting, after the morning milking at 5, 6, and, 7 days of adaptation of each experimental period.

The total mixed rations were fed two times daily (7.30 h and 16.30 h) after milking, and were adjusted daily to maintain 10% leftovers. Before providing the morning feed, the leftovers from each experimental unit were collected, weighed, recorded, sampled, and stored in a freezer (−10 ºC). Samples of the corn silage, elephant grass silage, chopped sugarcane, and concentrates were also processed and stored in a similar manner. At the end of the experimental period, a total composite sample per animal/treatment was obtained.

The composite samples of each material (corn silage, elephant grass silage, sugarcane, concentrates, and leftovers) were used to determine the dry matter (DM, method number 967.03; AOAC, 1990), crude protein (CP, method number 984.13; AOAC, 1990), ash (method number 942.05; AOAC, 1990), crude fat (CF, method number 2003.06; Thiex et al., 2003), and lignin contents by solubilizing cellulose with 72% w/w sulfuric acid as lignin (sa) (method number 973.18; AOAC, 1990). The neutral detergent fiber (aNDF) was assayed with a heat-stable amylase and expressed inclusive of residual ash (Van Soest et al., 1991). The energy content was calculated according to the NRC (2001) equations.

Samples of rumen fluid (500 mL) were collected using an esophageal tube, at 0, 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, and 24 h after the morning feeding for determination of the diurnal pattern of rumen ammonia nitrogen (NH_3-N). Rumen fluid collections were made in the minimum time required

Table 1 - Ingredients of the concentrates of the different diets

Ingredient	Concentrate (g kg^{-1} DM[1])		
	Conventional	RDP	RUP
Ground corn	646	749	685
Soybean meal	246	190	-
Corn gluten meal	-	-	886
Fish meal	-	-	120
Ground corn mature ears	-	-	58
Urea	-	10	-
Tallow	47	30	29
Premix	61	21	22
NaCl	19	11	11
Limestone	12	3	4
Mix1[2]	30	6	6
Mix2[3]	-	1	1

RDP - rumen degradable protein; RUP - rumen undegradable protein.
[1] Dry matter equal to 885.3 g kg^{-1} as fed.
[2] Contains: 230.00 g kg^{-1} Ca; 90 g kg^{-1} P; 20 g kg^{-1} Mg; 48 g kg^{-1} Na; 15 g kg^{-1} S; 100 mg kg^{-1} Co; 700 mg kg^{-1} Cu; 80 mg kg^{-1} I; 2,000 mg kg^{-1} Fe; 1,250 mg kg^{-1} Mn; 20 mg kg^{-1} Se; 2,700 mg kg^{-1} Zn; 200 IU g^{-1} vitamin A; 60 IU g^{-1} vitamin D3; 60 IU kg^{-1} vitamin E.
[3] Contains: 12,000 IU g^{-1} vitamin A; 1,800 IU g^{-1} vitamin D3; 54 IU kg^{-1} vitamin E.

Table 2 - Chemical composition of roughage and treatments (g kg^{-1} DM)

Constituent	Roughage			Treatment				
	CS	EGS	SC	CS	EGS		SC	
					RDP	RUP	RDP	RUP
Dry matter[1]	400.0	230.0	312.0	506.0	384.0	385.0	541.0	543.0
Crude protein	72.0	88.0	28.0	100.0	117.0	120.0	101.0	104.0
Rumen undegradable protein	-	-	-	26.0	34.0	50.0	34.0	60.0
aNDF	472.0	489.0	447.0	374.0	381.0	381.0	286.0	287.0
Lignin	62.0	89.0	92.0	50.0	69.0	69.0	57.0	58.0
Crude fat	19.0	25.0	10.0	29.0	31.0	32.0	22.0	23.0
Ash	48.0	89.0	32.0	56.0	78.0	83.0	36.0	46.0
Metabolizable energy[2]	9.74	8.31	8.68	10.54	9.63	9.58	10.73	10.64

RDP - rumen degradable protein; RUP - rumen undegradable protein; CS - corn silage; EGS - elephant grass silage; SC - chopped sugarcane; aNDF - neutral detergent fiber.
[1] g kg^{-1} as fed.
[2] MJ kg^{-1}.

to minimize saliva production by anxiety. Immediately after collection, the samples were filtered through a double-layer cotton mesh. After filtration, 50 mL of sample were transferred to a polyethylene flask containing 1 mL of 50% H_2SO_4 w/v, which was kept frozen at –10 ºC until the time of NH_3-N determination via displacing NH_3-N with excess KOH, steam distillation, and titration with 0.005 M HCl. The NH_3-N concentrations determined at different collection times were used to fit time series models to characterize the profile of diurnal NH_3-N (mg dL^{-1}) variation, as follows:

$$Y_i = A_0 + A_1 \times \sin(ct_i) + A_2 \times \cos(ct_i) + A_3 \times \sin(2ct_i) + A_4 \times \cos(2ct_i)$$

Eq. (1),

in which Y_i represents the level of rumen NH_3-N at time t_i; c is a constant fraction of the fundamental period, which corresponds to π rad h^{-1}; t_i is the i-th collection time; A_0 (mg dL^{-1}) represents the mean NH_3-N concentration; and A_1, A_2, A_3, and A_4 are scaling parameters that confer shape to the time profile. The circadian value of c was set at 0.1309 rad h^{-1}, corresponding to 1/24 of a fundamental period (Hopper et al., 1978).

On the 21st day of each experimental period, milk samples were collected during milking, and blood and urine samples were collected three hours after feeding to determine the levels of urea-N, glucose, and creatinine in the blood and urea-N in the urine and in milk. The urine sample was collected using a Foley catheter (Cunningham et al., 1955). Blood samples were collected from the mammary vein of each cow with vacuum tubes containing 100 µL of sodium fluoride. The analyses of urea and glucose were performed using Ureia Enzimática (enzymatic urea) – Analisa® and Glicose Enzimática (enzymatic glucose) – Analisa® enzyme kits. Fractions of composite milk samples were deproteinated using 25% trichloroacetic acid at a ratio of 5 mL to 10 mL of milk and filtered through filter paper to obtain 6 mL of serum. For the analyses of urea in milk serum and urine urea, a method similar to that employed for blood plasma was used, except that the urine samples were diluted at 1:50. The urea measurements were corrected for urea-N by multiplying by 0.47.

Morning and afternoon milk production was measured every day and pooled to obtain an average daily production. One aliquot of 2% of each milking production was collected at days 7, 11, 15, and 19 of each experimental phase and placed in a 600-mL plastic flask. The following parameters were determined in the milk samples: fat content, by the Gerber method (Kleyn et al., 2001); density, using a thermolactodensimeter calibrated to 15 ºC and corrected for sample temperature; and CP content, by the Kjeldahl method (method number 984.13; AOAC, 1990). A factor of 6.38 was used to determine the CP content of the milk samples. The total milk solids (TMS) were estimated using the Fleischmann formula (Jost, 2000):

$$TMS\ (g/kg) = 1.2 \times fat + 26.65 \times ((100 \times D) - 100/D)$$

Eq. (2),

in which fat = milk fat content (g kg^{-1}), and D = milk density (g cm^{-3}).

The DM and CP intake rates, urea-N concentrations, blood glucose, and creatinine, milk production, and milk constituents were analyzed by including treatments, square, period within square, and cow within square and the random error in the statistical model. The least-squares means for each treatment were requested. The treatments were compared using the following orthogonal contrasts: CS × Others, EGS × SC, EGS/RDP × EGS/RUP, and SC/RDP × SC/RUP. The linear model was fitted by using the GLM procedure of SAS® software (Statistical Analysis System, version 9.0). P-values were considered significant whenever $\alpha<0.05$.

The Pearson correlation coefficient was used to measure the intensity of the linear correlation between the CP intake and the concentrations of urea-N, glucose, and creatinine in

the blood plasma and urea-N in the urine, according to the CORR procedure of SAS® software (Statistical Analysis System, version 9.0).

Results

The lactation stage of the cows, represented by the square effect, did not affect the analyzed variables. The means of the recorded body weights of the cows were 526±64, 523±67, 542±65, 521±73, and 532±60 kg in the CS, EGS/RDP, EGS/RUP, SC/RDP, and SC/RUP diet groups (treatments), respectively.

Animals fed corn silage consumed less fiber than the animals fed EGS and more than those fed sugarcane. The EGS × SC contrast for DMI scaled to the body weight of the animals was significant, and the animals consumed more of the diets containing elephant grass silage than the diets containing sugarcane. More of the SC/RDP treatment was consumed by the cows than the SC/RUP treatment (Table 3). Paralleling to DMI, the fiber intake rate by the animals fed elephant grass silage was higher than in the animals fed sugarcane. However, the animals that were fed sugarcane supplemented with concentrate containing RDP consumed more fiber than the animals fed the sugarcane diet containing RUP concentrate (Table 3).

In the model describing the diurnal pattern of variation in rumen NH_3-N (Eq. 1), the intercept represents the mean rumen NH_3-N concentration. Thus, it can be observed that diets with EGS provided a higher rumen ammonia concentration compared with the other diets, while CS showed a higher concentration than SC (Table 4).

The level of plasma urea-N was higher in cows fed elephant grass-based diets (Table 5). Plasma glucose was not influenced by the treatments, while the CS × Others contrast was significant for plasma creatinine and urine urea-N (Table 5). The contrasts involving the different roughage sources (CS × Others and EGS × SC) and protein degradability of elephant grass-based diets (EGS/RDP × EGS/RUP) were significant for milk urea-N, while protein degradability did not affect the milk urea-N concentration in cows that consumed SC as roughage (Table 5).

The first peak in the ammonia concentration for all diets occurred 0 to 6 h after the morning and evening feedings, after which it began to decline, reaching a minimum point at between 12 and 18 h. The second peak occurred approximately between 24 and 30 h, and it was assumed that this cycle repeats over time, assuming steady-state conditions (Figure 1).

The CP intake and urine urea-N showed no correlation (P = 0.126). However, urine urea-N did show a positive correlation with blood urea-N of 0.55 (P<0.001) and with milk urea-N of 0.60 (P<0.001).

Milk production of cows receiving treatments containing EGS was higher than those cows receiving treatments containing SC; milk production of the animals consuming elephant grass silage with RUP was higher than in the animals consuming the same forage supplemented with RDP (Table 6). The forage type (CS × Others and EGS × SC) and rumen degradability of dietary protein in the diets with EGS (EGS/RDP × EGS/RUP) affected the milk fat content (Table 6). By contrast, the rumen protein degradability did not affect the milk fat content in the animals that consumed sugarcane (SC/RDP × SC/RUP). The milk protein content

Table 3 - Intakes of dry matter (DM), neutral detergent fiber (aNDF), and crude protein (CP) for cows fed diets with different roughage and protein sources with high and low ruminal degradability in g kg^{-1} of body weight

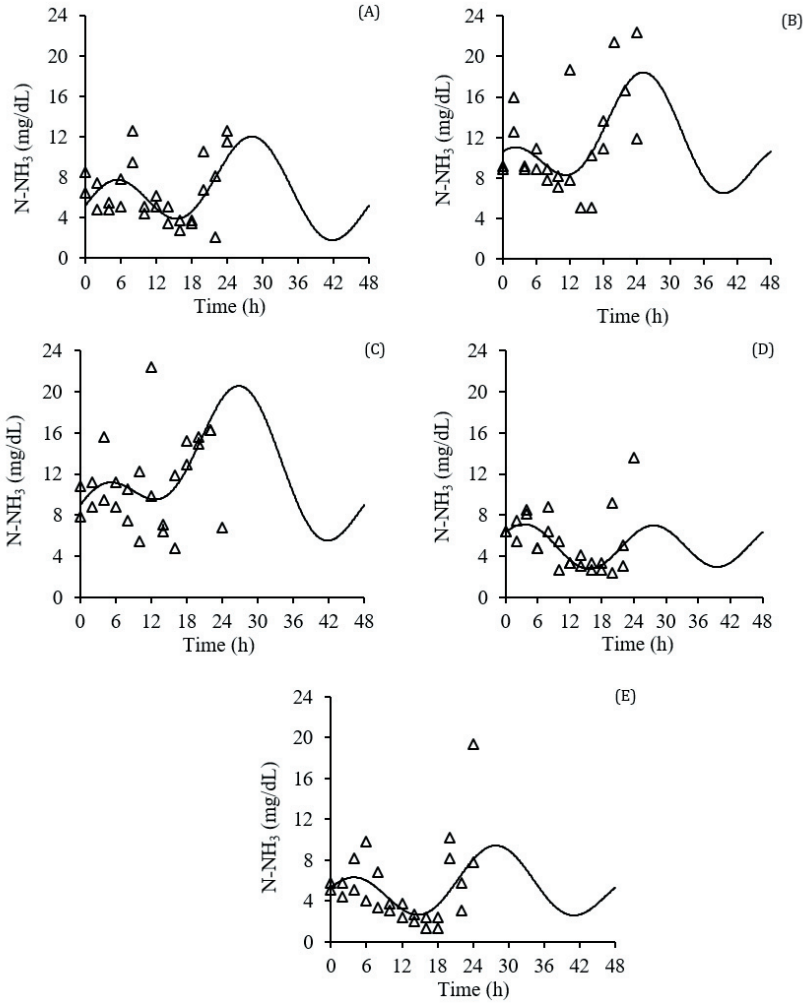
Variable	Treatment					SEM	P-value for the contrasts			
	CS	EGS		SC			1[1]	2[1]	3[1]	4[1]
		RDP	RUP	RDP	RUP					
DM	24.85	26.10	26.32	25.74	21.03	0.976	0.93	0.001	0.80	<0.001
aNDF	9.84	10.54	10.61	7.87	6.44	0.755	0.001	<0.001	0.83	0.001
CP	2.46	3.56	3.65	2.6	2.19	0.237	<0.001	<0.001	0.39	0.001

CS - corn silage; EGS - elephant grass silage; SC - chopped sugarcane; RDP - rumen degradable protein; RUP - rumen undegradable protein; SEM - standard error of the mean.
[1] 1 = CS × Others; 2 = EGS × SC; 3 = EGS/RDP × EGS/RUP; 4 = SC/RDP × SC/RUP.

Table 4 - Fitted equations of the time series models for the observed concentration of ammonia nitrogen in the rumen fluid (mg dL^{-1})

Treatment	Regression[1]	R^2
CS	$\hat{Y}_{T1} = 6.400 - 0.351 \sin(ct_i) - 2.376 \cos(ct_i) + 3.204 \sin(2ct_i) + 1.178 \cos(2ct_i)$	0.06
EGS/RDP	$\hat{Y}_{T2} = 11.281 + 0.130 \sin(ct_i) - 3.825 \cos(ct_i) + 1.400 \sin(2ct_i) + 3.106 \cos(2ct_i)$	0.06
EGS/RUP	$\hat{Y}_{T3} = 12.000 - 0.272 \sin(ct_i) - 5.184 \cos(ct_i) + 2.999 \sin(2ct_i) + 2.172 \cos(2ct_i)$	0.05
SC/RDP	$\hat{Y}_{T4} = 4.965 - 0.057 \sin(ct_i) + 0.100 \cos(ct_i) + 1.682 \sin(2ct_i) + 1.248 \cos(2ct_i)$	0.07
SC/RUP	$\hat{Y}_{T5} = 5.296 - 0.726 \sin(ct_i) - 1.377 \cos(ct_i) + 2.174 \sin(2ct_i) + 1.377 \cos(2ct_i)$	0.13

CS - corn silage; EGS - elephant grass silage; RDP - rumen degradable protein; RUP - rumen undegradable protein; SC - chopped sugarcane.
[1] c is the fundamental period equal to 0.1309 rad h^{-1}.



Δ - least squares means; solid lines - predicted values.
A - corn silage; B - elephant grass silage with rumen degradable protein; C - elephant grass silage with rumen undegradable protein; D - sugarcane with rumen degradable protein; E - sugarcane with rumen undegradable protein.

Figure 1 - Nycterohemeral patterns of ruminal ammonia nitrogen (NH_3-N, mg dL^{-1}) in cows fed diets with different roughage types combined with protein sources with high and low ruminal degradabilities.

Table 5 - Contrasts among and least squares means of levels of metabolites in blood plasma, urine, and milk of cows fed diets with different roughage types and protein sources with high (RDP) and low (RUP) ruminal protein degradabilities

Metabolite	Treatment					SEM	P-value for the contrasts			
	CS	EGS		SC			1[1]	2[1]	3[1]	4[1]
		RDP	RUP	RDP	RUP					
Blood plasma (mg dL^{-1})										
N-urea[2]	3.00	12.70	10.70	4.70	3.40	0.681	0.329	0.021	0.522	0.279
Glucose	42.40	43.80	43.30	43.30	44.00	3.007	0.253	0.892	0.724	0.597
Creatinine	1.16	1.09	1.04	1.04	1.03	0.139	0.035	0.495	0.456	0.899
Urine (mg dL^{-1})										
N-urea[2]	67.80	514.10	539.70	431.50	246.60	0.643	0.006	0.478	0.513	0.853
Milk (mg dL^{-1})										
N-urea	2.30	11.30	8.60	3.70	2.60	2.250	0.001	0.001	0.013	0.275

CS - corn silage; EGS - elephant grass silage; SC - chopped sugarcane; RDP - rumen degradable protein; RUP - rumen undegradable protein; SEM - standard error of the mean.
[1] 1 = CS × Others; 2 = EGS × SC; 3 = EGS/RDP × EGS/RUP; 4 = SC/RDP × SC/RUP.
[2] These variables were transformed to meet the homoscedasticity criteria as log(y) and then rescaled to be presented.

Table 6 - Milk yield and composition of cows fed diets with different roughage and protein sources with high (RDP) and low (RUP) ruminal protein degradability

Variable	Treatment					SEM	P-value for the contrasts			
	CS	EGS		SC			1[1]	2[1]	3[1]	4[1]
		RDP	RUP	RDP	RUP					
Production (kg day^{-1})										
Milk yield	14.70	13.76	16.40	14.44	13.92	1.226	0.868	0.03	0.001	0.35
4% fat corrected milk	13.66	13.84	16.03	13.46	13.16	1.086	0.235	0.001	0.001	0.547
Milk composition (kg day^{-1})										
Fat	0.52	0.56	0.63	0.51	0.51	0.045	0.049	0.001	0.001	0.78
Protein	0.41	0.38	0.45	0.42	0.38	0.033	0.833	0.066	0.001	0.033
Total milk solids	1.82	1.74	2.07	1.79	1.72	0.157	0.849	0.005	0.001	0.278
Composition (g kg^{-1} of milk)										
Fat	35.00	41.00	39.00	36.00	37.00	1.988	0.001	0.001	0.025	0.439
Protein	28.00	28.00	28.00	29.00	28.00	1.653	0.485	0.546	0.738	0.08
Total milk solids	124.00	127.00	127.00	125.00	124.00	3.576	0.186	0.028	0.557	0.661

CS - corn silage; EGS - elephant grass silage; SC - chopped sugarcane; RDP - rumen degradable protein; RUP - rumen undegradable protein; SEM - standard error of the mean.
[1] 1 = CS × Others; 2 = EGS × SC; 3 = EGS/RDP × EGS/RUP; 4 = SC/RDP × SC/RUP.

did not differ among the treatments, and the total milk solids content only differed when comparing the diets based on elephant grass silage and sugarcane (EGS × SC).

Discussion

The low intake of the SC-based compared with the EGS-based diets may be related to the lower degradation and passage rates of SC, as reported by Fernandes et al. (2001). Waldo et al. (1972) proposed a model of interdependence among the intake, passage, and degradation rates that reinforces the causal relationship between the ruminal dynamics of digesta and nutrient intake of SC diets. Intake estimates based on this model proportionally increase with disappearance of the ruminal digesta, which occurs via degradation or escape of the potentially degradable fraction or via passage of the indigestible fraction. According to Poppi et al. (1981), the particles present in the rumen are reduced via chewing, ruminal movements, and microbial degradation until they reach a critical size of 1.18 mm, at which point they escape the rumen. Low-degradability feeds can take longer times to disappear from the rumen, causing rumen fill and reduced intake rates due to the pressure that the fibrous matter exerts on ruminal mechanoreceptors, which in turn send appetite-inhibiting signals to the central nervous system (Van Soest, 1994; Forbes, 1996; Leek, 2004).

Another factor that affects the DMI is the synchronization between the ruminal degradation of carbohydrates and nitrogenous compounds (Sniffen et al., 1992; Van Soest, 1994; Fox et al., 2004). When the peak protein availability does not occur simultaneously with the peak production of volatile fatty acids in the rumen (because of a greater amount of available carbohydrates), the efficiency of microbial protein production decreases, which can reduce microbial growth, reduce fiber degradation and turnover, and cause rumen fill (Van Soest, 1994). Thus, use of a RDP source combined with sugarcane promoted a higher DMI (Table 3). The use of the CNCPS model to evaluate diets is useful to estimate the nutritive value of different forage resources and other tropical forage-based diets (Cabral et al., 2000; Vieira et al., 2000a,b,c). The dietary synchronization is important for microbial growth and, depending on the availability of non-fibrous carbohydrates, the microbial biomass uses degradable nitrogen more efficiently (Russell et al., 1992; Malafaia et al., 1998; 1999; Vieira et al., 2000c; Favoreto et al., 2008; Fernandes et al., 2014). Because of the nutritional imbalances of sugarcane, e.g., high soluble sugars and low protein content, it should be used with caution to prevent excess nutrient losses (Fernandes et al., 2001).

Broderick (2003) stated that in addition to synchronizing the availability between carbon and nitrogen in the rumen, it is important to provide high-quality protein to be directly digested by the intestinal enzymes of the host. And for a synchronized supply between non-fiber carbohydrates and RDP to maintain the needs of the rumen microorganisms, RUP sources with high intestinal digestibility should also be adequately supplied to meet the metabolizable protein requirement of lactating cows. The animals that received elephant grass silage had higher milk production and higher yields of fat and total milk solids than those that received sugarcane due to the higher DM and CP intakes. This is most likely due to a better concurrent availability of energy substrates and N compounds in the rumen (AFRC, 1993; NRC, 1996; Broderick, 2003).

In the EGS treatment, the animals that received RUP had higher milk production than those that received RDP, which may be associated with a higher availability of amino acids in the small intestine from digesting the protein that escaped microbial degradation in the rumen (Broderick, 2003). Huhtanen and Hristov (2009) and Ipharraguerre and Clark (2005) found that raising RUP levels of the diet is not necessarily associated with positive increments in milk production. Huhtanen and Hristov (2009) stated that one of the possible causes of these inconsistent responses to elevated dietary RUP levels may be related to an excess of metabolizable protein supply, i.e., in excess of the amount required. Therefore, actual amounts of RUP flowing to the intestines are usually in excess of the requirements of the cow. Castro et al. (2008) observed that large amounts of RUP are not associated with increases in either milk yield or N use by dairy cows fed alfalfa silage. It is possible that current models used to compute RUP amounts are biased to some extent by ignoring attributes (e.g., heterogeneity) of the ruminoreticular digesta (Huhtanen and Hristov, 2009).

Maximal rumen fermentation activity is achieved when rumen ammonia shows a concentration between 5 (Satter and Slyter, 1974) and 10 mg dL^{-1} (Van Soest, 1994). However, these values must not be considered fixed numbers because the uptake and use of ammonia for protein synthesis by bacteria depends on the rate of carbohydrate fermentation. Based on what was postulated by Van Soest (1994), the use of EGS-based feed results in an optimum rumen ammonia concentration (Table 4), which can be explained by higher protein intake (Tables 3). When a sugarcane-based diet was used, the mean concentration of NH_3-N was close to 5 mg dL^{-1} of rumen fluid, which is considered a minimum value to maintain optimal levels of microbial growth (Satter and Slyter, 1974). It is worth noting that any NH_3-N over this limit is not likely to be incorporated into microbial protein (Satter and Roffler, 1975).

The peak rumen ammonia concentration recorded in this study occurred from 3 to 6 h after feeding, a trend similar to that reported by Van Soest (1994). A drop in the rumen ammonia concentration occurred between 6 and 18 h after feeding, due either to the use of a portion of the ammonia for microbial protein synthesis or to absorption by the rumen epithelium, where ammonia is constantly absorbed into the bloodstream to be transferred to the liver and converted into urea, with associated energy expenditure. Urea can be recycled via saliva or it can be excreted in urine (Van Soest, 1994; Butler, 1998). Dairy cows fed diets containing dry rolled or pelleted barley combined with whole canola or whole flaxseed recycled from 58 to 65% of the urea N synthesized in the liver (Gozho et al., 2008). Claypool et al. (1980) found that higher levels of plasma urea-N resulted from the use of diets with higher CP levels. In the present study, SC-based diets presented lower levels of plasma urea-N if compared with EGS-based diets. Therefore, differences in the availability of nitrogen and carbohydrate fractions in the rumen possibly influence N use, and the greater amount of protein in the EGS resulted in luxuriant RDP intake. The high content of soluble carbohydrates in SC may have favored the use of ammonia released during protein degradation by rumen microbes. Both ammonia metabolism in the liver and urea excretion by the kidneys incur an energy cost for the animal (NRC, 1996), which can reduce its productive performance. However, the milk production of animals that consumed EGS was higher than that of animals that consumed SC, despite the higher plasma urea-N concentration of EGS. Sugarcane exhibits a low nutritional value and high availability of sugars in the rumen, which may limit the general use of N compounds by the animal (Fernandes et al., 2001).

Glucose is responsible for countless functions in ruminant species, and a change in blood glucose is indicative of problems in the animal. According to Kahn and Line (2010), the range of variation in normal levels of blood glucose in cows is 42 to 72 mg dL^{-1}. Therefore, the blood glucose levels observed in the cows of the present study were considered normal and were not influenced by the treatments.

Creatinine excretion is little affected by the dietary levels of protein, non-fiber carbohydrates, or non-protein nitrogen (Ørskov and MacLeod, 1982). However, the animals fed corn silage exhibited higher creatinine excretion, which may be associated with the mobilization or deposition of reserves because creatinine results from protein metabolism in muscle tissue and its excretion scales to the amount of muscular tissue (Brody, 1945). However, as we did not evaluate the body composition of cows and a Latin square as the experimental design was adopted, we cannot conclusively affirm what may have caused the significant difference in creatinine excretion observed for the CS × Others contrast. Nonetheless, the P-values obtained for this variable using the applied frequentist statistical analysis were, from a Bayesian point of view, close to false positive detection levels (Johnson, 2013).

The urinary excretion of urea showed a correlation with plasma urea-N, despite the lack of consistency between treatment effects on serum and urine responses measured in this study (Table 5). Harmeyer and Martens (1980) and Ferreira et al. (2009) reported that the amount of urea excreted in urine is mainly influenced by the plasma urea

concentration. Therefore, the N of EGS-based diets might have been used in a less conservative way by the cows in the present study.

According to Kauffman and St-Pierre (2001), an increase in the concentration of rumen ammonia results in a simultaneous increase in plasma urea and its consequent diffusion into the milk. The concentration of urea in milk is an indicator of the intensity of protein metabolism in cows (Jonker et al., 1998). In the present study, higher levels of milk urea-N were observed in association with the RDP diet only when EGS was used, most likely due to a rumen imbalance between nitrogen and energy availability resulting from the lower content of soluble carbohydrates in this forage source. The efficiency by which microorganisms in the rumen use nitrogen reflects on how the host also uses nitrogen; apparently, in this study, the corn silage-based diet contained a larger amount of synchronizable protein and carbohydrate fractions that might explain the lower observable plasma, milk, and urine urea-N.

Conclusions

Elephant grass silage combined with slow rumen degradable protein sources provides more nutrients so that lactating animals achieve a significant higher performance. This diet increases milk yield to approximately 18% more than diets based on sugarcane (rumen degradable and undegradable protein), elephant grass silage supplemented with rumen degradable protein, or corn silage-based diets.

The use of diets based on corn silage leads to better use of nitrogen compounds because these diets result in lower levels of urea nitrogen in plasma, urine, and milk at the same level as milk production, i.e., 14.7±0.97 kg day^{-1}, compared with diets containing elephant grass silage or sugarcane as forage sources. In sugarcane-based diets, greater nitrogen losses in the urine are observed, despite the greater availability of soluble and readily fermentable carbohydrates.

Acknowledgments

T. S. Oliveira thanks Fundação Carlos Chagas Filho de Amparo à Pesquisa do Estado do Rio de Janeiro – FAPERJ for the postdoctoral fellowship (case number E-26/101.429/2014) and Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – CAPES (E-45/2013-PARDPJ). R. A. M. Vieira thanks Conselho Nacional de Desenvolvimento Científico e Tecnológico – CNPq for the grant provided (case number 304920/2015-0).

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