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EFFICIENCY OF DIETARY NITROGEN UTILIZATION AND DIGESTIVE METABOLISM OF DAIRY COWS FED DIFFERENT NITROGEN SOURCES AND SUGARCANE

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ABSTRACT

The aim of this study was to evaluate four main sources of dietary nitrogen on the efficiency of nitrogen utilization and digestive metabolism of dairy cows fed diets with sugarcane as forage. Twelve multiparous Holstein cows, averaging 150 days in milk and 16.9 kg of milk/day, were distributed into three balanced and contemporary 4 × 4 Latin squares. The study consisted of four experimental periods of 21 days, with 14 days for diet adaptation and the remainder for sampling. Cows were fed isoproteic diets (157.0 g CP/kg DM) and sugarcane as the exclusive forage. The following main nitrogen dietary sources were evaluated: control - soybean meal and urea; soybean meal associated to high level of urea; raw soybean; and, corn gluten meal. Dietary nitrogen sources had no effect on ruminal pH and on microbial protein synthesis. However, the concentrations of ruminal ammonia, urea and blood urea nitrogen were lower for cows fed raw soybean than for cows fed the other nitrogen sources. In conclusion, the utilization of raw soybean as dietary main nitrogen source increases the efficiency of dietary nitrogen utilization of dairy cows fed diets with sugarcane as forage.

Key words: blood parameters; nitrogen balance; protein synthesis; ruminal fermentation and degradability

INTRODUCTION

Sugarcane (*Saccharum* spp.) is an important forage source that is used especially in tropical countries for dairy cows feeding, especially during winter period, in which there is a shortage of tropical pasture production. The increased use of sugarcane for ruminant feeding occurs mainly because of its high dry matter (DM) yield and its high concentration of sucrose, which is an important source of readily available energy in the rumen (Fernandes *et al.* 2003). However, feeding sugarcane for dairy cows has some limitations, such as low minerals and protein concentrations, and low digestibility of the fiber fraction, leading to reduced dry matter intake (DMI) (Magalhães *et al.* 2004).

The amino acids required for the metabolism in ruminants originate from the dietary rumen undegradable protein (RUP), rumen microbial protein and endogenous secretions (NRC 2001). Among these main sources of amino acids, the rumen microbial protein is the most important one, both qualitatively and quantitatively, because it has adequate amino acid profile independent of the diet (Dias *et al.* 2008). Adequate rumen degradable protein (RDP) supply can optimize microbial growth and increases the inflow of metabolizable protein to the intestines. Additionally, the availability of adequate amount of protein in the rumen, without exceeding the capacity of nitrogen utilization by microorganisms, can reduce nitrogen losses as urea. The reduction of nitrogen losses by endogenous and ruminal metabolism can increase the utilization efficiency of nitrogen sources, which reduces the nitrogen excretion to the environment and the costs of dairy cows feeding (Oba and Allen 2003).

Therefore, the rumen degradability of nitrogen sources in diets based on sugarcane as forage can affect the efficiency of dietary N use for microbial protein. Previous studies have evaluated the effect of different nitrogen sources in diets containing sugarcane as a substitute of corn silage on DMI, milk yield and composition and ruminal parameters (Mendonça *et al.* 2004, Magalhães *et al.* 2006, Sousa *et al.* 2009). However, few studies evaluated the degradability effect of nitrogen sources on the efficiency of nitrogen utilization in diets for dairy cows fed sugarcane as forage. Thus, the aim of the present study was to evaluate the effect of dietary nitrogen sources on ruminal fermentation parameters, microbial protein synthesis and efficiency of dietary nitrogen utilization.

MATERIALS AND METHODS

Twelve Holstein cows in the mid lactation (150 ± 46 DIM; mean ± SD), with an average body weight (BW) of 581±60 kg, and an average milk yield of 16.8±3.9 kg/day at the beginning of the study were randomly assigned to three contemporary 4 × 4 Latin squares. The experiment was conducted in four periods of 21 days, divided in 14 days of adaptation and 7 days of sample collection.

Diets were formulated according to NRC (2001) to be isoproteic (157.0 g CP/kg DM), and cows were assigned to receive one of the following diets: control - soybean meal and urea (104.0 g RDP/kg DM); soybean meal associated to high level of urea (112.0 g RDP/kg DM); raw soybean (109.0 g RDP/kg DM); and, corn gluten meal (98.0 g RDP/kg DM) (Table 1). Sugarcane (*Saccharum* spp.; variety IAC86-2480) was used as exclusive forage and the diets had a forage-to-concentrate (F:C) ratio based on DM of 45:55. The sugar content of sugarcane was determined by refractometry (Handheld®, refractometer model RHBO-90) which showed an average of 16º Brix®. Sugarcane was harvested manually each day prior to use and then it was chopped (Nogueira®, chopper model EM-9F3B) without straw removal to have an average particle size between 1.0 to 2.0 cm.

Throughout the experiment, cows were housed in individual stalls in a free-stall system and diets were fed as a TMR twice daily at 08:00am and 04:00pm. Amounts of feed offered and orts were weighed for each cow from d 14 to 21. Samples of all diet ingredients (0.5 kg), forage from each animal (0.5 kg) and orts from each cow were collected daily on d 15 to 18 and combined into 1 sample to represent 3 d for digestibility analysis (d 14 to 21). All samples were immediately frozen at -20°C. Cows were milked twice daily (06:00am and 03:00pm) during all the experiment.

Fecal samples (0.5 kg) were collected twice daily from d 15 to 18 at 08:00am and 04:00pm directly from the rectum, and so that as recombined for each animal each period. To estimate total fecal excretion of each animal, feeds, orts and feces samples were incubated for 288 h in the rumen of two cannulated Nellore steers to determination of the concentration of indigestible acid detergent fiber (iADF) used as internal marker (Casali *et al.* 2008).

Samples of orts, feed ingredients and feces were analyzed according to the methods described by AOAC (1995) for dry matter (DM, AOAC 950.15), ash (AOAC, 942.05), ether extract (EE, AOAC 920.39), CP (AOAC, 984.13). Non-fibre carbohydrates (NFC) were estimated by the method described by Hall (2000), where NFC = 100 - ([% CP - % urea CP + % UREA] + % EE + % NDF + % MM). Neutral detergent fiber (NDF), acid detergent fiber (ADF), neutral detergent insoluble nitrogen (NDIN), acid detergent insoluble nitrogen (ADIN) and lignin were obtained according to the method described by Van Soest *et al.* (1991). Analysis of NDF was determined using sodium sulphide and α-amylase, at Ankon® System. Diet ingredients and diets chemical composition are presented in Table 1.

Data and samples were collected to evaluate the efficiency of dietary nitrogen utilization, which were analyzed by ruminal microbial protein synthesis, blood metabolic parameters and nitrogen balance. Samples of ruminal fluid were collected with an esophageal tube three hours after the morning feeding to evaluate rumen fermentation parameters. Ruminal pH was determined using a potentiometer immediately after samples collection. The ammonia concentration of the ruminal fluid was determined according to the methodology described by Bergmeyer (1985).

The analysis of ruminal concentration of short-chain fatty acids was performed according to Erwin *et al.* (1961). For this analysis a gas chromatograph (model 9001; Finnigan/Tremetrics, San Jose, CA, USA) equipped with a glass column (2 m x ¼.) and packed with 80/120 Carbopack B-DA/4% Carbowax 20M was used.

Milk samples from both morning and afternoon milking were collected on two alternate days (18th and 20th) for determination of allantoin concentration in milk. Spot urine samples were collected in the day 16th of each experimental period for determination of urea, creatinine, allantoin, uric acid and total nitrogen. Concentrations of urine allantoin and uric acid and milk allantoin were determined by colorimetry according to the method of Fujihara *et al.* (1987), described by Chen and Gomes (1992). Total excretion of purine derivatives (PD) expressed in mmol/d was calculated by the sum of the excretions of allantoin and uric acid in the urine, and by the amount of allantoin excreted in the milk. The amount of absorbed microbial purines (absP) was calculated (mmol/d) using the PD excretion by the following equation: $absP = (PD - 0.236 * BW^{0,75})/0.84$, where 0.84 stands for the recovery of absorbed purines, and $0.236*PV^{0,75}$ is the endogenous excretion of PD (Orellana Boero *et al.* 2001). Absorbed purines were also evaluated by considering the endogenous excretion through the application of equation described by González-Ronquillo *et al.* (2003). The synthesis of rumen microbial nitrogen (micN, g N/d) was determined in terms of microbial purines absorbed (mmol/d) (Chen and Gomes 1992): $micN = (70*absP)/(0.83*0.134*1{,}000)$, where 70 is the N content of purines (mg N/mol); 0.83 is the intestinal digestibility of microbial purines; and 0.134 is the ratio between N content of purines and total N content of ruminal microorganisms (Valadares *et al.* 1999).

Daily total urine volume was obtained by the concentration of urine creatinine according to the method described by Valadares *et al.* (1999) and Rennó *et al.* (2008). Creatinine concentrations were determined using commercial kits (Laborlab®) and the enzyme kinetic was determined using a calorimetric device (SBA-200, CELM®). The obtained results were calculated by the following equation: Creatinine (mg/dL) = Creatinine (mg/dL)*0.020*50 (Biggs and Copper 1961). Daily total urine volume was estimated by dividing the daily urinary excretion of creatinine by the observed values of creatinine concentration in the urine spot samples, in accordance with Oliveira *et al.* (2001). The urinary excretion of creatinine was estimated from the 24.05 mg

creatinine/kg live weight (LW) proposed by Chizzotti *et al.* (2007). Thus, with the availability of the values of average daily creatinine excretion and creatinine concentration (mg/dL) presented in the spot urine samples, the total daily volume of urine (liter per cow/d) were estimated for further calculation of nitrogen balance, which was obtained by the difference among total N ingested and total N excreted in feces, milk and urine.

Table 1. Ingredients and composition of diets (g/kg) on a dry matter (DM) basis

Ingredients	Diets (main nitrogen sources)[9]			
	Control[1]	SBM[2] and high urea level	Raw soybean	Corn gluten meal
	DM g/kg			
Sugarcane	469.5	475	475.7	479
Ground corn	303.9	335	240.1	303
48% CP soybean meal	164	120	64.2	123
Raw soybean	-	-	160	-
Corn gluten meal	-	-	-	26.7
Urea	10.2	17.1	7.5	10.2
Ammonium Sulfate	1	1	1	0.5
Sodium Bicarbonate	7.5	7.5	7.5	6.9
Magnesium Oxide	2.7	2.7	2.7	2.7
Mineral mixture[8]	34.7	34.8	34.8	34.8
Limestone	3.7	3.7	3.7	3.7
Salt	2.7	2.7	2.7	2.7
	Chemical Composition g/kg			
Dry matter[3]	635.8	632	625.9	623.1
Organic matter[4]	907.3	902.6	916.9	910.5
Ash[4]	92.7	97.4	83.1	89.5
Crude protein[4]	156.8	158.1	153.1	155.3
RDP[4]	104	112	109	98
RUP[4]	53	46	49	59
ADIN[5]	16.7	14.4	15.9	14.9
NDIN[6]	50	48.4	46.6	46.7
Ether extract[4]	20.9	21.3	41.4	21.3
Neutral detergent fiber[4]	274.6	275.9	283.5	274.8
NDFa[4,10]	258.8	262.1	267.9	261
NDFp[4,11]	242.7	245.8	249.2	244.8
ADF[4]	169.3	167.5	185.4	168
iADF	60.9	61.6	61.5	62
Lignin[4]	31.1	30.7	32.7	31.4
Non-fibrous carbohydrate[4]	484	495.9	452.7	481.2
NE_L(Mcal/kg DM)[7]	1.63	1.63	1.72	1.65
TDN (Mcal/kg)[7]	706	703.8	736.9	710.2

[1]Control diet: soybean meal and urea (104.0 g/kg DM); [2]SBM: soybean meal associated to high level of urea; [3]natural matter basis; [4]dry matter basis; [5]Acid detergent insoluble nitrogen (related to total nitrogen); [6]Neutral detergent insoluble nitrogen (related to total nitrogen); [7]Estimate by NRC (2001); [8]Mineral mixture composition per kg: 180 g Ca, 90 g P, 20 g Mg, 20 g S, 100 g Na, 3,000 mg Zn, 1,000 mg Cu, 1,250 mg Mn, 2,000 mg Fe, 200 mg Co, 90 mg I, 36 mg Se, 900 mg F (maximum); [9]Isonitrogenous treatments (157 g/kg DM); [10]Corrected to ash; [11]Corrected to protein.

Blood samples were taken on day 16th of each experimental period, before the morning feeding, by vein or artery coccygeal puncture. Blood samples were analyzed to determine concentrations of NEFA, triglycerides, total cholesterol, HDL-cholesterol, LDL-cholesterol, VLDL-cholesterol, total protein, albumin, glucose, urea and ureic nitrogen. Analyses of blood parameters were performed using commercial kits (Laborlab® and CELM®) based on enzymatic colorimetric method and the data were analyzed by an automatic blood chemistry analyzer (Automatic Biochemistry System, SBA-200-CELM®). The concentrations of LDL-cholesterol and VLDL-cholesterol were determined indirectly by the following equations:

VLDL-cholesterol (mg/dL) = (concentration of triglycerides/5); and, LDL-cholesterol (mg/dL) = Total cholesterol - (HDL-cholesterol + VLDL-cholesterol) (Friedewald *et al.* 1972).

The data were analyzed using the Statistical Analysis System® (Version 9.2, SAS Institute, Cary, NC) after testing for normality of residuals and homogeneity of variance with the UNIVARIATE procedure. The data were analyzed according to the main effects of the treatments with the MIXED procedure of SAS according to the following model:

$$Y_{ijkl} = \mu + T_i + Q_j + A(Q)_k + Pl + e_{ijkl}$$

where Y_{ijkl} =dependent variable; μ = overall mean; Ti = fixed effect of i treatment (3 DF); Qj = fixed effect of Latin square j (j = 1 to 3; 2 DF); $A(Q)_k$ = random effect of cow k within each Latin square, k = 1 to 12 (9 DF); Pl = fixed effect of period l, l = 1 to 4 (3 DF); and e_{ijkl} = random error associated to each observation. The degrees of freedom were calculated according to the Satterthwaite (= DDFM Satterth) method.

Least squares means estimates were reported and the separation of least squares means was performed at α = 0.05 using PDIFF option of the LSMEANS statement. For all statistical analyses, significance was declared at $P \leq 0.05$ and trends at $P \leq 0.10$.

RESULTS

Ruminal parameters: Samples of ruminal fluid were collected to evaluate the rumen availability of nitrogen sources and the production of short-chain fatty acids (SCFA) by ruminal bacteria. Diets had no effect on ruminal pH and on SCFA measured either as concentration or as percentage of total SCFA (Table 2). The concentration of NH_3-N in ruminal fluid was higher for cows fed soybean meal associated with high levels of urea as nitrogen sources (12.17 mg/dL) than for cows fed the diet containing raw soybean (7.6 mg/dL) or corn gluten meal (9.23 mg/dL).

Table 2. Ruminal parameters according to main nitrogen dietary sources for dairy cows

Item	Diets (main nitrogen sources)				*SEM	*P*
	[1]Control	[2]SBM and high urea level	Raw soybean	Corn gluten meal		
pH	6.99	6.94	6.85	6.94	0.03	0.33
NH_3-N[3] (mg/dL)	9.51[a, b]	12.17[a]	7.60[b]	9.23[b]	0.58	0.02
			SCFA[4] *(% of total SCFA)*			
Acetic (C2)	65.10	65.06	64.24	64.30	0.32	0.38
Propionic (C3)	21.18	20.93	21.67	21.25	0.27	0.59
Butyric (C4)	13.72	14.01	14.09	14.45	0.22	0.53
			SCFA[4] *(mmol/L)*			
Acetic (C2)	45.32	48.10	48.76	45.86	1.48	0.75
Propionic (C3)	14.85	15.45	16.60	15.14	0.56	0.62
Butyric (C4)	9.62	10.44	10.44	10.44	0.37	0.73
Total SCFA	69.79	74.00	75.8	71.44	2.32	0.74
C2/C3[5]	3.10	3.16	2.98	3.05	0.05	0.33

Least squares means within a row with different superscripts letters are different (P < 0.05); *SEM= Standard error of mean; [1]Control diet: soybean meal and urea (104.0 g/kg DM); [2]SBM: soybean meal associated to high level of urea; [3]NH3-N: ammonia nitrogen; [4]SCFA: short-chain fatty acids; [5]C2:C3: Acetato/propionate ratio.

The effect of nitrogen sources on the microbial protein synthesis was estimated by analysis of purine derivatives. The production of purine derivatives (allantoin present in milk and urine and uric acid), absorbed purines, and total purine production were not affected by diets in this study (Table 3). Nitrogen metabolism and microbial protein synthesis did not differ according to diets. Likewise, microbial protein synthesis efficiency (grams of microbial protein per kg of TDN intake) was similar among diets. However, cows fed diets with high levels of urea and soybean as nitrogen source, tended (*P*=0.07) to eliminate less uric acid (mmol/d) than cows fed the others diets evaluated in this study.

Blood parameters: Plasma glucose concentration was not altered by diets (Table 4). Similarly, total serum protein concentration, albumin and non-esterified fatty acids did not differ among diets. Cows fed diets containing raw soybean had higher plasma total cholesterol concentration (185.3 mg/dL) than cows fed other diets (140.4 mg/dL). In another way, cows fed raw soybean diet had lower levels of blood urea (24.0 mg/dL) and blood urea nitrogen (11.10 mg/dL) compared with cows fed control diet (28.10 and 13.12 mg/dL, respectively) and diet containing corn gluten meal (29.42 and 13.75 mg/dL, respectively).

Table 3. Microbial production according to main nitrogen dietary sources for dairy cows.

Item	Diets (main nitrogen sources)				*SEM	*P*
	Control[1]	SBM[2] and high urea level	Raw soybean	Corn gluten meal		
			mmol/L			
Al-milk[3]	0.03	0.04	0.04	0.50	0.002	0.21
Al-urine[4]	19.27	19.20	21.90	18.98	1.14	0.72
Uric acid	0.58	0.72	0.52	0.74	0.04	0.07
			mmol/d			
Al-milk	0.65	0.80	0.74	0.87	0.05	0.35
Al-urine	199.14	228.55	239.54	198.66	14.85	0.58
Uric acid	6.49	8.65	6.40	7.82	0.56	0.32
Total purines	205.69	237.32	245.97	206.54	15.15	0.59
PD[5]	20.71	20.75	20.66	20.68	0.29	0.93
absP[6]	220.22	257.82	268.22	221.26	18.13	0.59
			g/d			
micN[7]	138.59	162.25	168.82	139.24	11.41	0.59
micP[8]	866.29	1014.13	1055.11	870.40	71.29	0.59
			g micP/Kg TDN			
Efficiency	68.50	81.00	79.30	68.20	5.90	0.71
			L/d			
Urine	10.96	11.82	11.61	10.57	0.55	0.77

*SEM= Standard error of means; [1]Control diet: soybean meal and urea (104.0 g/kg DM); [2]SBM: soybean meal associated to high level of urea; [3]Allantoin from milk samples; [4]Allantoin from urine samples; [5]PD: purine derivatives; [6]absP: absorbed purines; [7]micN: microbial nitrogen; [8]micP: microbial protein.

Table 4. Blood metabolites according to dietary protein sources for dairy cows

Item	Diets (main nitrogen sources)				*SEM	*P*
	Control[1]	SBM[2] and high urea level	Raw soybean	Corn gluten meal		
			mg/dL			
Glucose	54.90	54.90	55.00	53.90	2.42	0.98
Cholesterol	139.00[b]	139.00[b]	185.83[a]	143.33[b]	4.85	<0.0001
HDL-C[3]	35.50[b]	50.50[a]	44.75[a,b]	38.33[b]	2.07	0.02
Urea	28.10[a,b]	30.58[a]	24.00[b]	29.42[a]	0.99	0.04
BUN[4]	13.12[a,b]	14.29[a]	11.10[b]	13.75[a]	0.46	0.04
			g/l			
TP[5]	5.67	5.13	5.16	5.34	0.11	0.32
Albumin	2.35	2.2	2.29	2.22	0.05	0.7
			mmol/l			
NEFA[6]	0.281	0.293	0.308	0.3	0.02	0.89

Least squares means within a row with different superscripts letters are different (P < 0.05); *SEM= Standard error of mean; [1]Control diet: soybean meal and urea (104.0 g/kg DM); [2]SBM: soybean meal associated to high level of urea; [3]HDL-C: High-density lipid cholesterol; [4]BUN: blood urea nitrogen; [5]TP:Total protein; [6]NEFA: nonesterified fatty acids.

Nitrogen balance: Nitrogen intake and excretion of N via urine, feces and milk were determined to evaluate the effect of different nitrogen sources on N balance. In the present study, N intake was similar among diets. However, N excretion in feces (g N/d and g/100g of N intake) was lower for cows fed raw soybean than those fed the control diet or the diet containing corn gluten meal as a nitrogen source. Cows fed soybean meal and high urea content had higher nitrogen excretion in feces (g/100g of N intake) and urine (g N/d and g/100g of N intake). In another way, nitrogen excretion in milk did not differ among the diets, when results were expressed as g/d. However, when milk N excretion was evaluated as percentage of total N intake, a higher excretion of N in milk was observed for cows fed raw soybean (Table 5). As a consequence, cows fed diet with raw soybean had a higher efficiency of dietary nitrogen utilization for milk protein production (0.25 g N Milk /g N Intake) than for

cows fed diets with other nitrogen sources. In contrast, the highest N retained was observed for cows fed diets containing corn gluten meal (34.43 g/100 g of N intake).

Table 5. Nitrogen balance according to dietary protein sources for dairy cows

Item	Diets (main nitrogen sources)				*SEM	*P*
	Control[1]	SBM[2] and high urea level	Raw soybean	Corn gluten meal		
			g N/d			
Intake	478.19	472.88	476.38	505.09	6.75	0.15
Fecal excretion	143.29[a]	142.54[a,b]	127.32[b]	149.11[a]	3.27	0.04
Urinary excretion	118.62[b]	154.48[a]	130.53[b]	130.72[b]	5.70	0.01
Milk secretion	10.52	105.36	109.15	111.36	2.73	0.78
N retained	109.76	70.52	109.39	113.9	8.52	0.07
			g/100 g of N intake			
Fecal excretion	30.13[b]	33.44[a]	24.07[c]	29.96[b]	0.83	<0.0001
Urinary excretion	22.80[b]	35.68[a]	36.47[a]	16.72[c]	1.32	<0.0001
Milk secretion	22.37[b]	21.49[b]	25.51[a]	18.88[c]	0.48	<0.0001
N retained	24.69[b]	9.21[d]	13.93[c]	34.43[a]	1.66	<0.0001
			g N milk/g N intake			
Eficiency[3]	0.22[b]	0.22[b]	0.25[a]	0.19[c]	0.005	<0.0001

Least squares means within a row with different superscripts letters are different (P < 0.05).); *SEM= Standard error of mean; [1]Control diet: soybean meal and urea (104.0 g/kg DM); [2]SBM: soybean meal associated to high level of urea.

DISCUSSION

The results of this study indicated that the efficiency of dietary nitrogen utilization varied among the diets containing different nitrogen sources and sugarcane as forage. Cows fed raw soybean showed higher efficiency of converting dietary nitrogen to milk protein than cows fed other diets. Thus, the reduction of nitrogen losses in the endogenous and ruminal metabolism by partial replacement of soybean meal and urea by raw soybean increased the utilization efficiency of nitrogen sources, which might reduce the nitrogen excretion to the environment and the costs of dairy cows feeding.

Ruminal parameters: The average ruminal pH (6.93) observed in this study was considered adequate (between 5.5 and 7.0) for ruminal fermentation and proteolytic enzymes activity (Hoover and Stokes 1991). Nitrogen sources evaluated in this study had no effect on either total concentration or the proportion of SCFA, which also may explain the lack of difference in ruminal pH among diets. Similarly to the results of the present study, Silveira *et al.* (2009) found no effect of protein sources on either concentrations of acetate, propionate, butyrate and total concentration of SCFA or the acetate:propionate ratio when diets with sugarcane where provided to steers.

Other studies have also reported no change in ruminal pH when other sources of dietary protein were fed to cows, and it was suggested that these results were due to total mixed ration use (Mendonça *et al.* 2004, Magalhães *et al.* 2006, Sousa *et al.* 2009). Additionally, sugarcane is considered a source of effective fiber, which stimulates the rumination, and consequently, the salivation process. Pereira *et al.* (1996) reported average ruminal pH of 7.44 in cows fed diet containing only sugarcane and urea, whereas an average ruminal pH of 6.48 was reported in cows fed diets with sugarcane, urea and concentrate.

The use of raw soybean and corn gluten meal in the diets fed to dairy cows in this study reduced ruminal NH_3-N production, but it did not changed the microbial protein synthesis. The relationship between the concentration of ammonia in the rumen and optimization of microbial activity remains controversial among studies (Magalhães *et al.* 2006, Santos 2006). The inclusion of nitrogen sources of low rumen degradability can reduce the losses of nitrogen during rumen fermentation without impair rumen microbial growth and SCFA production, which can result in increased ruminal fermentation efficiency for use of the rumen degradable protein.

In this study, there was no effect of sources of dietary N on the microbial protein synthesis. Therefore, it can be estimated that none of the diets evaluated in this study had degradability limitation for microbial protein synthesis. This result can be attributed to two factors: 1) low average of daily milk yield per cow (17 kg); and 2) similar levels of TDN among diets (NRC 2001). However, there are lack of studies that evaluated the microbial protein synthesis and efficiency of use of dietary nitrogen in lactating dairy cows fed with different protein sources in diets based on sugar cane as forage.

Efficiency of microbial protein synthesis did not differ among diets fed to dairy cows in this study. The rate of rumen microorganism growth primary can be

altered by concentrations of TDN and non-fibrous carbohydrate in the diet (NRC 2001). In this study, the concentrations of TDN and non-fibrous carbohydrate were similar among diets, which may explain the lack of effect of diets on production and efficiency of microbial protein synthesis. The results of present study are in agreement with the study of Silveira *et al.* (2009), which reported no difference of microbial protein synthesis when evaluated diets with different N sources (urea, corn gluten meal, soybean meal) and sugarcane as forage. In this way, only the variation of nitrogen sources in the diet could not change the microbial protein synthesis, especially in cows of low milk production (Chizzotti *et al.* 2007).

Additionally, sugarcane is a forage source rich in sucrose and fibrous carbohydrates of low degradability and digestibility. Rapid solubility of sucrose and low degradability of the fiber carbohydrate can provide energy for rumen microbial utilization in different times of ruminal fermentation after feeding. The low availability of dietary nitrogen after the peak of fermentation can be supplied by the nitrogen recycled via saliva and ruminal epithelium, for balance of protein and energy in rumen. This may also explain the lack of effect on the efficiency and microbial protein synthesis when N sources with different rumen degradability were provided to the cows fed diets with sugarcane as forage (Santos *et al.* 1998, Santos 2006).

Dietary nitrogen fed to dairy cows should optimize microbial protein synthesis and to meet amino acids requirement of dairy cows with the minimum nutrient excretion in feces and urine (Santos *et al.* 1998). In the present study, higher urinary nitrogen excretion was observed for cows fed diets with soybean meal and high inclusion of urea compared with the other diets. This result suggests that cows fed diets containing soybean meal and high inclusion of urea had higher synthesis and absorption of ammonia in the rumen (above the optimum capacity to microbial utilization of rumen degradable protein), which is positively related to urinary nitrogen excretion (Santos 2006).

Blood parameters: In this study, blood glucose concentration did not differ among diets. The blood glucose is relatively constant in ruminants because the basal levels of glucose are maintained by the gluconeogenesis (Kreikemeier *et al.* 1991). No difference in plasmatic concentration of glucose and urea nitrogen was observed in a study that evaluated the use of diets containing soybean meal, urea, or starea (urea supplemented with a starch source) as nitrogen sources and sugarcane as forage (Oliveira Junior *et al.* 2004). Similarly, no difference in plasma concentration of glucose and urea were observed in other study when soybean meal was replaced by starea 150 S (0, 33, 66 and 100%) in diets fed to dairy cows with sugarcane as forage (Vilela *et al.* 2007).

The blood concentration of NEFA can be used to evaluate the energy balance of dairy cows, as it is influenced by the lipolysis, which is increased when the diet does not meet the energy requirements of cows. There was no effect of the diets on blood concentration of NEFA, which indicated that all diets evaluated in this study met the energy requirements of lactating cows. In the other way, plasma concentration of lipoproteins may be increased when cows are supplemented with lipid sources in the diet. Cows fed raw soybean had the highest levels of total cholesterol and HDL, which can be explained by the higher content of ether extract (41.4 g/kg DM) of the diet. However, the level of HDL observed in cows fed raw soybean did not differ from the HDL level observed in blood samples collected from cows fed soybean meal and high levels of urea as nitrogen sources.

Determination of serum concentrations of urea, hemoglobulin, globulins, albumin and total protein can be used to evaluate the protein metabolism. The concentration of blood urea can be used as an indicator of the efficiency of rumen-degraded protein utilization. Cows fed raw soybean diet had the lowest plasma ammonia concentration compared with cows fed other diets evaluated in this study. This result suggests lower ruminal protein degradability of raw soybeans, and thus less synthesis of ammonia in the rumen compared with the diet with soybean meal and high inclusion of urea.

Nitrogen balance: Higher conversion efficiency of dietary nitrogen in total milk protein was observed for cows fed diet containing raw soybean as the main nitrogen source. The diet with raw soybean decreased the concentration of ruminal NH_3-N, but did not limited microbial growth. The availability of ruminal NH_3-N according to the capacity of utilization by rumen microorganisms can reduce N loss improving the efficiency of dietary N utilization (Santos 2006).

Additionally, the diet with raw soybean in the present study may have increased the inflow of true protein to the intestines (in compere to soybean meal diet with high levels of urea), which could increase the amino acids flow to mammary gland, whereas microbial protein was not limited. These results agree with those observed by Silveira *et al.* (2009), which reported higher flow of total nitrogen and non-ammonia nitrogen to the duodenum when diets with protein sources with low (corn gluten meal) and medium (soybean) degradability were compared to a diet containing urea as nitrogen source.

The diet with corn gluten meal also resulted in lower concentration of ruminal ammonia without change microbial protein synthesis. However, differently of the results observed for cows fed the diet containing raw

soybean, the diet with corn gluten meal did not result in greater efficiency of dietary nitrogen utilization. Corn gluten meal is considered as a source of methionine amino acid, but is deficient in lysine. Lysine is the primary limiting amino acid for milk production, and, contrary to corn gluten, soybean has lysine levels similar to those required by dairy cows to milk production (NRC 2001, Santos 2006). For this reason, the efficiency of protein utilization for milk production of diets provided to dairy cows containing corn gluten meal can be lower than observed in diets containing raw soybean as the main dietary nitrogen source (Santos *et al.* 1998, NRC 2001). However, with the less excretion of N in milk, corn gluten meal had a higher efficient to retained the nitrogen (34.43g of N retained/100g of N intake) in compare with other diets.

Conclusion: Diet containing raw soybean as nitrogen source provides more efficient dietary nitrogen utilization for milk production during the digestive process of dairy cows fed diets with sugarcane as forage.

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