Nitrogen balance and milk composition of dairy cows fed with urea and soybean meal and two protein levels using sugar cane based diets

Balanço de nitrogênio e composição do leite de vacas alimentadas com ureia e farelo de soja e dois teores de proteína em dietas com cana-de-açúcar

Luís Henrique Andreucci CONTI[1]; Elmeson FERREIRA DE JESUS[1]; Angélica Simone Cravo PEREIRA[1]; Marcos André ARCARI[1]; Kleber da Cunha PEIXOTO JÚNIOR[2]; Francisco Palma RENNÓ[1]; Marcos Veiga dos SANTOS[1]

[1] Universidade de São Paulo, Faculdade de Medicina Veterinária e Zootecnia, Pirassununga – SP, Brasil
[2] Universidade Santo Amaro, Faculdade de Medicina Veterinária, São Paulo – SP, Brasil

Abstract

It was evaluated the effect of feeding two levels of crude protein (CP) (low: 142 g CP/kg DM; and high: 156 g CP/kg DM) and two nitrogen sources (soybean meal and urea) to dairy cows using sugar cane as forage on microbial protein synthesis, the composition of the milk nitrogen fraction, nitrogen (N) balance and blood parameters. Twelve Holstein cows with an average milk yield of 22.0 ± 2.3 kg/day, and with 235 ± 40 days in milk were included in this study. The animals were grouped into three balanced and contemporary 4x4 Latin squares for an experimental period of 21 days. On the 15th day of each period, milk and urine samples were collected for microbial protein synthesis determination. Total excretion of urine (L/day), milk urea nitrogen (MUN) and blood urea were higher for the diets with high CP, regardless of the nitrogen source. Nitrogen efficiency was higher for cows fed diets with low CP. Cows in the final third of lactation can be fed diets with reduced CP levels, regardless of the nitrogen source, soybean meal or urea, without influencing the synthesis of microbial protein or the composition of the nitrogen fraction of milk.

Keywords: Blood parameter. Dairy cows. Microbial protein synthesis. Soybean meal. Urea.

Resumo

Foi avaliado o efeito de dois teores de proteína bruta (PB) (baixa: 142 g de PB/kg de MS e alta: 156 g de PB/kg de MS) e de duas fontes nitrogenadas (farelo de soja e ureia) na dieta de vacas leiteiras, alimentadas com cana-de-açúcar como volumoso sobre a síntese de proteína microbiana, composição da fração nitrogenada do leite, balanço de nitrogênio (N) e parâmetros sanguíneos. Foram utilizadas 12 vacas Holandesas com produção média de 22,0 ± 2,3 kg leite/dia, no terço final de lactação (235 ± 40 dias em lactação), agrupadas em três quadrados latinos balanceados e contemporâneos 4 x 4, com período experimental de 21 dias, dos quais 14 para adaptação às dietas e os demais para coleta de amostras. A excreção total de urina (L/dia), o NUL e a ureia sanguínea foram maiores para as dietas com alta PB, independentemente da fonte nitrogenada. A eficiência de utilização do N foi maior para as vacas alimentadas com dietas com baixa PB. Vacas no terço final da lactação podem ser alimentadas com dietas com teores reduzidos de PB, independentemente de a fonte nitrogenada ser farelo de soja ou ureia, sem influenciar a síntese de proteína microbiana, a composição da fração nitrogenada do leite e obter maior eficiência de utilização do N da dieta.

Palavras-chave: Parâmetros sanguíneos. Vacas lactantes. Síntese de proteína microbiana. Farelo de soja. Ureia.

Introduction

To improve dairy cow nitrogen balance, microbial protein synthesis should be optimized, as the amino acid profile of microbial protein is similar to that of milk protein, and its intestinal digestibility is higher than that of plant proteins (COLMENERO; BRODERICK, 2006). A high concentration of CP in the diet, especially rumen degradable protein (RDP), can result in excess of ruminal ammonia, which passes through the rumen epithelium into the bloodstream and raises the urea nitrogen in plasma

Correspondence to:
Marcos André Arcari
Av. Duque de Caxias Norte, 225
CEP 13635-900, Pirassununga, SP, Brasil
e-mail: marcosarcari@usp.br

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(HRISTOV et al., 2004). Supplying adequate amounts of RDP and non-degradable protein (RUP) allows for the maximization of microbial protein synthesis and could allow for the reduction of CP levels in the diet. Additionally, the adequacy of the contents of RDP and RUP could decrease N excretion into the environment, improve the efficiency of nitrogen utilization and reduce the cost of milk production, without affecting animal performance (KALSCHEUR et al., 2006; REYNAL; BRODERICK, 2005).

Sugarcane is an alternative forage used in tropical areas for dairy cattle diets during periods of low availability of forage (AQUINO et al., 2008). Sugarcane has a low cost per unit of dry matter (DM) and a high productivity per hectare and maintenance of nutritional value through the year, in addition to its availability during periods of scarcity of forage (MENDONÇA et al., 2004). Some studies have evaluated the effects of different nitrogen sources in diets containing sugarcane as a replacement for corn silage on the dry matter intake, milk yield and composition and ruminal parameters (CORDEIRO et al., 2007; VOLTOLINI et al., 2008). However, few studies have assessed the effects of the appropriate dietary levels of CP and the degradability of protein sources in diets with sugarcane on the composition of the nitrogen fraction of milk and on the nitrogen balance in dairy cows.

The hypothesis of this study was that the reduction of dietary CP levels (from 156 to 142 g/kg DM) and using urea as the main nitrogen source in the diets of dairy cows with sugarcane as forage would allow for the maintenance of microbial protein synthesis, the composition of the nitrogen fraction of milk, the nitrogen balance and metabolic parameters. Thus, the aim of this study was to evaluate the effects of two main nitrogen sources (soybean meal and urea) and two levels of CP on microbial synthesis, the composition of the milk nitrogen fraction, the nitrogen balance and metabolic parameters when using sugarcane as forage in the diets of dairy cows.

Material and Methods

Twelve lactating Holstein cows, averaging 235 ± 40 days of milking (DIM), a body weight (BW) of 611 ± 35 kg, and a milk yield of 22 ± 2.3 kg/day at the beginning of the study, were divided according to DIM and milk production into three balanced and contemporaneous 4 × 4 Latin squares. The cows were milked twice daily and were housed in individual pens, and they had free access to water throughout the trial. The experiment consisted of four periods of 21 days each, 14 days for adaptation and 7 days for sample collection.

The diets were formulated according to the NATIONAL RESEARCH COUNCIL (2001) and were fed as total mixed rations (TMR) (Table 1). The

Table 1 – Ingredients and chemical composition of diets (g/kg), on a dry matter (DM) basis – Pirassununga – SP – Brasil – 2011

Ingredients (g/kg DM)	Diet[1]			
	Soybean meal		Urea	
	High CP	Low CP	High CP	Low CP
Sugarcane	450.2	450.8	449.9	450.6
Ground corn	268.1	232	316.9	275.1
Soybean meal 48% CP	151.	2109.9	79.9	54.9
Whole soybean *in natura*	120.1	126	139.8	123.2
Urea	2.2	2.2	10.0	10.0
Ammonium sulfate	-	-	1.1	1.1
Sodium	7.5	7.5	7.8	7.5
Magnesium oxide	2.5	2.5	2.5	2.5
Mineral supplement[2]	30	30	30	30
Limestone	1.1	1.1	1.1	1.1
Salt	2.5	2.5	2.5	2.5
Nutrient contents of diets				
Dry matter (DM)[3]	616.37	616.59	617.54	617.78
Organic matter (OM)[4]	916.32	916.65	913.22	912.15
Ash[4]	80.15	83.53	87.07	88.05
Crude protein (CP)[4]	155.70	142.07	156.25	142.29
Ether extract (EE)[4]	39.81	38.18	40.06	42.10
Neutral detergent fiber (NDF)[4]	325.63	327.59	321.56	324.07
Acid detergent fiber (ADF)[4]	189.55	190.89	186.30	189.32
Lignin (sa)[4]	48.96	49.83	47.23	49.02
Total digestible nutrients (TDN)[5]	73.94	71.94	73.24	71.73
Net energy of lactation (Nel) (Mcal/kg)[5]	1.98	1.96	1.95	1.95

[1] Diets with high (155.7 to 156.25 g CP/kg de DM) and low (142.07 to 142.29 g CP/kg DM) crude protein (CP) and soybean meal (SBM) or urea as nitrogen sources (NS). [2] Composition per kilogram of product: calcium – 190 g, phosphorus – 73 g, sulfur – 30 g, magnesium – 44 g, copper – 340 mg, zinc – 1350 mg, manganese – 940 mg, cobalt – 3 mg, iodine – 16 mg, selenium – 10 mg, iron – 1064 mg, vitamin A - 100.000 IU, vitamin D - 40.000 IU, vitamin E - 600 IU; [3] g/kg of natural matter; [4] g/kg of MS; [5] Values estimated by equations of the National Research Council (2001)

cows were fed ad libitum twice daily, and they had free access to water. Orts were collected and weighed once daily, and the diets were adjusted daily to yield orts of approximately 5 to 10% of the total feed offered. The two major protein sources of the diets were soybean meal and urea at two crude protein (CP) levels: low (142.1 to 142.3 g CP/kg DM) and high (155.7 to 156.3 g CP/kg DM). Sugarcane was the exclusive forage source, and the cows were fed a forage-to-concentrate (F:C) ratio of 45:55 based on DM.

During the sampling period, samples of sugarcane, diet concentrate ingredients and orts were collected to obtain composites for each sampled cow during each period. The samples were stored at -20°C until laboratory analysis. The samples of orts and feed ingredients were analyzed according to the Association of Official Analytical Chemists (1990) guidelines: for dry matter (DM) - method 934.01, ash - method 942.05, lignin – method 973.18, ether extract (EE) - method 920.39, and crude protein (CP) - method 988.05. The contents of neutral detergent fiber (NDF) and acid detergent fiber (ADF), exclusive of residual ash, were determined according to the procedures of Van Soest and Mason (1991) and the Association of Official Analytical Chemists (1990; method 973.18), respectively, using heat-stable α-amylase but without the addition of sodium sulfite. Total digestible nutrients (TDN) content was obtained according to the NATIONAL RESEARCH COUNCIL (2001) equation: NDT= dNFC + dCP + (dFA * 2.25) + dNDF - 7, where dCP, dNFC, dNDF and dFA represent total digestible nutrients.

To determine allantoin and milk urea nitrogen (MUN), milk samples were collected from the 14th to 18th day of each period. To determine milk nitrogen (total nitrogen - TN, non-protein nitrogen - NPN and non-casein nitrogen - NCN), milk samples were collected from two daily milkings from the 18th to 21st day of each period and were stored at -20ºC until analysis. Crude protein determination was based on the TN concentration by the Kjedahl method, according to Association of Official Analytical Chemists (1995) methods 33.2.11 and 991.20. To obtain the CP value, the TN was multiplied by a conversion factor of 6.38 (BARBANO et al., 1990). Non-casein nitrogen (NCN) and milk casein were determined by the method described by Lynch, Barbano and Fleming (1998). The true protein concentration was obtained by subtracting the concentrations of TN and NPN, and whey protein was determined by subtracting casein from true protein.

To determine blood parameters, blood samples were collected on the 16th day of each period by puncturing the coccygeal artery or vein before feeding, to determine glucose, total cholesterol, HDL cholesterol, urea and urea nitrogen in serum, as well as total protein, albumin and serum aspartate amino transferase and gamma glutamyl transferase enzymes. For the dosage of plasma glucose, samples were collected in tubes with sodium fluoride anticoagulant. The blood parameters were analyzed using commercial kits (Laborlab®, São Paulo, Brazil), using enzymatic colorimetric endpoints with the readings performed with an automated blood biochemistry analyzer (SBA-200 - Celm®, Barueri, Brazil).

To determine the nitrogen balance, the purine derivatives were determined (creatinine and uric acid) from urine, according to the method described by Rennó et al. (2008). To determine the microbial protein synthesis, milk and urine samples were collected on the 15th day of each period to analyze allantoin concentrations in the milk and creatinine and purine derivatives in the urine. Spot samples of urine were collected on the 15th day of each experimental period, 4 hours after morning feeding, by stimulation of the vulva. Urine samples were filtered, and aliquots of 10 mL were diluted in sulfuric acid at 0.036 N to avoid bacterial degradation of purine derivatives and uric acid precipitation. The determination of creatinine concentration in urine was performed using commercial kits (Laborlab®, São Paulo, Brazil), by the colorimetric kinect enzymatic reaction in an automated blood biochemistry analyzer (SBA-200 Celm®, Barueri, Brazil), according to the method described by Biggs and Cooper (1961). The analysis of purine derivatives (allantoin and uric acid) was performed by the colorimetric method described by Chen and Gomes (1992). The total excretion of purine derivatives was calculated according to the sum of the quantities of allantoin and uric acid excreted in urine and the quantity of allantoin excreted in milk, expressed in mmol/L and mmol/day. The total daily urine volume was estimated according to the method described by Oliveira et al. (2001), and the creatinine excretion in urine was estimated based on the composition of the excretion of 24.05 mg/kg body weight of creatinine (CHIZZOTTI et al., 2008).

Microbial purines absorbed were evaluated according to González-Ronquillo et al. (2004). The ruminal synthesis of nitrogenous compounds was evaluated (Pabs, mmol/day) using the following equation: Nmic = (70*Pabs) / (0.83*0.134*1.000), where 70 is the N content in purines (mg of N/mol) (CHEN; GOMES, 1992); 0.134 is the ratio of purine N to total N in bacteria; and 0.83 is the intestinal digestibility of microbial purines.

The results were analyzed using the Statistical Analysis System® (2008), after testing for the normality of residuals and the homogeneity of variance with the UNIVARIATE procedure. The data were analyzed according to the main effects of the nitrogen source, CP content and interaction using the MIXED procedure in SAS and adopting a significance level of 5%, according to the following model:

$$Y_{ijklm} = \mu + F_i + T_j + F_i{*}T_j + Q_k + A(Q)_l + P_m + e_{ijklm},$$

where Y_{ijklm} = dependent variable; μ = overall mean; F_i = fixed effect of nitrogen source i, i = soybean meal or urea; T_j = fixed effect of CP level j, j = 14.5 or 16%; $F_i{*}T_j$ = interaction between nitrogen source i and CP level j; Q_k = fixed effect of Latin square k, k = 1 to 3; $A(Q)_l$ = random effect of cow 1 within Latin squares, l = 1 to 12; P_m = fixed effect of period m, m = 1 to 4; and E_{ijklm}= random error associated to each observation. The calculated degrees of freedom were performed according to the Satterthwaite method (DDFM = Satterth).

Results and Discussion

There were no effects of the diets on the concentrations of NCN, casein, and whey protein or on the CN-to-true protein ratio (Table 2). These results showed that regardless of the main nitrogen source (urea or soybean meal), dairy cows in the final third of lactation, with a milk yield averaging 19.6 kg/day, can be fed diets containing low CP levels (142 g CP/kg DM) without changing the concentration of the milk nitrogen fractions. Reducing dietary CP from 156 to 142 g/kg DM also did not reduce the production of true protein in milk. Similar results were found by Aquino et al. (2008), in whose study the inclusion of increasing levels of urea (0, 0.75 and 15 g/kg DM) in diets containing sugarcane for dairy cows did not influence CP concentration, true protein, non-protein nitrogen (NPN), non-casein nitrogen, casein, whey protein or milk urea nitrogen.

Table 2 – Effect of feeding urea or soybean meal as the main nitrogen source and two CP levels on composition of milk nitrogen fractions of dairy cows – Pirassununga – SP – Brasil – 2011

Item	Diets[1]				SE	Probability		
	Soybean meal		Urea					
	High CP	Low CP	High CP	Low CP		Source	Level	Int
	%							
CP[2]	3.32	3.35	3.41	3.28	0.068	0.81	0.24	0.08
NPN[3]	0.22	0.20	0.22	0.21	0.003	0.28	0.01	0.43
NCN[4]	0.80	0.81	0.81	0.79	0.014	0.52	0.66	0.17
TrueP[5]	3.10	3.15	3.19	3.07	0.067	0.93	0.39	0.06
CN[6]	2.52	2.54	2.60	2.49	0.055	0.69	0.25	0.11
WheyP[7]	0.58	0.61	0.59	0.58	0.014	0.25	0.32	0.13
CN:TrueP[8]	0.81	0.81	0.81	0.81	0.002	0.44	0.23	0.91
	mg/dL							
MUN[9]	14.59	11.45	13.41	13.06	0.415	0.64	0.001	0.005

[1] Diet with high (155.7 to 156.25 g CP/kg DM) and low (142.07 to 142.29 g CP/kg DM) CP and soybean meal (SBM) or urea as nitrogen sources (NS). [2] Crude protein; [3] Non-protein nitrogen; [4] Non-casein nitrogen; [5] True protein; [6] Casein; [7] Whey protein; [8] Casein-to-true protein ratio; [9] Milk ureic nitrogen

In the present study, the concentration of milk NPN (%) was higher for cows fed diets with a high CP level (0.22%) than in cows fed a low CP level (0.20%) (Table 2). There was an effect of the interaction (P = 0.005) between nitrogen source and CP levels on the concentration of MUN (mg/dL). Cows fed diets with soybean meal showed a greater variation in MUN between diets with high and low CP levels than cows fed diets with urea as the main nitrogen source. However, Kalscheur et al. (2006) reported an increased concentration and an increased yield of milk protein with increasing levels of RDP in the diet of dairy cows (68-110 g/kg DM). Likewise, the true protein concentration and MUN showed linear decreases with decreasing concentrations of RDP in the diet (132 - 106 g/kg DM) fed to cows in the middle of lactation (REYNAL; BRODERICK, 2005).

The average concentrations of MUN in the present study were in the range of those recommended by Broderick (1995), from 12 to 17 mg/dL, indicating the adequacy of the diets in terms of CP levels. Among the diets evaluated, only those with low CP and with soybean meal as the main nitrogen source resulted in MUN concentrations of 11.45 mg/dL. Moreover, increasing the dietary CP (156 to 176 g/kg DM) resulted in increased MUN concentration (8.9 to 12 mg/dL) in diets containing corn silage and alfalfa (COLMENERO; BRODERICK, 2006). There was a linear increase of MUN (9.5 to 16.4 mg/dL) when lactating cows were fed increasing levels of RDP in diets with corn silage (68 and 110 g/kg DM) (KALSCHEUR et al., 2006). Milk production with high MUN concentrations is indicative of an excess of CP and RDP in the diet, which increases the feed cost and reduces production efficiency.

The results of this study indicate that the use of low CP (142 g/kg DM) in diets with soybean meal or urea as the main nitrogen source fed to cows in late lactation with sugarcane as the forage source does not alter the composition of the milk nitrogen fractions and reduces the concentration of NPN in milk.

There was a higher consumption of total nitrogen in the cows fed high-CP diets (482.24 g/day) than in the cows fed low-CP diets (428,99 g/day), which can be explained by the higher amount of nitrogen in the high-CP diets (Table 3). The excretion of N in milk was higher for cows fed diets with soybean meal (104.36 g/day) compared to cows fed diets with urea

Table 3 – Effect of feeding two main nitrogen sources and two crude protein (CP) levels on intake, excretion and balance of nitrogen in dairy cows – Pirassununga – SP – Brasil – 2011

	Diets[1]							
	Soybean meal		**Urea**			***P*-value**		
Item	**High CP**	**Low CP**	**High CP**	**Low CP**	**SE**	**Source**	**Level**	**Int**
Total N[2] Intake (g/d)	483.84	436.94	480.63	421.03	8.34	0.45	0.001	0.61
Fecal excretion								
Total N, g/d	68.74	67.29	74.64	78.47	3.75	0.18	0.85	0.68
Total N, % of intake	14.17	15.07	15.47	18.68	0.853	0.06	0.13	0.39
Urinary excretion								
Total N, g/d	151.48	163.77	188.54	167.28	9.95	0.28	0.81	0.37
Total N, % of intake	31.08	38.67	39.70	38.41	2.16	0.29	0.45	0.28
Milk excretion								
Total N, g/d	105.01	103.70	99.74	99.45	2.58	0.01	0.66	0.78
Total N, % of intake	21.89	24.37	21.19	24.00	0.692	0.49	0.001	0.69
N balance								
N retained, g/d	158.26	104.63	121.74	77.58	11.90	0.11	0.02	0.81
N retained, % de N intake	32.85	22.84	24.51	19.48	2.52	0.17	0.08	0.56
Efficiency, g milk N/g N intake	0.22	0.24	0.21	0.24	0.007	0.45	0.001	0.80

[1] Diet with high (155.7 to 156.25 g CP/kg DM) and low (142.07 to 142.29 g CP/kg DM) CP and soybean meal (SBM) or urea as nitrogen sources (NS).
[2] Total nitrogen

(99.60 g/day). The relative excretion of N in milk (% total N) was higher in cows fed a low CP-diet (24.4%) than in those fed a high-CP diet (21.54%).

The results of this study indicate that it is possible to reduce the excretion of nitrogen and the crude protein content of diets for dairy cows without altering milk composition or yield. In a recent review of the efficiency of N use in the diets of dairy cows, Castillo et al. (2000) reported that when the intake of N of lactating cows was greater than 400 g N/d, N excretion in urine increased exponentially, but the rate of increase of N excretion in feces and milk decreased linearly. These authors suggested the use of 150 g/kg DM of CP to produce a reduction in urinary N excretion, without altering milk production.

The urinary volume was greater for cows fed higher CP content, which suggests that cows fed higher CP concentration possibly need excrete higher urinary volumes to eliminate plasma urea that is in highest concentration in plasma of cows fed higher CP content. However in the present study, there was no effect of the diet on urinary nitrogen excretion (Table 3). There is a correlation between urine N and MUN (KOHN; KALSCHEUR; RUSSEK-COHEN, 2002). In a previous study conducted by Mendonça et al. (2004), there was no difference in urinary nitrogen excretion (g/d) between cows fed corn silage and cows fed sugarcane. Agle et al. (2010) described decreased urinary nitrogen excretion in diets with low CP (124 g/kg DM) compared to diets with high CP (154 g/kg DM), but no effects of CP levels on milk were observed.

In this study, there were no effects of the diets on the excretion of N in feces (g/d and total %) (Table 4). Mendonça et al. (2004) reported no increase in the excretion of N in feces with an increase of urea (0.35 or 1% DM) in the diet. An increase in the CP content of the diet also had no effect on fecal N excretion (AGLE et al., 2010). Cows fed diets with low CP showed higher efficiency in nitrogen utilization (0.240 g milk N/g consumed N) than cows fed diets with high CP (0.215 g milk N/g consumed N) (Table 4). The highest amount of nitrogen in milk as

Table 4 – Effect of feeding urea or soybean meal as the main nitrogen source and two CP levels on concentrations of purines derivatives, microbial protein synthesis and total urine excretion in dairy cows – Pirassununga – SP – Brasil – 2011

	Diets[1]							
	Soybean meal		Urea			Probability		
Item	High CP	Low CP	High CP	Low CP	SE	Source	Level	Int
				mmol/L				
Milk-Al[2]	0.02	0,02	0.03	0.03	0.003	0.18	0.43	0.26
Urine-Al-[3]	16.22	18.45	13.08	20.51	0.991	0.70	0.001	0.08
Urine-UA4	0.87	1.07	0.66	1.00	0.096	0.40	0.12	0.71
				mmol/day				
Milk-Al[2]	0.50	0.48	0.70	0.57	0,072	0.15	0.48	0.61
Urine-Al-[3]	261.65	256.20	261.43	263.92	9.37	0.81	0.92	0.79
Urine-UA[4]	12.13	14.53	12.80	12.71	0.83	0.70	0.45	0.41
TP[5]	95.29	94.63	95.20	95.52	0.37	0.48	0.76	0.39
PD[6]	273.78	270.73	274.24	276.65	9.73	0.84	0.98	0.86
Abs-P[7]	302.23	298.48	302.23	305.71	11.5	0.85	0.99	0.85
				g/day				
Nmic[8]	190.21	187.86	190.22	192.39	7.24	0.85	0.99	0.85
CPmic[9]	1188.88	1174.08	1188.91	1202.53	45.2	0.85	0.99	0.85
				g PBmic/kg TDN				
PE[10]	84.61	88.81	93.91	87.01	0.979	0.92	0.80	0.86
				L/day				
TEU[11]	17.94	15.85	21.32	14.04	3.70	0.55	0.001	0.05

[1] Diet with high (155.7 to 156.25 g of CP/kg DM) and low (142.07 to 142.29 g of CP/kg DM) CP and soybean meal (SBM) or urea as nitrogen source (NS); [2] Milk allantoin; [3] Urine allantoin; [4] Uric acid; [5] Total purines; [6] Purine derivatives; [7] Absorbable purines; [8] Microbial nitrogen; [9] Crude protein; [10] Productive efficiency (milk N/TDN consumed); [11] Total excretion of urine

a proportion of total N consumed, in cows fed diets with low CP, resulted from greater utilization of nitrogen intake and its conversion into milk protein. The results of this study are different to those reported by Kalscheur et al. (2006), who reported a elevation in nitrogen efficiency from 0.282 to 0.365 g milk N/g N intake when PDR levels were reduced from 11 to 6.8% DM. Similarly in the present study, Colmenero and Broderick (2006) reported a reduction in nitrogen efficiency use from 0.365 to 0.254 g milk N/g N intake when the CP content of diets increased from 135 to 194 g CP/kg. These results indicate that for cows in late lactation with average milk production of 19.6 kg/d and sugarcane as forage, the use of diets with low CP was more efficient in terms of nitrogen use.

The nitrogen balance was positive and was higher for cows fed high CP level diets (140 g/d) compared to cows fed low CP level diets (91.1 g/d) (Table 4). None of the diets had a negative balance of N, indicating that the CP intake met the requirements of RDP and RUP for the cows. These results differ from those of the National Research Council (2001) because the National Research Council model predicted negative values of RDP (-133 g/d) for diets with low CP.

There was no influence of diets on purine derivative concentration (mmol/L or mmol/day) (Table 4). There was an effect of protein content (P=0.001) on the excretion of allantoin in urine (mmol/day). Cows fed diets with low CP had higher excretion of allantoin in urine (19.48 mmol/L) than cows fed diets with high CP, regardless of the nitrogen source (Table 4). These results could be associated with the higher amount of urine excreted by the cows fed diets with high CP (19.63 L urine/d), compared to the amount excreted by cows fed diets with high CP (14.94 L urine/d). Therefore, the lower urine excretion in cows fed with low CP diets could have resulted in a higher concentration of allantoin. In another study, an increase in the CP content of diets for dairy cows (156 to 176 g CP/kg DM) did not affect the excretion of allantoin in urine (COLMENERO; BRODERICK, 2006). Allantoin is the primary component for the urinary excretion of purine derivatives, and it indicates microbial nucleic acid absorption in the small intestine and is related to microbial protein synthesis in the rumen (RENNÓ et al., 2008).

The average excretion of allantoin in milk in this study, was 0.56 mmol/day, lower than the level found by Souza et al. (2006) in a study performed using cows with 70 days in milk and a forage-to-concentrate ratio 60:40 (11.8 mmol/day). Similarly, in the present study, the average excretion of uric acid in urine was 13.04 mmol/day, similar to the results reported by Mendonça et al. (2004) (15 mmol/day), who evaluated cows with 84 days in milk and an average milk production of 20.36 kg/day that were fed sugarcane as forage. The total excretion of allantoin was 95.4% of the excretion of purine derivatives. Mendonça et al. (2004) reported that the total excretion of allantoin was 90.8%, and Oliveira et al. (2001) reported that allantoin was 87.8% of the total excretion.

There was an effect of the interaction between the nitrogen source and CP content on total urine excretion (L/day). The average urine excretion was 24% higher in cows fed a high-CP diet (19.63 L/day) than in cows fed a low-CP diet (14.95 L/day). Dairy cows fed increasing levels of CP in the diet (156 – 176 g CP/kg DM) had an increase in total urine excretion (COLMENERO; BRODERICK, 2006). According to Dinn, Shelford and Fisher (1998), for each increase of one percentage unit in CP, there was an increase of 2 L of urine/cow/day. In this study, the average variation in CP between diets was 1.37% and provided an increase of 4.7 L of urine/cow/day for diets with high CP or 3.43 L of urine/cow/day for each increase of one percentage unit of CP. Therefore, feeding cows in late lactation diets with low CP content reduced total urine excretion, with no effect on microbial protein synthesis.

Plasma concentrations of urea, albumin and total protein have been used to adjust dietary protein levels, while β-hydroxybutyrate, free fatty acids and cholesterol have been used to evaluate the energy content of diets. The plasma urea concentration indicates the metabolism of ammonia in the liver, while high concentrations of nitrogen in the blood and of the end products of protein metabolism indicate inefficiency or an excess of N in the diet (KOHN; KALSCHEUR; RUSSEK-COHEN, 2002). The average value for blood glucose was 75.72 mg/dL (Table 5), which might be indicative of an excess of energy in the diet for cows in late lactation with average milk production of 19.6 kg/d. There were no effects of nitrogen source, CP content or interaction on blood glucose, total proteins (TP) or aspartate amino transferase (AST) and gamma glutamyl transferase (GGT) enzymes, which were within the normal range for the species in question (KANEKO, 1989).

Cows fed diets with high CP had higher plasma urea concentrations (30.79 mg/dL) than cows fed diets with low CP (25.13 mg/dL), and the same was observed for serum urea nitrogen (Table 5). However, no significant difference between CP sources, which suggests that the amount of RUP present in the soybean meal was sufficient to generate the same effect on plasma compared with urea and urea as a source of CP was observed. These findings could have been the result of the higher rates of the rumen protein degradation of ammonia in cows fed diets with high CP. Ammonia passes through the rumen epithelium and reaches the bloodstream, to be metabolized by the liver and then excreted in urine as urea (REYNAL; BRODERICK, 2005). In the present study, the average concentration of plasma urea nitrogen was 27.69 mg/dL. These results are lower than those (52 mg/dL) described by Mendonça et al. (2004) and higher than those (19.42 mg/dL) reported by Voltolini et al. (2008) and (18.25 mg/dL) by Souza et al. (2006). Cows fed sugarcane had plasma urea nitrogen concentrations ranging from 15 to 40 mg/dL (VOLTOLINI et al., 2008). A blood concentration of urea less than 11 mg/dL indicates that the amount of RDP in the diet is insufficient (BRODERICK, 1995), while concentrations greater than 19 mg/dL can be associated with reductions in the pregnancy rates of dairy cows (FERGUSON et al., 1993). In the present study, blood urea nitrogen was higher in cows fed diets with high CP (14.39 mg/dL) than in cows fed diets with low CP (11.74 mg/dL) (Table 5).

The higher total excretion of urine (Table 4), the NPN and MUN content of milk (Table 3), the lower

Table 5 – Effect of feeding urea or soybean meal as the main nitrogen source and two CP levels on blood parameters of dairy cows – Pirassununga – SP – Brasil – 2011

	Diets[1]							
	Soybean meal		Urea			Probability		
Item	High CP	Low CP	High CP	Low CP	SE	Source	Level	Int
				mg/dL				
Glucose	75.33	74.75	77.71	75.10	1.08	0.51	0.45	0.63
Total-Col[2]	187.40	192.90	202.20	195.30	4.85	0.03	0.85	0.10
HDL-C[3]	56.42	40.08	37.92	57.5	3.95	0.92	0.77	0.01
PU[4]	31.00	22.92	30.58	27.33	1.36	0.28	0.01	0.19
SUN[5]	14.49	10.71	14.29	12.77	0.63	0.28	0.01	0.19
				g/L				
TP[6]	60.00	59.4	63.2	60.6	0.08	0.12	0.25	0.46
Alb[7]	24.4	24.4	25.2	24.9	0.01	0.02	0.58	0.50
				U/L				
AST[8]	54.67	55.97	58.83	54.92	1.14	0.26	0.34	0.06
GGT[9]	2.59	2.59	2.83	2.67	0.11	0.17	0.49	0.49

[1] Diets with high (155.7 to 156.25 g of CP/kg DM) and low (142.07 to 142.29 g of CP/kg DM) CP and soybean meal (SBM) or urea as nitrogen source (NS); [2] Total cholesterol; [3] HDL cholesterol – high density lipoprotein; [4] Plasma urea; [5] Serum urea nitrogen; [6] Total protein; [7] Albumin; [8] Aspartate aminotransferase; [9] Gamma glutamyl transferase

efficiency of nitrogen utilization (Table 4) and the higher nitrogen concentrations in plasma and in blood (Table 4) in cows fed diets with high CP indicate that there was excess nitrogen in the diets with high CP content fed to cows in late lactation and with average production of 19.6 kg/d. Similar results were reported by Ghorbani et al. (2011), in whose study dairy cows fed diets containing 234 g CP/kg DM had higher concentrations of blood urea compared to cows fed 195 and 214 g CP/kg DM. Reductions in blood urea concentrations could be associated with increased production efficiency, reductions in production costs and lower excretion of nitrogenous compounds into the environment. However, insufficient concentrations of nitrogen in the diet can also compromise productive performance (KALSCHEUR et al., 2006).

Cows fed diets with high CP levels had higher blood concentrations of albumin (2.51 g/L) compared to cows fed soybean meal (2.44 g/L) (Table 5). Plasma urea concentrations are related to short-term protein metabolism, while albumin concentrations are related to long-term metabolism (PAYNE; PAYNE, 1987). Lactating cows tend to have reduced blood albumin concentration after calving because amino acids being directed to milk protein synthesis can reduce other protein synthesis, which promotes the reduction of albumin during the course of lactation.

Conclusion

Feeding diets with low CP levels (142 g CP/kg DM), with soybean meal or urea as the main nitrogen source and sugarcane as forage for cows in late lactation, does not change ruminal microbial protein synthesis, milk protein composition, nitrogen balance, or blood metabolic parameters, and these diets result in higher efficiency of nitrogen use compared to diets with a high CP content (156 g CP/kg DM).

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