Journal of Animal and Veterinary Advances 11 (22): 4135-4142, 2012
ISSN: 1680-5593

Intake, Nutrient Digestibility and Milk Yield of Dairy Cows Fed Urea and Two Levels of Crude Protein in Diets with Sugar Cane

Elmeson Ferreira de Jesus, Luis Henrique Andreucci Conti, Tiago Tomazi,
Marina Elena Diniz, Amaral Migliano, Luis Felipe Prada e Silva,
Francisco Palma Renno and Marcos Veiga dos Santos
Department of Nutrition and Animal Production,
School of Veterinary Medicine and Animal Sciences,
University of Sao Paulo, Sao Paulo, Brazil

Abstract: The objective of this study was to evaluate the effect of two Crude Protein (CP) levels and two nitrogen sources in the diets of dairy cows on DM and nutrient intake, total apparent digestibility, ruminal fermentation, milk yield and composition and energy balance using sugarcane as forage. Twelve multiparous Holstein cows, averaging 235 days of milking and 22.0 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. The cows were fed isocaloric diets with *ad libitum* intake and forage based on sugarcane in a 2×2 factorial arrangement of treatments: two main nitrogen sources (soybean meal andurea) and two levels of CP and 156 g CP kg^{-1} DM). Milk samples for compositional analysis were collected on the 14-17th days of each period. Ruminal fluid samples were collected by an esophageal tube, 3 h after morning feeding, for pH, short chain fatty acid and ammonia nitrogen analysis. Apparent digestibility was determined by means of an internal indicator (ADFi). No effect of diet was observed on Dry Matter Intake (DMI) (kg/day), net energy for lactation (Mcal/day) or total nutrientapparent digestibility. Interactions between nitrogen source and diet crude protein content were observed on rumen pH ($p = 0.007$) and acetate to propionate ratio ($p = 0.003$). A tendency was observed ($p = 0.052$) toward an effect of CP levels on milk protein yield (kg/day) as the cows fed diets with soybean meal produced more milk protein than those fed diets with urea. The results indicate that diets with lower levels of CP do not alter the milk yield and composition of dairy cows in the final third of lactation.

Key words: Degradability, nitrogen source, milk yield and composition, diary cows, Brazil

INTRODUCTION

Sugarcane (*Saccharum* sp.) is widely used as forage for ruminant feeding intropical conditions, primarily during the winter because sugarcane has high yield of Dry Matter (DM) and easy to grow. Furthermore, the use of sugarcane occurs mainly duringperiods of tropical pasture scarcity (Fernandes *et al*., 2003). However, there are limitations for sugarcane's use in the diets of dairy cows, mainly due to its low protein level, low fiber digestibility and the consequence of a reduction in feed intake (Magalhaes *et al*., 2004). Partial or total substitution of sugarcane by other roughage (Pires *et al*., 2010) or by greater inclusion of concentrate in the diet is a strategy that can increase the productive performance of cows that are fed sugarcane.

To optimize microbial protein synthesis and consequently to improve animal performance with a minimum amount of dietary crude protein, it is necessary to provide an adequate amount of Rumen Degradable Protein (RDP) (NRC, 2001). However, there have been only a few studies regarding the adequacy of crude protein dietary levels and the degradability of nitrogen sources in dairy cows fed sugarcane as forage. Although, the use of high levels of dietary CP can increase milk production (Wu and Satter, 2000), CP can also increase ruminal ammonia and blood urea concentrations and consequently increase N excretion (Castillo *et al*., 2001). Nitrogen sources constitute a large share of the total cost of feeding dairy cows thus, replacing soybean meal with urea could be analternative to reduce feed costs without reducing the productive performance of dairy cows

Corresponding Author: Elmeson Ferreira de Jesus, Department of Nutrition and Animal Production, School of Veterinary Medicine and Animal Sciences, University of Sao Paulo, Sao Paulo, Brazil

(Pina *et al*., 2006). Researchers hypothesized that sugarcane-based diets for dairy cows in the final third of lactation could be fed with reduced levels of CP and with urea as the main nitrogen source without reducing milk yield or altering the milk's composition. The use of reduced levels of CP might reduce production costs and environmental N losses. Therefore, the aim of this study was to evaluate the effects of two dietary crude protein levels and two nitrogen sources (soybean meal and urea) on nutrient intake and total apparent digestibility, ruminal fermentation, milk yield and composition and energy balance, using sugarcane as forage in the diet of dairy cows.

MATERIALS AND METHODS

The study was conducted at the Campus of University of Sao Paulo in Pirassununga, SP, Brazil. Twelve lactating Holstein cows, averaging 235 Days in milk (DIM), an average Body Weight (BW) of 611 kg and an average milk yield of 22 kg day^{-1} at the beginning of the study were grouped according to DIM and milk production into three balanced and contemporary 4×4 Latin squares. The cows were milked twice daily and were housed in individual pens and they had free access to water through out the study. The experiment consisted of four experimental periods of 21 days which were divided in 14 days for diet adaptation and 7 days for sample collection.

Diets were formulated according the NRC (2001) and the cows were fed Total Mixed Rations (TMRs) (Table 1). The cows were fed *ad libitum* with isoenergetic diets and sugarcane (variety IAC86-2480) as exclusive forage. A factorial arrangement of treatments (2×2) with two main protein sources (soybean meal and urea) and two CP levels, low (142.07-142.29 g of CP kg^{-1} of DM) and high (155.7-156.25 g of CP kg^{-1} of DM) was used in this study. The Forage to Concentrate (F:C) ratio of the diets based on DM was 45:55. Sugarcane was harvested manually and was stored for 1 day until chopping it into particles of 0.5-1.0 cm. The sugar content of the sugarcane was determined by refract ometry (Handheld®, Refractometer Model RHBO-90) to be anaverage of 18° Brix.

Diets were offered twice daily after the morning and afternoon milking. Orts were collected and their weights recorded once daily and the feeding rate was adjusted daily to yield orts of approximately 5-10% of intake. 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.

Table 1: Ingredients and composition of diets (g/kg) on a Dry Matter (DM) basis

	Diets[1]			
	Soybean meal		Urea	
Ingredients	High CP	Low CP	High CP	Low CP
DM (g kg^{-1})				
Sugarcane	450.80	450.20	450.60	449.90
Corn meal	232.00	268.10	275.10	316.90
48% CP soybean meal	151.20	109.90	79.90	54.90
Whole soybean seed	120.10	1260.00	19.80	123.20
Urea	2.20	2.20	10.00	10.00
Ammonium sulfate	-	-	1.10	1.10
Sodium bicarbonate	7.50	7.50	7.50	7.80
Magnesium oxide	2.50	2.50	2.50	2.50
Vitamin-mineral mix[2]	30.00	30.00	30.00	30.00
Limestone	1.10	1.10	1.10	1.10
Salt	2.50	2.50	2.50	2.50
Nutrient content of diets				
Dry Matter (DM)[3]	616.60	616.40	617.80	617.50
Organic Matter (OM)[4]	916.60	916.30	912.20	913.20
Mineral Matter (MM)[4]	83.50	80.20	88.00	87.10
Crude Protein (CP)[4]	155.70	142.10	156.20	142.30
NIDN[5, 7]	113.90	113.90	113.90	113.90
ADIN[6, 7]	26.70	15.24	15.10	15.40
Ether Extract (EE)[4]	38.20	39.80	42.10	40.10
Neutral Detergent Fiber (NDF)[4]	327.60	325.60	324.10	321.60
Nonfiber Carbohydrates (NFC)[4]	395.00	416.30	389.70	409.90
Acid Detergent Fiber (ADF)[4]	190.90	189.50	189.30	186.30
Lignin[4]	49.80	49.00	49.00	47.20
Total Digestible Nutrients (TDN)[7]	71.94	72.94	71.73	73.24
Net Energy of Lactation (NE_L) (Mcal/kg)[7]	1.96	1.97	1.96	1.85

[1]Diets with high (155.7-156.25 g CP/kg DM) and low (142.07-142.29 g CP/kg DM) crude protein and with Soybean Meal (SBM) or urea as the Nitrogen Source (NS). [2]Provided (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 40000 IU; vitamin E 600 IU; [3]g/kg of natural matter; [4] /kg of DM; [5]Neutral detergent insoluble nitrogen; [6]Acid detergent insoluble nitrogen; [7]Values estimated by equations from the NRC (2001)

The samples of orts and feed ingredients were analyzed according to the AOAC (1990) for Dry Matter (DM) Organic Matter (OM), Mineral Matter (MM), Ether Extract (EE), Crude Protein (CP), Neutral-Detergent Insoluble Nitrogen (NDIN), Acid-Detergent Insoluble Nitrogen (ADIN) and lignin. Nonfiber Carbohydrates (NFC) levels were estimated according to Hall (1998) using the following equation:

$$\text{NFC} = 100 - ([\%\text{CP} - \%\text{UreaCP} + \%\text{Urea}] + \%\text{EE} + \%\text{NDF} + \%\text{MM})$$

Total Digestible Nutrient (TDN) content was obtained according to the NRC (2001) equation as follows:

$$\text{NDT} = \text{dCNF} + \text{dCP} + (\text{dFA} \times 2.25) + \text{dNDF} - 7$$

where, dCP, dNFC, dNDF and dFA represent total digestible nutrients. The contents of Neutral Detergent

Fiber (NDF) and Acid Detergent Fiber (ADF) were obtained with an Ankon® System using α-amylase without sodium sulfite addition for NDF determination (Van Soest and Mason, 1991). Samples of sugarcane, feces and orts were collected in amounts of approximately 200 g and were subsequently dried in a forced draft oven (60°C/72 h), ground through a 2 mm screen and then stored as composite samples of eachanimal and period. Feces were collected from the rectum of each cow or immediately after defecation on the 14, 15 and 16 days of each experimental period, before the morning and afternoon milking and then they were stored at -20°C. At the end of the sample collection, the fecal subsamples were mixed to obtain one composite sample foreach cow for each period. To determine nutrient digestibility and the total apparent digestibility of DM, the amount of DM excreted in the feces was estimated by the concentration of indigestible Acid Detergent Fiber (iADF). To evaluate the contents of indigestible components, the orts and concentrate ingredient samples were conditioned in bags of non-woven fabric (TNT 100 g/m^2) with dimensions of 4×5 cm, based on the relationship of 20 mg of DM/cm^2 (Nocek, 1988) and were incubated in the rumen for a period of 288 h, according to an adaptation of the technique described by Casali *et al.* (2008). After incubation, the bags were washed with water until they were totally clear and were dried to obtain the indigestible DM. The bags were treated with acid detergent for 1 h using an Ankon® fiber analyzer (Mertens, 2002). After this period, the samples were washed with hot water and acetone, dried and weighed according to the earlier procedure for the determination of iADF.

On day 21 of each experimental period, samples of ruminal fluid were collected with an esophageal tube, 3 h after the morning feeding, to evaluate the rumen fermentation and pH parameters. Ruminal fluid samples were centrifuged at 3000 rpmfor 15 min and formic acid (0.2 mL) was added to 1 mL of supernatant and themixture was stored at -20°C to determine the short-chain fatty acid concentration. From the same sample, 1 mL of 1 N sulfuric acid was added to 2 mL of supernatant to determine the concentration of ammonia nitrogen.

The analysis of short chain fatty acids was performed according to Erwin *et al.* (1961). A gas chromatograph (Model 9001; Finnigan/Tremetrics, San Jose, CA, USA) equipped with a glass column (2 m×1/4) and packed with 80/120 Carbopack B-DA/4% Carbowa x 20M was used. In this analysis, nitrogen was used as the carrier gas (25 mL min^{-1} of flow rate), oxygen was used as the oxidant gas (175 mL min^{-1}) and hydrogen was used as the fuel gas (15 mL min^{-1}). The temperatures used were 220°C in the vaporizer, 250°C in the flame ionization detector and 195°C for 3 min in the separation column, increased by 10°C min^{-1} to reach 200°C. The ammonia nitrogen concentration of the ruminal fluid was determined with a salicylic acid procedure, in accordance with the methodology described by Bergmeyer (1985).

Milk yield was measured daily and was adjusted to 3.5% fat (FCM) according toSklan *et al.* (1992) in which:

$$FCM = (0.432+0.1625\times \text{Fat milk content})\times \text{kg of milk yielded}$$

The Body Condition Score (BCS) and the BW of the cows were assessed on the 7th adaptation day and at the end of each period. The BCS evaluations were performed according to Edmonson *et al.* (1989). Individual samples for milk composition analysis were collected from 15-18th day of each period. Analyses of milk fat, protein and lactose were performed by infrared absorption with MilkoScan TM FT + equipment (Foss Electric, Hillerod, Denmark).

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 nitrogen source, CP content and the interaction with the MIXED procedure in SAS and adopting a significance level of 5%, according to the following model:

$$Y_{ijklm} = \mu+N_i+L_j+N_i\times L_j+S_k+C\,(S)\,l+P_m+e_{ijklm}$$

Where:

Y_{ijklm}	=	Dependent variable
μ	=	Overall mean
N_i	=	Fixed effect of nitrogen source i (i = soybean meal or urea)
L_j	=	Fixed effect of CP level j (j = low or high)
$N_i\times L_j$	=	Interaction between N_i and L_j
Sk	=	Fixed effect of Latin square k (k = 1-3)
C (S)l	=	Random effect of cow l within Latin square S (l = 1 a 12)
P_m	=	Fixed effect of period m (m = 1 a 4)
E_{ijklm}	=	Residual error

The degrees of freedom were calculated according to the Satterthwaite (= DDFM Satterth) Method.

RESULTS AND DISCUSSION

There were no effects of nitrogen source or diet crude protein content on DMI (kg/day and BW %), nutrients consumption (kg/day and BW %), NFC, TDN or Net Energy of Lactation (NEL) (Table 2). Similarly, the

Table 2: Adjusted means of intake and total apparent digestibility of nutrients and drymatter according to Crude Protein (CP) level and main nitrogen source in diets of dairy cows

	Diets							
	Soybean meal		Urea			Probability[2]		
Items	High CP	Low CP	High CP	Low CP	SE[8]	CP level	N source	Int
Intake (kg day^{-1})								
Dry matter	19.40	17.55	17.14	18.99	0.30	0.990	0.780	0.260
Organic matter	17.81	16.28	15.76	17.32	0.28	0.987	0.708	0.308
Crude protein	2.88	2.40	2.78	2.79	0.05	0.369	0.557	0.356
Ether extract	0.44	0.82	0.45	0.44	0.01	0.748	0.786	0.861
NDF[3]	6.42	5.68	5.81	6.25	0.11	0.773	0.969	0.311
NFC[4]	7.97	7.56	6.63	7.70	0.12	0.574	0.300	0.207
TDN[5]	14.05	13.32	12.66	13.82	2.20	0.842	0.670	0.384
Mcal/day								
NEL	37.87	35.79	34.09	37.63	0.59	0.798	0.728	0.370
Coefficient of digestibility (%)								
Dry matter	67.20	60.40	57.06	63.32	0.71	0.920	0.278	0.082
Organic matter	68.65	60.90	57.33	65.44	0.92	0.978	0.461	0.097
Crude protein	69.20	60.12	60.50	60.20	0.69	0.508	0.582	0.076
Ether extract	88.30	87.80	87.32	87.28	0.57	0.847	0.584	0.883
NDF	55.80	51.92	59.88	57.61	0.84	0.425	0.835	0.268
NFC	74.06	71.42	64.04	68.75	1.17	0.847	0.223	0.523
Obs TDN[6]	67.66	63.32	60.00	65.15	0.73	0.904	0.383	0.207
Intake (% BW)[7]								
Dry matter	3.45	3.32	3.06	3.37	0.05	0.739	0.513	0.446
NDF	1.14	1.01	0.97	1.11	0.02	0.937	0.671	0.153

[1]Diets with high (155.7-156.25 g CP/kg DM) and low (142.07-142.29 g CP/kg DM) crude protein and with Soybean Meal (SBM) or urea as the Nitrogen Source (NS). [2]Probable effects of diet on crude protein content, the two nitrogen sources and their interaction. [3]NDF: Neutral Digestible Fiber. [4]NFC: Nonfiber Carbohydrate. [5]TDN: Total Digestible Nutrients. [6]Obs TDN: Observed Total Digestible Nutrients. [7]BW: Body Weight. [8]SE: Standard Error

diets had no influence on DMI (18.27 kg day^{-1}). The treatments met the requirements of metabolizable energy, regardless of CP or protein source. Furthermore, by observing the cows' nutritional requirements and considering that cows were in their final third of lactation with a milk yield averaging, 22 kg day^{-1}, it appears that any diet with 16.0% CP might exceed the metabolizable protein requirements.

According to Allen (2000), the low digestibility of the fiber from sugarcane reduces DMI. Correa *et al.* (2003) reported that high-producing cows (34.6 kg milk day^{-1}) that were fed sugarcane had a tendency toward reduced DMI during the experimental period. These researchers suggested that digestive tract filling can lead to decreased consumption of sugarcane but only long experimental periods could characterize this effect.

Voltolini *et al.* (2008) found no effect of diet on DMI in cows averaging 93 Days in Milking (DIM) yielding 18 kg milk day and eating sugarcane as forage. However, the results of DMI in that study were lower (14.24 kg day^{-1}) than those observed in thepresent study (18.27 kg day^{-1}). The researchers reported that the high proportion of indigestible NDF present in sugarcane resulted in greater retention of this fraction in the digestive tract which limited the DMI.

Aquino *et al.* (2007) evaluated cows in the middle stage of lactation that werefed sugarcane as forage and were fed increasing levels of urea (0.75 and 1.5% of DM) and they found no effect of diet on DMI (16.37 kg day^{-1}) with either diet. The DMI observed in that study was lower than that found in the present study (18.27 kg day^{-1}). Magalhaes *et al.* (2006) reported a difference in DMI when corn silage was replaced with sugarcane (33.3, 66.6 and 100%). Cows fed diets in which corn silage was ompletely replaced by sugarcane showed a DMI of 17.26 kg day^{-1}. In the present study, the DMI was higher (18.27 kg day^{-1}) but no difference was observed between the diets.

Magalhaes *et al.* (2006) reported a reduction in the ruminal passage rate (5.84-5.27% h^{-1}) of cows averaging 53 DIM and a milk yield of 19.0 kg day^{-1} that were fed diets in which sugarcane had completely replaced corn silage. These results indicate that the higher accumulation of indigestible material in the rumen led to physical satiety which might explain the reduction of DMI in diets with sugarcane instead of corn silage.

A tendency toward an effect of nitrogen sources and crude protein content on the apparent digestibility of DM ($p = 0.08$), OM ($p = 0.09$) and CP ($p = 0.07$) was observed in this study (Table 2). Magalhaes *et al.* (2006) reported no differences in the digestibility of DM (64.45%), OM (65.93%) and CP (67.35%) as a result of replacing (33.3, 66.6 and 100%) corn silage with 100% sugarcane.

The mean CP digestibility of cows that were fed diets with high CP and soybean meal as the main nitrogen source was higher (69.20%) than that of cows that were fed diets with low CP and urea as the nitrogen source (60.16%). The use of increasing levels of CP (12.7-18.4%) resulted in increases in DM, OM and CP digestibility in the studies by Cunningham *et al.* (1996) and Broderick (2003), a result that was not observed in the present study by increasing dietary CP from 14.2-15.6%.

Cordeiro *et al.* (2007) observed no effects of increasing levels of CP (11.5-16.0%) on the nutrient digestibility of DM (69.13%), OM (70.19%), CP (70.48%), NDF (37.82%) or NFC (94, 10%) in the diets of crossbred cows with an average milk yield of 15.0 kg day^{-1}. These results were similar to those found in the present study, except for the results regarding NDF digestibility (56.3%) which were lower and regarding NFC, (69.57%) which were higher. These researchers reported that it was probable that the lack of an effect of the CP content of diets on NFC digestibility occurred due to the high amount of sugarcane-soluble carbohydrates. Interactions between nitrogen sources and dietary CP levels had effects on ruminal pH (Table 3) that were observed in this study.

Table 3: Adjusted means of ruminal fermentation parameters according to crude protein levels and main nitrogen sources in diets of dairy cows

	Diets[1]							
	Soybean meal		Urea			Probability[2]		
Items	High CP	Low CP	High CP	Low CP	SE[5]	Content	Source	Int
pH	6.98	7.54	7.14	6.91	0.03	0.228	0.083	0.007
NH_3-N[3] (mg dL^{-1})	10.27	9.83	11.19	10.81	0.46	0.839	0.632	0.989
M (mol L^{-1})								
Acetate	52.09	35.30	47.88	50.22	1.24	0.414	0.414	0.178
Propionate	16.80	9.46	14.00	16.42	0.43	0.286	0.365	0.059
Butyrate	10.88	6.56	9.72	10.05	0.29	0.202	0.450	0.151
Total SCFA[4]	79.81	50.65	71.09	76.72	1.88	0.245	0.389	0.111
Percentage								
Acetate	65.23	69.32	66.26	65.57	0.25	0.113	0.193	0.027
Propionate	21.04	18.24	20.24	21.35	0.22	0.344	0.190	0.045
Butyrate	13.72	12.32	13.20	13.07	0.20	0.313	0.872	0.436
Acetate/Propionate ratio	3.11	3.91	3.36	3.09	0.05	0.108	0.080	0.003

[1]Diets with high (155.7-156.25 g CP/kg DM) and low (142.07-142.29 g CP/kg DM) crude protein and with Soybean Meal (SBM) or urea as the Nitrogen Source (NS). [2]Probable effects of diets on crude protein content, two nitrogen sources and their interaction. [3]NH_3-N: Ammonia Nitrogen. [4]Total SCFA: Total Short-Chain Fatty Acids. [5]SE: Standard Error

Cows fed low CP and urea as the nitrogen source showed a ruminal pH reduction (6.91) compared with cows fed diets with low CP and SBM as the nitrogen source (7.14). The opposite effect occurred in the ruminal pH of cows fed diets with high CP content and with urea (7.54) compared to cows fed high CP and SBM as the nitrogen source (6.98) (Table 3). These results suggest that increasing the CP content of diets with urea as the nitrogen source can increase pH, compared to diets containing SBM as the nitrogen source.

Cows fed SBM as the nitrogen source tended to have higher average ruminal pH levels (7.26) than those fed SBM (7.02). These results suggest that a higher rumen ammonia concentration can alkalinize the rumen environment. The ruminal pH observed in this study (7.14) was higher than that described by Pires *et al.* (2008) who evaluated the effects of replacing maize silage with sugarcane (0, 25, 50, 75 and 100%) in diets given to lactating cows. These researchers found a pH value of 6.48 in the cows givena diet with 100% sugarcane and they suggested that the lower pH observed in the cows given diets with sugarcane as the only forage was a consequence of the higher fiber percentage of these diets which resulted in greater ruminating stimulation. In a study by Mendonca *et al.* (2004), no variation in ruminal pH (6.7) was reported 3 h after feeding cows a diet containing 60% of DM as corn silage or sugarcane as forage.

Another study evaluated different levels of substitution (0, 33.3, 66.6 and 100%) of corn silage by sugarcane in diets with an F:C ratio of 60:40. A pH of 6.78 was observed in cows fed diets with 100% sugarcane (Magalhaes *et al.*, 2006). The mean ruminal pH observed in this study, after feeding the animals was close to the acceptable range recommended by Hoover and Stokes (1991) for maximum microbial growth and maximum ruminal fiber digestion (6.7-7.1). When ruminal pH reaches values <6.2 there is inhibition of structural carbohydrate degradation and consequently a decrease in the efficiency of using this energy source by ruminants (Orskov, 1982).

There were no effects of diet on ammonia nitrogen (NH3-N) concentrations inthe ruminal fluid (Table 3). In the present study, the concentration of NH_3-N (10.52 mg dL^{-1}) was lower than that described by Pires *et al.* (2008) in cows given diets with 100% sugarcane (18.59 mg 100 mL^{-1}). These researchers suggested that the decrease in ammonia concentration as a result of the inclusion of sugarcane was caused by the increased use of NPN for microbial protein synthesis. This finding might also have been due to the high content of readily fermentable carbohydrates in sugarcane.

Studies evaluating the minimum concentration of ruminal NH_3-N have been controversial. Slyter *et al.* (1979) recommended a minimum of 5 mg dL^{-1} of NH_3-N concentration in the ruminal fluid to obtain maximum microbial growth. Thus, the mean ruminal ammonia concentration (10.52 mg dL^{-1}) in this study was greater than the minimum value suggested and did not limit the growth of rumen micro organisms. Moreover, Leng and Nolan (1984) recommended N-NH_3 concentrations in the ruminal fluid between 15 and 20 mg dL^{-1}.

There were no effects of diet on the concentrations of acetate, propionate butyrate or total short chain fatty acids (mmol L^{-1}). There was a tendency toward an effect of the interaction ($p = 0.059$) between CP and dietary nitrogen source on ruminal propionate concentration. Cows fed urea had a higher propionate concentration (15.21 mmol) than cows fed SBM (13.13 mmol) which might have occurred due to the larg eramount of corn in the urea-based diets.

An interaction effect ($p = 0.02$) of dietary nitrogen source and CP levels on the concentration of acetate was observed in the present study. Cows fed low CP and ureaas the nitrogen source had a lower acetate percentage (65.57%) than cows fed low CP and SBM (69.32%). Furthermore, cows fed diets with high CP and urea showed an acetate level (66.26%) higher than that of cows fed high CP diets and SBM (65.23%) (Table 4). These results indicate that the use of urea as the main nitrogen source increased the percentage of acetate when the dietary CP content was increased.

Significant interactions ($p = 0.04$) were found between the main nitrogen source and dietary CP content on propionate percentage. Cows fed urea and a low-CP diet

Table 4: Adjusted means of milk yield and milk composition according to crude protein level and main nitrogen source in diets of dairy cows

	Diets[1]					Probability[2]		
	Soybean meal		Urea					
Items	High CP	Low CP	High CP	Low CP	SE[5]	Content	Source	Int
3.5% FCM[3] (kg day^{-1})	20.72	18.23	19.48	20.24	0.66	0.455	0.734	0.185
Milk yield (kg day^{-1})	20.23	19.65	17.04	19.71	0.72	0.271	0.100	0.114
Milk fat (kg day^{-1})	0.73	0.61	0.75	0.73	22.88	0.122	0.149	0.284
Milk true protein (kg day^{-1})	0.66	0.65	0.55	0.63	15.84	0.247	0.052	0.163
Milk lactose (kg day^{-1})	0.87	0.84	0.76	0.85	32.15	0.527	0.297	0.192
Milk fat (%)	3.70	3.30	4.38	3.75	0.08	0.001	0.005	0.472
Milk true protein (%)	3.38	3.38	3.28	3.34	0.07	0.809	0.565	0.829
Milk lactose (%)	4.33	4.29	4.35	4.33	0.04	0.703	0.680	0.682
BCS[4]	2.62	2.96	2.90	2.62	0.05	0.805	0.817	0.081
Body weight (kg)	608.00	616.00	609.00	612.00	11.06	0.675	0.847	0.725

[1]Diets with high (155.7-156.25 g CP/kg DM) and low (142.07-142.29 g CP/kg DM) crude protein and with Soybean Meal (SBM) or urea as the Nitrogen Source (NS). [2]Probable effects of diet on crude protein content, two nitrogen sources and their interaction. [3]FCM: 3.5% fat corrected milk. [4]BCS: Body Condition Score. [5]SE: Standard Error

had a higher propionate concentration (21.35%) than cows fed diets with SBM (18.24%). This difference might have occurred due to the greater proportion of corn grain in this diet because corn grain's higher starch availability increases propionate production. The opposite effect occurred in cows fed high-CP diets as the cows fed urea showed propionate concentrations lower (20.24%) than those fed SBM (21.04%) (Table 3).

There were interactive effects (p = 0.003) of nitrogen sources and the content of dietary crude protein on the Acetate to Propionate (A:P) ratio. When low-CP diets were considered, the cows fed urea had a lower A:P ratio (3.09) than the cows fed SBM (3.91). However, the cows fed high-CP diets and urea as the main N source had a higher A:P ratio (3.36) than those fed SBM (3.11) (Table 3). The concentrations of ruminal acetate (66.59%) and propionate (20.21%) in the present study were lower than thoseobtained by Pires *et al.* (2008) who observed concentrations of acetate and propionateof 69.07% and 22.12%, respectively. Increasing the proportion of forage in diets led to areduction in propionate yield and an increase in the rumen acetate to propionate ratio (Bauman *et al*., 1985).

There were no effects of the diets on milk yield (kg/day) either with or without milk fat correction (3.5%) or on fat yield, protein or lactose (Table 4). These results indicate that the nutrients supplied to the cows and especially to the mammary glands were not limited by nitrogen source or levels of dietary CP. Mendonca *et al.* (2004) evaluated cows fed sugarcane based-diets and increased levels of SBM by partial replacement of urea and no effects of the diet were observed on milk yield. The average milk yield in this study was similar to the range (8-22 kg day^{-1}) described in several studies in which diets based on sugarcane were used (Mendonca *et al*., 2004; Magalhaes *et al*., 2006; Aquino *et al*., 2007; Pires *et al*., 2008).

A tendency toward an effect (p = 0.052) of CP level on protein yield (kg/day) was observed. This result might have been due to the increased availability of dietary protein sources for rumen microorganisms with a diet based on sugarcane.

Cows fed diets with urea as the main nitrogen source had higher milk fat content (4.06%) than those fed SBM (3.5%). Contrary results werere ported by Mendonca *et al.* (2004) who found no changes in milk composition when soybean meal in the diet was replaced with urea. According to the NRC (2001), the maintenance of rumen ammonia nitrogen concentrations can increase ruminal fiber degradation, the proportion of acetate and the synthesis of milk fat by the mammary glands.

Costa *et al*. (2005) evaluated the substitution of corn silage with different proportions (60, 50 and 40%) of sugarcane. The average milk yield reported for cows fed a diet based on sugarcane was 18.5 kg day^{-1}. These results were similar to those described by Valvasori *et al*. (1995) who compared the substitution of corn silage with sugarcane (0, 50 and 100%) in the diets of cows with milk yields averaging 18.95 kg day^{-1}. Both results were similar to the milk yield average in this study (19.15 kg day^{-1}). Magalhaes *et al*. (2004) reported an average milk yield of 20.36 kg day^{-1} in cows fed 100%sugarcane and an F:C ratio of 60:40. In other study in which the researchers increased the levels of CP concentrate (12.7, 14.1, 15.5 and 16.9% based on DM) in a diet with 60% corn silage and 40% concentrate, the milk yield averaged 26.13 kg day^{-1} (Pereira *et al*., 2005). However, in the latter study, the researchers observed that the level of 15.5% CP (%DM) resulted in the best responses regarding milk yield. This finding indicates that the level of 16.9% CP in the DM of diets with sugarcane as forage could be excessive and could lead to increased costs and greater N losses to the environment. According to the results of the study, it is possible to use diets with 14.5% crude protein and urea asthe main nitrogen source to feed cows in the final third of lactation without decreases in yield or milk composition.

The BCS (2.77) and BW (611.25) indicated that the diets met the cows'nutritional requirements during the experiment. Another relevant factor that favored the BW maintenance of animals used in this experiment was all of the animals having already completed the negative energy balance period thus, suggesting that the nutritional requirements were met by the diets.

CONCLUSION

The use of 142 g of CP/kg of DM in dairy cows that were fed diets withsugarcane does not change the in take or digestibility of DM and nutrients, rumen fermentation or energy balance, compared to diets with 156 g of CP/kg of DM. For cows in late lactation with an average milk yield of 20 kg day^{-1}, the use of sugarcane as for age and urea as the main nitrogen source in the diet does not affect milk yield or milk composition except for favorably influencing the milk fat content.

ACKNOWLEDGEMENTS

The reasearchers are grateful to Fundacao de Amparo a Pesquisa do Estado de Sao Paulo, Brazil (FAPESP) for research funding (Proc. 08/11140-0) and to Gilmar Edson Botteon, Jose Garcia Moreno Franchini and Lucineia Mestieri for technical assistance.

REFERENCES

AOAC, 1990. Official Methods of Analysis. 12th Edn., AOAC International, Washington, DC., USA., pp: 1098-1990.

Allen, M.S., 2000. Effects of diet on short-term regulation of feed intake by lactating dairy cattle. J. Dairy Sci., 83: 1598-1624.

Aquino, A.A., B.G. Botaro, F.D. Ikeda, P.H.M. Rodrigues, M.D. Martins and M.V. dos Santos, 2007. Effect of increasing dietary urea levels on milk yield and composition of lactating cows. Braz. J. Vet. Res. Anim. Sci., 36: 881-887.

Bauman, D.E., S.N. McCutcheon, W.D. Steinhour, P.J. Eppard and S.J. Sechen, 1985. Sources of variation and prospects for improvement of productive efficiency in the dairy-cow: A review. J. Anim. Sci., 60: 583-592.

Bergmeyer, H.U., 1985. Methods of Enzymatic Analysis. 3rd Edn., Vol. 9, VCH Publishers, Florida, USA., pp: 449-453.

Broderick, G.A., 2003. Effects of varying dietary protein and energy levels on the production of lactating dairy cows. J. Dairy Sci., 86: 1370-1381.

Casali, A.O., E. Detmann, S.C.V. Filho, J.C. Pereira, L.T. Heriques, S.G. Freitas and M.F. Paulino, 2008. Influence of incubation time and particles size onindigestible compounds contents in cattle feeds and feces obtained by *in situ* procedures. Braz. J. Vet. Res. Anim. Sci., 37: 335-342.

Castillo, A.R., E. Kebreab, D.E. Beever, J.H. Barbi, J.D. Sutton, H.C. Kirby and J. France, 2001. The effect of protein supplementation on nitrogen utilization in lactating dairy cows fed grass silage diets. J. Anim. Sci., 79: 247-253.

Cordeiro, C.F.A., M.L.A. Pereira, S.S. Mendonca, P.J.P. Almeida, L.V. Aguiar and M.P. Figueiredo, 2007. Intake and total digestibility of nutrients and milk production and composition in dairy cows fed with increasing crude protein levels in the diet with sugar cane and concentrates. Braz. J. Vet. Res. Anim. Sci., 36: 2118-2126.

Correa, C.E.S., M.N. Pereira, S.G. de Oliveira and M.H. Ramos, 2003. Performance of Holstein cows fed sugar cane or corn silages of different grain textures. Sci. Agric., 60: 621-629.

Costa, M.G., J.M.D. Campos, S.D. Valadares, R.F.D. Valadares, S.D. Mendonca, D.D. Sousa and M.D. Teixeira, 2005. Effects of feeding corn silage or different dietary ratios of sugarcane and concentrate on production of lactating dairy cows. Braz. J.Vet. Res. Anim. Sci., 34: 2437-2445.

Cunningham, K.D., M.J. Cecava, T.R. Johnson and P.A. Ludden, 1996. Influence of source and amount of dietary protein on milk yield by cows in early lactation. J. Dairy Sci., 79: 620-630.

Edmonson, A.J., I.J. Lean, L.D. Weaver, T. Farver and G. Webster, 1989. A body condition scoring chart for Holstein dairy cows. J. Dairy Sci., 72: 68-78.

Erwin, E.S., G.J. Marco and E. Emery, 1961. Volatile fatty acid analysis of blood and rumen fluid by gas chromatography. J. Dairy Sci., 44: 1768-1771.

Fernandes, A.M, A.C. Queiroz, J.C.J. Pereira, R.P. Lana and M.H. Pereira *et al*., 2003. Chemical composition of sugar cane varieties (*Saccharum* spp L.) with different cycles of production in three cut time. Braz. J. Vet. Res. Anim. Sci., 32: 977-985.

Hall, M.B., 1998. Making nutritional sense of nonstructural carbohydrate. Proceedings of the 9th Florida Ruminant Nutrition Symposium, January 15-16, 1998, Gainesville, pp: 108-121.

Hoover, W.H. and S.R. Stokes, 1991. Balancing carbohydrates and proteins for optimum rumen microbial yield. J. Dairy Sci., 74: 3630-3644.

Leng, R.A. and J.V. Nolan, 1984. Nitrogen metabolism in the rumen. Symposium: Protein nutrition of the lactating dairy cow. J. Dairy Sci., 67: 1072-1089.

Magalhaes, A.L.R., J.M.D. Campos, L.D. Cabral, R. Mello and J.A. de Freitas *et al*., 2006. Effects of replacing corn silage with sugarcane on production and ruminal metabolism of lactating dairy cows. Braz. J. Vet. Res. Anim. Sci., 35: 591-599.

Magalhaes, A.L.R., J.M.D. Campos, S.D. Valadares, R.D. Torres, J.M. Neto and A.J. de Assis, 2004. Sugar cane as a substitute for corn silage in diets for milking cows: Performance and economical viability. Braz. J. Vet. Res. Anim. Sci., 33: 1292-1302.

Mendonca, S.D., J.M.D. Campos, S.D.V. Filho, R.F.D. Valadares and C.A. Soares *et al*., 2004. Intake, apparent digestibility, milk production and composition and ruminal variables of dairy cows fed sugar cane based diets. Braz. J. Vet. Res. Anim. Sci., 33: 481-492.

Mertens, D.R., 2002. Gravimetric determination of amylase-treated neutral detergent fiber in feeds with refluxing in beakers or crucibles: Collaborative study. J. AOAC Int., 85: 1217-1240.

NRC, 2001. Nutrient Requirements of Dairy Cattle. 7th Edn., National Academies Press, Washington, DC., USA., ISBN: 0309069971, Pages: 381.

Nocek, J.E., 1988. *In situ* and other methods to estimate ruminal protein and energy digestibility: A review. J. Dairy Sci., 71: 2051-2069.

Orskov, E.P., 1982. Protein Nutrition in Ruminants. Academic Press, New York, Pages:162.

Pereira, M.L.A., S.C.V. Filho, R.F.D. Valadares, J.M.S. Campos and M.L. Leao *et al*., 2005. Intake, total apparent digestibility, milk production and composition in early lactation cows fed with increasing levels of crude protein in the concentrate. Braz. J. Vet. Res. Anim. Sci., 34: 1029-1039.

Pina, D.S., S.C.V. Filho, R.F.D. Valadares, J.M. Campos and E. Detmann *et al*., 2006. Intake, apparent total tract digestibility of nutrients and milk yield and composition of dairy cows fed diets supplemented with different protein sources. Braz. J. Vet. Res. Anim. Sci., 35: 1543-1551.

Pires, A.V., I.M. Susin, F.A.P. Santos, C.Q. Mendes, R.C. Oliveira, J.J.R. Fernandes and J.M.C. Simas, 2008. Effect of starch sources and processing on performance and nitrogen metabolism in lactating Holstein cows. Braz. J. Vet. Res. Anim. Sci., 37: 1456-1462.

Pires, A.V., I.M. Susin, J.M.C. Simas, R.C. Oliveira, J.J.R. Fernandes, R.C. Araujo and C.Q. Mendes, 2010. Replacement of corn silage by chopped sugar cane and whole cottonseed on the performance of lactating Holstein cows. Braz. J. Vet. Res. Anim. Sci., 11: 251-257.

Sklan, D., R. Ashkenazi, A. Brun, A. Devorin and K. Tabori, 1992. Fatty acids, calcium soaps of fatty acids and cottonseeds fed to high yielding cows. J. Dairy Sci., 75: 2463-2472.

Slyter, L.L., L.D. Satter and D.A. Dinius, 1979. Effect of ruminal ammonia concentration on nitrogen utilization by steers. J. Anim. Sci., 48: 906-912.

Valvasori, E., C.S.L. Lucci and J.R.P. Arcaro, 1995. of whole sugar cane and corn silage as roughage for milking cows. Braz. J. Vet. Res. Anim. Sci., 32: 224-228.

Van Soest, P.J. and V.C. Mason, 1991. The influence of Maillard reaction upon thenutritive value of fibrous feeds. Anim. Feed Sci. Technol., 32: 45-53.

Voltolini, T.V., F.A.P. Santos, J.C. Martinez, C.M.M. Bittar, H. Imaizumi and C.S. Cortinhas, 2008. Different metabolizable protein levels in sugar cane diets to lactating dairy cows. Braz. Anim. Sci., 9: 309-318.

Wu, Z. and L.D. Satter, 2000. Milk production during the complete lactation of dairy cows fed diets containing different amounts of protein. J. Dairy Sci., 83: 1042-1051.