Effect of dietary nitrogen source and crude protein content on nitrogen balance and lactating performance of dairy cows

Efeito da fonte de nitrogênio e teor de proteína bruta da dieta sobre o balanço de nitrogênio e o desempenho produtivo de vacas leiteiras

Marina Elena Diniz Amaral MIGLIANO[1]; Camila SILANO[1]; Cristian Marlon de Magalhaes Rodrigues MARTINS[1]; Marcos André ARCARI[1]; Marcos Veiga dos SANTOS[1]

[1]Universidade de São Paulo, Faculdade de Medicina Veterinária e Zootecnia, Departamento de Nutrição e Produção Animal, Pirassununga – SP, Brazil

Abstract

The aim of the present study was to evaluate the effect of two crude protein contents (CP) and two main nitrogen sources in the diet of dairy cows, using sugarcane as forage, on intake, total apparent digestibility, milk yield and composition, and nitrogen balance. Twelve Holstein cows in late lactation were assigned in three contemporary square, in a Latin Square design 4 × 4, with 2 × 2 factorial treatment arrangement, in 21-day trial by period, 14 for diet adaptation and the last seven for sampling and analysis. Cows were housed in individual stalls and fed a total mixed ration (TMR) "ad libitum" composed of two main nitrogen sources (cottonseed meal or whole raw soybean grain) and two levels of CP (130g or 148g/kg dry matter - DM) in the diet. In the present study, there was an interaction effect between nitrogen source and crude protein on DM intake (DMI). Cows fed whole raw soybean had higher DMI when the CP content of the diet was 130 g CP/kg DM, while cows fed cottonseed meal had higher DMI when the CP content of the diet was 148 g CP/kg DM. A similar result of DMI was observed for organic matter intake, whereas there was no interaction effect between dietary nitrogen source and crude protein content on intake of other nutrients. Total apparent digestibility of NDF and TDN was higher when soybean was used as the main diet nitrogen source; whereas the digestibility of dietary CP was not changed by the nitrogen sources. Although there were changes in the DMI and the apparent digestibility coefficient, in the current study, there was no effect of dietary crude protein content and nitrogen source on milk yield. In conclusion, the use of low dietetic concentrations of crude protein (130 g/Kg of DM) does not change the lactating performance of dairy cows and may reduce diet cost, while the choice between whole raw soybean grain or cottonseed meal as a protein ingredient may depends only on the availability and/or price, as both ingredients result in similar performance.

Keywords: Blood metabolism. Milk yield and composition. Sources of protein. Digestibility. Nitrogen efficiency.

Resumo

O objetivo do presente estudo foi avaliar o efeito de dois teores proteicos (PB) e duas fontes nitrogenadas na dieta de vacas leiteiras, utilizando cana-de-açúcar como forragem, sobre o consumo, digestibilidade aparente total, produção e composição do leite, e o balanço de nitrogênio. Doze vacas leiteiras Holandesas em estágio intermediário de lactação foram distribuídas em três quadrados contemporâneos, em um delineamento em quadrado latino 4 × 4, com arranjo fatorial de tratamentos 2 × 2, em períodos de 21 dias, onde os primeiros 14 dias foram destinados para a adaptação às dietas e os últimos sete para as coletas de dados e análises. As vacas foram alocadas em baias individuais em um sistema f*ree-stall,* e alimentadas com dietas totalmente misturada (TMR) "ad libitum" compostas por duas fontes nitrogenadas principais (farelo de algodão ou grão de soja crú integral) e dois níveis de proteína (130 g ou 148 g/kg de matéria seca- MS). No presente estudo, houve efeito de interação entre a fonte nitrogenada e o teor de PB sobre o consumo de MS (CMS). As vacas alimentadas com grão de soja crú integral tiveram maior CMS quando o teor de PB da dieta foi de 130 g CP/kg MS, enquanto que as vacas alimentadas com farelo de algodão apresentaram maior CMS quando o teor de PB da dieta foi de 148 g PB/kg MS. Resultado semelhante ao CMS foi observado para o consumo de matéria orgânica, enquanto que não houve efeito de interação entre fonte nitrogenada e teor de PB sobre o consumo dos demais nutrientes dietéticos. A digestibilidade aparente total da FDN foi maior quando grão de soja crú integral foi utilizado como principal fonte nitrogenada da dieta; enquanto que a digestibilidade da PB não foi alterada pelas fontes nitrogenadas. Apesar de haver alterações no CMS e na digestibilidade aparente total, no presente estudo, não houve efeito de teor de PB e fonte nitrogenada sobre a produção de leite. Como conclusão, o uso de baixo teor dietético de PB (130 g PB/kg de MS) não altera o desempenho produtivo de vacas leiteiras e pode reduzir os custos da dieta, enquanto que

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a escolha entre grão de soja cru integral e farelo de algodão como principais fontes nitrogenadas da dieta pode depender apenas da disponibilidade e custo, uma vez que ambos ingredientes apresentam desempenhos semelhantes.

Palavras-chave: Metabolismo sanguíneo. Produção e composição do leite. Fonte de proteína. Digestibilidade. Eficiência do nitrogênio.

Correspondence to:
Cristian Marlon de Magalhães Rodrigues Martins
Rua Duque de Caxias Norte, 225 – Jardim Elite
CEP 13635-900, Pirassununga, SP, Brazil
email: cristian.martins@usp.br

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Introduction

Feeding of dairy cattle is the highest individual cost component in dairy production systems, especially protein sources, which are one of the costliest dietetic ingredients. Additionally, efficient use of dietary protein may not only reduce costs of dairy cow feeding, but also environmental contamination due to reduced nitrogen in feces and urine. When the capacity of rumen microorganisms to utilize dietary N for microbial protein synthesis is exceeded, the N in excess is converted to ammonia, absorbed by the ruminal epithelium, converted into urea in the liver and excreted by renal system or through milk production. Thus, previous studies evaluated the efficiency of dietary CP utilization by optimizing rumen degradable protein (RDP) to maximize microbial protein synthesis with low nitrogen losses, and, additionally, supplying of rumen undegradable protein (RUP) (VOLTOLINI et al., 2008) and/or bypassing essential amino acids (BRODERICK et al., 2009) to improve metabolizable protein that reaches the gut.

For cows producing 20 l/d of milk and grazing elephant grass as pasture (18.6% CP), Danes et al. (2013) studied three CP levels of concentrate (8.7%, 13.4% and 18.1% CP), and did not observe effect of CP concentrate on DMI, milk yield, and fat and crude. However, to meet the requirements of dairy cows for amino acids balancing RDP and RUP, the nitrogen sources, crude protein content, and ruminal carbohydrate digestibility may be considered in diet formulation. In Brazil, among the main sources of true protein available for feeding dairy cows, soybean meal is widely used due to its high CP content and purchase availability. However, the price oscillations of protein sources make it necessary to use alternative ingredients for dairy cow feeding, as dietary protein is the single most expensive nutrient per unit used. Among the alternative protein ingredients to soybean meal, raw whole soybean grain can be used because it does not need additional industrial processing and can be included in the diet of dairy cows as protein and lipid source. Besides soybean, cottonseed meal can also be used as protein source, although it contains higher RUP and lower energy and protein content (NATIONAL RESEARCH COUNCIL - NRC, 2001). Thus, the choice of protein ingredients used in dairy cows may also change according to availability, cost, milk yield level, and available sources of forage and energy concentrate, so that dietary N can be used efficiently by ruminal microorganisms (NRC, 2001).

Sugarcane is an important forage used in tropical countries for feeding cows a with low or medium yield, since it presents high dry matter (DM) production in seasons of low forage availability, but low CP content and low NDF digestibility. In addition, sugarcane has a high concentration of soluble carbohydrates, an energy source readily available for microbial growth in the rumen. Previous studies have evaluated the substitution of corn silage by sugarcane (MENDONÇA et al., 2004; COSTA et al., 2005; MAGALHÃES et al., 2006; SOUSA et al., 2009), the protein content (VOLTOLINI et al., 2008; JESUS et al., 2012), and inclusion of urea (AQUINO et al., 2007a,b) in sugarcane-based diets for dairy cows. However, there are few studies using sugarcane as forage that evaluated different protein sources and the optimum CP content for maximum efficiency of N use without reducing milk yield. Thus, this study aimed to evaluate the effect of using whole raw soybean grain or cottonseed meal associated with two dietary CP content, and their interactions, on the digestive metabolism, lactating performance and efficiency of dietary N utilization in dairy cows fed sugarcane as forage.

Material and methods

At the beginning of the trial, 12 multiparous Holstein cows averaging (mean ± SD) 19 ± 6 kg of milk/d, 2.6 ± 0.8 of parity, 155 ± 65 DIM, and 550 ± 46 kg of BW, were assigned for treatment of 4 periods of 21 days and 4 treatments, using three contemporary 4 x 4 Latin square design arrangement. Each experimental period was divided into 14 days of adaptation and 7 days for data and sample collection. Cows were housed in individual stalls in a free-stall system and diets were fed as a TMR once daily at 07:00 a.m. and at 2:00 p.m. The arrangement of treatments was factorial 2 x 2, with two main nitrogen sources (cottonseed meal 38 and whole raw soybean grain) and two levels of CP (130g and 148g/Kg DM) (Table 1).

Cows were milked twice daily (at 07:00 am and at 3:00 pm) throughout the experiment. Milk yield was corrected to 3.5% fat (FCM), according to the formula proposed by Sklan et al. (1994): FCM = (0.1625 + 0.432 × Milk fat) × kg milk. Milk samples were collected from d 14 to 17 of each period, chilled, and preserved with 2-bromo-2-nitropropane-1,3-diol (0.05%, wt/vol) for determination of fat and lactose by infrared absorption (BENTLEY INSTRUMENTS, 1995a). The individual milk samples were collected from 18 to 21 experimental days and immediately frozen at -20°C for later determination of the nitrogen fractions of milk (total nitrogen - TN, non-protein nitrogen - NPN and non-casein nitrogen - NCN). The determination of milk protein concentration was based on the measurement of total nitrogen (TN), according to methodology described by the American Association of Official Analytical Chemists (AOAC, 1995) by the method 33.2.11; 991.20. To obtain the CP results, NT was multiplied by the conversion factor 6.38 (BARBANO; CLARCK, 1990). The fractions of NCN and milk casein were determined by the method described by Lynch et al. (1998). The milk urea nitrogen (MUN) concentration was performed by spectrophotometric enzymatic trans-reflectance method using 150® Speck-Chem Equipment (BENTLEY INSTRUMENTS, 1995b).

Body condition score (BCS) and body weight were assessed on the seventh day of adaptation and at the end of each trial period to assess the variability of body weight of cows. The weight of cows was obtained by averaging two successive weighing's taken before supply of feed and after milking for two days. Weights at the seventh day of adaptation and at the last day of each experimental period were used to calculate the variation of BCS and body weight. The Brix® degree value of the sugarcane was determined in portable refractometer (brand Handheld®, RHBO-90 *refractometer model*) and averaged 18º brix. The sugarcane (variety IAC86-2480) was harvested manually with the removal of excess straw, on the day before use, stored until the next day and chopped by Pinheiro chopper brand, model 3610, in particle sizes from 0.5 to 1.0 cm. For dry matter intake (DMI) estimation, amounts of feed and orts offered were weighed to each cow from 14 to 21 d. The cows were fed according to DMI on the previous day in order to keep the daily orts between 5 and 10%. Samples of the feed supplied, orts of the diets and feces (for determination of dry matter and nutrient digestibility) were collected during the last 4 days of each experimental period, and were stored at -20°C, in order to compose a pool of samples per period for each cow based on dry weight for chemical analysis. Samples of ingredients of diets, orts and feces were pre-dried in an oven with forced ventilation (60°C / 72 hours), processed on knife mill with sieves with 2 mm pores. The DM, ash, CP and EE content in samples were determined according to AOAC (1995). The ADIN, NDIN, ADL, NDF and ADF contents were obtained according to Van Soest et al. (1991). The NDF analysis was done according to adaptation methodology described by Mertens (2002), using α-amylase without sodium sulfite.

Table 1 – Chemical composition and ingredients proportions of diets (g/Kg of DM) according to N source and two CP contents – Pirassununga, SP, Brazil – 2012

Ingredients	**Diets**			
	Whole Raw Soybean		**Cottonseed Meal**	
	Low[1]	**High[2]**	**Low[1]**	**High[2]**
	g/Kg DM			
Sugarcane	449.90	449.90	450.10	450.00
Corn Grain	340.00	299.20	345.10	303.80
Soybean Meal 48% CP	30.20	50.10	30.40	50.10
Whole Raw Soybean	120.10	139.90	-	-
Cottonseed Meal 38% CP	-	-	113.70	135.10
Urea	8.00	9.10	8.00	9.10
Ammonium Sulfate	1.70	2.30	1.70	2.30
Mineral Mixture	19.90	19.90	20.00	19.90
Sodium Bicarbonate	8.00	8.00	8.00	8.00
Magnesium Oxide	2.30	2.30	2.30	2.30
Dicalcium Phosphate	8.00	8.00	8.00	8.00
Limestone	6.30	6.30	6.40	6.30
Sodium Chloride	2.80	2.30	3.70	2.30
Potassium Chloride	2.80	2.80	2.80	2.80
	Chemical Composition g/Kg MS			
Dry Matter	609.5	616.6	619.2	617.3
Organic Matter	930.3	934.2	962.0	961.7
Mineral Matter	38.2	41.8	38.7	42.0
Crude Protein	129.0	146.0	130.2	146.0
Ether Extract	47.0	50.0	24.0	23.0
Neutral Detergente Fiber	323.0	322.0	343.0	348.0
Acid Detergente Fiber	200.0	203.0	205.0	215.0
Neutral Detergent Insoluble Nitrogen	27.9	30.2	28.5	25.9
Acid Detergent Insoluble Nitrogen	21.0	26.2	19.0	2.29
Indigestible ADF	103.3	106.1	107.3	109.1
Lignin	27.5	32.2	27.6	29.5

[1] Diets with low crude protein concentration (130 g/Kg CP on DM)
[2] Diets with high crude protein concentration (148 g/Kg de CP on DM)

Fecal samples were collected from all cows during the last 4 days of each experimental period approximately at prefeeding (0600 h) and 6 h post-feeding (1200 h), transferred to aluminum pans, and stored at -20°C until the analysis procedure. Fecal samples were analyzed for DM, ash, EE, OM, CP, ADIN, NDIN, NDF and ADF as described earlier, and indigestible ADF was used as an internal marker to estimate apparent nutrient digestibility and fecal N output. To evaluate the content of indigestible components, the processed samples were placed in "non-woven" cloth bags (100 g/m2), with dimensions of 4 x 5 cm. Aliquots were placed in all bags, according to the relationship of 20 mg of dry matter per square centimeter of surface (NOCEK; RUSSEL, 1988). Prior to incubation of the samples, two Holstein cows were adapted for 7 days with the soybean meal and ground corn diet, and sugarcane as roughage. Subsequent to the adaptation period, samples were incubated in the rumen for a period of 288 h according to the adaptation technique described by Casali et al. (2008). After removal from rumen, bags were washed with running water until the water ran clear and immediately taken to the forced air oven (60°C / 72 h). Then, bags were dried in an oven (105°C / 45 min), and thereafter put in a dessicator and weighed to obtain the indigestible DM. The bags were subjected to acid detergent treatment for 1 h (MERTENS, 2002). They were then washed with hot water and acetone, dried and weighed according to the previous procedure. At the end of this treatment, iADF were obtained.

The collection of blood samples was performed on the 16th day of each period by puncturing the coccyx vein or artery prior to the feed supply in the morning. Samples were collected in vacuum tubes containing 10 ml of blood for determination metabolic glucose, total cholesterol, HDL cholesterol, urea, and blood urea nitrogen (BUN), total protein and albumin. For dosage of glucose in plasma, samples were collected in vacuum tubes containing 4 ml sodium fluoride anticoagulant. Immediately after collection, the samples were chilled and centrifuged at 2000 g for 15 min to separate plasma. The centrifuged obtained was transferred to plastic tubes, identified and stored at -20°C until the completion of laboratory tests.

Analyses of blood parameters were performed using commercial kits (Laborlab® and CELM®) based on enzymatic colorimetric method endpoint, and the reading was performed in automatic analyzer for blood biochemistry (Automatic System of Biochemistry SBA-200 - CELM®).

To perform the analysis, the HDL-C concentration, 200μL of sample was pipetted into 2.5 mL tubes with 100mL single reactive precipitation (CELM-1763) at a ratio of 2:1 and manually mixed by gentle inversion for 20 sec. After standing for 10 min, samples were centrifuged for 15 min at 2700 G. In the supernatant separated by centrifugation, the remaining molecules are bound to HDL cholesterol.

To estimate the nitrogen balance calculation: creatinine, total nitrogen compounds, urea and creatinine concentration in urine was performed (VALADARES et al., 1999; RENNÓ et al., 2008) from spot urine samples of 50 mL. Urine samples were collected from all cows at d 15 of each experimental period, 4 h after the morning feeding, and urination was stimulated by vulva massage and immediately diluted into 40 mL of 0.036 N sulfuric acid and stored at -20°C until analysis. Urinary creatinine concentrations were determined using commercial kits (Laborlab®) based on colorimetric enzymatic reaction kinetics in SBA-200 CELM® device. To perform this analysis, 100 uL of urine were diluted in 4,900 uL of deionized water. The results were calculated by the following formula: creatinine (mg/dL) = creatinine (mg/dL) × 0.020 × 50 (COOPER; BIGGS, 1961).

Total daily urine volume was estimated by dividing daily urinary creatinine excretions by the observed values of creatinine concentration of spot urine samples according to Oliveira et al. (2001). The daily urinary creatinine excretion was estimated from the proposition of 24.05 mg/Kg body weight (BW) (CHIZZOTTI et al., 2007). Thus, based on the daily average excretion of creatinine and creatinine concentration (mg/dL) in spot urine samples, the total daily urine volume in liters per cow/day were estimated by calculating the nitrogen balance. The total nitrogen intake was determined by dividing the CP value of samples by 6.25. The same calculation was performed to CP values of feces resulting in total nitrogen excretion in g/Kg DM. For the determination of total nitrogen in urine and milk samples, the amount in grams of nitrogen per 100 ml of urine or milk was obtained by dividing the CP value of samples by 6.25 for urine and 6.38 for milk samples (BARBANO; CLARK, 1990). Nitrogen balance was obtained by subtracting the values obtained for nitrogen in urine, feces and milk from the total grams of nitrogen intake, obtaining the retained nitrogen values in grams and percentage of total nitrogen.

The experimental design was a 4 x 4 Latin Square, with factorial arrangement of treatments 2 × 2: two nitrogen sources (cottonseed meal and whole raw soybean grain), two crude protein contents (130g or 148g/Kg DM) and the interaction between the two factors. The results were statistically analyzed by using SAS (Version 9.1.3, SAS Institute, Cary, NC, USA, 2004), testing for residual normality and homogeneity of variances by Proc-UNIVARIATE. Data were analyzed according to the main effects for source and crude protein content and the interaction among two factors, by Proc-MIXED command of SAS, adopting 5% of significance level, according to the following model:

$$Y_{ijklm} = \mu + N_i + C_j + N_i{*}C_j + S_k + C_{k(S)l} + P_m + e_{ijklm}$$

Y_{ijkl} = dependent variable,
μ = overall mean;
N_i = Fixed effect of N source (1 DF);
C_J = Fixed effect of CP content / j = 130 g or 148 g/Kg DM (1 DF);
$F_i{*}T_j$ = Fixed effect of interaction between N_i and C_J (1 DF);
S_k = Fixed effect of Latin Square (2 DF);
$C(_k)_l$ = Random effect of cow $_k$ Within each Latin square (9 GL);
P_m = Fixed effect of period (3 GL);
e_{ijklm} = random error associated with each observation.

Results

In the present study, there was an interaction effect between source and CP content of the diet (P = 0.045) on DMI and OM intake (OMI). Cows fed soybean and low crude protein content in the diet (130 g/Kg DM CP) ingested 0.97 kg more DM than cows fed soybean and 148 g CP/Kg DM; while cows fed cottonseed meal and high CP content in the diet ingested 0.27 Kg DM more than cows fed cottonseed meal and high CP content in the diet. Similar results were observed for OMI, which was higher when cows were fed soybean at lower CP content and cottonseed meal at higher crude protein content in the diet. On the other hand, only dietary CP content effect was observed (P = 0.0002) on CP intake, as cows fed diets with high CP content had higher CP intake. Conversely, the NDF intake was changed only according to CP source (P < 0.0001), since cows fed whole raw soybean diet had a NDF intake of 0.54 Kg/day higher than cows fed cottonseed meal as the main diet protein source. The ether extract intake changed according to dietary nitrogen source and CP content, as cows fed whole raw soybean grain as the main protein source and high crude protein content of diet ingested more EE than cows fed low CP and cottonseed meal as the main nitrogen source (Table 2).

In this study, the total apparent digestibility of DM, EE, OM and CP were not altered by nitrogen sources and CP concentration. However, the NDF digestibility was changed according to the nitrogen source, since cows fed whole raw soybean had a 7.56% increase of NDF digestibility compared to cows fed cottonseed meal as the main nitrogen source. Similar results were observed for the total apparent digestibility of TDN, which there was a tendency of effect for higher TDN digestibility when cows where fed with whole raw soybean in comparison with cows fed cottonseed meal (Table 3).

Table 2 – Least square means for effect of dietary crude protein content and nitrogen sources on dry matter and nutrient intake of lactating dairy cows – Pirassununga, SP, Brazil – 2012

Item	Diets				SEM[3]	*P*-value		
	Whole Raw Soybean		Cottonseed meal					
	***Low*[1]**	***High*[2]**	***Low*[1]**	***High*[2]**		**Content**	**Source**	**Int[4]**
			kg / day					
DM	19.28	18.31	18.43	18.7	0.56	0.249	0.432	**0.045**
OM	17.47	16.81	17.49	18.01	0.53	0.800	**0.041**	**0.047**
CP	2.56	2.61	2.44	2.71	0.07	**0.0002**	0.761	0.109
NDF[6]	6.31	6.2	5.60	5.81	0.19	0.682	**< 0001**	0.171
EE	0.53	0.58	0.27	0.35	0.02	**0.0003**	**< 0001**	0.398
			g / kg Body Weight					
DM	31.4	28.1	30.0	30.6	0.09	0.104	0.471	**0.015**
NDF[5]	10.0	9.80	8.90	9.20	0.03	0.711	**< 0001**	0.146
			g / kg 0,75					
DM	153.8	132.6	146.8	150.1	4.27	0.409	0.220	**0.006**

[1]Low dietary concentration of CP (130 g/Kg of DM); [2] High dietary concentration of CP (148 g/Kg of DM); [3] Standard error of means; [4]Interaction effect between CP content (130 g/Kg vs. 148 g/Kg) and nitrogen sources (whole raw soybeans meal vs. cottonseed meal);[5]Neutral detergent fiber

Table 3 – Least square means for effect of dietary crude protein content and nitrogen sources on total apparent digestibility coefficients of dry matter and nutrients of lactating dairy cows – Pirassununga, SP, Brazil – 2012

Item	Diets				SEM[3]	*P*-value		
	Soybean grain		Cottonseed meal					
	***Low*[1]**	***High*[2]**	***Low*[1]**	***High*[2]**		**Content**	**Source**	**Int[4]**
			g/Kg DM					
Dry Matter	673.4	685.5	675.2	665.6	4.68	0.891	0.321	0.235
Organic Matter	677.3	691.2	683.2	677.2	4.86	0.657	0.649	0.264
Crude Protein	753.6	769.0	766.7	769.4	7.19	0.430	0.557	0.577
Neutral Detergent Fiber	593.4	598.6	510.8	518.9	12.41	0.777	**0.001**	0.949
Ether Extract	891.6	902.5	862.1	893.8	6.90	0.097	0.134	0.405
TDN[5]	757.14	773.7	744.1	744.9	6.38	0.456	**0.078**	0.495

[1]Diets with high (148 g/Kg CP of DM) and low (130 g/Kg CP of DM) concentration of crude protein; [1] Low dietary concentration of CP (130 g/Kg of DM); [2] High dietary concentration of CP (148 g/Kg of DM); [3]Standard error of means; [4] Interaction effect between CP content (130 g/Kg vs. 148 g/Kg) and nitrogen sources (whole raw soybeans meal vs. cottonseed meal);[3]Standard error of means; [4] Interaction between CP content (130 g/Kg vs. 148 g/Kg) and sources (whole raw soybeans meal vs. cottonseed meal); [5] Total digestible nutrients

Nitrogen sources and dietary CP content also affect the blood variables studied. In the present study, there was an interaction effect between the nitrogen source and the crude protein content of the diet on the blood glucose concentration (P = 0.02). Cows fed whole raw soybean and low CP concentrations showed higher blood glucose concentration compared to cows fed soybean and high CP content in the diet; while cows fed cottonseed meal had higher blood glucose concentration when the CP content was 148 g/Kg DM. There was also an interaction effect between the source and the CP content of the diet on the blood total cholesterol concentration: Cows fed soybean and high dietary CP content showed higher blood cholesterol compared to cows fed soybean and low protein content; while cows fed cottonseed meal and low protein content had higher blood cholesterol concentration compared to cows fed cottonseed meal at high protein content. Additionally, there was a nitrogen source effect (P < 0.0001) on total cholesterol and HDL blood concentration: Cows fed whole raw soybean grain showed higher blood cholesterol than those fed cottonseed meal as main dietary nitrogen source. In the present study, there was also an interaction effect between the nitrogen source and the crude protein content of diet on plasma concentration of urea and BUN, so that when cows were fed whole raw soybean and low CP, the blood concentrations of urea and BUN increased by 20.55% and 20.51%, respectively, than when cows were fed whole raw soybean and high dietary crude protein content. However, when cows were fed cottonseed meal and high dietary levels of CP, blood concentrations of urea and BUN increased by 10.995 and 34.20%, respectively, when compared to cows fed cottonseed meal and low levels of CP (Table 4).

Table 4 – Least square means for effect of dietary crude protein content and nitrogen sources on concentration of plasma metabolites of lactating dairy cows – Pirassununga, SP, Brazil – 2012

Item	**Diets**				**SEM[3]**	***P*-value**		
	Whole Raw Soybean		**Cottonseed meal**					
	130[1]	**148[2]**	**130[1]**	**148[2]**		**Source**	**Content**	**Int[4]**
			mg/dL					
Glucose	75.75	69.33	71.75	76.5	1.4	0.44	0.68	0.02
Total Colesterol	160.19	172.41	132.7	131.07	5.09	<.0001	0.09	0.004
HDL	51.25	56.05	43.79	43.2	1.49	<.0001	0.33	0.21
Urea	29.33	24.33	28.83	32,00	1.23	0.46	0.28	<.0001
BUN[5]	13.69	11.36	11.14	14.95	0.57	0.44	0.28	<.0001
			g/L					
Albumin	3.37	3.36	3.36	3.36	0.03	0.91	0.87	0.94
			mmol/L					
NEFA[6]	0.14	0.14	0.13	0.15	0.006	0.54	0.37	0.37

[1]Low dietary concentration of CP (130 g/Kg of DM); [2]High dietary concentration of CP (148 g/Kg of DM); [3]Standard error of means; [4]Interaction effect between CP content (130 g/Kg vs. 148 g/Kg) and nitrogen sources (whole raw soybeans meal vs. cottonseed meal); Standard error of means; [4]Interaction between CP content (130 g/Kg vs. 148g/Kg) and sources (whole raw soybeans meal vs. cottonseed meal); [5]Blood urea nitrogen; [6]Non-esterified fatty acids

In the present study, there was no effect of nitrogen source, CP content, or interaction between CP and nitrogen source on milk yield, FCM, fat, crude protein, true protein, casein, whey protein, non-casein nitrogen and lactose contents in milk. Similarly, the daily production of fat, protein and lactose did not change according to diets. However, there was a significant effect of CP content (P = 0.02) on casein/true protein ratio, since cows fed diets containing 148 g/Kg of CP showed higher casein/true protein ration than cows fed diets containing 130 g/Kg of CP. Otherwise, there was a nitrogen source effect and an interaction between CP content and nitrogen source on the concentration of NPN in milk, so that cows fed whole raw soybean and low CP showed higher NPN in milk compared to cows fed whole raw soybean and high levels of CP; while the opposite was observed for cows fed cottonseed meal and low dietary CP concentration, which had lower NPN concentration in milk than cows fed diets high in CP and cottonseed meal as the main nitrogen source. Similarly, there was a nitrogen source effect (P < 0.0001) and interaction between nitrogen source and CP content (P < 0.0001) on the MUN concentration. Cows fed soybean grain and low CP content presented 1.47 mg/dL more MUN than cows fed soybean and high CP levels; while cows fed cottonseed meal and high crude protein produced 3.31 mg/dL more MUN than cows fed cottonseed meal and low crude protein content in the diet (Table 5).

Table 5 – Least square means for effect of dietary crude protein content and nitrogen sources on milk yield and composition of lactating dairy cows – Pirassununga, SP, Brazil – 2012

Variable	Diets				SEM[3]	*P*-value		
	Whole Raw Soybean		Cottonseed meal					
	Low[1]	*High*[2]	*Low*[1]	*High*[2]		Content	Source	Int[4]
			kg / Day					
Milk yield	18.28	17.46	19.00	18.05	0.61	0.084	0.219	0.405
FCM[5]	17.21	16.79	17.17	17.33	0.81	0.709	0.485	0.416
Fat	0.62	0.62	0.60	0.63	0.03	0.421	0.787	0.421
Crude protein	0.54	0.50	0.55	0.55	0.02	0.446	0.082	0.208
Lactose	0.67	0.65	0.68	0.70	0.03	0.850	0.193	0.349
			g /100 g DM					
Fat	3.8	3.92	3.69	3.83	0.09	0.137	0.227	0.883
Crude protein	3.24	3.23	3.26	3.34	0.047	0.27	0.08	0.22
NPN[6]	0.15	0.14	0.15	0.16	0.004	0.93	0.01	0.03
NCN[7]	0.85	0.84	0.87	0.89	0.02	0.7	0.06	0.07
Tprot[8]	3.24	3.23	3.26	3.34	0.047	0.27	0.08	0.22
CAS[9]	2.51	2.53	2.53	2.61	0.036	0.12	0.08	0.31
Wprot[10]	0.72	0.7	0.72	0.7	0.018	0.19	0.63	0.21
Cas:Tprot	0.77	0.78	0.77	0.78	0.004	0.02	0.4	0.24
Lactose	41.3	41.1	40.5	41.3	0.05	0.494	0.494	0.308
			mg/dL					
MUN[11]	9.83	8.40	9.88	13.19	0.55	0.710	<0.0001	<0.0001

[1]Low dietary concentration of CP (130 g/Kg of DM); [2]High dietary concentration of CP (148 g/Kg of DM); [3]Standard error of means; [4]Interaction effect between CP content (130 g/Kg vs. 148 g/Kg) and nitrogen sources (whole raw soybeans meal vs. cottonseed meal); [5]FCM = Fat corrected milk to 3.5%; [6]NPN = Not-protein nitrogen; [7]NCN = Not-casein nitrogen; [8]Tprot = True protein; [9]CAS = Casein; [10]Wprot = Whey protein; [10]MUN = Milk urea nitrogen

In the current study, diets did not affect N intake and daily excretion of N in g/d. However, when N excretion was expressed as % of total N intake, there was nitrogen source effect on the N fecal excretion and milk secretion, so that cows fed whole raw soybean showed higher N excretion in feces when compared to cows fed cottonseed meal as the main dietary nitrogen source; while N excretion in milk was higher when cows were fed cottonseed meal. Thus, there was neither effect of nitrogen source, CP content nor their interaction on N balance or efficiency of dietetic N utilization (Table 6).

Table 6 – Least square means for effect of dietary crude protein content and nitrogen sources on nitrogen balance in lactating dairy cows – Pirassununga, SP, Brazil – 2012

Item	Dieta				*SEM*[3]	*P*-value		
	Cottonseed meal		Whole Raw Soybean					
	Low[1]	High[2]	Low[1]	High[2]		Source	Content	Int[4]
N, intake (g/dia)	406.73	417.04	414.75	401.26	12.26	0.918	0.748	0.097
N, fecal excretion								
Total N, g/d	99.28	110.35	130.04	114.25	6.15	0.123	0.305	0.110
Total N, % N intake	25.1	26.35	28.86	26.6	0.82	0.0282	0.185	0.306
N, urinary excretion								
Total N, g/d	0.13	0.14	0.15	0.14	0.01	0.206	0.701	0.252
Total N, % N intake	6.01	6.47	6.38	5.76	0.32	0.902	0.944	0.251
N, milk secretion								
Total N, g/d	86.5	83.64	79.67	81.13	3.91	0.229	0.174	0.249
Total N, % N intake	21.67	20.42	19.69	19.36	0.87	0.003	0.053	0.234
N, balance								
N retido, g/d	212.81	204.24	188.36	219.4	6.87	0.262	0.416	0.204
N retido, % N intake	51.71	50.74	47.12	52.07	1.58	0.459	0.440	0.272
Efficiency, g N milk/g N intake	0.21	0.2	0.19	0.2	0.01	0.150	0.700	0.240

[1]Low dietary concentration of CP (130 g/Kg of DM); [2]High dietary concentration of CP (148 g/Kg of DM); [3]Standard error of means; [4]Interaction effect between CP content (130 g/Kg vs. 148g/Kg) and nitrogen sources (whole raw soybeans meal vs. cottonseed meal); Standard error of means; [4]Interaction effect between CP content (130 g/Kg vs. 148 g/Kg) and nitrogen sources (whole raw soybeans meal vs. cottonseed meal)

Discussion

Dry matter and nutrient intake, total apparent digestibility and blood metabolism

In the present study, there was an interaction effect between nitrogen source and CP content of the diet on the DMI. The DMI increased when cows were fed low CP diets (130 g/Kg of CP: 13 g of CP/kg DM below the NRC (2001) recommendation for the cows used in this study) and whole raw soybeans as the main nitrogen source. This result may be associated with the composition of whole raw soybean, as it has high lipid content (22% EE of DM), which suggests that increasing inclusion of whole raw soybean, in order to raises the dietetic CP content, can reduce the DMI. However, when cottonseed meal was used as the main nitrogen source, DMI increased as CP content increased in the diet, indicating a positive association between dietary CP content and DMI for this nitrogen source. Broderick et al. (2009) studied cows averaging (mean ± SD) 43 ± 6 kg of milk/d and reported an increase in DMI when CP content of the diet was raised from 15.8 to 17.1%. Broderick et al. (2009) described that increasing dietary crude protein content may raise ruminal fermentation, which can result in a higher DMI. However, Danes et al. (2013) described no effect of 3 CP contents of the concentrate (8.7, 13.4 and 18.1% DM) on DMI of cows with milk yield of 19 kg/day, and a grazing system based on elephant grass. Other studies using temperate (BARGO et al., 2001; MCCORMICK et al., 2001) or tropical (WALES et al., 2000; PEREIRA et al., 2009) grasses also did not observe increases on forage intake by increasing the concentrate content of CP.

Studying different nitrogen sources (urea, urea and cottonseed meal, urea and corn ground grain, urea and wheat bran) in diets for dairy cows with sugarcane as forage, Vilela et al. (2003) observed an increase in DMI when they used cottonseed meal and wheat bran associated with urea as dietary nitrogen source. According to hepatic oxidation theory (ALLEN et al., 2009), increasing total of fuels metabolized by ruminant liver are the main factor contributing to reduce DMI, despite palatability and physical limitation by NDF from forage. Thus, total of fuels (as propionate and fatty acids), as well as hepatic energy status, milk yield and feed sources, in a multifaceted with alternate and redundant mechanisms, can affect the DMI.

In the current study, there was an interaction effect between nitrogen source and CP protein content of the diet on blood glucose concentration. Cows fed cottonseed meal had higher blood glucose concentration when the CP content of the diet was 148 g CP/kg DM, while cows fed whole raw soybean had higher blood glucose concentration when the dietary content of CP was 130 g CP/kg DM. This result can be associated with DMI, since there was a similar effect of interaction between nitrogen source and CP content of the diet on the DMI. When the DMI increases, the availability of nutrients for ruminal fermentation can also increase, resulting in higher concentrations of ruminal SCFA, especially of propionic acid, which can be converted to glucose in the liver during gluconeogenesis (KREIKEMEIER et al., 1991; NRC, 2001).

Cows fed whole raw soybean had higher blood concentrations of total cholesterol and HDL, which may be due to the higher lipid content of this ingredient when compared to cottonseed meal as the main dietetic nitrogen source (NRC, 2001). In the present study, there was an interaction effect between nitrogen source and CP content of the diet on the blood concentration of urea and BUN (blood urea nitrogen). When cows were fed cottonseed meal, the blood concentration of urea and BUN was higher in cows fed diets with 148 g of CP/kg DM, whereas when cows were fed with whole raw soybeans, the higher blood urea concentration and BUN were observed when the dietetic CP content was 130 g/kg DM. This result is similar to those found for blood glucose and may also be associated with increased availability of nutrients to rumen fermentation, which in turn, can increase concentration of microbial products fermentation such as SCFA, gas (NRC, 2001) and ammonia (MAGALHÃES et al., 2006). The increase in ruminal ammonia nitrogen concentration is directly associated with the blood concentration of urea and BUN, which may explain the results observed in this study.

Milk yield and composition, and nitrogen balance

The dietary CP content may be reduced, without causing losses to lactating performance of dairy cows, when microbial protein synthesis is maximized by the efficient use of dietary N during ruminal fermentation, and also by supplying dietary RUP or bypassing essential amino acids (BRODERICK et al., 2009). In the current study, there was no effect of nitrogen source, CP content and the interaction between them on milk yield, fat-correct milk (FCM) and daily production of fat, protein and lactose. Similar results were reported by Jesus et al. (2012), who studied the use of soybean meal or urea as the main dietary nitrogen source associated with two dietary CP levels, low (142.07 to 142.29 g of CP/kg DM) or high (155.7 to 156.25 g of CP/kg DM), for cows fed sugarcane as forage. As a result, Jesus et al. (2012) found no effect of nitrogen source, CP content and interaction effect between them on milk yield, FCM, and daily production of protein, fat and lactose. Danes et al. (2013) studied the effect of three levels of dietary crude protein diet for cows grazing on elephant grass as pasture, and similar to the present study, there was no observed effect of dietary protein on milk yield and fat and protein daily production. However, Vilela et al. (2003) reported that cows fed with only urea as main nitrogen source reduced fat-correct milk yield when compared with urea plus cottonseed meal, urea plus corn ground grain and urea plus wheat bran, but found no effect on milk fat and protein content.

Broderick et al. (2009) studied, in a factorial arrangement of treatments 2 × 2 × 2, the effect of two protein levels (15.8 and 17.1%) with or without supplementation RUP and 0 or 9 g/d of protected methionine in diet of dairy cows. There was no interaction effect between treatments, but it was reported that the use of high crude protein content (17.1%) in the diet increased 1.1 kg/d of DMI, 1.7 kg/d of milk production, 2.2 kg/d of 3.5% FCM, 0.10 kg/d of fat production, and 0.05 kg/d of true protein production, when compared to diets with 15.8 % CP. Also, in the study of Broderick et al. (2009), supplementation with RUP and protected methionine increased the FCM and the daily production of fat. Thus, the effects of CP in the diet on dairy cow lactating performance may depend on the ingredients used, and also on the level of milk production. Therefore, in this study and the study of Danes et al. (2013), for cows with average milk production of 17-19, the increase of dietary CP content may not change the productive performance, whereas it can increase the diet cost and excretion of nitrogen into the environment.

Milk fat content plays an important role in the yield and texture of some products, such as cheese; whereas the true protein content of the milk can directly affect the yield of the industrial production of cheese and other dairy products rich in protein. In this study, there was no effect of protein content, nitrogen source, and their interactions on milk fat content. However, cows fed cottonseed meal as the main nitrogen source in the diet tended ($P = 0.082$) to produce milk with higher crude protein content compared to cows fed whole raw soybean grain.

Crude protein content of milk may not be a useful evaluation of the quality of milk protein, because it include urea nitrogen, which has no important role on cheese yield. Moreover, the increased concentration MUN is associated with low efficiency of N dietary utilization during digestive metabolism of dairy cows. In this study, cows fed cottonseed meal as protein source showed higher NPN and MUN than cows fed whole raw soybeans. The protein fractions of milk are mainly affected by the supply of metabolizable protein (MP), the amino acid profile of the protein, and the excess of nitrogen excreted into the milk as urea (SANTOS et al., 1998). Thus, the results of this study indicate that the increase in CP content of milk observed with the use of cottonseed meal may be due mainly to the increase of non-protein nitrogen milk. In contrast to the present study, Pina et al. (2006) reported increased milk protein content of cows fed soybean meal compared to cottonseed meal, while Voltolini et al (2008) found no effect of different metabolizable protein levels in dairy cow diet on the content of milk protein. Aquino et al. (2007b) reported that the addition of urea (0; 0.75 and 1.5% DM) in sugarcane-based diets of dairy cows did not affect the crude protein content and nitrogen fractions of milk.

Increased food efficiency utilization for animal production is necessary because there is decreased availability associated with increased prices of agricultural inputs, and also due to the need to reduce excess nutrient excretion into the environment. These factors can contribute to greater efficiency and sustainable livestock production. Utilization efficiency of dietary crude protein may depend on several factors, such as sources used, diet protein content (BRODERICK et al., 2009), and additionally, the availability of energy in the rumen for microbial growth and productive performance of each animal (BACH et al., 2005; SANTOS, 2006). The available nitrogen in the rumen, when it is not used for the microbial protein synthesis due to energy loss or to limited microbial growth, can be converted into ammonia, which can be converted into urea by the liver and excreted by the renal system (SANTOS, 2006).

Thus, the balance of protein and energy availability in the rumen, as well as providing RUP, according to the choice of the ingredients and dietary level of energy and protein, can optimize dietary nitrogen efficiency use for milk production. However, in this study, there was no effect of diets on N intake and excretion in feces and urine, as well as the daily dietary balance, and the efficiency of N utilization for the milk production (milk N g/g N intake). These results indicate that for cows with an average production of 17-19 l/d, using cottonseed meal or whole raw soybean does not change the efficiency of dietary N utilization, probably due to the low milk production cows evaluated in this study. However, the decreased protein content of the diet by 1.3% below the NRC (2001) recommendations can reduce feed costs. Unlike the present study, Broderick et al. (2009) reported that the high protein content of the diet increased the N balance, but the MUN and urinary N excretion increased, reducing the efficiency of the N use (N milk g/g N consumed) to produce milk.

In this study, there was a tendency for the interaction effect between nitrogen source and CP content of the diet on the N intake, so that cows fed cottonseed meal showed higher N intake when CP content of the diet was 148 g of CP/kg DM, while cows fed whole soybean as the main protein source had higher N intake when the CP content of the diet was low. This trend interaction effect may have contributed to the absence of protein content effect on the N intake, and thereafter on milk secretion and N use efficiency for milk yield.

Conclusion

For feeding dairy cows with an average milk yield of 17-19 l /d, fed with sugarcane as forage, diets with 130 g/d of crude protein do not change the milk yield and composition, and nitrogen balance when compared to cows fed diets containing 148 g/kg of CP. This result indicates that the reduction of diet CP by 1.3% below the NRC recommendations (2001) for medium production cows can reduce diet cost and increase the N conversion efficiency for milk protein, without changing milk yield. Likewise, the use of whole raw soybean or cottonseed meal as a main dietary nitrogen source does not affect the milk yield, which indicates that the choice of one of the two nitrogen sources evaluated in this study depends only on the availability and cost of these ingredients.

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