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PROTEIN WITH INCREASING LEVELS OF UREA IN THE DIET OF DAIRY COWS AT PASTURE

(Substituição da proteína bruta por níveis crescentes de ureia na dieta de vacas leiteiras em pastagem)

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RESUMO

Este estudo teve como objetivo avaliar o efeito da substituição da proteína bruta da dieta por níveis crescentes de ureia nos parâmetros digestivos, produtivos e econômicos de vacas leiteiras mestiças em lactação, em condições de pastejo (Urochloa brizantha cv. Xaraés). Cinco vacas mestiças Holandês x Zebu foram distribuídas em delineamento quadrado latino 5 x 5, com cinco períodos experimentais de 21 dias cada. Os níveis de reposição de proteína bruta na dieta por ureia foram de 0, 7%, 14%, 21% e 28%. A ingestão de matéria seca, proteína bruta, fibra em detergente neutro e nutrientes digestíveis totais e o coeficiente de digestibilidade, não foram afetados pelo aumento dos níveis de ureia (p>0,05), exceto pela ingestão de carboidratos não fibrosos que aumentaram linearmente com os níveis suplementares de ureia (p<0,05). A produção de leite, a sua composição química, a eficiência alimentar, o peso corporal e a eficiência no uso de nitrogênio não apresentaram diferença significativa (p>0,05) para vacas alimentadas pelo aumento dos níveis de ureia. Os indicadores financeiros (margem bruta, margem líquida, rentabilidade e taxa de retorno) foram positivos, superando as despesas operacionais e o custo operacional total. A substituição parcial da proteína bruta da dieta pela ureia em vacas leiteiras em lactação sob pastejo não interfere nos parâmetros produtivos. Os resultados deste estudo demonstram que a substituição de 28% da proteína bruta da dieta por ureia pode ser feita para reduzir os custos de alimentação sem impacto negativo no desempenho de vacas em lactação mantidas em pastagens de grama tropical.

Palavras-chave: Consumo de ração, nitrogênio não proteico, produção animal, *Urochloa brizantha*.

ABSTRACT

This study aimed to evaluate the effect of replacing dietary crude protein with increasing levels of urea on the digestive, productive and economic parameters of crossbred mid-lactation dairy cows under grazing conditions (*Urochloa brizantha* cv. Xaraés). Five crossbred cows (Holstein x Zebu) were distributed in a Latin square design (5 x 5) at five experimental periods of 21 days each. Replacement levels of dietary crude protein by urea were 0%, 7%, 14%, 21% and 28%. The intakes of dry matter, crude protein, neutral detergent fiber, and total digestible nutrients, as well as the coefficient of digestibility, were not affected by increasing levels of urea (p>0.05), except for the intake of non-fibrous carbohydrates that increased linearly with supplemental urea levels (p<0.05). Milk yield and

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chemical composition, feed efficiency, body weight, and nitrogen use efficiency did not show a significant difference (p>0.05) for cows fed by increasing levels of urea. The financial indicators (gross margin, net margin, profitability, and rate of return) were positive, exceeding operating expenses and the total operating cost. The partial replacement of dietary crude protein by urea in mid-lactation dairy cows under grazing conditions does not interfere with the productive parameters. The results of this study demonstrate that replacing 28% of dietary crude protein by urea could be done in order to reduce feeding costs without negative impact on the performance of lactating cows grazing tropical grass pastures.

Key words: Animal production, feed intake, non-protein nitrogen, Urochloa brizantha.

INTRODUCTION

Dairy farming in the tropics is a low-technology agricultural system predominantly dominated by small farmers, with herds mostly composed of crossbred cows. Having an efficient and profitable dairy farming the quantity and quality of feed are the major factors (WANAPAT *et al.,* 2018).

The variability in forage nutritional value and supply throughout the year due to the seasonality of production may require the use of concentrate supplementation, which assumes greater or lesser importance depending on the milk yield potential and lactation stage of the cow.

Feeding has a significant impact on the production costs in dairy systems. Therefore, new formulations based on the use of alternative feedstuffs in replacement of traditional products are necessary. This strategy is particularly important for protein supplements, minimizing total protein intake as well as reducing feed costs (BAHRAMI-YEKDANGI *et al.,* 2014). Thus, to reduce the cost of conventional ingredients in dairy farms, the use of alternative suppliers to feed ruminant in tropical countries is extremely necessary (CERUTTI *et al.,* 2016).

Urea is an alternative feedstuff that can partially replace dietary protein due to the ability of ruminal microorganisms hydrolyzed the non-protein nitrogen (NPN) by bacterial urease and utilize by micro-organisms for microbial protein synthesis (PHILLIPSON, 1964; HIGHSTREET *et al.,* 2010). In addition, to having a low acquisition cost, high availability and an ease of handling (ALMORA *et al.,* 2012; GONÇALVES *et al.,* 2014).

The dietary inclusion of urea as a supplemental source of non-protein nitrogen for dairy cows is common practice. However, recommendations under specific conditions are necessary to improve the conditions of management and economic return, especially for cows under grazing conditions. This study aimed to evaluate the effect of partially replacing dietary crude protein for urea on the productive and economic performance of crossbred mid-lactation dairy cows under grazing conditions.

MATERIAL AND METHODS

Experimental area, animals, diets, and experimental design

Procedures involving animals were approved by the Committee for Ethics in Animal Use under protocol number 131 (April 28, 2016) from State University of Southwest of Bahia, Brazil.

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The experiment was conducted at the Bela Vista farm at Macarani, State of Bahia, Central-Southern Bahia, Brazil, located at 15°40’35” South latitude and 40°45’06” West longitude, at an altitude of 310 m, from June 06 to September 14, 2014. The climate in the region is tropical (Aw), with a dry season, according to the Köppen (Year) classification. During this dry period in the region, low temperatures and rainfall are common.

Five crossbred Holstein (H)/Zebu (Z) cows (with proportions varying from 3/4 to 1/4 of H × Z), of third or fourth lactation were used with approximately 120±15.33 days in milk at the beginning of the experiment were distributed in a 5x5 Latin square, five urea levels and five experimental periods. The experiment lasted 105 days and was divided into five experimental periods of 21 days each, with the first 16 days for adaptation to diets and the last five days for data collection. Five dietary treatments were formulated in an attempt for them to be isoenergetic and isoproteic for cows with an average body weight of 450 kg and milk yield of 12 kg day^{-1} adjusted to 3.5% fat according to NRC (2001). Diets were based on increasing levels of urea in replacement of dietary crude protein at 0.7%, 14%, 21% and 28%. The supplement was provided daily after milking at 8 a.m. in individual and covered stalls at 3 kg per cow. The animals also had access to twelve paddocks of 0.5 ha each, with *Urochloa brizantha* cv. Xaraés, and an rest area, provided with natural shade and fresh water. The cows were weighed two days at the beginning and three days at the end of each experimental period to verify body weight variations for each treatment.

Sampling procedure, data collection, and data analysis

In order to estimate forage production, 5 samples were randomly taken from the exact paddock were being used at the time of data collection, at 5 cm above the ground, using pruning shears and a square of 1.0 x 1.0 m, as well as using the technician of the hand-plucked 5 samples were randomly taken to chemical composition analyses at the 5 days over the data collection time. Samples of the ingredients and diet were analyzed as well (Tab. 01).

In order to estimate fecal production, chromium oxide (Cr_2O_3) was used as an external indicator, supplied daily at 7:00 a.m. in a single 10 g dose, which was packed in paper cartridges and introduced orally for 12 days. The feces were collected directly from the rectum during the last 5 days of the experimental period in alternating shifts (alternating morning or afternoon). Subsequently, the fecal output was calculated according to Smith and Reid (1955), using the following formula: FO= OP/CMF where FO = daily fecal output (g day); OP = amount of chromic oxide provided (g day); and CMF = concentration of chromic oxide in the feces (g g DM). The concentration of the chromium oxide in the feces was performed by the method of atomic absorption, according to the methodology described by Williams *et al.* (1962), using the Model GBC Avant E Atomic Absorption Spectrometer apparatus.

Samples of feces, feeds and forages were pre-dried at 55 °C for 72 h, ground using a Willey mill (Tecnal, Piracicaba, São Paulo, Brazil) with a 1 mm sieve, stored in plastic bags, and storage until the analysis of chemical composition according to the Association of Analytical Chemists (AOAC, 1990): levels of the dry matter (DM) (Method 967−AOAC, 1990), ash (Method 942−AOAC, 1990), crude protein (CP) (Method 981.10−AOAC, 1990), and ether extract (EE) (Method 920−AOAC, 1990). To determine the neutral detergent fiber

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(NDF), acid detergent fiber (ADF) contents, neutral detergent insoluble nitrogen (NDIN), and acid detergent insoluble nitrogen (ADIN) the methodology of Van Soest *et al.* (1991) was used with the modifications that were proposed in the Ankom device manual (Ankom Technology Corporation, Macedon, NY, USA). The percentage of total carbohydrates (TC) was assessed using the equation provided by Sniffen *et al.* (1992): TC (% DM) = 100 − (%MM + %CP + %EE). The non-fiber carbohydrates (NFC) in the samples that did not contain urea were calculated by using the equation proposed by Detmann and Valadares Filho (2010): NFC = 100 – (% CP + % EE + % ash + % NDFap), in which % CP = crude protein content, % EE = ether extract content, % ash = ash content, and % NDFap = concentration of neutral detergent fiber corrected for ash and protein. Therefore, the NFC of the samples that contained urea was determined by using the following formula: NFC = 100 – {(% CP – % CPU + % U) + % ash + % EE + % NDFap}, in which %CPU = crude protein from urea and %U = urea content. Total digestible nutrient (TDN) content was calculated according to NRC (2001): TDN = DCP + DEE × 2.25 + DNDF + DNFC, where DCP = digestible crude protein, DEE = digestible ether extract, FDND = digestible neutral detergent fiber, and DNFC = digestible non-fibrous carbohydrates (Tab. 01).

Table 01: Diets composition and chemical composition of ingredients, forage: concentrate, and the hand-plucked pasture samples of *Urochloa brizantha* cv. Xaraés.

		Urea levels				
Diets composition (%)	**Forage**	**0%**	**7%**	**14%**	**21%**	**28%**
Corn	-	40.8	50.5	60.1	69.2	77.8
Soybean meal	-	54.0	42.9	32.0	21.7	11.7
Mineral Blend	-	5.20	5.11	5.00	4.88	5.11
Urea	-	0.00	1.49	2.90	4.22	5.46
Nutrients (%)						
DM	28.5	86.8	86.0	86.1	85.5	86.2
CP	10.4	23.6	20.3	25.3	22.9	21.5
Lignin	5.70	0.50	0.60	0.60	0.60	0.60
EE	2.10	2.40	2.70	2.70	2.90	2.70
ADF	38.2	6.90	4.10	3.80	4.30	4.10
aNDF	69.5	39.1	33.9	32.6	33.7	28.9
NFC	7.60	32.5	40.2	38.3	40.8	48.5
iNDF	16.7	3.70	2.50	2.60	2.50	2.50
MM	8.60	5.10	4.50	4.10	4.00	4.20

DM = dry matter; CP = crude protein; EE = ether extract; ADF = acid detergent fiber; aNDF = neutral detergent fiber adjusted for ash and protein; NFC = non-fiber carbohydrates iNDF = neutral detergent fiber indigestible; MM = mineral matter.

The dry matter intake (DMI) was obtained by the equation:DMI= {[(FO*MCF) – MS] /MCFO} + DMIS, where DMI is the dry matter intake (kg day^{-1}), FO is the fecal output (kg day^{-1}), MCF is the marker concentration in feces (kg^{-1}), MS is the marker in supplement (kg day^{-1}), MCFO is the marker concentration in the forage (kg^{-1}), and the DMIS is the dry matter intake of supplement (kg day^{-1}).

Indigestible neutral detergent fiber (iNDF) was used as an internal indicator obtained after ruminal incubation (DETMANN *et al.,* 2012). Subsequently, the material was

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subjected to neutral detergent extraction following the methodology proposed by Mertens (2002).

Feed efficiency was calculated by dividing the average milk yield (kg day) by the DM intake (kg day) (VALADARES FILHO *et al.,* 2000). The cows were milked once a day in the presence of calves. Milk yield was evaluated from the 17th to the 21st day of each experimental period. The cows were milked at 05h00 and the milk was weighed immediately after. On the 19^{th} day of each experimental period, aliquots of 350 mL of milk were collected and submitted to the composition analysis using the Lactoscan® digital device to determine the contents of fat, lactose, minerals and total solids. Fat-corrected milk (FMC) (3.5 g kg^{-1}) was calculated as proposed by Sklan *et al.* (1992) by the following equation: FCM= (0.432 + 0.1625 x percentage of milk fat) x kilograms of milk.

To estimate the balance of nitrogen compounds, spot urine samples were collected in spontaneous urination from all animals, on the 21^{st} day of each experimental period, about 4 hours after delivery of the supplement, as described by Barbosa *et al.* (2006). Samples were filtered through gauze and a 10 mL aliquot was separated and diluted with 40 mL sulfuric acid (0.036 N) according to the methodology of Valadares *et al.* (1999) intended for quantification of urinary concentrations of urea, nitrogen, and creatinine. Blood samples were taken by jugular vein puncture, also from all animals, on the 21^{st}day of each experimental period, about 4 hours after providing the supplement, using 5mL tubes (VacutainerTM) with EDTA. Then, the blood samples were centrifuged at 2,400 rpm for 15 minutes, and the plasma stored in 5 mL micro tubes and frozen at –20 °C until analysis.

Creatinine concentrations in urine and urea concentrations in urine and plasma were estimated using commercial kits (Bioclin). The conversion of urea into nitrogen ureic was made by multiplying the values by a factor of 0.4667. Nitrogen balance (N retained, g day) was calculated using the following formula: N retained (g) = {N intake (g) – N fecal (g) – N urine (g)} Where: N retained = nitrogen retained in the animal organism; N intake = nitrogen intake by the animal; N fecal = nitrogen excreted in the feces; N urine = nitrogen excreted in the urine. Creatinine excretion (mg day) used to estimate the urine volume through spot samples was obtained for each animal according to the equation described by Chizzotti *et al.* (2004): CE = {32,27 – 0,01093 x BW} Where: CE = daily creatinine excretion (mg day); BW = body weight (kg). Urine volume was estimated from the relationship between creatinine excretion (mg day) obtained in the previous equation, and the average concentration in urine samples (mg dL).

An economic feasibility study was performed; methodologies based on operating costs were used to evaluate the cost of production (AGUIAR e RESENDE, 2010), because it was a dairy activity, some economic indicators were converted into milk equivalent -ME.

Statistical analysis

Analyses of variance were performed for all the variables using a 5x5 Latin Square Design, totaling five cows, five urea levels and five experimental periods. An effect was considered significant at a probability level of 5% or lower. When a significant effect was detected, an analysis of regression was conducted to check out which is the most appropriate equation (linear or quadratic) to fit the data. The data were submitted to analyses of variance and regression using the Statistical and Genetics Analyses System - SAEG (RIBEIRO

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JUNIOR, 2001). The indices of economic viability were compared through descriptive analyzes using the software Microsoft Excel 2013.

RESULTS AND DISCUSSION

The intakes by cows of almost all components were not affected for increasing levels of urea ($p>0.05$), except for the intake of NFC that increased ($p<0.05$) linearly with supplemental urea showing values of 1.46 kg day^{-1} with the 0% of urea in the diet reaching 1.93 kg day^{-1} with 28% of urea (Tab. 02). The average of daily DM intake was 11.07 kg^{-1}, 2.46% of BW, the DMF average was 8.49 kg day^{-1}, or 1.88% of BW, the average of CP, aNDF and TDN intake were 1.46 kg day^{-1}; 6.77 kg day^{-1} and 6.88 kg day^{-1} respectively (Tab. 02). The average of daily DM intake is in accordance with the recommendations of NRC (2001) for a 450-kg cow producing 10 kg of milk containing 3.5 g kg^{-1} fat. It demonstrates that urea, regardless of its inclusion level, had no influence on dry matter intake in crossbred mid-lactation dairy cows under grazing conditions. Although urea is used to limit intake due to its metabolic effects and low palatability (HUBER and COOK, 1972) cows fed with urea responded similarly to those fed with plant-derived sources of dietary protein.

Table 02: Nutrient intake and digestibility of mid-lactation cows supplemented on pasture in increasing levels of crude protein replacing of the diet by urea.

	Urea levels						
Digestibility	**0 (%)**	**7 (%)**	**14 (%)**	**21 (%)**	**28 (%)**	**CV%[1]**	***P value*[2]**
TDM (g kg^{-1})	61.57	61.05	64.05	63.89	61.97	3.51	0.1636
CP (g kg^{-1})	62.67	61.67	63.24	62.55	63.74	8.54	0.9785
EE (g kg^{-1})	55.68	52.83	60.43	58.8	56.91	8.53	0.1921
aNDF (g kg^{-1})	65.09	63.08	68.21	66.39	64.35	5.08	0.2036
NFC (g kg^{-1})	66.93	71.41	75.67	74.63	79.34	10.76	0.2052
Nutrient intake							
TDM (kg day^{-1})	11.45	11.25	10.53	10.71	11.41	7.44	0.3199
TDM (% BW)	2.56	2.52	2.33	2.35	2.56	8.42	0.2560
DMF (kg day^{-1})	8.85	8.67	7.95	8.14	8.83	9.58	0.3188
DMF (% BW)	1.97	1.94	1.75	1.78	1.98	10.79	0.2601
CP (kg day^{-1})	1.51	1.49	1.40	1.43	1.46	6.06	0.3635
aNDF(kg day^{-1})	7.16	6.90	6.37	6.52	6.88	8.43	0.2491
aNDF(% BW)	1.60	1.54	1.40	1.43	1.54	9.46	0.2057
NFC (kg day^{-1})	1.46	1.62	1.65	1.67	1.93	3.93	0.0023
TDN (kg day^{-1})	6.92	6.73	6.76	6.79	7.22	9.15	0.7269

[1]CV% = Coefficient of variation (as percentage); [2]Using F test at 5% significance level; TDM = total dry matter; DMF = dry matter from forage; CP = crude protein; EE = Ether Extract; aNDF = ash and protein-free neutral detergent fiber; NFC = non-fibrous carbohydrates; TDN = total digestible nutrients.

The rates of 0%, 7%, 14%, 21% and 28% of urea in replacement of dietary crude protein corresponded to 0.0; 0.31; 0.62; 0.93; 1.24 g kg^{-1} of urea in a total dry matter basis. All urea levels allowed satisfactory synchrony between the supply of fermentable carbohydrates and nitrogen compounds without influencing the dry matter intake.

The proportion of soybean meal in the concentrate decreased as urea levels

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increased. Consequently, the proportion of corn also increased, resulting in a linear increase in the content of non-fibrous carbohydrates.

In pasture-based systems, the dry matter intake is directly associated with forage availability, quality, and allowance, which can significantly interfere with the success of the livestock company. The average of dry matter availability was 2,700 kg DM ha^{-1}, value 60% below than the recommended by the national literature (PAULINO *et al.,* 2008; SILVA *et al.,* 2009) of 4,500 kg DM ha^{-1}. Forage availability did not reduce the dry matter intake, and the results were consistent with the genetic potential and lactation stage of cows. According to Hodson (1990), the dry matter intake from pasture reaches a maximum when the forage allowance is between 10 to 12% of the body weight, where excellent performances in grazing animals are reported. The average of dry matter allowance reached 6% of the body weight, which is below the recommended limits. However, this restriction was not enough to negatively influence the productive performance of cows.

The replacement of the dietary crude protein for urea for all levels tested did not influence ($p>0.05$) the digestibility of DM, CP, EE, aNDF, and NFC with average values of 62.51; 62.77; 56.93; 65.42 g kg^{-1} respectively (Tab. 02). According to the report by Pessoa *et al.* (2009), the positive effects of the dietary inclusion of urea on nutrient digestibility in ruminants depend on the ability of ruminal microorganisms to assimilate the final fermentation products. The partial replacement of total dietary protein with five levels of urea contributed to the synchrony between the release of nitrogen compounds and energy, boosting the development of ruminal microbiota.

Milk yield, feed efficiency, crude protein efficiency, and body weight condition were not influenced ($p>0.005$) by the increasing levels of urea in replacement of dietary crude protein. The average of the milk yield was 8.9 kg day^{-1}, FE was 0.78 kg milk DMI^{-1} and CPE 5.94 kg milk CP^{-1} (Tab. 03). The milk composition as well were not influenced ($p>0.05$) by the increasing levels of urea with average values of protein, fat, lactose, mineral and total solids of 3.22; 4.42; 4.88; 0.76; 13.3 g kg^{-1} respectively (Tab. 03). The fat-corrected milk yield averaged was below the expected production of 12 kg day^{-1} according to the diet formulation. However, the milk yield was satisfactory and consistent with the genetic potential of cows, lactation stage, climatic conditions, and forage intake.

The quality of the diet favored weight gain due to the reduction in the energy requirement for milk production as lactation progressed. Therefore, cows did not mobilize body reserves to sustain milk production.

Feed efficiency and CP use efficiency were similar between treatments because there was no difference intakes of dry matter and other nutrients and their digestibility.

Milk fat concentration in cows fed NPN is close to 3.5 g kg^{-1} (GONÇALVES *et al.*, 2014), a value lower than the one found in this study (4.4 g kg^{-1}). This result is important because the dairy industry is tending to pay more for milk with higher concentrations of solids, such as fat. The concentration found can be explained by the high proportion of fiber in the diet and by the lactation stage of cows. According to Reis *et al.* (2012), the milk fat content is inversely proportional to milk yield and generally decreases as production increases. Cows were studied after peak production in mid-lactation, the point in which milk yield starts to decrease.

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Table 03: Performance and milk composition of mid-lactation cows supplemented on pasture in increasing levels of crude protein replacing of the diet by urea.

	Urea levels						
Milk composition	**0 (%)**	**7 (%)**	**14 (%)**	**21 (%)**	**28 (%)**	**CV%[1]**	***P value*[2]**
Protein (g kg^{-1})	3.30	3.20	3.20	3.20	3.20	2.07	0.1329
Fat (g kg^{-1})	4.80	4.30	4.20	4.30	4.50	10.77	0.4099
Lactose (g kg^{-1})	5.00	4.80	4.90	4.90	4.80	1.62	0.1532
Minerals (g kg^{-1})	0.80	0.80	0.80	0.70	0.70	7.91	0.3639
Total solids (g kg^{-1})	13.80	13.10	13.10	13.20	13.30	3.97	0.2118
Performance							
Milk yield (kg day^{-1})[3]	9.10	8.80	9.00	8.30	9.30	13.07	0.7263
FE (kg milk DMI^{-1}) CMS)	0.80	0.80	0.80	0.70	0.80	12.62	0.8677
CPE (kg milk CP^{-1})	5.90	5.80	6.10	5.60	6.30	12.82	0.6894
BWC (kg day^{-1})	0.40	0.30	0.40	0.40	0.40	89.40	0.9640

[1]CV% = Coefficient of variation (as percentage); [2]Using F-test at 5% significance level; [3] milk yield corrected to 3.5% of fat; FE = Feed Efficiency; CPE: crude protein efficiency; BWC = body weight condition.

Milk protein content was not changed by urea inclusion due to the synergy between ruminal fermentation processes, allowing the microorganisms to capture available nitrogen in the rumen and to convert N into microbial protein regardless of the protein source.

Milk lactose content usually ranges from 4.5 to 5 g kg^{-1} the value obtained in the present study (4.9 g kg^{-1}) is within this range. Lactose is the milk component least affected by diet. The average of total solids reached 13.3 g kg^{-1}, demonstrating that there was no effect of adding urea to the diet on milk total solids under the conditions of the present study.

The blood urea nitrogen (BUN) did not influence ($p>0.05$) by the urea levels tested with an average value of 12.17 mg dL^{-1}, as well as milk urea nitrogen (MUN) did not influence ($p>0.05$) by the urea levels with average value of 11.52 mg dL^{-1} (Tab. 04).

Table 04: Ureic nitrogen concentrations of mid-lactation cows supplemented on pasture in increasing levels of crude protein replacing of the diet by urea.

	Urea levels						
	0 (%)	**7 (%)**	**14 (%)**	**21 (%)**	**28 (%)**	**CV%[1]**	***P value***
BUN (mg dL^{-1})	10.93	11.51	12.19	12.41	13.79	15.49	0.2297
MUN (mg dL-^{1})	10.32	10.88	11.55	11.76	13.11	16.02	0.2097

[1]CV% = Coefficient of variation (as percentage); [2]Using F-test at 5% significance level; BUN = Blood urea nitrogen; MUN = Milk urea nitrogen.

Nitrogen levels did not affect ($p>0.05$) the content of nitrogen in the feces, milk and the urine (g day^{-1}), nor the N intake (g day^{-1}), N retention (g day^{-1}) and nitrogen retention (% N intake), whose average values were 90.07; 45.98; 32.49; 238.91; 70.36; 29.34 g kg^{-1} (Tab. 05). According to Chizzotti *et al.* (2007), blood urea nitrogen cannot exceed the interval from 13 to 15 mg dL^{-1} since values above this limit would represent losses of dietary proteins. Jonker *et al.* (1998) define as acceptable for blood urea nitrogen BUN the range from 10 to 16 mg dL^{-1}. The average value obtained for cows fed diets with supplemental urea was considered adequate, indicating good nutritional management and efficient use of nitrogen. According to Vasconcelos *et al.* (2010), blood urea nitrogen values below 10 mg dL^{-1} suggest protein and/or energy-deficient diets. In this study, none of the cows fed

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increasing levels of urea had values below 10 mg dL^{-1}. Regarding the MUN levels, values between 10 and 14 mg dL^{-1} are recommended (JONKER *et al.,* 1998; JOHNSON and YOUNG 2003; RAJALA-SCHULTZ and SAVILLE 2003). According to Leão *et al.* (2014), although several non-nutritional factors such as milk yield, age, lactation stage, and breed may influence the MUN concentration, the most significant factor is protein nutrition.

Table 05: Nitrogen compounds of mid-lactation cows supplemented on pasture in increasing levels of crude protein replacing of the diet by urea.

	Urea levels						
	0 (%)	**7 (%)**	**14 (%)**	**21 (%)**	**28 (%)**	**CV%[1]**	***P value***
N intake (g day^{-1})	246.15	239.53	236.42	237.30	235.17	2.90	0.1633
N feces (g day^{-1})	92.62	91.97	89.31	91.17	85.30	14.22	0.8954
N milk (g day^{-1})	48.09	44.43	46.36	43.02	48.00	14.01	0.6711
N urine (g day^{-1})	27.97	28.99	32.11	39.03	34.39	41.68	0.7094
N retained (g day^{-1})	77.47	74.14	68.65	64.08	67.48	33.02	0.8935
N retained (% N intake)	31.38	31.06	29.12	26.39	28.74	31.13	0.9084

[1]CV% = Coefficient of variation (as percentage); [2]Using F-test at 5% significance level.

There was no difference in nitrogen intake with increasing urea levels. This result is explained by the lack of change in dry matter and protein intakes between treatments (Tab. 02) and the similarity in CP levels since the diets were formulated to be isonitrogenous. The positive nitrogen balance indicates protein retention in the animal's body, avoiding losses in cow performance. Therefore, this means that the protein requirements have been met (VASCONCELOS *et al.,* 2010).

Operating expenses decreased with increasing urea levels, the total cost (cow day) reduced to 5.50% compared to the levels of 0% to 28% of replacement of dietary crude protein in the diet by urea. Profitability and rate of return were positive, with an increase of 13.37% and 38.39% respecpetively (Tab. 06).

Table 06: Economic viability of milk production of mid-lactation cows supplemented on pasture in increasing levels of crude protein replacing of the diet by urea.

	Urea Levels				
Gross income (R$* cow day^{-1})	**0 (%)**	**7 (%)**	**14 (%)**	**21 (%)**	**28 (%)**
Total Gross Income	10.68	10.87	11.06	10.39	11.06
Milk price equivalent (l)	11.24	11.44	11.64	10.93	11.64
Cost (R$ cow day^{-1})					
Operating Expenses	5.10	4.97	4.79	4.70	4.65
Total Operating Cost	5.22	5.09	4.92	4.82	4.77
Opportunity cost	3.31	3.31	3.31	3.31	3.31
Total cost	8.55	8.40	8.23	8.13	8.08
Financial indicators					
Gross margin (R$ cow day^{-1})	5.58	5.90	6.27	5.69	6.41
Net margin (R$ cow day^{-1})	5.46	5.78	6.14	5.57	6.29
Profit (R$ cow day)	2.15	2.47	2.83	2.26	2.98
Profitability (%)	20.00	22.72	25.58	21.75	26.94
Rate of return (%)[(8/IC)]**	3.36	3.86	4.42	3.54	4.65

* R$: Brazilian real (Portuguese: real, pl. reais; sign: R$; code: BRL) is the official currency of Brazil. It is subdivided into 100 centavos. ** Including the land. IC–Invested capital.

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The operating expenses were directly influenced by the partial replacement of protein from soybean meal for urea. The high nitrogen content in urea reduces the price per kilogram of protein compared to other traditional crude protein sources. Then, the cost of the concentrate is inversely proportional to the inclusion of non-protein nitrogen sources, positively influencing the financial indicators: gross margin, net margin, profitability, and rate of return (Tab. 06). This result allows dairy farmers to reduce feeding costs by using urea as an alternative source to the traditional soybean meal.

CONCLUSION

The replacement of crude protein with urea in diets for mid-lactation dairy cows under grazing conditions does not interfere with the productive parameters. Therefore, we recommend replacing 28% of dietary crude protein with urea due to better financial results.

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