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Production and marginal analysis of dairy cows fed sorghum silage diets with urea levels

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Palavras chave adicionais
Amônia.
Bovinocultura.
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Proteína microbiana.
Ruminante.

SUMMARY

The objective of this study was to evaluate productive, nutritional and economic traits of dairy cows fed sorghum silage diets with urea levels. Five ¾ Holstein × ¼ Gir lactating dairy cows in the middle third of lactation, at an average age of 60 months and average body weight of 518 kg ± 52 kg, were allocated to five treatments in a 5 × 5 Latin square experimental design. Treatments (U-50, U-75, U-100, U-125 and U-150) consisted of different urea levels in the concentrate (5.7, 8.6, 11.4, 14.3 and 17.1 g urea/kg concentrate, respectively). The significance level of 0.5 was adopted. Urea levels did not influence the feed intake, digestibility, or metabolic parameters of dairy cows. No significant differences were observed for 3.5% fat-corrected milk yield. Milk composition was not significantly affected. Gross revenue from the sale of milk was not significantly affected, averaging BRL 28.65. Marginal rate of return did not differ significantly. The different urea levels did not influence the dairy cows' milk yield. In conclusion, urea can be included in the concentrate of sorghum silage diets for dairy cows at the levels of 5.7 to 17.1 g/kg of concentrate without compromising production performance.

Produção e análise marginal em vacas de leite submetidas a níveis de ureia na dieta à base de silagem de sorgo

Additional keywords
Ammonia.
Cattle farming.
Microbial protein.
Non-protein nitrogen.
Ruminant.

RESUMO

Objetivou-se avaliar as características produtivas, nutricionais e econômicas de vacas de leite submetidas a níveis de ureia com dietas à base de silagem de sorgo. Foram utilizadas 05 vacas lactantes ¾ Holandês x ¼ Gir Leiteiro, no terço médio de lactação, com idade média de 60 meses e peso corporal médio de 518 kg ± 52 kg, que foram distribuídas em cinco tratamentos, em delineamento experimental de quadrado latino 5x5. Os tratamentos (U-50, U-75, U-100, U-125 e U-150) foram constituídos de diferentes níveis de ureia no concentrado (5,7; 8,6; 11,4; 14,3 e 17,1 g de ureia/kg de MS do concentrado, respectivamente). Adotou-se como nível de significância 0,05. Não houve efeito dos níveis de ureia no consumo alimentar, digestibilidade e parâmetros metabólicos de vacas de leite. Não foram observadas diferenças significativas na produção de leite corrigida para 3,5% de gordura. A composição do leite não sofreu efeitos significativos. O custo total com volumoso, concentrado e alimentação não sofreu efeitos significativos. A receita bruta com a venda de leite não sofreu efeito significativo, apresentando média de BRL 28,65. A taxa de retorno marginal não apresentou diferenças significativas. Os diferentes níveis de ureia não afetaram a produção de vacas de leite. Conclui-se que na dieta de vacas de leite à base de silagem de sorgo pode-se realizar a inclusão de ureia no concentrado com níveis de 5,7 a 17,1 g/kg do concentrado, sem redução do desempenho produtivo dos animais.

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INTRODUCTION

The Brazilian dairy farming activity has evolved continuously in the last decades, providing a consistent growth in production that has placed the country fourth in the rank of milk producers worldwide. However, yield per cow remains around 1,700 L/year, which represents approximately 5.5 L/cow/day (IBGE 2017).

In addition to production, milk consumption per capita in Brazil has also risen in the last decade. Estimates indicate that in the year 2017, Brazilians consumed an average of 173 L of milk per person. Despite this consumption increase, this value is still below indicators observed in developed countries, which lie in the range of 250 to 300 L/person (EMBRAPA 2018).

Milk yield is directly linked to the cows' nutrition, which requires diet formulations that meet their maintenance and production requirements. When herbage cannot meet those requirements, especially in medium- to high-yield animals, grain-based concentrates must be provided to correct the nutritional deficit and allow for constant production on the dairy farm (Teixeira et al. 2015, p. 281).

However, such concentrates are costly, with protein being the most expensive macronutrient in them,

which impacts the final production cost per liter of milk. Therefore, diets must be balanced, since excessive protein intake causes the animal to excrete the surplus through urine and milk. This leads to economic losses and a decline in production, as this excretion process incurs energy expenditure besides environmental contamination (Fessenden et al. 2019, p. 3023).

Dietary protein plays a critical role as one of the main essential nutrients for successful production, since, in addition to providing amino acids to the animal, it is also a source of nitrogen for microbial protein synthesis. After energy, protein is the nutrient required in largest quantity by ruminants. The final supply of protein to the small intestine consists of rumen-undegradable protein and microbial protein, the latter of which is synthesized in the rumen and considered of high biological value, supplying more than 50% of the amino acids absorbed by ruminants (Schwab & Broderick 2017, p. 10097).

It is essential to optimize microbial protein synthesis in ruminants. To this end, rumen microorganism growth should be stimulated, which requires protein degradation and ammonia ($N\text{-}NH_3$) availability in addition to energy availability in the rumen environment (Brooks et al. 2012, p. 4985).

Urea is widely used in the feeding of dairy cows due to its low cost, high availability and ease of use. This organic compound improves microbial protein synthesis capacity. Upon reaching the rumen, urea is hydrolyzed and converted to $N\text{-}NH_3$ and carbon dioxide (CO_2). Together with sources of rapidly fermentable carbohydrates, this ammonia is utilized for microbial protein synthesis, as it supplies protein and energy to those microorganisms (Alves et al. 2014, p. 58).

The objective of this study was to evaluate productive, nutritional, metabolic and economic traits of dairy cows fed sorghum silage diets with urea levels (**Table I**).

MATERIAL AND METHODS

This study was conducted after approval by the Ethics Committee on Animal Use (CEUA) of the State University of Piauí (UESPI) (approval no. 0356/19).

The experiment was conducted on Branquinha Farm, located in Corrente/PI, Brazil (10° 26' 30'' S and 45° 9' 52 '' W). According to the Köppen classification system, the climate of the region is an Aw type (tropical with dry winters). Minimum and maximum temperatures in the region are 17.6 and 32.2 ºC, respectively. Average annual precipitation is 1035 mm, and the rainy period is concentrated between November and March, possibly extending through May.

Five ¾ Holstein × ¼ Gir lactating dairy cows in the middle third of lactation, at an average age of 60 days and average body weight of 518 kg ± 52 kg, were allocated to five treatments in a 5 × 5 Latin square experimental design (five animals, five periods and five treatments). The animals were kept in individual 30-m^2 stalls arranged side by side, where they were fed a diet formulated to meet the requirements for maintenance and production of 20 L of milk with 3.5% fat, following the NRC (2001), with a roughage-to-concentrate ratio of approximately 60:40.

Treatments corresponded to different concentrations of urea in the diet: U-50 (5.7 g urea/kg concentrate DM); U-75 (8.6 g urea/kg concentrate DM); U-100 (11.4 g urea/kg concentrate DM); U-125 (14.3 g urea/kg concentrate DM) and U-150 (17.1 g urea/kg concentrate DM) (**Table II**).

The animals were allowed a 20-period of acclimation to diets and management prior to the start of the experimental period. The experiment lasted 105 days, which were divided into five 21-day periods consisting of 14 days of acclimation to the experimental diets and seven days for data collection.

The daily management of the cows started at 02h00, when they were removed from the stalls for the first milking, which was performed mechanically (bucket-at-foot, single-file model with pit). Before each milking session, the teats were sanitized (pre-dipping) with a sodium hypochlorite (NaClO)-based antiseptic solution and, after milking, the teats were immersed in an iodine glycerin-based solution (post-dipping). Subsequently, the diet was provided in half-drum feeders with 100 cm (linear) per animal. Water was supplied *ad libitum* in drinkers with automated supply. The second milking began at 13h00, followed by feed supply.

Fecal output was estimated using 500 mg of LIPE® (isolated, purified and enriched lignin from *Eucalyptus grandis*; UFMG, Minas Gerais) as an external marker. One capsule was provided daily after the first milking, for five days (two for adaptation and regulation of marker excretion flow and three for fecal collection) (Saliba 2013, p. 352). The feces were harvested directly from the rectal ampulla once daily, at the time the marker was administered, and stored in a freezer at -10 ºC. To determine the internal marker, indigestible neutral detergent fiber (iNDF), samples of silage, feces and concentrates were incubated in the rumen of five fistulated animals for 240 h (Casali et al. 2008, p. 335) and the residue was considered indigestible. Apparent digestibility was estimated from fecal output, which was determined using LIPE® as an external marker, and dry matter intake (DMI) was measured from the 16th to the 21st day as the difference between the amount supplied and orts.

Samples of concentrate, silage and feces were predried in a forced-air oven at 55 ºC for 72 h. The dry matter (Method 967.03), total nitrogen (Method 981.10), mineral matter (Method 942.05) and ether extract (Method 942.05) contents were determined according to methods described in AOAC (1997, p. 369-406). The neutral detergent fiber content corrected for ash and protein (NDFap) was estimated according to Mertens (2002, p. 1217). Non-fibrous carbohydrates (NFC) were calculated as proposed by Detmann & Valadares Filho (2010, p. 980: 100 – [% CP – % CP from urea + % urea) + % NDFap + % EE + % ash).

Milk yield was evaluated from the 15th to the 19th day of each experimental period. 3.5% fat-corrected milk yield (3.5%FMY) was determined by the follow-

Table I. Chemical composition of sorghum silage and supplement provided to dairy cows fed sorghum silage diets with urea levels (Composição química da silagem de sorgo e suplemento fornecido a vacas leiteiras alimentadas com dietas de silagem de sorgo com níveis de uréia).

Item	Chemical composition of silage and concentrates					
	Silage	Urea level				
		U-50	U-75	U-100	U-125	U-150
DM[1] (g/kg)	308	753	789	796	852	875
MM[2] (g/kg DM)	93	78	83	66	96	101
CP[3] (g/kg DM)	77	262	286	283	320	334
NDFap[4] (g/kg DM)	706	225	210	208	185	190
ADF[5] (g/kg DM)	430	88	107	100	102	92
EE[6] (g/kg DM)	80	56	51	52	50	51
NFC[7] (g/kg DM)	53	379	329	390	363	305

[1]Dry matter; [2]Mineral matter; [3]Crude protein; [4]Neutral detergent fiber corrected for ash and protein; [5]Acid detergent fiber; [6]Ether extract; [7]Non-fibrous carbohydrates.

Table II. Proportion of ingredients in the supplements and chemical composition of sorghum silage diets with urea levels fed to dairy cows (Proporção de ingredientes nos suplementos e composição química de dietas de silagem de sorgo com níveis de ureia alimentados com vacas leiteiras**).**

Ingredient	Proportion of ingredients in the supplement				
	U-50	U-75	U-100	U-125	U-150
Ground sorghum grain (g/kg)	513.3	520.7	528.4	536.3	544.5
Soybean meal (g/kg)	441.4	430.4	419.1	407.4	395.4
Mineral salt* (g/kg)	23.2	23.6	23.9	24.2	24.6
Calcitic limestone (g/kg)	15.0	15.2	15.4	15.7	15.9
Urea (g/kg)	5.7	8.7	11.7	14.9	18.1
Dicalcium phosphate (g/kg)	1.4	1.4	1.5	1.5	1.5
Chemical composition of the diets					
CP (g/kg DM)	174.7	178.3	179.6	184.3	186.9
NDF (g/kg DM)	504.3	493.8	493.3	476.8	477.4
TDN (g/kg DM)	767.9	726.5	779.0	742.1	751.0
EE (g/kg DM)	69.7	67.3	67.9	66.7	66.8
NFC (g/kg DM)	190.0	171.4	197.4	189.4	165.0

*Composition: calcium 200 g/kg; phosphorus 100 g/kg; sodium 68 g/kg; magnesium 15 g/kg; sulfur 12 g/kg; zinc 6285 mg/kg; manganese 1960 mg/kg; copper 1650 mg/kg; fluorine (maximum) 1000 mg/kg; cobalt 200 mg/kg; iron 560 mg/kg; iodine 195 mg/kg; nickel 40 mg/kg; selenium 32 mg/kg. CP – crude protein. NDF – neutral detergent fiber. DM – dry matter. TDN – total digestible nutrients. EE – ether extract. NFC – non-fibrous carbohydrates.

ing formula (Tyrrell & Reid 1965, p. 1215): 3.5%FMY = 12.82*P + 7.13*F+ 0.323*MY, where 3.5%FMY = milk yield (kg/day); F = fat yield (kg/day); and P = protein yield (kg/day).

Milk samples for composition analysis were collected individually during the morning milking on the 18th day of each period and sent to the Laboratory of the Milk Clinic at the Department of Animal Science of ESALQ/USP. The fat, protein, lactose and total solid contents were analyzed by the infrared method, using a Bentley 2000 analyzer (Bentley Instruments®); urea nitrogen was measured using a ChemSpec 150 analyzer (Bentley Instruments®); and somatic cell count (SCC) was determined by the flow cytometry method, using Somacount 300 (Bentley Instruments®), at the Laboratory of the Milk Clinic at the Department of Animal Science of ESALQ/USP, following their respective standard methods.

Body condition score was measured by a visual assessment performed by only one properly trained observer, using a 5-point scale (1 = lean and 5 = fat) with 0.25-unit increments (Edmonson et al. 1989, p. 68). Body weight change was measured by individually weighing the animals on an electronic scale at the start and end of each period. Three weighing sessions were carried out simultaneously to obtain the average weight. Both body condition score and individual weight were measured on the 1st day of each experimental period.

On the 21st day of each period, 4 h after the morning feed, approximately 100 mL of rumen fluid were harvested using an esophageal probe. After collection, the pH was immediately read using a digital pH meter. Next, the rumen fluid was filtered through gauze and aliquots were used to evaluate ammoniacal nitrogen ($N\text{-}NH_3$), short-chain fatty acids (SCFA) and protozoa. The rumen fluid aliquots were packed in labeled plastic bags that were then frozen at -20 ºC for later laboratory analysis.

To evaluate $N\text{-}NH_3$, 1 mL sulfuric acid (H_2SO_4, 1:1) was added to 10 mL rumen fluid to interrupt fermentation. The concentration was determined at the Animal Physiology Laboratory at Southwest Bahia State University and the solution was processed by the potassium hydroxide (KOH) distillation method, as described by Dettmann et al. (2012, p. 214). Aliquots of 9 mL of rumen fluid were acidified with 1 mL phosphoric acid (H_3PO_4, 25%) for analysis of SCFA, which were performed at the Federal University of Viçosa (MG, Brazil). Based on the SCFA data, ruminal CO_2 and CH_4 production was determined using Wolin's stoichiometry (1960, p. 1452), assuming that the oxidation balance of all ruminal products is zero:

$$\text{Acetate} + \text{Propionate} + \text{Butyrate} + CO_2 + CH_4 = 0$$

where CO_2 and CH_4 are produced exclusively through the acetate and butyrate production pathways, based on the following equations:

$CO_2 + CH_4 = \text{Acetate} + 2\ \text{Butyrate}$; after a few replacements:

$$CO_2 = \text{Acetate}/2 + \text{Propionate}/4 + 1.5 * \text{Butyrate}$$

Thus, the produced CO_2 moles can be calculated from the amounts and molar ratios of acetate, propionate (p) and butyrate. Once the CO_2 moles are known, the CH_4 moles can be calculated by the equation below:

$$CH_4 = (\text{Acetate} + 2\ \text{Butyrate}) - CO_2$$

The samples for the count and identification of protozoa consisted of 1 mL of rumen fluid diluted in 9 mL formalin (CH_2O, 37% formaldehyde). The observation took place at the Cellular Laboratory at UESB, where 10 µL of the sample were placed on a slide with slip cover under an optical microscope under 100 x magnification (Wolht et al. 1976, p. 459).

Rectal temperature (ºC) was measured using a digital clinical thermometer, which was inserted into the animal's rectum after cleaning in a way that the bulb would be in contact with the mucosa. The equipment was held in place for approximately 60 s, until a beep sound would indicate the stabilization of temperature.

For marginal analysis, the partial budget method was adopted, considering the elements that varied with the animals' milk yield and the feeding system of each tested treatment, e.g., sorghum silage, concentrate (grain sorghum, soybean meal, urea, calcitic limestone and dicalcium phosphate) and mineral salt. The concentrate costs were obtained considering the intake and price of ingredients quoted during the experiment.

Subsequently, the revenues from the sale of milk per treatment were evaluated using the price of milk corresponding to the amount paid in the south of Piauí State, as quoted by the Center for Advanced Studies on Applied Economics at ESALQ/USP. The following variables were evaluated: gross revenue from the sale of milk (GRSM) and revenue minus feeding costs (RMFC: difference between GRSM and total feeding cost). The marginal rate of return (MRR) was calculated by the methodology proposed by Evans (2005), using the following formula:

$$MRR = (RMFC_{standard} - TFC_{standard} / TFC_{test} - TFC_{standard}) * 100.$$

Data was analyzed as a Latin square experimental design (5 × 5) by the variance and regression analysis procedure of SAEG statistical computer program (Sistema para Análises Estatísticas, version 9.0). Polynomial contrasts (L and Q) were used in the analysis of the means of variables dependent on the urea inclusion levels in the total diet (5.7, 8.6, 11.4, 14.3 and 17.1 g urea/kg concentrate DM). The 5% probability level of significance was adopted.

RESULTS

The dietary urea levels ($P<0.05$) (**Table III**) did not affect the feed intake, digestibility or metabolic parameters of the lactating cows (**Table III**).

There was no effect ($P<0.05$) of urea levels (**Table III**) on the apparent digestibility of dry matter or nutrients (CP, NDF, EE, NFC and TDN). It is essential to know the chemical composition and digestibility of feedstuffs so that balanced diets can be formulated for the animals to express their maximum production potential.

The urea levels also did not affect ($P<0.05$) rumen pH (**Table III**) or ammoniacal nitrogen ($N\text{-}NH_3$).

No significant differences were observed for milk yield, milk composition or 3.5% fat-corrected milk yield (3.5%FMY) as affected by the urea levels ($P<0.05$). Milk protein was not influenced by the urea concentration ($P<0.05$) (**Table IV**).

Body weight change and body condition score were not influenced by the urea levels ($P<0.05$) (**Table IV**), due to the lack of effects on DMI.

The total roughage (TRC), concentrate (TCC) and feeding (TFC) costs were not significantly affected ($P<0.05$), averaging BRL 2.00, 9.17 and 11.17, respectively.

Gross revenue from the sale of milk (GRSM) did not differ significantly ($P<0.05$), averaging BRL 28.65, with a fixed paid value of BRL 1.50 per liter of milk.

Marginal rate of return (MRR) also did not show significant differences ($P<0.05$) (**Table IV**).

DISCUSSION

The lack of effects on DMI is related to a balance between the chemical and physical interaction of nutrients, especially between the protein and carbohydrate fractions. This is a consequence of the addition

Table III. Feed intake, apparent digestibility and metabolic parameters of dairy cows fed sorghum silage diets with urea levels and respective coefficients of variation (CV) and regression equations (RE) (A ingestão alimentar, digestibilidade aparente e parâmetros metabólicos das vacas leiteiras alimentaram dietas de silagem de sorgo com níveis de ureia e respectivos coeficientes de variação (CV) e equações de regressão (RE)).

Item	Urea level					CV (%)	RE	R^2
	U-50	U-75	U-100	U-125	U-150			
Intake								
RDM[1]	12.37	12.02	12.20	12.51	12.70	16.19	Ŷ =12.36	--
CDM[2]	8.98	9.04	9.13	9.85	10.15	16.46	Ŷ = 9.43	--
TDM[3]	21.34	21.06	21.33	22.36	22.85	16.30	Ŷ = 21.79	--
CP[4]	3.31	3.52	3.52	4.12	4.38	16.14	Ŷ =3.77	--
NDF[5]	10.76	10.39	10.53	10.65	10.92	16.29	Ŷ = 10.65	--
EE[6]	1.47	1.41	1.48	1.51	1.53	24.19	Ŷ = 70.56	--
NFC[7]	4.07	3.63	4.20	4.23	3.75	17.83	Ŷ = 3.98	--
TDN[8]	16.48	15.34	16.71	16.73	17.25	21.42	Ŷ = 16.50	--
Digestibility								
DM[9]	58.69	59.65	64.28	62.23	62.61	12.98	Ŷ= 61.49	--
CP[10]	74.21	77.88	77.73	81.07	80.61	5.62	Ŷ= 78.30	--
NDF[11]	73.77	71.94	76.01	72.77	72.69	8.06	Ŷ= 73.44	--
EE[12]	70.94	64.45	78.49	63.57	75.33	15.12	Ŷ= 70.56	--
NFC[13]	89.68	82.70	90.09	78.93	82.18	9.48	Ŷ= 84.71	--
TDN[14]	76.79	72.65	77.90	74.21	75.10	6.14	Ŷ= 75.33	--
Metabolic parameters								
pH[15]	6.51	6.63	6.61	6.64	6.65	3.94	Ŷ= 6.61	--
Sedimentation[16]	172	177	185	170	134	22.35	Ŷ= 167.35	--
Reduction[17]	133	145	194	132	172	33.14	Ŷ= 155.15	--
$N\text{-}NH_3$[18]	39.2	26.7	31.7	21.9	22.9	17.33	Ŷ =28.48	---
Glucose[19]	66	66	66	58	61	6.29	Ŷ= 63.6	--
RT[20]	38.6	38.7	38.1	38.6	38.6	0.81	Ŷ= 38.5	--

[1]Roughage dry matter (kg¹d); [2]Concentrate dry matter (kg¹d); [3]Total dry matter (kg¹d); [4]Crude protein (kg¹d); [5]Neutral detergent fiber (kg¹d); [6]Ether extract (kg¹d); [7]Non-fibrous carbohydrates (kg¹d); [8]Total digestible nutrients (kg¹d); [9]Dry matter (%); [10]Crude protein (%); [11]Neutral detergent fiber (%); [12]Ether extract (%); [13]Non-fibrous carbohydrates (%); [14]Total digestible nutrients in the diet (%); [15]pH of rumen fluid; [16]Rumen fluid sedimentation time (s); [17]Rumen fluid reduction time (s); [18]Ammoniacal nitrogen (mg/dL); [19]Blood glucose (mg/dL); [20]Rectal temperature (°C).

of small amounts of urea to the diet, which not only complemented the dietary protein but was also capable of improving rumen microbial activity due to its rapid and high availability in the rumen environment. Therefore, the small differences between the urea levels used in the present study were responsible for the lack of effects between the treatments.

Antunes et al. (2016, p. 176) used diets based on sorghum silage, the same roughage source tested in the present study, with different urea levels (0, 3.6, 7.3 and 10.1 g/kg diet DM), to feed lactating cows. As in the present study, no difference was detected for total DMI, and urea intake (g/day) was similar between the two experiments.

Fessenden et al. (2019, p. 3028) used 4 g urea/kg DM in the diet of lactating cows and also found no changes in total DMI, which the present findings corroborate. This confirms the hypothesis that including low urea levels in the diet of dairy cattle does not change their dry matter intake.

Although urea was used as a physical controller of intake, with higher inclusion levels in cattle diets typically reducing their intake (Lima et al. 2013, p. 712), the present results show that Girolando cows, with a daily milk yield of up to 20 kg, respond similarly in production as when cereal protein sources are used. Thus, nutritional challenges can be imposed with higher levels of the evaluated compound in diets. Wilson et al. (1975, p. 1431) attributed the decreased dietary DM intake to intermediate catabolites of the urea metabolism only when inclusion was above 2.3%. In the present study, the treatment with the highest urea level contained only 0.8% of the ingredient in the DM.

The urea levels were not able to alter ($P<0.05$) CP intake, as it depends on residence time in the rumen for greater degradation by microbial action. Furthermore, the experimental diets were formulated to be isonitro-

Table IV. Yield and marginal analysis of lactating cows fed sorghum silage diets with urea levels and respective coefficients of variation (CV) and regression equations (RE) (A produção e a análise marginal das vacas lactadoras alimentaram dietas de silagem de sorgo com níveis de ureia e respectivos coeficientes de variação (CV) e equações de regressão (RE)).

Item	Urea concentration					CV (%)	RE	R^2
	U-50	U-75	U-100	U-125	U-150			
Milk yield and composition								
MY[1]	18.95	19.38	19.44	18.55	19.16	28.04	Ŷ= 19.10	-
3.5%FMY[2]	21.96	21.95	22.98	20.02	21.59	32.70	Ŷ= 21.70	-
SNF[3]	94.0	94.3	95.2	94.1	96.2	3.40	Ŷ= 94.74	-
PTN[4]	38.2	39.3	39.1	39.0	40.7	7.18	Ŷ= 39.24	-
Lactose ($g.kg^{-1}$)	46.3	45.5	46.6	45.6	46.1	2.07	Ŷ= 45.99	-
Fat ($g.kg^{-1}$)	45.8	46.2	46.8	47.0	48.4	12.20	Ŷ= 46.84	-
F:P[5]	1.20	1.13	1.20	1.16	1.19	7.22	Ŷ= 1.17	-
TS[6]	139.8	138.5	142.0	139.4	144.6	5.41	Ŷ= 140.85	-
SCC[7]	760	350	264	423	404	101.03	Ŷ= 400.25	-
MUN[8]	19.88	19.68	21.80	21.16	22.65	14.19	Ŷ= 21.03	-
BWC[9]	0.122	-0.222	-0.167	-0.022	0.444	1966.72	Ŷ= 0.03	-
BCSC[10]	-0.05	-0.05	-0.05	0.00	0.00	-390.87	Ŷ= -0.03	-
Cost								
TRC[11]	2.00	1.95	1.98	2.03	2.06	16.21	Ŷ= 2.00	-
TCC[12]	8.77	8.77	8.81	9.46	9.66	18.21	Ŷ= 9.17	-
TFC[13]	10.77	10.72	10.78	11.49	11.71	17.75	Ŷ= 11.17	-
Economic indicator								
GRSM[14]	28.43	29.08	29.16	27.82	28.74	30.70	Ŷ= 28.65	-
RMFC[15]	17.66	18.36	18.38	16.34	17.03	48.18	Ŷ= 17.55	-
MRR[16]	0.00	589.10	583.43	591.64	596.27	3221.84	Ŷ=472.09	-

Milk yield (kg^1d); [2]3.5% fat-corrected milk yield (kg^1d); [3]Solids non-fat ($g.kg^{-1}$): [4]Protein ($g.kg^{-1}$); [5]Fat:protein ratio (g/g); [6]Total solids ($g.kg^{-1}$); [7]Somatic cell count (1000 SC/mL); [8]Milk urea nitrogen (mg/dL); [9]Daily body weight change (kg^1d); [10]Body condition score change (Points); [11]Total roughage cost ($BRL.day^{-1}$); [12]Total concentrate cost ($BRL.day^{-1}$); [13]Total feeding cost ($BRL.day^{-1}$); [14] Gross revenue from the sale of milk ($BRL.day^{-1}$); [15]Revenue minus feeding cost ($BRL.day^{-1}$); [16]Marginal rate of return (%).

genous and isoenergetic, according to the NRC (2001). The non-variation in CP intake is explained by the lack of changes in DMI.

No changes were observed for NDF intake with the different urea concentrations in the diet (P<0.05). However, average NDF intake was 10.65 kg DM, or 2% body weight. This high intake may be explained by the fact that all treatments had urea as a source of non-protein nitrogen (NPN), which, when associated with the soluble carbohydrates from the concentrate, improves the ruminal environment, providing optimal conditions for the microorganisms to degrade the fibrous fraction of the diet. As a result, the animal is stimulated to consume more feed, as it was available 24 h daily in the trough.

La Ossa et al. (2013, p. 145) worked with confined dairy cows fed diets with roughage-to-concentrate ratios similar to those used in the present study and found a lower NDF intake. Therefore, factors related to the roughage used in our experiment might have led to this result, regardless of the urea level.

In the present study, the NFC content of the diet was approximately 183 g/kg DM. The NRC (2001) recommends a maximum limit for NFC according to the dietary fibrous content (NDF and ADF), which, in the current experiment, was around 200 to 230 g NFC per kg DM. Thus, at values higher than those, there would be alterations in DMI.

Ether extract intake was not significantly affected by the urea concentration (P<0.05), because the largest source of EE in the total diet was the silage (79 g EE per kg of DM). However, intake is restricted by the type of supply adopted—total mixture of concentrate with silage—, which did not allow for the animals to select ingredients.

Total digestible nutrient intake was not affected by the urea concentrations (P<0.05). The NRC (2001) recommends the intake ratios of 335 g TDN per kg DM and 90 g CP per kg DM, both per kilogram of milk pro-

duced. Very similar results were found in the present study: 365 g TDN per kg DM for every 94 g CP per kg DM. Thus, an increase in this nutrient would not be interesting for milk yield due to the loss of energy that would result from the imbalanced energy-to-protein ratio. In this way, the changes in dietary urea levels were not sufficient to cause differences in DMI.

There were no alterations (P<0.05) in NDF digestibility. The non-alteration of this variable may be explained by the lack of changes in the intake of CP, a nutrient responsible for increasing the availability of rumen nitrogenous compounds, resulting in stimulus to fiber digestion. However, in the present study, the nutrient digestibility coefficients were higher than values presented in studies using similar roughage-to-concentrate ratios (La Ossa et al. 2013, p. 147), which is likely due to the interaction between the roughage source used and RDP that provided substrates for a greater microorganism action in the rumen.

The higher uptake of nitrogen compounds in the rumen may stimulate fiber digestion, as it results in greater growth of fibrolytic microorganisms and an increase in the concentration of volatile fatty acids, such as acetate, isobutyrate and isovalerate (Olmos et al. 2006, p. 1709).

However, the positive effects of urea on nutrient digestibility when included in ruminant diets depend on the ability of rumen microorganisms to assimilate the final products of fermentation (Pessoa et al. 2009, p. 941). It is known, therefore, that animal production is defined by voluntary intake, which determines the amount of nutrients ingested, and that digestibility is a qualitative description of the feed. Thus, despite being low, the urea levels studied here did not compromise the intake and digestibility of the diets.

The dietary urea levels did not influence rumen pH, because this variable was associated with the concentrate levels of the diet, since a complete mixture (roughage and concentrate) was provided. This allowed for a limitation of concentrate intake in a short time, preventing high SCFA concentration peaks and maintaining rumen stability.

The rumen fluid pH should be higher than 6 and lower than 7.2 for the ruminal environment to have favorable conditions for the proliferation of cellulolytic bacteria, consequently improving fiber digestibility (Alves et al. 2018, p.330). In the current experiment, the average rumen fluid pH was 6.61, which is within the ideal range for the proliferation of those bacteria.

When consumed by the animals, urea is immediately attacked by bacterial ureases and degraded, increasing ammoniacal nitrogen production. Ammoniacal nitrogen is utilized immediately for microbial protein synthesis; however, excess urea or energy imbalance results in an increase in rumen ammonia concentration and its escape through the rumen wall (Ítavo et al. 2016, p.452). The lack of an effect on the $N\text{-}NH_3$ concentration in the present study may be explained by the slight differences in urea levels between the animals, which was slightly above 11 g/kg concentrate DM between the lowest and highest levels.

There was no difference (P<0.05) for blood glucose (**Table III**). This was an expected finding, since the amount of glucose circulating in the bloodstream of ruminants is little affected by the feeding level (Mota et al. 2018, p. 371). Most part of the circulating glucose in ruminants originates in hepatic gluconeogenesis, with propionate being its main precursor. Propionate, in turn, originates from the degradation of dry matter, which is influenced by the degradation of protein fractions (Marques et al. 2011, 1088). However, because the diets tested in the present study were isonitrogenous, dry matter degradation was similar in all treatments, and so the circulating glucose rates were not affected.

Some studies show that the circulating glucose in ruminants is not affected when the crude protein source is partially or fully replaced, as reported by Silva et al. (2016, p. 177) in dairy cows; Mota et al. (2018, p. 375) in dairy heifers; and Bezerra (2018, p. 101) in dairy cows.

The urea levels (P<0.05) did not influence milk yield or composition (**Table IV**). This lack of effects on milk yield may be related to the fact that the nutritional requirements were met—not only for crude protein, but also rumen degradable and undegradable protein.

Hu et al. (2007, p. 3355) associated changes in 3.5%CMY with fat ($kg.day^{-1}$) and protein ($kg.day^{-1}$) yields, because they are part of the equation that corrects the actual milk-yield value including fat, which is one of the parameters determining the change in 3.5%CMY. Souza et al. (2015, p. 564) and Antunes et al. (2017, p. 176) replaced plant-derived protein sources with NPN and also observed no significant effects on fat-corrected milk yield.

Although the fat content was the milk component that varied the least as affected by the diet, with changes in the rumen fermentation processes (Santos et al. 2012, p. 1025), no significant effects were observed for this variable in response to the urea levels (P<0.05). This lack of effect may be associated to a lack of changes in fermentation pattern resulting from the diets, which directly affects the substrate generated for the synthesis of fat in the mammary gland. According to Marques et al. (2011, p. 1088-1094) and Barbosa et al. (2012, p. 621), the constituents of milk can be directly altered by the nutritional and metabolic state of dairy cows.

The increasing levels of protein in milk are correlated with higher amounts of dietary NFC (Santos et al. 2012, p.1025). Once associated with the NPN, non-fibrous carbohydrates improve the capture of N and energy by the microorganisms, optimizing the synthesis of microbial protein, which is rich in amino acids. These amino acids are then absorbed in the small intestine, increasing the synthesis of milk protein (Tacoma et al. 2017, p. 7246). In the present study, no changes were observed in the NFC content and all treatments contained urea, which explains the lack of effects for this variable.

Furthermore, despite being at low concentrations, urea was present in all treatments. As such, it provided NPN, optimizing the rumen microorganisms and

increasing microbial protein, which is high in AA essential for the synthesis of milk and milk protein (Clark et al. 1992, p. 2304).

The urea levels did not influence the solids not-fat or total solids contents, due to the lack of variation in their main constituents. Total solids is an important indicator of milk quality, as it represents the sum of all solid parts of milk, especially fats and proteins, which are considered by the dairy industry as the components that promote yield in milk-derived products and through which the producer is paid for the product delivered to the industry.

Similarly to the present reports, Faleiro Neto et al. (2013, p. 52) evaluated two sources of NPN in the diet of Holstein/Zebu crossbred cows, offered at three levels (0.3, 0.6 and 0.9%, as-fed basis), and a control diet without a NPN source and found no differences in total solids content, which averaged 11.19%.

Milk urea nitrogen (MUN) was not influenced by the urea levels ($P<0.005$). When not fully used by rumen microorganisms, excess protein in the diet of dairy cows, especially in the degradable and soluble forms, is absorbed by the rumen epithelium as ammonia into the bloodstream, converted to urea by the liver and excreted in urine and milk (Fessenden et al. 2019, p. 3031). As such, it constitutes an important tool to evaluate the balancing of dairy cattle diets (Tripathi, 2014, p. 1). In the current study, not even the diet with the highest urea level was able to cause excessive N in the rumen, and thus MUN was not changed.

Milk somatic cell count (SCC) was not affected ($P<0.05$) by the urea levels. Somatic cell count is influenced by several factors related to the milk, e.g., calving order, age, lactation period, month and season of the year, gestation, nutritional state and, mainly, mammary gland health (Teixeira et al. 2015, p. 284). Normative Instruction (NI) no. 76 determines that SCC must be at maximum 500,000 SC/mL (BRASIL, 2018). The SCC found in this study (425,000 SC/mL) is below the maximum level established by that NI. This low SCC is related to milking management and equipment hygiene, which were strictly observed in all milking sessions.

It should be stressed that despite the lack of significant differences in milk composition between the treatments, the cows produced milk with high levels of solids non-fat, proteins, fat and lactose, which averaged 94.74, 39.24, 46.84 and 45.99 g/L of milk, respectively, exceeding their required minimum (solids not-fat: 84 g/L, proteins: 29 g/L, fat: 30 g/L and lactose: 43 g/L) as established by NI no. 76 for milk (BRASIL, 2018).

Body condition score is associated with the maximum genetic milk production capacity of lactating cows. When this limit is reached, energy reserves start to be deposited in the animal body in the form of tissue. The NRC (2001) reports that BCS is related to fat, protein and energy contents in the body and is responsible for variations of the orders of 65, 52 and 66% in those components, respectively. In the present study, no change was observed in the animals' BCS.

The dietary urea levels also did not have significant effects ($P<0.05$) on gross margin (revenue minus feeding cost), which averaged BRL 17.55/day. The lack of effects for both cases was due to the lack of changes in milk yield, which is the main factor responsible for variations in revenues. The lack of effect is explained by the fact that those costs were related to daily intake, which was not changed by the dietary urea levels. The objective of the present study was not to replace the CP source, but rather provide a source of rapidly degradable protein, aiming to improve the rumen environment; hence the lack of effects on the final price (BRL) of the kilogram of concentrate across the treatments and, consequently, on TFC. Total concentrate cost, in turn, was the item that most significantly impacted TFC.

Marginal rate of return represents the difference obtained with the increase in return, in percentage terms, of the total additional costs. It is a useful tool to make recommendations of new technologies for producers (Evans, 2005). In the present study, despite the lack of significant effects between the treatments, a positive MRR was obtained in both cases, meaning the use of any of the concentrates studied here can be indicated (for medium-yield lactating cows fed sorghum silage).

Higher yield does not always translate into higher profit. For this reason, the production activity must be efficient in both technical and economic terms. That is, the activities should be performed at a minimum cost (Lopes et al. 2012, p. 458). The production cost is considered a necessary expense for the generation of the product in both the technical and economic terms, the latter of which are considered an opportunity cost.

The exchange ratio observed in this study was BRL 2.56 for every BRL 1.00 invested in feeding. Although other costs such as vaccination, labor and depreciation were disregarded, the exchange ratio was considered high. Feeding cost represents 50 to 68% of the total cost per liter of milk produced (Rodrigues et al. 2012, p.109).

CONCLUSION

Urea can be added to the concentrate of sorghum silage diets for dairy cows at the levels of 5.7 to 17.1 g/kg of concentrate without reducing the animals' productive performance. Despite the lack of statistical differences between the treatments, there was a BRL 0.99 difference between the highest and lowest total daily feeding costs, which were achieved with the treatments including 8.6 and 17.1 g urea/kg concentrate DM, respectively.

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