Sugarcane with Elephant grass replacing sugarcane with urea in the diet of crossbred dairy cows

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ABSTRACT. The objective was to evaluate the partial substitution of sugarcane treated with urea by elephant grass in the feeding of crossbred cows in lactation. Eight crossbred cows (Holstein x Gyr) were used, with 474 kg of body weight (standard deviation = 46.15 kg) and 8.15 kg of milk per day. The animals were housed in individual stalls, distributed in two 4 x 4 Latin squares, and the treatments were: sugarcane; sugarcane corrected with 0.5% of urea and ammonium sulfate (9:1); 75% of sugarcane + 25% of elephant grass and 50% of sugarcane + 50% of elephant grass, based on natural matter. In addition to the treatments, it was provided water and mineral salt ad libitum and 3 kg of concentrate supplement (24% crude protein), based on corn meal and soybean meal, supplied in two portions during the morning and afternoon milking. There was a treatment effect on the intake and digestibility of dry matter and nutrients, except for non-fibrous carbohydrate intake, and there was no effect on milk production and composition. It can be concluded that the substitution of sugarcane by elephant grass (25 and 50%) increases the intake and decreases the digestibility of DM and OM, without affecting milk production, when the cows are supplemented with 3 kg of concentrate, containing 24% of protein per day.

Keywords: animal nutrition; cattle, digestibility; feed evaluation; intake; milk.

Received on May 21, 2019. Accepted on June 7, 2019.

Introduction

The state of Minas Gerais, Brazil, accounts for 30% of the national milk production, with most of this volume coming from properties relying on family agriculture and producing less than 200 liters per day (Azevedo, Felix, Júnior, Almeida, & Duarte, 2011). Additionally, production systems are basically characterized by low adoption of technology and predominant use of crossbred Dutch x Zebu cattle, with different blood fractions (Costa et al., 2016).

Within this reality, sugarcane (Saccharum spp.) stands out among the most frequently used roughages in the region, especially during the dry period of the year, for being low-cost, having a high production potential and being well accepted by animals (Mendonça et al., 2004). However, it has nutritional limitations, mainly because of slow fiber degradation and low protein content, which may compromise voluntary consumption and cattle performance (Pereira et al., 2000). For this reason, associating sugarcane with other feeds is recommended to raise the nutritional quality of the diet. Adding urea to sugarcane is a practice that is being employed for a long time in cattle feeds. However, some producers are resistant to this procedure, especially due to the possibility of intoxicating the animals when it is supplied inappropriately. Moreover, urea is not used by organic or agro ecological cattlemen, which requires the use of an alternative source of nitrogen. In practical contexts, sugarcane is being associated with roughages containing higher levels of crude protein (CP), such as elephant grass (Pennisetum purpureum) at early growth stage (31 to 45 days), which may reach approximately 15% of CP in dry matter (Voltolini et al., 2010). However, it is necessary to assess the effect of this practice on feed consumption and digestibility, as well as on animal performance.

Therefore, this study aimed to analyze the effect of replacing sugarcane treated with urea for different proportions of a mixture of sugarcane and elephant grass at early growth stage to feed lactating crossbred cows.
Material and methods

The experiment was conducted at Boa Vista farm, district of Cachoeirinha, belonging to the Federal University of Viçosa (UFV), Viçosa, Minas Gerais, Brazil.

Eight crossbred cows were used (Dutch × Gir), with initial average body weight of 474 + 46.15 kg and average production of 8.15 + 1.16 kg of milk per day. The animals were housed in individuals stalls, distributed into four 4 x 4 Latin squares; the treatments were composed of: sugarcane; sugarcane corrected with 0.5%, in natural matter (NM), of an urea and ammonium sulfate mixture (9:1); 75% of sugarcane + 25% of elephant grass and 50% of sugarcane + 50% of elephant grass, based on NM. In addition to the treatments, every day all animals were given water and mineral salt ad libitum and 3 kg of concentrated supplement, with approximately 24% of CP, made of cornmeal and soy bran, split into two portions and provided during the morning and afternoon milkings. Leftovers between 5 and 10% of the total supplied diet were allowed, ensuring ad libitum consumption by the animals during the experimental period.

The chemical composition of the treatments and of the concentrated supplement is displayed in Table 1.

| Item | Sugarcane | Elephant grass | Concentrated supplement | SC | SC + U3 | 75% SC + 25% EG | 50% SC + 50% EG |
|------|-----------|----------------|-------------------------|----|--------|----------------|----------------|
| DM²  | 27.60     | 12.1           | 82.25                   | 38.98 | 38.98 | 35.91           | 32.84          |
| OM³  | 97.72     | 87.54          | 94.49                   | 97.05 | 95.68 | 95.05           | 93.02          |
| CP⁴  | 2.06      | 11.19          | 24.19                   | 6.67 | 10.25 | 8.48            | 10.28          |
| NDF⁵ | 48.69     | 71.44          | 12.69                   | 41.19 | 40.61 | 45.69           | 50.20          |
| EE⁶  | 1.44      | 2.26           | 2.49                    | 1.66 | 1.64  | 1.82            | 1.98           |
| NFC⁷ | 45.53     | 2.65           | 55.12                   | 47.53 | 45.46 | 39.58           | 31.07          |
| iNDF⁸| 19.88     | 18.89          | 1.06                    | 15.96 | 15.73 | 15.76           | 15.57          |

¹Total composition of treatments, including the concentrated supplement; ²5 kg day⁻¹ provided during milkings; ³Sugarcane corrected with 0.5% of a mixture of urea and ammonium sulfate (9:1) in natural matter, with 260% of CP in the mixture; ⁴natural matter; ⁵dry matter; ⁶SC: sugarcane; EG: Elephant grass; U: Urea; DM: Dry matter; OM: Organic matter; CP: Crude protein; NDF: Neutral detergent fiber; EE: Ethereal extract; NFC: Non-fibrous carbohydrates; iNDF: Indigestible neutral detergent fiber.

The experimental periods lasted 14 days, with the first 10 being intended for the animals to adapt to the facilities and the diet, while the four last days were dedicated to consumption and milk production measurements, as well as sample collection.

To assess and quantify voluntary consumption, the supplied diet and leftovers of each animal were weighed and sampled daily, from the 11th to the 14th day of each experimental period. The samples were put inside plastic bags and frozen at -20°C for further processing and analysis.

To estimate digestibility coefficients, feces were collected directly from the animals’ rectal ampulla, on three consecutive days (12th, 13th and 14th) and at different times, as follows: 12th day - 8h, 13th day - 12h, and 14th day – 16h. The samples were put inside plastic bags and frozen (-20°C) for further processing and analysis.

The animals were weighed at the beginning of the experiment and at the end of each experimental period so that the effect of the treatment on body weight variance was known.

From 12th to 114th day of each experimental period, milk production was recorded. Milk samples were sourced from morning and afternoon milking on the 12th and 13th days of each experimental period, poured into flasks containing Bronopol®, stored at 2 and 6°C and sent to the Animal Nutrition Laboratory of the Federal University of Viçosa for analyses concerning protein, fat, lactose and total solid contents, according to methodology described by the International Dairy Federation (IDF, 1996).

For laboratory analyses, samples of the provided feed, leftovers and feces were unfrozen, pre-dried in forced-ventilation oven (60°C/72-96h), homogenized, processed in a Willey-type mill, first with a 2-mm mesh sieve, after which a portion for analyses on indigestible neutral detergent fiber (iNDF) was stored, and the remaining was reprocessed through a 1-mm mesh sieve for the other analyses. Samples were composed of feeds, leftovers and feces, representatively by animal and by period, based on the weight of the air-dried sample; they were stored in plastic containers.

In the samples of feeds, leftovers and feces, these were the contents assessed: dry matter (DM), according to method INCT-CA G-003/1; mineral matter (MM), according to method INCT-CA M-001/1; crude protein (CP), according to method INCT-CA N-001/1; neutral detergent fiber (NDF), according to...
method INCT-CA F-001/1, as described by Detmann et al. (2012) and non-fibrous carbohydrates (NFC), calculated as proposed by Detmann and Valadares Filho (2010).

To estimate fecal excretion, the fraction of indigestible neutral detergent fiber (iNDF) was used as internal indicator. The samples of feeds, leftovers and feces, dried and passed through a 2 mm mesh sieve, were stored in F57 bags (Ankom®), in triplicates, with a ratio of 20 mg of DM cm$^2$ of surface. The bags were incubated in a rumen-cannulated heifer for 288 hours, which was fed with a balanced diet containing roughage and concentrate (Detmann et al., 2012). After the incubation period, the bags were washed in running water until the water was completely clear. Then, the bags with samples were subjected to extraction with neutral detergent for one hour (Mertens, 2002).

Data were subjected to statistical analyses using the MIXED procedure from software Statistical Analysis System (SAS, 2004), adopting a significance level of 0.05.

**Results and discussion**

Increase was found in DM (DMI, p < 0.05) with the substitution of sugarcane by elephant grass (Table 2). The treatment with 50% replacement of sugarcane for elephant grass (50% SC + 50% EG) resulted in higher DMI compared to the other treatments, in line with the findings of Cabral et al. (2015), who observed that the addition of sugarcane to goat diets caused a linear decrease in DMC. Low DMI reported in diets with sugarcane as source of roughage has been frequently related to the low digestibility of sugarcane fiber (Cabral et al., 2015; Salomão et al., 2015). In the present study, the digestibility coefficients of NDF (NDFD) between the abovementioned treatments were similar (p > 0.05%; Table 3). However, in addition to digestibility, other factors may influence DMI. Sugarcane has a higher iNDF content (Table 1), and its digestible fraction is usually degraded in the rumen more slowly (Pereira et al., 2000). These characteristics cause the food to stay longer in the rumen, leading to ruminal fill and, consequently, to limited DMI (Cabra et al., 2015; Sampaio et al., 2009). This theory corroborates with the research conducted by Magalhães et al. (2004) with dairy cows, in which the addition of sugarcane to their diets decreased ruminal passage rates, which directly affected DMI.

Among the study treatments, only the one with the highest amount of elephant grass (50% SC + 50% EG) reached an ingestion of 1.2% of body weight in NDF, which is defined by Mertens (1987) as a physiological restrictor to DMI, with all the other treatments presenting NDF ingestion (NDFI) below this value. This confirms that, in addition to NDF amounts, other factors, such as fiber quality, also must be taken into consideration when estimating DMI; the amount that limits NDFI must not be taken as a static and absolute value, in accordance with Detmann et al. (2003).

Ingestion of DM components (OMI, CPI, NDFI and TDNI) also increased with partial substitution of sugarcane for elephant grass (50% SC + 50% EG; p < 0.05), as a reflection of higher DMI (Table 2).

The assessed treatments had no effect (p > 0.05) on NFCI (Table 2). Despite the smaller NFC content present in elephant grass compared to sugarcane (2.65 and 45.53%, respectively), in the treatments with elephant grass addition (75% SC + 25% EG and 50% SC + 50% EG), NFCI was compensated with increased DMI.

As expected, due to a higher nitrogen input, the addition of 0.5% of urea to sugarcane (SC + U) resulted in higher CPI (p < 0.05), which also led to a higher TDNI (p < 0.05) compared to the treatment with sugarcane (SC) as the only roughage food (Table 2). According to Xin, Schaefer, Liu, Axe, and Meng (2010), diets with sugarcane and urea have higher amounts of NPN and NFC and a better synchrony between urea hydrolysis and carbohydrate degradation by ruminal microorganisms. This synchrony stimulates the growth of ruminal microbiota, resulting in increased production of microbial protein and higher DM digestibility, which indeed happened in the present study (p > 0.05; Table 3). According to Silva and Leão (1979), 7% of CP is considered the minimum necessary to meet ruminal microbiota requirements and keep the rumen operating properly. In this study, only the treatment with sugarcane as the sole source of roughage (SC) contained the value close to 7%, with the CP percentage of the other treatments being higher (Table 1).

Within the assessed treatments, sugarcane with addition of 0.5% of urea (SC + U) showed higher digestibility coefficients for DM, OM and NFC (p < 0.05; Table 3) compared to the other treatments. There was no difference (p > 0.05) in digestibility values for CP and NDF between the treatment with sugarcane treated with 0.5% of urea (SC + U) and the treatment with a 50% substitution of sugarcane for elephant grass (50% SC + 50% EG), although said treatments virtually presented the same CP contents (Table 1), and the treatment with 50% of sugarcane (50% SC + 50% EG) presented higher NDF content.
Table 2. Ingestion of dry matter and of its constituents by low-producing dairy cows as a function of different forms of roughage supplementation.

| Item          | SC     | SC + U | 75% SC + 25% EG | 50% SC + 50% EG | SE     | P value |
|---------------|--------|--------|-----------------|-----------------|--------|---------|
| DMI, kg d⁻¹   | 8.50a  | 8.86a  | 9.98b           | 11.49c          | 0.24   | 0.001   |
| OMI, kg d⁻¹   | 8.15a  | 8.49a  | 9.48b           | 10.79c          | 0.23   | 0.001   |
| CPI, kg d⁻¹   | 0.75a  | 0.81a  | 0.84b           | 1.01c           | 0.01   | 0.001   |
| NDFI, kg d⁻¹  | 3.34a  | 3.51a  | 4.42b           | 5.72c           | 0.12   | 0.001   |
| EEI, kg d⁻¹   | 0.15a  | 0.15a  | 0.18b           | 0.22c           | 0.00   | 0.001   |
| NFCL, kg d⁻¹  | 3.99a  | 4.01a  | 4.10a           | 3.91a           | 0.12   | 0.713   |
| TDNI, kg d⁻¹  | 5.36a  | 5.81a  | 5.96b           | 6.60c           | 0.16   | 0.001   |

Means followed by equal letters in the same row do not differ from each other by Tukey’s test at 5% of probability; SC = Sugarcane; U = 0.5% of urea and ammonium sulfate mixture (9:1) in natural matter; EG = Elephant grass; SE = Standard error of the mean; DMI = Dry matter ingestion; OMI = Organic matter ingestion; CPI = Crude protein ingestion; NDFI = Neutral detergent fiber ingestion; EEI = Ethereal extract ingestion, with EE values estimated using the CQBAL 3.0 platform; NFCL = Non-fibrous carbohydrate ingestion; TDNI = Total digestible nutrient ingestion.

The treatments that had sugarcane as exclusive roughage (SC and SC + U) showed the highest TDN% values compared to the treatments with elephant grass (75% SC + 25% EG and 50% SC + 50% EG). This behavior most likely results from the higher NFC (Table 1) and NFCD values in the diets containing sugarcane as exclusive roughage, since these components refer to TDN% (Table 3).

Table 3. Diet digestibility of low-producing dairy cows as a function of different forms of roughage supplementation.

| Item | SC     | SC + U | 75% SC + 25% EG | 50% SC + 50% EG | SE     | P value |
|------|--------|--------|-----------------|-----------------|--------|---------|
| DMD, % | 69.24b | 72.52a | 66.54bc         | 65.06c          | 0.79   | 0.001   |
| OMD, % | 70.71b | 73.86a | 68.19b          | 66.58b          | 0.75   | 0.001   |
| CPD, % | 52.08b | 60.73a | 51.75b          | 53.58ab         | 2.16   | 0.026   |
| NDFD, % | 41.54b | 47.77a | 42.00b          | 46.14ab         | 1.57   | 0.025   |
| EED, % | 71.65  | 71.65  | 72.84           | 74.03           | -      | -       |
| NFCD, % | 86.44b | 87.66a | 84.53b          | 80.00c          | 0.98   | 0.001   |
| TDN, % | 65.35a | 66.06a | 59.89b          | 57.57b          | 0.81   | 0.001   |

Means followed by equal letters in the same row do not differ from each other by Tukey’s test at 5% of probability; SC = Sugarcane; U = 0.5% of urea and ammonium sulfate mixture (9:1) in natural matter; EG = Elephant grass; SE = Standard error of the mean; DMD = Dry matter digestibility; OMD = Organic matter digestibility; CPD = Crude protein digestibility; NDFD = Neutral detergent fiber digestibility; EED = Ethereal extract digestibility; NFCD = Non-fibrous carbohydrate digestibility; TDN = Total digestible nutrients.

Despite the higher digestibility coefficient, the treatment with sugarcane and 0.5% of urea (SC + U) did not result in higher productivity (p > 0.05; Table 4), corroborating with Rayburn and Fox (1995), to whom DMI has more influence on productive variations than food digestibility.

There was no difference (p > 0.05) between the studied treatments as to milk production and milk production corrected to 3.5% of fat (Table 4). The treatment with 50% replacement of sugarcane for elephant grass (50% SC + 50% EG), despite increasing TDNI and its components (Table 2), did not result in higher milk production. However, there was numerical increase in production (Table 4), which may indicate that this treatment provided more substrate to meet the animals’ needs.

No differences were found (p > 0.05) in milk composition between the studied treatments (Table 4). These results were also observed by Aguilar et al. (2015), Souza et al. (2015) and Teixeira et al. (2015), in investigations with urea addition to sugarcane. Jesus et al. (2012) found higher fat content. According to these authors, urea promoted greater fiber degradation, higher acetate proportion in the rumen and, consequently, higher fat synthesis by the mammary gland. Valadares, Broderick, Valadares Filho, and Clayton (1999) reported lower milk fat levels in diets based on sugarcane, with proportion of concentrated food addition higher than 65%. In the present research, milk fat percentage stood around 3.5%, which is above the minimum fat content required by the Brazilian law (NI 76). However, with the addition of elephant grass, the percentage of fat in the milk was expected to rise because of the larger amount of fibers in its composition compared to sugarcane, and due to the higher roughage:concentrate ratio consumed by the animals subjected to these treatments.

According to Santos et al. (2012), for milk protein content to increase, there should be a higher amino acid input for absorption in the small intestine. These amino acids may have dietetic origin (rumen undegradable protein - RUP) or microbial origin (micP), with the second one being the most important, for having an aminoacidic profile similar to the aminoacidic profile necessary for the synthesis of milk protein.
Thus, to increase milk protein, a greater stimulation to microbial growth is necessary, especially with the synchronism between NFC and rumen degradable protein (RDP) availability and higher dietetic supply of high-quality RUP. According to the National Research Council (NRC, 2001), milk protein percentage has low correlation with the amount of CP supplied in the diet ($r = 0.14$), confirming the hypothesis that milk protein content has more to do with qualitative aspects than quantitative ones as to diet CP supply. In the present research, the protein contents of all treatments surpass the minimum amount required by the Brazilian law (NI 76, Table 4).

Among the main milk components, lactose is the one with the smallest variation and, usually, constant percentage (Santos et al., 2012). The lactose contents presented by all treatments stood below the minimum value (4.5%) established by Normative Instruction number 76, of 2018).

The animals subjected to diet with sugarcane (SC); sugarcane corrected with 0.5% of urea (SC + U) and 75% of sugarcane and 25% of elephant grass (75% SC + 25% EG) lost body weight ($-2.04; -0.29; -0.06$ kg day$^{-1}$; respectively) during the experimental period, while the treatment with 50% of sugarcane and 50% of elephant grass (50% SC + 50% EG) promoted a weight gain of 0.5 kg day$^{-1}$ (Table 4). According to the NRC (2001), the daily DMI requirement for Dutch-bred animals, with the weight and milk production of the animals used in this study, is approximately 12 kg. Among the assessed treatments, none met this DMI requirement; the treatment with 50% substitution of sugarcane for elephant grass (50% SC + 50% EG) in roughage was the closest to this value (11.49 kg day$^{-1}$; Table 2). Moreover, TDNI and CPI requirements are, according to the NRC (2001), approximately 5.77 and 1.51 kg day$^{-1}$, respectively. In this experiment, none of the treatments met the CPI requirement. However, the treatment with 50% substitution of sugarcane for elephant grass (50% SC + 50% EG) was the closest to this value. The TDNI requirement was met by all treatments, except for the treatment with sugarcane (SC) as only source of roughage. These results explain the expressive weight loss ($-2.04$ kg day$^{-1}$) of the animals fed with sugarcane (SC) - as a result of a great body reserve mobilization to meet lactation maintenance needs - and the weight gain (0.50 kg day$^{-1}$) of the animals receiving treatment with 50% of sugarcane and 50% of elephant grass (50% SC + 50% EG), especially due to the excessive energy present in said treatment.

### Table 4. Milk production and composition, and body weight variation of low-producing dairy cows as a function of different forms of roughage supplementation.

| Treatments                      | SC   | SC + U | 75% SC + 25% EG | 50% SC + 50% EG | SE  | P value |
|---------------------------------|------|--------|-----------------|-----------------|-----|---------|
| Milk, kg d$^{-1}$               | 6.86 | 6.97   | 7.15            | 7.57            | 0.28| 0.309   |
| MPC, kg d$^{-1}$                | 6.88 | 6.96   | 6.89            | 7.47            | 0.30| 0.470   |
| Fat, %                         | 3.55 | 3.48   | 3.28            | 3.50            | 0.27| 0.907   |
| Protein, %                     | 5.15 | 5.05   | 5.17            | 5.10            | 0.10| 0.809   |
| Lactose, %                     | 4.10 | 3.96   | 4.28            | 4.22            | 0.14| 0.389   |
| Total solid, %                 | 11.9 | 11.6   | 11.9            | 11.9            | 0.27| 0.811   |
| BWV, kg d$^{-1}$               | -2.04|-0.29   |-0.06            | -0.06           | -   | -       |

SC = Sugarcane; U = 0.5% of urea and ammonium sulfate mixture (9:1) in natural matter; EG = Elephant grass; SE = Standard error of the mean; BW = Body weight; MPC = Milk production corrected to 3.5% of fat; BWV = Body weight variation.

### Conclusion

Replacing sugarcane for elephant grass, at the proportions of 3:1 (25% of elephant grass) and 1:1 (50% of elephant grass), increases consumption and decreases DM and OM digestibility, without harms to milk production, when the cows are supplemented with 5 kg of concentrate containing 24% of protein per day. Therefore, this substitution can be used for correcting the nutritional deficiencies of sugarcane in the feeding of lactating crossbred cows by producers wanting to avoid using urea.

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