CONCENTRATION OF UREA NITROGEN IN BUFFALO MILK DURING DIFFERENT SEASONS OF THE YEAR IN NORTHEASTERN BRAZIL¹

ADRIANO HENRIQUE DO NASCIMENTO RANGEL^{2*}, ADRIANA DIOCLECIANO SOARES², TÁBATTA CRISTINE CHAVES DE LIMA², THALITA POLYANA MONTEIRO ARAÚJO², DORGIVAL MORAIS DE LIMA JÚNIOR¹

ABSTRACT: This study aimed at assessing the influence of different seasons of the year (dry and rainy) on urea nitrogen (MUN) concentration of buffalo milk in a commercial herd from Rio Grande do Norte state. The region is characterized by a rainy tropical climate with a dry and wet season from August to January and February to July, respectively. Samples were collected monthly from the cooling tank between February 2010 and February 2011, in the morning. Next, they were homogenized and packed in 40-mL plastic flasks, containing the preservative Bronopol[®] and later sent to the laboratory for urea nitrogen analysis. Although March 2010 exhibited greater ureanitrogen concentration ($25.4 \pm 4.4 \text{ mg/dL}$) and November the lowest mean ($12 \pm 0.4 \text{ mg/dL}$), there was no significant variation in milk urea nitrogen in samples collected from cooling tanks during the rainy and dry seasons.

Keywords: Buffalo. Urea Nitrogen. Rainy and Dry.

CONCENTRAÇÃO DO NITROGÊNIO URÉICO NO LEITE DE BÚFALAS NAS DIFERENTES ÉPOCAS DO ANO NO NORDESTE BRASILEIRO

RESUMO: Este estudo teve como objetivo avaliar a influência de diferentes épocas do ano (seca e chuvosa) em nitrogênio da uréia (MUN) concentração de leite de búfala em um rebanho comercial do Rio Grande do Norte. A região é caracterizada por um clima tropical chuvoso com uma estação seca e chuvosa de agosto a janeiro e fevereiro a julho, respectivamente. As amostras foram coletadas mensalmente a partir do tanque de resfriamento entre fevereiro de 2010 e fevereiro de 2011, na parte da manhã. Em seguida, foram homogeneizadas e embalada em frascos de 40 mL de plástico, que contém o conservante Bronopol ® e depois enviado para o laboratório para análise de azoto da ureia. Embora março 2010 exibiu maior concentração de nitrogênio da uréia ($25,4 \pm 4.4 \text{ mg/dL}$) e novembro a menor média ($12 \pm 0.4 \text{ mg/dL}$), não houve variação significativa em nitrogênio uréico no leite em amostras coletadas em tanques de resfriamento durante a estação chuvosa ea seca estações.

Palavras-chave: Buffalo. Uréia. Chuvosa e seca.

^{*} Autor para correspondência

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² Universidade Federal do Rio Grande do Norte, Unidade Acadêmica Especializada em Ciências Agrárias – EAJ, CEP: 59000-000, Macaíba-RN; e-mail: adrianohrangel@yahoo.com.br.

³ Universidade Federal de Alagoas, Campus Arapiraca, Arapiraca-AL; e-mail: juniorzootec@yahoo.com.br

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INTRODUÇÃO

Buffalo milk production is an important activity in several countries, such as India, Pakistan, Bulgaria, among others. Its introduction in Brazil, albeit recent, has awakened growing interest in breeders and researchers, since it offers a new alternative for milk production.

One problem in buffalo breeding is the absence of nutritional requirement tables, mainly for lactating females. This condition makes the formulation of diets for these animals quasi-empirical, and may cause losses due to protein-energy imbalance, as well as reproductive difficulties (SARWAR et al., 2009; ROY et al., 2011).

A number of tools have been proposed to assess the nutritional status of ruminants (CORASSIM et al., 2004), milk urea nitrogen being one of the most promising. Milk urea nitrogen (MUN) is positively correlated ($r^2 = 0.96$) with plasma urea nitrogen (BAKER et al., 1995), which is affected by a series of processes, primarily nutritional.

Microbial protein synthesis is dependent on the balance between carbon and nitrogen sources in the rumen. Normally, when few carbon skeletons are released or when there is excess nitrogen, the latter is absorbed in the form of ammonia and converted into urea in the liver and kidneys. Urea circulating in plasma is therefore an excellent indicator of nitrogen balance in the animal (HALL; HEREJK, 2001; JEN-KINS; MCGUIRE, 2005; BUCHOLTZ; JOHSON, 2007).

Urea nitrogen concentration in milk is a tool to assess both individual members of herds and the balance between nitrogen and carbon consumption. However, it is difficult to accurately estimate the nutritional status of a single cow, based on one determination of nitrogen. Nevertheless, when mean milk urea concentrations are determined in groups of animals or in herds, direct inferences regarding adequate diet seem possible (OLTNER et al., 1985; CARLSSON; PEHRSON, 1994).

However, to correctly interpret MUN concentrations, it is important to consider other factors that influenced the different results presented in the literature. In addition to diet-related factors, milk production, age of the cow, lactation stage, live weight and concentrations of milk fat and protein can also alter MUN concentration (ROY et al., 2003; ROY et al., 2005; MEYER et al., 2006a).

In addition to these factors, seasonal variations in MUN were also recorded in cows by Carlsson and Pehrson (1993) and Carlsson et al., (1995). The authors found that MUN concentration in grazing animals was higher than when they were confined. According to Moller et al., (1993), greater variation in milk urea nitrogen among grazing cows is due to seasonal variations in pasture protein and amount of fibrous carbohydrates. However, Godden et al., (2001) suggest that MUN fluctuations, mainly in cooling tanks, may be mistaken with lactation stage and nutritional effects.

Given the absence of reference values in the literature for the MUN of buffalo and the need to understand different factors influencing the content of this component, the present study aimed to conduct an exploratory survey of urea nitrogen concentration in buffalo milk stored inside cooling tanks during different seasons of the year.

MATERIAL E MÉTODOS

The study was carried out on private land in the municipality of Taipú, in the Agreste region of Rio Grande do Norte state, about 50 km from Natal. The area is characterized by a rainy tropical climate, with two well-defined seasons: the dry period from August to January and the rainy season from February to July. Mean yearly rainfall in the wet season is 855 mm. According to the Brazilian Agricultural Research Corporation (EMPARN), mean rainfall recorded during the study period up to August was 1221.8 mm. Mean temperature and relative humidity are 25.3 °C and 79.0%, respectively (IDEMA, 2011).

Nutritional management of animals was characterized by rotating pastures of *Brachiaria brizantha* and *Panicum maximum* cv. Massai. During milking, buffaloes were supplemented, according to production, with corn, soybean meal and cottonseed concentrate (Table 1). In the dry season, in addition to supplying concentrate during milking time, buffaloes received sugar cane corrected with urea and ammonium sulfate (9:1), based on natural matter.

Samples were collected monthly from the cooling tank between January 2010 and March 2011. Milk samples were homogenized by activating the tank agitator for five minutes. These were then packed in 40-mL plastic flasks containing the preservative Bronopol[®], and sent to the Milk Laboratory of the Luiz de Queiroz School of Agriculture (ESALQ/USP), Piracicaba (SP). Milk urea nitrogen (MUN) concentrations were determined by the enzymatic spectrophotometric method using the Chem-Spec 150[®] analyzer (BENTLEY INSTRUMENTS, 1998). Statistical analyses were conducted using the Statistical Analyses System (SAS, 2002) and means were compared by Tukey's test, at a significance level of 5%.

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| Ingredients | Dry Season | Rainy Season |
|-------------------------------------|--------------------|--------------|
| Soybean meal | 42.07 | 26.67 |
| Com grain | 30.81 | 32.78 |
| Cottonseed | 15.09 | 29.32 |
| Cottonseed oil | 3.80 | 3.00 |
| Sugar cane yeast Mineral mixture | 1.00 3.08 | 1.00 3.08 |
| Urea | 1.00 | 1.00 |
| Sodium bicarbonate | 1.00 | 1.00 |
| Bicalcium phosphate | 0.40 | 0.35 |
| Lime | 1.75 | 1.80 |
| Total | 100.00 | 100.00 |
| Ch | emical composition | |
| Crude protein (%) | 28.00 | 23.60 |
| Either extract | 8.44 | 10.00 |
| Acid detergent fiber (%) | 3.53 | 1.18 |
| Total digestive nutrients (%) | 78.00 | 78.00 |
| Calcium (%) | 1.62 | 1.62 |
| Phosphorous (%) | 0.70 | 0.70 |
| Selenium (mg) | 0.84 | 0.84 |
| Vitamin A (U.I.) | 6,600.00 | 6,600.00 |

Tabela 1. Percentage and chemical composition of concentrates.

RESULTS AND DISCUSSION

Monthly distribution of data collected during the study period is illustrated in Figure 1. Values ranged from 12 to 25.4 mg/dL, with a mean of 17.8 ± 4.4 mg/dL, in line with those obtained in the state of São

Paulo by Fernandes (2004), who found a variation between 5.6 and 27.3 mg/dL, with a mean of 15.9 mg/dL. By contrast, in Italy Campanile et al., (1998) provided a diet with 9% to 12% of crude protein, obtaining MUN values from 29.4 to 37.3 mg/dL, respectively



Figura 1. Monthly means of milk urea nitrogen (MUN) analyse

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March 2010, in the rainy season, had the highest urea nitrogen concentration with 25.4 ± 4.4 mg/dL, whereas November, in the dry season, had the lowest mean, with 12 ± 0.4 mg/dL. Typically, as the dry season advances, and owing to the action of animals, there are significant alterations in forage structure, particularly in the most digestible portion, which decreases drastically. As a result of the reduced availability of green leaves and increased culm content, crude protein levels of forage decreases (LIMA et al., 2004; VOLTOLINI et al., 2010 and PAULA et al., 2011). Moreover, some authors suggest that environmental factors can modify buffalo milk composition even more than cow's milk

(CAMPANILE et al., 1998; RANGEL et al., 2010)

Plotting rainfall (mm) and MUN (mg/dL), graphs shows a peak in MUN (19.9 mg/dL) immediately after May (112 mm), likely caused by improved pastures with a larger amount of young leaves (GARCIA et al., 2010; PAULA et al., 2011). In 2/3 of the dry season MUN was probably maintained by sugar cane supplementation with urea and concentrate, given that the pasture (non-irrigated) could not likely sustain microbial growth (CRUZ et al., 2006; VOLTOLINI et al., 2008; VASCONCE-LOS et al., 2010). In December, an association between improved pasture and supplementation may explain the MUN peak of 19.7 mg/dL (FERNANDES, 2004).



Figura 2. Relationship between rainfall and milk urea nitrogen (MUN).

Table 2 shows MUN values in different seasons of the year. No reference values for milk urea nitrogen of buffaloes were found (OLIVEIRA et al., 2009). These authors reported mean values of 18.96 mg/dL of urea nitrogen in the blood serum of buffaloes. Javaid et al., (2008) studied different levels of degradable protein in buffalo rumen, observing means between 19.42 and 35.43 mg/dL after 3 h of feeding.

With respect to MUN, Roy et al., (2003) investigated buffaloes at different orders of delivery and lactation stages, obtaining values between 55.67 and 64.03 mg/dL. In a study assessing different supplementation and milking periods, Roy et al. (2005) observed values ranging from 40.10 to 47.53 mg/dL. Both studies show high values when compared to those obtained in the present investigation.

According to Grant et al., (2007), mean MUN in a herd of dairy cows should be between 12 and 16 mg/dL. Mean values above 16 mg/dL would indicate deficiency in non-fibrous carbohydrate fermentation, excessive protein in diet and/or imbalance between energy and nitrogen availability in the rumen (GRANT, 2005).

Lucci et al., (2006) state that amounts of protein equivalents above those recommended by nutritional requirement tables in lactating cow diets increases milk production and concentrations of plasma and milk urea nitrogen. Since MUN means of 19.5 mg/dL suggest inadequate buffalo diet, studies are needed to establish reference values for these animals, given reports of differences in nitrogen recycling between this species and other ruminants (OBITSU; TANIGUCHI, 2009).

Research also indicates that changes in MUN, with regard to protein content in pasture, may be related to the seasons. Due to the constant offer of concentrate to lactating buffaloes, in addition to the sugar cane mixture corrected with urea in the dry season, no difference in MUN concentration between seasons could be observed. Arunvipas et al., (2002) reported high MUN concentrations in cows at the end of winter/beginning of spring (March-April) in Canada, with the highest values occurring in July and August (13.10 mg/dL). Working with confined

Table 2. Analysis of urea nitrogen in the dry and rainy seasons.

| Season | Buffalo milk urea nitrogen concentration (md/dL) | |
|-----------------------|--|--|
| Rainy (February-July) | 19.5 ± 4.8^{a} | |
| Dry (August-January) | 15.9 ± 2.6^{a} | |

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cows, Godden et al., (2001) observed that milk urea concentration was higher in Canada in summer (July to September). Yoon et al., (2004) studied the influence of seasons on the MUN of cows in South Korea and found higher values in spring and summer. In a recent study, Botaro et al., (2011) assessed bulk tank milk samples from commercial cattle herds in the state of São Paulo, observing that the season influenced non-protein nitrogen concentration of cow's milk, in contrast to the present study.

CONCLUSION

There was no variation in buffalo milk urea nitrogen concentration collected from cooling tanks in the rainy and dry seasons;

There is a need to establish milk urea nitrogen reference values for buffaloes.

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