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Original

Variation of milk ureic nitrogen according to season and days in milk

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ABSTRACT

Objective. Determine the variation of MUN according to the seasons of the year (rainy and dry season) and the days in milk (DIM) of dairy cows from two herds in the Boyacá highlands of Colombia. **Materials and methods.** A descriptive quantitative study was carried out for convenience. 4,901 milk samples were included from 112 animals located at a height of 2,600 meters above sea level during a period of 24 months, grouping the samples according to the season and the days in milk production. The samples were processed through a spectrophotometric enzymatic methodology for the determination of MUN. An analysis of variance was carried out for the groups by climatic season, while for the days in milk a quantitative description was made. **Results.** The MUN varied according to the season of the year ($p<0.05$), the values being higher during the rainy season. The MUN was lower during the first 60 DIM in most of the conformed groups and the peaks appeared from 180 DIM. The lowest MUN values according to the days in milk were determined in the first 60 days in most of the conformed groups, likewise, the main peaks were determined from 180 DIM. **Conclusions**. The MUN varied according to the season, being higher in rains, the MUN was lower during the first 60 DIM in most of the groups formed and the peaks appeared from 180 DIM.

Keywords: Dairy cows; protein; nutritional balance; urea; energy (*Sources: DeCS, CAB*).

RESUMEN

Objetivo. Determinar la variación de NUL según las temporadas del año (época de lluvia y seca) y los días en leche (DEL) de vacas lecheras de dos ganaderías en el altiplano boyacense de Colombia. **Materiales y métodos.** Se realizó un estudio de tipo descriptivo cuantitativo por conveniencia. Se incluyeron 4.901 muestras de leche de 112 animales ubicados a una altura de 2600 msnm durante un período de 24 meses, agrupando las muestras de acuerdo con la temporada y los días en producción de leche. Las muestras fueron procesadas a través de metodología enzimática espectofotométrica para la determinación del NUL. Se realizó un análisis de varianza para los grupos por temporada climática, mientras que para los días en leche se hizo una descripción cuantitativa. **Resultados.**

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El NUL varió según la temporada del año ($p<0.05$), siendo mayores los valores durante la época lluviosa. El NUL fue menor durante los primeros 60 DEL en la mayoría de los grupos conformados y los picos se presentaron a partir de los 180 DEL. Los menores valores de NUL de acuerdo a los días en leche se determinaron en los primeros 60 días en la mayoría de los grupos conformados, así mismo los principales picos se determinaron a partir de los 180 DEL. **Conclusiones.** El NUL varió de acuerdo con la temporada siendo más alto en lluvias, el NUL fue menor durante los primeros 60 DEL en la mayoría de los grupos conformados y los picos se presentaron a partir de los 180 DEL.

Palabras clave: Vacas lecheras; proteína; balance nutricional; urea; energía (*Fuentes: DeCS, CAB*).

INTRODUCTION

Urea is the end product of nitrogen conversion and has two origins, the first being unused ammonium, which is formed in the rumen and converted to urea in the liver, and the second being the catabolism of amino acids in the body. When urea is found in the blood, it is called blood urea nitrogen (BUN) (1,2,3).

Due to their small size, urea molecules can cross cell membranes (including those of the mammary gland) and reach the milk, where it takes the name of milk urea nitrogen (MUN), which constitutes 2.5 to 3% of total milk nitrogen. MUN is therefore mainly derived from and strongly related to BUN (4,5,6).

In cattle, MUN is related to crude protein intake, the degradability of crude protein in the ration and the energy balance of the cow. As the level of production increases, the protein-energy ratio of the feed equals, resulting in an increase in ammonium that cannot be used by ruminal bacteria, which leads to an increase in urea production, followed by an increase in BUN and MUN concentrations. Dietary supplementation with urea-type nitrogen affects the ruminal microbiological synthesis and hepatic urea synthesis in dairy cattle (2,3).

The MUN may vary due to external conditions such as sampling season, type of sampling, sample storage, sample processing, lactation status of the female, age at calving, number of calvings and production level (7,8,9). In addition, they vary between herds, groups of cows and cows in the same group (10).

MUN is an important and effective indicator to establish urea nitrogen status in dairy cows, as this tool allows easy identification of protein underfeeding or overfeeding (11,12). Its monitoring is important because MUN is related to reproductive disorders, increased somatic cell count and increased nitrogen excretion with a negative environmental impact (10,13,14,15). In Colombia, few studies are related to the analysis of MUN in dairy herds (16,17), and the variation of MUN concerning days in milk (DIM) in the tropical climate conditions of the country. It is evident, in the scientific literature, the absence of studies on the variations of MUN associated with the climatic seasons and the days in milk production of cows in national herds. The municipality of Duitama is characterized by its milk production, being part of the so-called "boyacense dairy cordon".

Therefore, the objective of this study was to determine the variation of MUN according to the season (rainy and dry seasons), and the days in milk of dairy cows from two dairy farms in the municipality of Duitama in the highlands of Boyacá, Colombia.

MATERIALS AND METHODS

Type and site of study. A convenience, descriptive, quantitative and descriptive sampling was carried out. The study was carried out in two farms in the municipality of Duitama, Boyacá, which has an average temperature of 14ºC in the rainy season (RS) and 18ºC in the dry season (DS) (18). The farms included in the study are two kilometers apart and have similar environmental conditions of temperature, rainfall, pastures and receive water from the same river.

Animals included in the study. A total of 112 dairy cows of the Holstein breed, between two and three calvings, and between 5 and 305 DIM were selected. From farm one, 50 animals were selected, with an average weight of 460.6±55.7 kg and an average production of 17.5 kg milk/day/cow. On the other hand, 62 animals were selected from farm two, with an average weight of 452.5±42.3 kg and an average production of 18.3 kg milk/day/cow.

Only cows with optimal health conditions were included in the study, i.e. without lameness, respiratory, reproductive and udder diseases. Mammary gland counts below 200,000 cells/mL were considered healthy. Somatic cell count analysis was performed at each sampling. The presence of somatic cell counts higher than stipulated constituted the discarding of the sample for that analysis.

Feeding. The animals were grazed on pastures with Kikuyo (Cenchrus clandestinum). They received during each milking (a.m. and p.m.) 2 kg of commercial balanced feed, with moisture values of 13%, crude protein of 14%, ash of 10%, fat of 2.5% and crude fiber of 25%.

Milking. On both farms, milking is mechanical and was carried out twice a day, the first at 3:00 a.m. and the second at 14:00 p.m.

Sampling. Samples were collected before the start of milking and after teat cleaning, which was performed with 10% iodine solution (20), collected from all four teats and mixed. One sample was considered to be the mixture of one teat dip from each teat at each milking, i.e. the morning sample was stored in a refrigerator at a temperature of 6°C to be mixed with the afternoon sample and then transported at a temperature of 4°C until processing (7,19). Two samples per month were collected throughout the study period, for a total of 5,376 samples in total, however, 475 samples were excluded due to increases in somatic cell counts above 200,000 cells/mL, therefore, the total number of samples included in the study was 4,901.

Processing. Samples were analyzed on the same day of collection at the Laboratory of Milk Quality Analysis and Mastitis Control at the Universidad Pedagógica y Tecnológica de Colombia. MUN analysis was performed using the enzymatic spectrophotometric methodology using a Mindray BS120 kit using the UREA kit from LAB TEST at a wavelength of 450nm (21). In addition, somatic cell counting was performed with a Fossomatic™ 7 based flow cytometry.

Farm data collection. The farms included in the study organized data on days in lactation and the number of calvings in an Excel® for Microsoft 2010 database. Data from the years 2018 and 2019 were taken and included in the statistical analysis performed.

Category of analysis. The quarters were selected according to data from the Institute of Hydrology, Meteorology and Environmental Studies for the area(18), where the climatological seasons are separated into two, with two periods for each: DS, which comprises the first period from January to March (which will be referred to hereafter as DS1 and according to the year, 18 or 19, and a second period from October to December (DS2); and RS (which will be referred to hereafter as RS1 and according to the year 18 or 19), which comprises the first period from April to June, and the second from July to September (RS2), the difference between these two periods for each season and between seasons is the intensity of the rainfall.

Data for DIM were pooled from the first 5 days in milk until day 30, and thereafter, data were pooled every 30 days until day 330.

Data analysis. For the analysis of variation across seasons, an average of MUN of both farms was performed, for each quarter, without discrimination of the DIM and analysis of variance was performed for each season by year, including as a blocking factor the farms, using the Fisher's least significant difference comparison of means procedure, with a confidence level of 95%, using Statgraphics Centurion® software, version for Windows 10. On the other hand, a descriptive analysis of the average MUN for the DIM groups (every 30 days) was carried out for each of the quarters.

RESULTS

Statistically significant differences were found between the quarters included for the two years assessed. The lowest MUN values were identified in the periods DS1-18, DS2-18 and DS2-19. On the other hand, the highest values were found in the RS1-18, RS2-18, RS2-19 season.

The average MUN results for each quarter over the two years are shown in Figure 1.

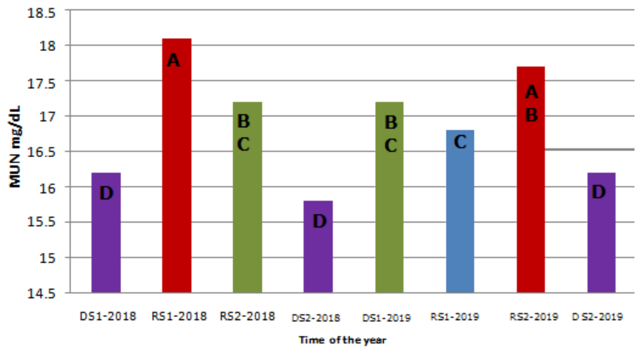

Figure 1. Average MUN according to the season of the year (rainy and dry seasons) of dairy cows in two dairy farms in the highlands of Boyacá, Colombia. Columns with different letters indicate statistically significant differences (p<0.05).

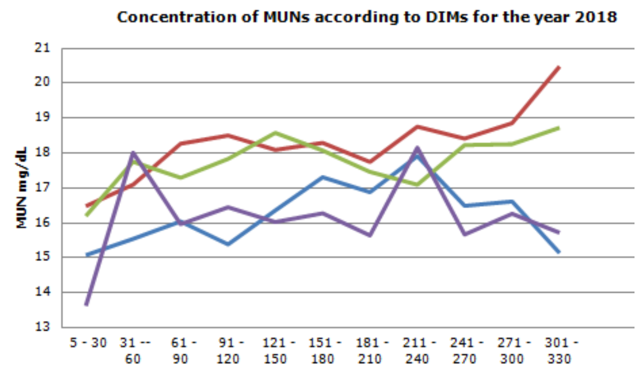

Figure 2. Average MUN according to DIM on dairy cows on two farms in the Boyacá highlands of Colombia in 2018. Blue line: DS1-2018. Red line: RS1-2018. Green line: RS2-2018. Purple line: DS2-2018.

There was a statistically significant effect of the time of year on the concentration of MUN (p<0.05) in the first year (2018) the highest results were obtained in the rainy season, with statistical differences between groups, when this is compared with 2019, it does not show the same behavior, finding that the first quarter of the dry season was statistically the same compared to the second rainy period in 2018 (p>0.05), and the first rainy period of 2019 has a lower value compared to the first quarter of the same year and the same period in 2018 (p>0.05). However, most quarters of the dry season had the same average percentage for MUN (p>0.05).

Variation of MUN according to DIM. Figure 2 shows the year 2018, for the first quarter (DS1-18) the average MUN has three increases, the first between 61 and 90 DIM same as RS1; the second between 151 and 180 DIM, and the third between 211 and 240 DIM, the interesting thing is the exception of the period RS2, the other quarters had the almost the same behavior for the last increase (211 and 240 DIM).

The DS2 and RS2 quarters had their first increase between 31 and 60 DIM, but RS2 increases again between 121 and 150 days, and the last major increase, together with RS1, was between 301 and 330 days, while the DS1 and DS2 periods decreased in the same period.

Compared to the 2018 data, the average MUN according to DIM was different (Figure 3). The DS1 epoch only has two increases, the first one between 91-120 DIM together with DS2, while the RS1 and RS2 periods decreased the same period. S1l has the second increase between 241 and 270 DIM, while the rest of the periods decreased. On the other hand periods RS1 and RS2 had their first increase between 61 and 90 DIM, with a second increase between 151 and 210 DIM, with a decrease between 241 and 270 DIM, and there RS2 increased while RS1 decreased.

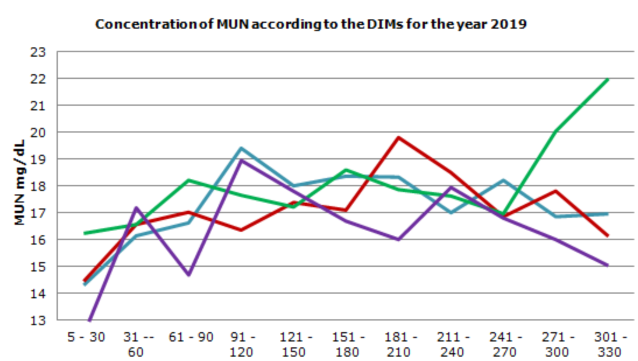

Figure 3. Average MUN according to DIM on dairy cows on two farms in the Boyacá highlands of Colombia in 2019. Blue line: DS1- 2019. Red line: RS1-2019. Green line: RS2-2019. Purple line: DS2-2019.

DISCUSSION

Normal MUN values vary according to the literature consulted, for example, studies in Brazil mention ideal values of MUN in dairy cows between 10 and 14 mg/dL, being so, the values determined in this study are above this range, indicating possible deficient nutritional management in the two herds evaluated. And even the values presented here are higher than those reported by the same researchers in the same breed (10). However, it was not possible to relate the results presented due to a lack of information related to paddock management, the composition and variation of the diet fed to the animals, and the general productive behavior of the animals within the farm.

On the other hand, for the Colombian case, researchers present a MUN classification where values above 18 mg/dL can generate reproductive alterations, values between 15 and 18 mg/dL there is an underutilization of nitrogen, and that the optimal values that every herd should manage are between 12 and 15 mg/dL (22). The results show that none of the values reported for any of the trimesters is within the optimal values for production and reproduction. And that for the DIM the only ones that show the majority to be in the optimal group are in the first 30 DIM. There are even reports of normal MUN values in certain Holstein herds above 40 mg/dL (23).

For the conditions in the department of Antioquia (Colombia), a constant increase in MUN concentration was found up to 115 DIM, reaching a value of 17.75 mg/dL (17). These results are contrary to most of the behavior's found throughout the two years of study, because the only two periods where the growth in MUN concentration was constant was in the RS1-2018 and DS1-2019 periods, the other concentrations revealed increases and decrease with no visible pattern during the first 120 DIM, the maximum value determined in this study was in the DS1-2019 period with a result of 19.42 mg/dL.

Subsequently, the researchers evidenced a constant decrease up to 190 DIM until reaching a concentration of (17.40mg/dL); the only period in which this behavior was evidenced in DS2-2019, the other periods presented inconsistent behaviors of increases and decreases that are contrary to what was reported by Henao et al (17). Finally, the researchers mentioned a constant increase in concentration up to day 300 until reaching a concentration of (18.50 mg/dL), this same behavior was evident in the RS1-2018, RS2-2018 and RS2-2019 epochs.

Some research mentions that the MUN content on Dutch farms varies according to the type of housing in which the animals are kept, with those on smaller and less organized farms showing a much higher value (5,24), and also mentions that in these regions in the summer months the urea content is significantly higher (24). Due to the climatic conditions of this country, it is important to highlight that the feeding regime during this period changes, offering the animals more digestible feed and better concentrations of nutrients, which affects the behavior of the MUN in these herds, due to the tropical condition of Colombia, there is not a summer season, but two dry periods, in which, due to the low technification of the national farms, highly usable feed is not offered, as it is offered in Europe.

In a study carried out in Brazil during the rainy season (January to May), they subjected several lactating females of the "Mantiqueira" breed to different amounts of crude protein in the diet (450, 600 and 750 grams CP), and analyzing up to 60 DIM they found MUN values of 14.15 mg/dL, 16.50 mg/dL and 20.71 mg/dL respectively (25). These values are similar to those found in the same trimesters of this investigation. Similarly, the results are similar to those determined for the groups under 60 DIM in this study, showing similar behavior despite the geographical distance and climatic conditions between the two countries.

Sources of MUN variation in dairy cows of different breeds have also been determined, finding that regardless of breed, number of calvings and amount of milk produced, MUN values will increase in rainy periods, while the lowest values will occur in the summer season (10). These changes are explained by the structural alterations that the pastures undergo, particularly in the digestible portion, which decreases drastically.

It has been established that MUN has a decrease in the first 60 days of lactation and a significant peak mainly near the middle of lactation due to factors of quantity of feed intake because in the puerperium the intake decreases drastically in the animals, and as lactation progresses this intake normalizes and, therefore, MUN values begin to increase (10). Behaviors similar to those reported were determined in the present investigation, where the majority of the lowest values occurred in the first 60 days of lactation and peaks of increase are shown from day 181 and not from day 151 as in other studies (7,10).

A contradictory result was found for MUN values worldwide, where researchers determined that for Holstein cattle the highest MUN values occurred in the summer (28 mg/dL) compared to the winter months (24.95 mg/dL), attributing these results to the ingredients with which the diets received by the animals were prepared concerning the harvest season (11).

On the other hand, in Brazil, a similar study was carried out in buffaloes according to the months of the year, determining for the rainy period (August to January) a higher average value (18.56 mg/dL) than in the dry period (February to July) where an average value of 15.91 mg/dL was determined (26); these values show similar behavior to that reported in this study where the highest values for MUN were determined for the rainy period in general in both years, with values similar to those proposed in the aforementioned study.

In conclusion, the highest MUN values were determined, throughout the two years of study, in the rainy season concerning the dry season ($p<0.05$). The MUN was lower during the first 60 DIM in most of the groups formed and the peaks occurred from 180 DIM onwards.

Conflict of interest

All authors declare that there is no conflict of interest for the publication of this manuscript.

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