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COMUNICACIÓN

Evaluating nitrogen release rates of commercial slow-release urea products using Brix value analysis: a validation study comparing two methods

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ABSTRACT
The stability of three slow-release urea products (SRU) and a non-protected granulated urea (U) product was assessed based on their Brix value and total Nitrogen (N) Kjeldahl concentration in water. Each SRU and U products (10 g) was mixed with 50 ml of distilled water at 23.5 °C for various durations: 1, 3, 6, 12, and 24 h. After each period of time, Brix values and total N concentrations were measured in the aliquots. The N concentrations in all products exhibited time-dependent changes. A positive and linear correlation was observed between the total N in water and Brix values. The R^2 between both methods was 0.97. Thus, the Brix value serves as a convenient and effective method to evaluate the relative rate of N release for different SRU products.

Keywords: effectiveness, non-protein nitrogen, release characteristics, tool

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RESUMEN
La estabilidad de tres productos de urea de liberación lenta (ULL) y un producto de urea (U) granulada no protegida se evaluó en función de su valor Brix y la concentración total de nitrógeno (N) Kjeldahl en agua. Cada producto ULL y U (10 g) se mezcló con 50 ml de agua destilada a 23,5 °C durante varios períodos: 1, 3, 6, 12 y 24 h. Después de cada período de tiempo, se midieron los valores Brix y las concentraciones de N total en las alícuotas. Las concentraciones de N en todos los productos mostraron cambios dependientes del tiempo. Se observó una correlación positiva y lineal entre el N total en agua y los valores Brix. El R^2 entre ambos métodos fue de 0,97. Por lo tanto, el valor Brix surge

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como un método conveniente y eficaz para evaluar la tasa relativa de liberación de N de diferentes productos de ULL.

Palabras clave: efectividad, nitrógeno no proteico, características de liberación, herramienta

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INTRODUCTION

Urea, upon ingestion by an animal, undergoes rapid hydrolysis, resulting in the production of N-NH_3 (ammonia) and carbon dioxide. However, if the levels of N-NH_3 absorbed by the rumen exceed the capacity of the liver to convert it into urea, it can lead to the accumulation of N-NH_3 in the bloodstream, which can be toxic and potentially fatal for the animal (De Azevedo et al., 2008). To mitigate these risks, alternative sources of non-protein nitrogen have been investigated, including slow-release urea (SRU), which releases N-NH_3 into the rumen at a slower rate than regular urea (Oliveira et al., 2004).

In addition to reducing the risk of N-NH_3 intoxication, the use of SRU offers other benefits in ruminants. One notable advantage is its improved N utilization efficiency. The gradual release of N-NH_3 from SRU allows for a more synchronized availability of N, matching the animal's metabolic needs and optimizing its utilization in rumen microbial protein synthesis. This can result in enhanced feed efficiency, improved rumen health, and ultimately better animal performance (Broderick et al., 2007).

Furthermore, SRU can contribute to a more balanced rumen fermentation process. By providing a steady and controlled release of N, it helps maintain a favorable rumen pH and promotes the growth of beneficial microbial populations. This can lead to improved fiber digestion, increased production of volatile fatty acids, and better overall rumen function (De Azevedo et al., 2008; Kohn et al., 2002; National Research Council, 2001).

The use of SRU in ruminants not only helps mitigate the risk of N-NH_3 intoxication but also offers advantages such as improved N utilization efficiency and a more balanced rumen fermentation process. These benefits can contribute to enhanced animal performance and overall herd health.

Refractometry is a technique commonly used for the optical determination of liquid density. By utilizing refractometers, it becomes possible to determine the concentration of various mixtures, both simple and compound, in industrial processes such as food and beverage production (Chang & Yeh, 2011; Dongare et al., 2014). This method relies on measuring the refractive index of a substance, which can be expressed as a Brix value. The Brix value represents an optical characteristic of the substance and indicates the number of dissolved particles or the total soluble solids in a solution. Researchers have successfully used refractive index and Brix value to predict the compositional characteristics of single and multi-component mixtures, including solutions containing amino acids (Chang & Yeh, 2011).

Determining protein degradation kinetics *in vivo* can be both expensive and time-consuming. Therefore, it would be highly beneficial to have a simple, cost-effective, and static method for quantifying the relative differences in the release of N-NH_3 from SRU. Such a method would provide valuable information for farmers in the field, allowing them to assess the stability of urea before feeding it to cattle.

This study aimed to develop and validate a practical, economical, and class method based on Brix values for measuring the rate of urea release from different SRU products and raw urea (U) as control. The research included a comprehensive comparison between the Brix-based method and the total N Kjeldahl concentration method to ensure the accuracy and reliability of method.

MATERIALS AND METHODS

This study utilized three SRU products commercially available in Argentina, namely, Nitrosafe, Nitrum24®, and Optigen®, designated as A, B, and C, respectively. These products, coated with different technologies to ensure controlled N release, exhibited nitrogen percentages of 42, 42, and 41 for A, B, and C, respectively. Additionally, U, with 45 % nitrogen content, was included as a control. Each SRU product was handled identically throughout the experiment.

For each treatment, 10 ± 0.01 g of both SRU and U were mixed with 50 ml of distilled water. The mixtures were manually shaken for 15 seconds and then stored at 23.5 °C for various durations: 1, 3, 6, 12, and 24 h to simulate field conditions easily replicable by farmers. Four replicates were conducted for each h and product set.

After each period of time, the Brix value was measured in duplicate using a Milwaukee MA871 digital refractometer, calibrated before each measurement to ensure accurate readings. Furthermore, an aliquot of each SRU and U solution was immediately frozen to halt any possible reaction and preserve sample integrity for subsequent total N analysis using the Kjeldahl technique.

The release kinetics of SRU and U were determined based on the total N concentration and the Brix value of each sample.

Statistical analyses

The results were analyzed using ANOVA (analysis of variance) to assess the differences between means. Post-hoc comparisons were performed using the LSD (least significant differences) test. The correlation between methods was also examined. A significance level of $p \leq 0.05$ was used to determine statistical significance. For the statistical analyses, InfoStat software was utilized (Di Rienzo et al., 2018).

RESULTS AND DISCUSSION

A positive and significant linear relationship was observed between the total N values in the water (total N concentration) and the corresponding Brix values (R^2 = 0.97, $p \leq 0.05$, n = 35; Figure 1). As anticipated, at the first hour, the U treatment exhibited a significantly higher N release ($p \leq 0.05$; Figure 2) compared to the other treatments, while no significant differences ($p > 0.05$) were found between the Brix values of treatments B and C. Treatment A displayed intermediate values between these groups and U. At the third hour of incubation, treatments A and U demonstrated high Brix values and a rapid, significantly higher release ($p \leq 0.05$) of N, in contrast to treatments B and C, which showed analogous Brix values ($p > 0.05$) and a relative low N release. During the 6, 12, and 24 h treatments, U and A exhibited the highest Brix values ($p \leq 0.05$), with no significant differences between them ($p > 0.05$). When comparing both methods (Brix values and total N values), a significant and high correlation was observed ($p < 0.05$; R^2 = 0.97). These findings indicate that the release of urea and the rate of release of N of different products can be relatively and precisely estimated using Brix values obtained through a simple, low-cost

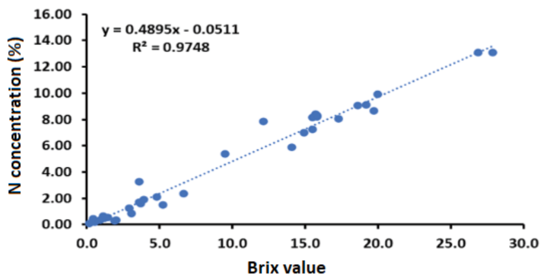

Figure 1. Correlation between the total N concentration (Kjeldahl technique) in water and Brix values.

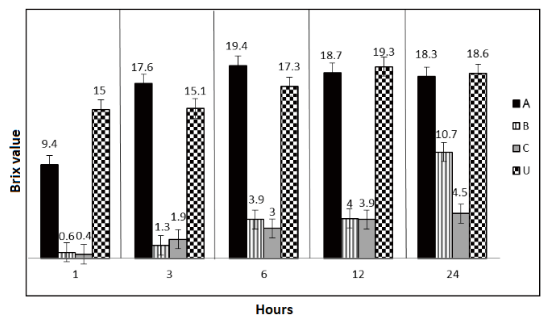

Figure 2. Brix values between the unprotected granulated urea (U) and slow-release urea product treatments (A, B and C) at the fixed times.

and static test employing distiller water as the solvent. In this regard, the Brix value can serve as a predictive measure for the rate of dissociation of SRU products.

CONCLUSION

This study examined the rate of nitrogen release from different commercially available SRU products and evaluated the effectiveness of Brix values as a measure of N release. The comparison between SRU treatments revealed clear differences in N release rates. Treatment U and A exhibited the highest initial N release (by 3 h of incubation), while treatments B and C demonstrated a more controlled and gradual release, suggesting that they are designed to release N at a slower rate. The study also established a positive and significant linear relationship between Brix values and total N concentrations in the water. This finding validates the use of Brix values as a simple and reliable method to estimate the stability and release characteristics of SRU products. By employing Brix values, farmers and researchers can easily assess the relative rate of N release from different SRU products in a cost-effective manner. This information is crucial for decision-making regarding the selection and utilization of SRU products in livestock feeding practices. In summary, the results of this study provide valuable insights into the differences in N release among SRU products and highlight the utility of Brix values as an effective tool for evaluating the release characteristics of urea products.

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