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Original paper

Performance of a colorimetric method for milk urea evaluation in dairy farms: the screening study

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Summary

The aim of this study, conducted in the Blida region in north-central Algeria was to evaluate a colorimetric method (CM) for measuring milk urea concentration and to assess its relevance as an indicator of dietary balance, thus addressing the lack of nutritional management tools for dairy cows in Algeria. The approach was first to validate the colorimetric method (CM) by testing 50 milk samples and comparing the results with those obtained by a reference enzymatic method (RM). Then, we analyzed 52 Holstein cow milk samples to correlate urea concentration with milk yield and protein content (PC). The results confirmed a high reliability of CM for on-farm monitoring, demonstrating an excellent correlation with the reference method (r = 0.8589; p < 0.001). The herd analysis revealed an optimal average urea concentration (236.92 mg/L). A positive and statistically significant correlation was found between urea and protein content (r = 0.42; p = 0.03), while no association was observed with milk yield (r = 0.14; p = 0.31). Cross-analysis of urea-protein profiles showed that 61.5% of the animals had a nutritionally balanced diet, while 38.5% exhibited imbalances. The predominant issue, affecting 21.15% of the cows, was an excess of fermentable nitrogen in the diet, revealed by elevated urea levels (> 300 mg/L) associated with normal protein content. This profile indicates an oversupply of degradable protein, which may contribute to metabolic stress. In conclusion, the colorimetric method is a reliable and relevant monitoring tool. However, the findings highlight the need to combine it with other indicators to obtain a comprehensive and accurate assessment of dietary balance.

Keywords: milk, cow, urea, protein, nutritional monitoring, colorimetric method

Bovine milk production is a cornerstone of food security and agricultural economies in many countries, including Algeria (30). Optimizing the performance of modern dairy farms depends on a delicate balance between maximizing production, preserving animal health and welfare, and controlling production costs. At the center of this equation, nutritional management plays a critical role, as it directly affects the metabolic health of cows, their reproductive capacity, and, consequently, the profitability of the farm (13, 17).

A precise balance between protein and energy intake in the diet is essential for effective rumen function and optimal milk production. Globally, milk urea nitrogen (MUN) concentration has, for several decades, emerged as a non-invasive and cost-effective tool

for nutritional monitoring (19, 31). This biochemical indicator, measurable in both individual and bulk tank milk samples, reflects the animal's efficiency in utilizing dietary nitrogen (20, 29). The measurement of urea in bulk tank milk is a rapid and accessible method to evaluate the nitrogen balance of dairy herds (10, 11, 21). Although this collective approach does not provide a precise diagnosis at the individual animal level, it is a form of a screening study capable of identifying nutritional imbalances that may influence milk production performance and udder health (24). Elevated MUN levels may indicate an excess of rumen-degradable protein relative to available energy, resulting in nitrogen waste, increased feed costs, negative environmental impacts, and potentially impaired reproductive performance (4, 8). Conversely, low

MUN levels may suggest a protein deficiency, thereby limiting milk production potential (27)

The Algerian dairy cattle sector faces a major structural challenge. The absence of a generalized and systematically implemented milk recording system (22) deprives farmers and technical advisors of objective indicators to assess the impact of feeding practices on herd health and productivity (5). This situation often leads to an empirical approach to ration management, which can be both economically suboptimal and detrimental to animal health. Therefore, the need for simple, reliable, and low-cost diagnostic tools adapted to the local context is of utmost importance.

It is within this context of limited reliable data that our study was initiated. It aims to contribute to the evaluation of milk urea as a relevant and accessible criterion for managing the dietary regimen of dairy cows under Algerian farming conditions. By introducing this parameter, we seek to provide stakeholders in the dairy sector with a tool to optimize the relationship between nutrition and production, with potential zootechnical and economic benefits. This study has a dual objective: first, to assess the performance of a colorimetric method (CM) for quantifying urea in raw milk known for its low cost and availability on the Algerian market (BioSystems, UREA/BUN-COLOR, Spain); second, to explore relationships between milk yield, protein content, and milk urea concentration.

Material and methods

Evaluation of the colorimetric method

Milk sampling. Fifty bulk tank milk samples were collected from farms registered with the milk collection center of a dairy facility in Blida. The samples were transported to the laboratory in a cooler at +4°C and analyzed within 24 hours of collection.

Sample preparation and milk analysis. A 1 mL aliquot of milk was placed in polypropylene tubes and mixed with 4 mL of trichloroacetic acid (TCA). The tubes were then centrifuged at 4,000 rpm for 3 minutes, and the urea concentration in the supernatant was measured by both the kinetic and colorimetric methods.

Kinetic method (reference method – RM). A commercial urea assay kit (BioSystems, UREA/BUN-UV, Spain) was used to quantify the milk urea concentration. Spectrophotometric readings were taken at 340 nm after 30 seconds (T1) and again after 60 seconds (T2), following the AFNOR standard (1992). Urea concentrations were expressed in g/L.

Colorimetric method (CM): Berthelot reaction. A commercial urea assay kit (BioSystems, UREA/BUN-COLOR, Spain) was used. The reaction produces a stable greencolored derivative that remains stable for up to 8 hours. Spectrophotometric readings were taken at 578 nm. Milk urea concentrations were expressed in mg/L.

Analysis of milk protein and milk urea content. The approach adopted was based on the milk recording method described by Rombach (29), which relies on three parameters: bulk tank and individual milk urea content, individual milk yield, and individual protein content.

Farm and animals

The study was conducted on a dairy farm representative of the Blida region, selected based on its regular collaboration with the milk collection center, the availability of reliable production records, and the homogeneity of the herd. The latter consisted of 62 Friesian Holstein dairy cows. To limit the effects of variability related to age, parity, and physiological stage, only cows in their second lactation and in early lactation (< 120 days in milk) were included. All animals underwent a prior clinical examination to exclude any cows presenting mastitis (negative CMT test), metabolic diseases (ketosis, hypocalcemia), or clinical signs of energy or mineral deficiencies. Only 52 clinically healthy cows were retained for the trial.

Feeding and ration

The diet of the dairy cows in the studied farm was structured according to typical feeding practices in central Algeria, especially in the Blida region. The diet was delivered as separate feed components through a fractionated feeding system twice daily, commonly coinciding with milking times. The ration comprised forages (40% of dry matter intake), consisting of 20% local alfalfa hay, 15% maize silage, and 5% barley straw; and concentrates (60% of dry matter intake), composed of 30% soybean meal, 15% maize grain, 15% barley grain, 5% minerals, vitamins, and feed additives.

The diet provided an estimated daily dry matter intake ranging from 15 to 18 kg per cow, which corresponds to approximately 13 to 15 lactation feed units (UFL) of net energy and supplies between 1200 and 1400 grams of digestible protein in the small intestine.

Milk sampling and analyses

A composite milk sample was collected from all four quarters of each cow during the evening milking. The samples were transported in a cooler at +4°C to the laboratory of the Institute of Veterinary Sciences (University of Blida 1). The milk yield of each cow was recorded on the day of sampling.

Protein content was determined with a LACTOSCAN device (Type MCC) by a method routinely used for standard analyses (ISO 9622:2013). Urea concentrations were measured by the colorimetric method, following the procedure described in the first experiment.

Ethical statement

All animal studies were conducted with the utmost regard for animal welfare, and all animal rights were appropriately observed. No animal suffered during the course of the work. All experiments were carried out according to the guidelines of the Institutional Animal Care Committee of the Algerian Higher Education and Scientific Research (Agreement Number 45/DGLPAG/DVA.SDA. 14).

Statistical analyses

Statistical analyses were performed with the SYSTAT software (version 10). The results were expressed as mean ± standard deviation. Two types of statistical analyses were used. For the bulk tank milk (from farms), correlation analysis and least-squares linear regression were employed to determine the relationship between urea concentrations measured by the two methods: reference method (RM) and colorimetric method (CM).

For individual cow milk samples, the results were categorized based on three reference urea thresholds, following the classification described by Rombach (24) and Guliński (10): optimal (comfort) urea (150-250 mg/L), high urea (> 250 mg/L), and low urea (< 150 mg/L). Lactating cows were grouped according to milk yield levels: Group 1: low producers (\leq 20 L/day), Group 2: high producers (\geq 20 L/day), and according to milk protein percentage: Group 1: \leq 3.4%, Group 2: \geq 3.4%.

A correlation analysis was conducted to examine relationships between milk protein content, daily milk production, and individual urea concentrations. A one-way analysis of variance (ANOVA) was performed using Fisher's Least Significant Difference (LSD) test for mean comparisons. A 9-cell grid plot was constructed to visualize the distribution of individual urea values relative to milk protein percentages. The optimal group of cows was defined as those with milk protein content between 3.2% and 3.6% and urea concentrations between 150 mg/L and 250 mg/L. The results were considered statistically significant at P < 0.05.

Results and discussion

This study had a dual objective: first, to validate a colorimetric method (CM) for the determination of milk urea concentration in comparison to a reference method (RM); and second, to assess the usefulness of this parameter as a nutritional management tool by analyzing its relationships with milk yield and protein content.

Colorimetric method validation. The urea analysis results obtained by the two methods are presented in Table 1. The mean \pm standard deviation of urea concentration for all milk samples amounted to 283.80 \pm 75.73 mg/L according to the reference enzymatic method (RM) and 299.80 \pm 80.78 mg/L according to the colorimetric method (CM).

Tab. 1. Descriptive analysis of urea content in bulk tank milk samples

Urea content (mg/L)	Enzymatic method (RM)	Colorimetric method (CM)	
Mean ± SD	283.80 ± 75.73	299.80 ± 80.78	
Median	270	305	
Maximum	480	520	
Minimum	150	150	
Coefficient of variation (%)	26.95	26.69	

The distribution of percentage values for bulk tank milk samples, categorized by urea concentration ranges and analyzed by both assay methods, is shown in Figure 1. For urea levels \geq 350 mg/L, 310-340 mg/L, 200-300 mg/L, 160-190 mg/L, and \leq 150 mg/L, the respective percentages obtained by the enzymatic method (RM) and the colorimetric method (CM) were 18% and 11%, 22% and 28%, 8% and 10%, 38% and 46%, and 6% versus 2%. A bilateral comparison of the percentage distributions across the urea concentration

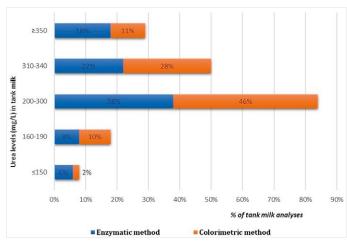


Fig. 1. Distribution of bulk tank milk analysis according to urea concentration

classes (RM vs. CM) revealed no statistically significant differences (p > 0.05).

The simple linear regression analysis for RM and CM showed a correlation coefficient (r) of 0.8589, with a 95% confidence interval of [0.763, 0.9178], and a high level of significance (p < 0.001) (Fig. 2). These results demonstrate a strong agreement between the two methods of analyzing urea concentration in bulk tank milk, supporting the validity of the colorimetric method under the local farming and laboratory conditions.

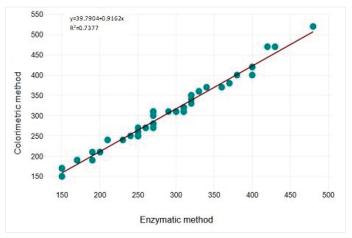


Fig. 2. Relationship between bulk tank milk urea concentrations determined by the enzymatic method (X-axis) and the colorimetric method (Y-axis)

Our results confirm the relevance of urea measurement in bulk tank milk as a global and early indicator of nutritional imbalances that can affect productivity and udder health. However, as highlighted, this method is a screening test and does not replace analyses at the individual cow or quarter level. The quality and applicability of screening data must be considered in light of the intended use and inherent limitations of a collective measurement (2, 36). An approach combining collective screening and targeted individual analyses would thus be the most effective strategy for the sanitary and nutritional management of dairy herds (24, 36).

The validation of alternative, more accessible analytical methods is essential for routine laboratory work and on-farm monitoring. The results of this study demonstrate excellent agreement between the colorimetric method and the reference technique. This conclusion is strongly supported by a high and statistically significant correlation coefficient. Such a correlation is a key success criterion in validation studies and is consistent with numerous other works that have validated alternative methods, such as infrared spectroscopy, against standard chemical reference methods (12, 32).

Although the colorimetric method (CM) slightly overestimated the mean concentration (299.80 \pm 80.78 mg/L) compared to the reference method (RM) (283.80 \pm 75.73 mg/L), this difference is not unusual. It may be attributed to the increased sensitivity of the colorimetric method to interferences from the complex milk matrix, such as lipids or pigments, which can affect optical readings (15). However, this discrepancy has no major practical impact, as the distribution of samples across different concentration classes did not show any statistically significant differences (p > 0.05) between the two techniques. This suggests that, in categorizing milk samples for the purpose of nutritional alerts, the performance of CM is comparable with that of RM. These findings are consistent with those reported by Kohn et al. (23) and Wattiaux and Ranathunga (33). Thus, the colorimetric method can be considered valid and reliable for routine use in Algerian dairy farming systems, where accessibility and cost are critical factors.

Exploration of relationships between milk yield, milk protein content, and milk urea concentration. The individual values for milk urea concentration, protein content, and milk yield of the cows are presented in Table 2.

Tab. 2. Individual milk urea concentrations, protein content, and milk yield of cows

Milk from all cows (n = 52)								
Parameter	Urea concentration (mg/L)	Protein content (%)	Milk yield (L)					
Mean ± SD	236.92 ± 52.28	3.37 ± 0.13	21 ± 6.98					
Median	230	3.37	20					
Maximum	370	3.72	40					
Minimum	160	3.15	12					
Coefficient of variation (CV%)	22.06	3.91	33.11					

Individual milk urea concentrations ranged from 160 to 370 mg/L, with a herd average of 236.92 ± 52.28 mg/L. Milk protein percentages in the 52 samples ranged from 3.15% to 3.72%, with an average of 3.37 ± 0.13 g/L. Individual milk yields ranged from 12 to 40 liters, with an average of 21 ± 7 liters.

The distribution of milk samples according to defined urea concentration thresholds (Fig. 3) revealed

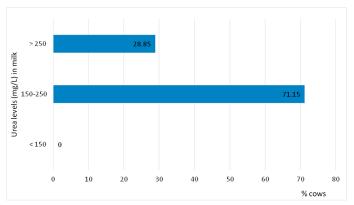


Fig. 3. Distribution of cow milk samples according to urea concentration

that 28.85% of the samples had high urea levels (> 250 mg/L), while 71.15% fell within the optimal range (150-250 mg/L).

Once the validity of the colorimetric method was established, attention could be turned to the second part of the study: interpreting urea concentrations as a nutritional indicator. The average urea concentration in individual milk samples $(236.92 \pm 52.28 \text{ mg/L})$ fell within the range generally considered optimal by many authors (typically 150-250 mg/L or approximately 10-16 mg/dL of Milk Urea Nitrogen, MUN), suggesting an overall adequate protein-to-energy balance in the given herd (7, 19). The observed distribution, with 71.15% of samples within this optimal range, supports this interpretation. Nevertheless, the presence of nearly 29% of samples with elevated urea levels (> 250 mg/L) indicates a significant proportion of cows potentially experiencing nutritional imbalances, a common challenge in modern dairy farming.

The correlation coefficient between individual urea concentrations and milk protein percentage (r = 0.4812) was statistically significant (p = 0.0003). In contrast, no significant correlation was found between individual urea concentrations and milk yield (r = 0.1419; p = 0.3155). Urea concentrations differed significantly between groups based on milk protein percentage (p < 0.05) and tended to differ between milk yield levels (p = 0.1) (Tab. 3).

Tab. 3. Mean milk urea concentrations (mg/L) in relation to milk yield (L/day) and milk protein percentage (%) in Friesian Holstein cows

			Cows (n/%)	Mean urea (mg/L)	SD	Р
Milk yield	Group ¹	1	29/55.76	226.90	41.94	0.1
		2	23/44.23	249.57	61.97	
Milk protein (%)	Group ²	1	28/53.84	217.50°	40.59	0.03
		2	24/46.15	259.58b	56.37	

Explanations: a, b – Different superscript letters indicate statistically significant differences (P < 0.05). Group¹ 1: ≤ 20 L/D, Group² 2: ≥ 20 L/D; Group² 1: $\leq 3.4\%$, Group² 2: $\geq 3.4\%$

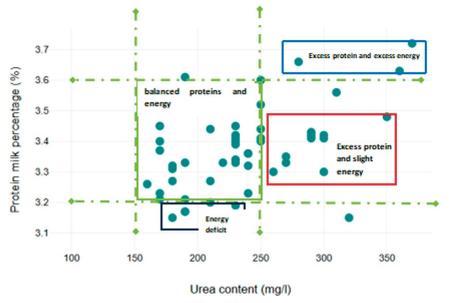


Fig. 4. Classification of cows according to milk urea concentration and milk protein percentage. Group 1: green box, Group 2: red box, Group 3: black box, Group 4: blue box

Based on the nine-cell grid plotting urea concentration against milk protein percentage, four groups of cows were identified as having either balanced or unbalanced protein and energy levels. The first group, representing 61.53% of the cows, exhibited a normal protein content (3.2-3.6%) and a normal urea concentration (150-250 mg/L), indicating a balanced nutritional status. The second group, comprising 21.15% of the cows, had a normal protein content (3.2-3.6%) but elevated urea levels (> 250 mg/L), suggesting an excess of degradable nitrogen relative to available energy. The third group, representing 7.69% of the cows, showed a low protein content (< 3.2%) and normal urea levels (150-250 mg/L), potentially indicating a protein deficiency. The fourth group included 5.76% of the cows with both a high protein content (> 3.6%) and an elevated urea concentration (> 250 mg/L), which suggests a possible imbalance due to excessive protein intake.

A positive and statistically significant correlation was established between milk urea concentration and milk protein content. This finding is consistently reported in the literature (3, 27, 28) and continues to be confirmed by recent research (13). The physiological explanation lies in the fact that an excess of rumendegradable protein relative to the energy available for microbial synthesis leads to increased hepatic urea production. The nuance introduced by Gustafsson and Palmquist (14) and Rombach (29), who emphasized the importance of synchronizing fermentable energy and protein availability, remains a central concept in interpreting variability in this relationship.

In contrast, our study did not reveal a significant correlation between milk urea concentration and milk yield (r = 0.1419; p > 0.05). This lack of a direct relationship is also well-documented in the literature

and suggests that the association is complex and often non-linear. Various studies have reported conflicting results – negative, positive, or no correlation at all (16, 35). As noted by Carlsson and Berglund (9), milk yield is more strongly influenced by energy-related and genetic factors than by urea levels alone. Therefore, milk urea concentration should not be viewed as a direct predictor of milk volume, but rather as a more reliable indicator of nitrogen utilization efficiency and the nutritional balance of the ration (36).

Beyond analyzing individual correlations, the practical value of milk urea concentration becomes fully apparent when combined with milk protein percentage to establish a precise nutritional diagnosis. The use of a nine-cell grid that cross-references urea and protein levels has proven to be a practical and relevant

diagnostic tool, as demonstrated by Wattiaux and Ranathunga (33) and Rombach (29). The identification of 61.53% of cows within the "balanced" profile is a positive indicator of overall herd management. However, analyzing the unbalanced profiles provides targeted avenues for improvement. The high urea and normal protein levels in Group 2 (21.15% of cows) indicate an excess of ruminal nitrogen – a classic scenario that leads to low feed efficiency, economic losses, and increased environmental burden. These profiles confirm the value of jointly monitoring milk urea and protein to fine-tune dietary management, optimize rations, and improve profitability – an approach emphasized by Broderick and Clayton (6) and by Mulligan et al. (26), and increasingly reaffirmed in modern strategies aimed at reducing the environmental footprint of livestock production (1, 5).

It is important, however, to place this study in context and acknowledge its limitations. Although the sample size was adequate, it could be increased to strengthen the findings. The main limitation remains the lack of detailed data on feed rations, which prevents establishing a direct link between dietary inputs and milk-based indicators. For future research, longitudinal studies conducted in Algeria are needed to establish reference values tailored to the local context. These should incorporate detailed ration analysis – a recommendation widely supported in the scientific literature to enhance the precision of decision-making tools (31). Furthermore, evaluating the economic and environmental impact of ration optimization through milk urea monitoring is a promising direction for future research.

In conclusion, this study validates the colorimetric method for the quantification of milk urea and confirms its relevance as an indicator of protein-energy nutritional efficiency under Algerian dairy farming

conditions. The method demonstrated excellent agreement with the reference technique and diagnostic interchangeability, qualifying it as a precise and economically viable alternative for routine monitoring. Beyond this methodological validation, the study highlights a direct zootechnical application: the identification of a substantial proportion of cows (~40%) experiencing nutritional imbalance, primarily due to suboptimal management of dietary nitrogen. By decoupling milk urea concentration from milk yield and instead linking it to protein metabolism, our findings reinforce its role as an indicator of nutritional efficiency rather than productivity. This work thus provides stakeholders in the dairy sector with a valuable decision support tool for the proactive management of feed rations – an essential step toward optimizing both the performance and sustainability of dairy farming operations.

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