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Protein supplementation for lactating cows in pasture of Mombaça grass (*Megathyrsus maximum* cv. Mombaça)

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ABSTRACT. The objective of this study was to evaluate the performance and metabolic parameters of crossbred lactating cows under intermittent grazing on *Megathyrsus maximum* cv. Mombaça with protein supplementation in three different proportions. Twelve lactating crossbred multiparous cows, grouped in randomized blocks, received supplementation in three proportions of crude protein (CP): 9, 15 and 21% DM in the concentrate, for 60 days. Milk yield, fat, protein, casein, lactose, defatted dry extract and total solids content were not influenced ($p>0.05$) by the crude protein content of the concentrates. Milk yield corrected for 4% fat, linearly decreased ($p<0.05$) with the increase in CP content of the concentrates. Values of urea and urea nitrogen in plasma were higher for treatments with 15 and 21% CP. The values of albumins, globulins, hemoglobin, and total proteins were not influenced ($p>0.05$) by the crude protein levels. It is concluded that the increase in crude protein levels in the concentrate in the diet of lactating cows grazing on Mombaça grass influences productive and metabolic parameters. It is recommended a level of 9% Crude Protein.

Keywords: crossbred cows, milk production, sustainability, tropical grass

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Introduction

Protein is one of the most important nutrients for feeding dairy cows and it is the costliest in formulations. Tropical pastures show a wide variation in protein composition during their development (Silva et al., 2020; Barbero et al., 2021; Uzcátegui-Varela et al., 2022). Animal production on pasture is optimized when the positive interaction between forage and supplementation is maximized (Franco et al., 2021).

Reducing dietary protein can reduce feed intake, fiber digestibility and, consequently, milk yield and quality (Bossche et al., 2022). However, some cows may tolerate low-protein diets better, as they may be more efficient at microbial protein synthesis. Results obtained by Sairanen and Huhtanen (2024), highlight the difference in response between animals, considering that some may be metabolically more efficient at synthesizing milk protein.

Since large amounts of nitrogen cannot be retained in the body's tissues, the largest proportion of excess nitrogen ingested in relation to milk nitrogen is excreted, mainly in the urine. Dairy cows secrete around 21 to 33% of the nitrogen they consume in their milk, with almost all of the remaining nitrogen being excreted in feces and urine (National Academies of Sciences, Engineering, and Medicine, 2021). Franco et al. (2021) observed greater nitrogen losses in the urine as protein supplementation increased.

Nitrogen losses represent an environmental concern, as nitrate volatilization, leaching and denitrification of nitrogen compounds result in a negative environmental impact. In animal terms, the main losses are associated with inefficient nitrogen utilization in the rumen (National Academies of Sciences, Engineering, and Medicine, 2021).

It is important to note that the protein composition of Mombaça grass is influenced by the season of the year, with studies reporting crude protein contents ranging from 6.7 to 19% (Guerra et al., 2021; Franciscatti et al., 2023). Moreover, the genetic potential of the animals also affects their response to supplementation, as demonstrated by Franciscatti et al. (2023), who evaluated protein supplementation in Curraleiro Pé Duro cows increasing the crude protein levels in the concentrate feed which promoted greater intake and digestibility of crude protein, as well as higher levels of milk protein, lactose, and non-fat milk solids. However, it did not influence milk fat content or milk yield. In this context, studies that assess, evaluate the hypothesis across different genetic groups, whether animal performance improves with increasing crude protein levels are essential to achieve greater productive efficiency within livestock systems.

As an alternative to increase the productivity of the production system, the study of protein supplementation in grazing tends to determine the best combination of forage and protein supplementation to maximize the use of the potentially digestible portion of dry matter, given that there is a supply of energy and nitrogen to the rumen environment, thus favoring microbial growth, increasing digestibility and, consequently, dry matter intake. It is hypothesized that, in a pasture with high nutritional value, increasing the protein level in the supplement improves milk production and quality.

The objective of this study was to evaluate the effect of different levels of protein supplementation on milk yield and milk quality of crossbred cows managed on Mombaça grass pasture.

Material and methods

Site, animals and experimental design

The experimental trial was carried out in the dairy sector of the Specialized Academic Unit in Agricultural Sciences at the Jundiaí Agricultural School of the Federal University of Rio Grande do Norte - UFRN, which is located in the town of Macaíba - RN, from January to March 2015.

Twelve lactating crossbred multiparous cows producing on average 14 kg per day were used. The cows were distributed in a randomized block design, considering milk yield and dry matter intake as grouping criteria. The animals underwent a 14-day adaptation period to the experimental diets before being separated into blocks.

The experiment lasted 60 days, during which the productive performance and metabolic parameters of the cows were evaluated under three levels of protein supplementation: the control with low protein (9% CP), medium (15% CP), and high (21% CP).

Feed and experimental diets

Analyses of the bromatological composition of the ingredients in the diets that composed the concentrate and roughage supplementation were carried out at the Animal Nutrition Laboratory (UFRN), where the samples were thawed at room temperature, partially dried in a forced ventilation oven (55ºC for 72 hours) and then ground through a sieve with mesh size of 1 mm for analysis of dry matter (105 °C; method 967. 03), crude protein (method 990.03), ether extract (method 920.39) and mineral matter (method 942.05) according to Association of Official Analytical Chemistry (1990). Neutral detergent fiber (NDF) and acid detergent fiber (ADF) were analyzed according to Mertens (2002) and Van Soest (1963), respectively.

Concentrate supplementation (Table 1) was supplied twice a day, during milking in the morning at 6 am and in the afternoon at 2 pm. The cows received 1 kg of concentrate for every 3 kg of milk produced (Campos & Miranda, 2012).

Table 1. Inclusion of ingredients in the experimental concentrate.

Ingredients (g kg^{-1} DM)	Protein levels		
	Low	Medium	High
Ground corn	960	660	490
Soybean meal	0	130	300
Wheat bran	0	170	170
Mineral mix	40	40	40
Total	1000	1000	1000

The animals were managed in a 1.65-hectare area established with irrigated and fertilized Mombaça grass (*Megathyrsus maximus* cv. Mombaça), divided into 17 paddocks. They had *ad libitum* access to water and shade. Grazing was managed using the intermittent stocking method, where the experimental animals occupied a paddock for one day, while another group adjusted the forage height. The chemical composition of the feed used in the diet is detailed in Table 2.

Table 2. Nutritional value of the feed used (g kg^{-1} DM).

Nutrient	Mombaça Grass	Ground Corn	Soybean Meal	Wheat Bran
DM	176.1	904.2	897.2	900.8
OM	874.6	983.9	928.6	960.1
CP	147.1	92.9	450.0	165.0
EE	31.7	40.7	17.0	30.7
MM	125.4	16.1	71.4	39.9
NDF	599.5	140.7	140.1	351.3
ADF	351.4	16.6	114.8	98.3
LIG	21.0	-	-	-

DM: Dry Matter (g kg^{-1} NM); OM: Organic Matter, CP: Crude Protein, EE: Ether Extract, MM: Mineral Matter, NDF: Neutral Detergent Fiber, ADF: Acid Detergent Fiber, LIG: Lignin.

Characterization of the forage canopy structure

Grazing height was assessed by measuring 20 spots per paddock, distributed along four rows, with five random spots, before the animals entered the paddock (pre-grazing) and after they left (post-grazing). Height was also monitored weekly, as a criterion for grazing management.

The height of each spot corresponded to the average height of the canopy around the ruler, and the pre-grazing height was maintained at 70 cm. The average stocking rate, considering only the animals in the experiment, was 7.2 AU ha-1.

To estimate forage mass, a 0.5 m^2 frame was used, and the material contained within the frame was cut at ground level, with 4 samples per grazing cycle. Forage accumulation rate was calculated by the difference between pre-grazing and post-grazing forage mass of the previous grazing cycle divided by the number of days between measurements. The volumetric density of dry matter was obtained by dividing the dry mass of the sample by the total volume of the sample in an area of 0.5 m^2.

The forage mass eliminated between pre-grazing and post-grazing was approximately 2.6 tons of DM ha^{-1}. As the paddocks had an average area of 0.09 ha, the mass removed was 234 kg per paddock, or 19.5 kg DM per cow, given that the experimental plot contained 12 cows. Simulated grazing was carried out weekly throughout the experiment.

Forage samples were weighed and divided into two aliquots: the first to determine the bromatological composition, and the other to separate the leaves, stem + sheath and senescent material; in which the dry matter analysis of each fraction was carried out. Composite samples were taken every three collections. The characteristics of the pasture structure are described in Table 3.

Table 3. Average structural characteristics of Mombaça grass when the animals entered and left the paddock.

Parameters	Pre-grazing	Post-grazing
	Average	Average
Height (cm)	70.36	50.02
Forage mass (kg DM ha^{-1})	18.540	15.856
Component participation		
Leaves (g kg^{-1} MS)	410	210
Stem + sheath (g kg^{-1} DM)	310	260
Senescent material (g kg^{-1} DM)	280	530
Leaf:stem ratio	1.32	0.80

Production and composition of the milk

The cows were mechanically milked twice a day and milk yield was measured twice a week. Milk yield was corrected to 4% fat, through the equation: CMY = (0.4 × MY) + (15 × (MY × FAT), following the methodology described by the National Research Council (2001), where CMY = milk yield corrected for 4% fat (kg day^{-1}); MY = milk yield (kg day^{-1}); FAT = fat content in milk (kg day^{-1}) (FAT = MY × %FAT), where %FAT is the percentage of fat in the milk.

Every seven days, milk samples were collected proportionally in the morning and afternoon, based on the individual production of each animal. In the morning, the sample was stored in a plastic bottle and kept refrigerated; after the afternoon collection, the milk was homogenized. Part of the milk sample was placed in a 40 mL jar and sent for analysis of the milk components: protein, fat, lactose, total solids, casein and urea nitrogen using the Bentley 2000 equipment, through infrared absorption, at Laboleite - Milk Quality Laboratory, at the Federal University of Rio Grande do Norte - UFRN.

Another fraction of the composite milk sample was deproteinized with 25% trichloroacetic acid at a ratio of 10 mL of milk:5 mL of acid, filtered through filter paper and stored at -20 °C, for subsequent urea analyses, carried out on the filtrate using the Labtest® kit in a commercial laboratory.

Metabolic profile

Blood was collected three times during the 60-day experiment, at intervals marking the beginning, middle and end of the experimental period, four hours after feeding, through jugular venipuncture with Vacutainer® tubes, with and without anticoagulant (EDTA) to obtain serum and plasma, respectively. The blood samples without anticoagulant were refrigerated and those with anticoagulant were homogenized, refrigerated and taken to the laboratory for further processing. All the tubes were centrifuged for 15 minutes at 500 rpm. The serum and plasma aliquots were placed in Eppendorf® microtubes and stored at -20 °C for later analysis. The biochemical and metabolic indicators determined in the blood were: urea, urea nitrogen, total proteins, albumin, globulin and hemoglobin.

Statistical analysis

The randomized block design was used and the statistical model was $Y = B_i + D_j + E_{ij}$. Where, Y = the overall mean; B_i = the random effect of the block; D_j = the fixed effect of the diet; and E_{ij} = the residual error.

The PROC MIXED procedure of the Statistical Analysis System (2009) was used. The means were obtained through the LSMEANS command. The data were subjected to Tukey's mean comparisons at 5% significance level.

Results and discussion

There were no differences (p>0.05) in milk yield, which averaged 13.91 kg day^{-1} during the study. There was also no effect on milk fat content, which averaged 39.6 g kg^{-1}. Additional milk constituents such as protein, casein, lactose, defatted dry matter and total milk solids also showed no difference.

The milk yield results indicate that grass intake in this experiment, combined with supplementation with a low level of crude protein, was enough to meet the nutritional needs of the crossbred cows for the level of milk yield presented. This may have been due to the chemical composition of the Mombaça grass (Table 2), as it was managed using fertilization and irrigation, in addition to adjustments in the stocking rate, and grazing using a top group and a second group, which made it possible to improve the quality of the pasture ingested by the lactating cows, as evidenced by the high protein content of the pasture (147.1 g kg^{-1} DM).

There was an effect on milk yield corrected for 4% fat, with higher production in the treatments with low and medium protein supplementation, showing values of 14.73 and 13.62 kg cow-day^{-1} of milk, respectively. The lowest milk production was observed for the high protein inclusion treatment (13.10 kg cow-day^{-1}).

The lower milk yield corrected for 4% fat observed in the cows that consumed the concentrate with the highest level of crude protein may have been due to the excess protein in relation to the cows' nutritional requirements, as the cows will have to expend energy to metabolize the excess nitrogen. In order to eliminate the excess nitrogen from the body, the energy allocated to milk production is reduced and consequently the efficiency of protein use (Correa-Luna et al., 2020). This is justified by the higher levels of nitrogen compounds in milk and blood (National Academies of Sciences, Engineering, and Medicine, 2021).

There was an effect on urea nitrogen content in milk, which averaged 20.79 mg dL^{-1} in the treatment with high protein supplementation, while there was no difference in the treatments with medium and low supplementation (Table 4).

Table 4. Production and composition of milk from cows fed different levels of protein in the concentrate.

Parameters	Protein levels			SEM	Pr >F
	Low	Medium	High		
MY (kg day^{-1})	14.46	13.99	13.27	1.1418	0.2542
$MY_{4\%}$ (kg day^{-1})	14.73a	13.62a	13.10b	1.0849	0.0241
Fat (g kg^{-1})	41.2	38.6	39.1	0.1491	0.2111
Protein (g kg^{-1})	30.3	30.5	30.2	0.1159	0.9786
Casein (g kg^{-1})	23.5	23.7	23.6	0.07566	0.9746
Lactose (g kg^{-1})	49.4	48.7	49.5	0.05909	0.5330
DDE[5] (g kg^{-1})	89.9	89.3	89.6	0.12	0.9146
Total solids (g kg^{-1})	132.5	128.8	129.0	0.229	0.4337
MUN (mg dL^{-1})	16.89b	19.33b	20.79a	1.1038	0.0315

SEM: Standard error of the mean; MY: Milk yield, $MY_{4\%}$: Milk yield corrected for 4% fat; DDE: Defatted dry extract; MUN: Milk urea nitrogen. Means followed by different letters in the line are different according to the Tukey's test at 5% probability.

Excess CP in the diet is quickly converted into urea to avoid damage caused by excessive ammonia, so that urea is transported from plasma to other fluids such as saliva to be recycled or excreted in the urine, but it can also be found in milk as MUN, and the relationship between CP and MUN is positive, as it used as an indicator of excess CP in the diet (Correa-Luna et al., 2020). In this study, the treatment with high protein supplementation indicates the imbalance between protein and energy by evaluating MUN, which shows similar results in the blood parameters evaluated.

The lower MUN levels for low and medium protein supplementation treatments are within the parameters established by Almeida et al. (2021), who reported that MUN levels should range 15,5 mg dL^{-1}. However, the high level of MUN for the treatment with high protein supplementation suggests that there was an excess of nitrogen intake or an excess of degradable protein in the rumen. This increase can contribute to cases of excess urea in the circulation and, in more extreme cases, can lead to a drop in reproductive rates (Zhao et al., 2025).

Changes were observed in blood urea content in cows fed different levels of protein supplementation, which averaged 43.37 mg dL^{-1} for high protein supplementation, and that was similar to the medium supplementation treatment which averaged 32.62 mg dL^{-1}, while the lowest blood urea content was found in the low protein supplementation treatment. Blood urea nitrogen showed a similar behavior, with lower values in the low protein supplementation treatment (12.05 mg dL^{-1}).

The total protein, albumin, globulin and hemoglobulin values did not differ according to the protein levels evaluated and are shown in Table 5.

Table 5. Protein metabolic profile of cows fed different levels of protein in concentrate.

Parameters	Protein levels			SEM	Pr >F
	Low	Medium	High		
Urea (mg dL^{-1})	25.87b	32.62ab	43.37a	2.8483	0.0021
Urea Nitrogen (mg dL^{-1})	12.05b	15.20ab	20.21a	1.3274	0.0021
Total proteins (g dL^{-1})	7.76	8.30	7.72	0.4609	0.6066
Albumin (g dL^{-1})	2.85	2.75	2.94	0.1158	0.5418
Globulins (g dL^{-1})	4.90	5.54	4.78	0.4852	0.5052
Hemoglobin (g dL^{-1})	9.57	9.83	9.53	0.4981	0.8986

SEM: Standard error of the mean; Means followed by different letters in the line are different according to the Tukey's test at 5% probability.

The results found for milk urea nitrogen follow the same pattern as the blood tests. Thus, the results for blood urea are higher than those considered a reference for dairy cattle (5 to 20 mg dL^{-1}) mentioned by Arruda, et. al. (2008). However, similar plasma urea nitrogen values at lower supplementation levels were found in other studies (Dall-Orsoletta et al., 2020). These values, among other factors, can be attributed to the excessive supply of dietary protein, which can generate productive, reproductive and environmental losses, and suggests that the CP content in the DM of the concentrate supplied was excessive.

Providing a diet that contains crude protein above the animal's requirement will also slightly increase the energy expenditure to recycle nitrogen compounds in the form of urea. As protein is generally the most expensive nutrient, this also increases formulation costs (National Academies of Sciences, Engineering, and Medicine, 2021).

The amount of urea in blood and milk dependents on the energy: protein ratio of the diet. A low protein intake is associated with lower urea rates in blood and milk, while high urea rates indicate excess of rumen-degradable proteins, or inefficient contribution of energy (National Academies of Sciences, Engineering, and Medicine, 2021).

Reducing the inclusion of protein in the diet reduces the nitrogen excreted and can improve the efficiency of nitrogen use. However, a reduction below the requirement limits productivity. Nitrogen use efficiency is affected by the availability of energy and milk yield generally increases as the concentration of energy in the diet increases. Feeding diets with lower protein contents with the supplementation of amino acids protected from rumen degradation should reduce N excretion in the urine. (National Academies of Sciences, Engineering, and Medicine, 2021). Further studies should be carried out exploring the reduction of protein supplementation without limiting milk protein synthesis in lactating cows, considering the essential amino acids methionine and lysine followed by histidine (Bossche et al., 2022).

Conclusion

Increasing levels of crude protein in the concentrate diet, for low-producing crossbred cows grazing on Mombaça grass, negatively impacts production and metabolic parameters. A concentrate supplement with 9% CP is recommended for cows with an average milk yield of 13.91 kg day^{-1}.

Data availability

Does not apply.

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