

Strategic Protein Supplementation to Increase Milk Production in Crossbred Cows Grazing on Tropical Pastures

Research Article

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ABSTRACT

This study aimed to evaluate the production and milk composition of crossbreed F1 Holstein × Zebu (1/2 H×Z) cows grazing on Marandu grass (*Urochloa brizantha* cv. Marandu) or Tifton-85 (*Cynodon* spp.) supplemented with different protein concentrate. 36 H × Z cows (±514 kg of body weight) were used. The cows had ± 65 days in milk and were distributed in a randomized block design, in a 2 × 3 factorial arrangement, i.e., two types of grass (Marandu and Tifton-85 grass), three types of protein supplementation (2.0 kg of concentrate with low rumen degradable protein; 2.0 kg of concentrate with high rumen degradable protein; and no concentrate feed). Both grass produced similar amounts of forage mass, kg.h⁻¹. The nutritional value for both grasses was also similar in all the variables analyzed, such as crude fat, crude protein, carbohydrates, non-fiber carbohydrates, neutral detergent fiber, and, acid detergent fiber. The milk yield was not affected by the different grasses used for grazing. Only the lactose content was higher in cows grazing on marandu grass than in Tifton-85 grass (P=0.032). The cows that did not receive concentrate had lower daily milk yield (P=0.010) and a lower percentage of lactose (P=0.011). In conclusion, both grasses can be used for grazing F1 ½ H × Z cows, and protein supplementation, regardless of its ruminal degradability, in this situation is necessary to increase milk production.

KEY WORDS lactose content, Marandu grass, rumen degradable protein, Tifton-85 grass.

INTRODUCTION

Dairy cattle feeding in tropical and subtropical areas is based on extensive grazing of native grasses (Corsi *et al.* 2001; Ramírez-Rivera *et al.* 2019), and its use as a source of roughage requires knowledge of the factors that interfere on its nutritional quality (energy and protein), which can thus affect animal consumption (Jefferson *et al.* 2004). Tropical pastures include grasses of the genera *Brachiaria* and *Cynodon* (da Silva *et al.* 2015; Pequeno *et al.* 2015;

Lara *et al.* 2021). The *Brachiaria* grass is highly adaptable to tropical conditions, as well as being tolerant of low-fertility soils (Lara *et al.* 2021). The Marandu palisade grass is widely used in forage-livestock systems due to its tolerance to spittlebugs (*Deois flavopicta* and *Zulia entreriana*), high forage accumulation and nutritional value if properly cultivated and managed, and high production of viable seeds (Pequeno *et al.* 2015). The *Cynodon* species and cultivars, as well as their hybrids, are creeping grasses that are propagated by stolons, rhizomes or both (da Silva *et al.*

2015), and are characterized by a high nutritional value if properly managed (Rezende *et al.* 2021).

The nutrition of dairy cattle in tropical and subtropical regions is one of the main challenges farmers must consider due to the dependence on pasture intake (Edson *et al.* 2018), whose growth is directly related to environmental factors such as temperature and rainfall. According to Detmann *et al.* (2014), tropical grasses hardly balance the availability of nutrients for cattle throughout the production period and also present nutritional limitations. During the dry season, the quality of pasture decreases, with low crude protein content ($<80 \text{ g}\cdot\text{kg}^{-1}$), low apparent, and high digestibility coefficient of neutral detergent fiber content (Reyes Sánchez *et al.* 2006).

This period of low pasture quality causes a nutritional imbalance in cattle due to a deficit in energy and protein (Madzimore *et al.* 2011; Mwendia *et al.* 2018). Therefore, some protein reserves of the animal body must be mobilized to support the synthesis of milk components (Reyes Sánchez *et al.* 2006). As a result, milk production decreases, limiting production potential (Mwendia *et al.* 2018). Commercial protein concentrates supplementing generally mitigates this seasonal nutritional deficit (Reyes Sánchez *et al.* 2006; Hills *et al.* 2015).

In this way, supplementation in the dry season to complement the cow's diet to achieve productivity gains represents the greatest expense for milk yields (Albarrán-Portillo *et al.* 2015). Concentrate supplements with a high crude protein (CP) content in the diet improves intake (Detmann *et al.* 2014). The use of suitable supplementation is an alternative for providing the nutritional requirements of different animal species/categories, avoiding restrictions on animal production, and minimizing production costs. The advantage of balancing the levels of rumen-degradable protein (RDP) and rumen-undegradable protein (RUP) has been demonstrated, leading to the rational use of N, contributing to the economic success of cattle rearing and reducing environmental pollution (Kaufman *et al.* 2017; Savari *et al.* 2018; Martins *et al.* 2019; Alves *et al.* 2020; Rehman *et al.* 2020).

In addition, crossbreeding different cattle breeds is an important tool for dairy farmers in tropical regions, as it promotes the combination of breed advantages, such as productivity and adaptability to environmental conditions (Fraga *et al.* 2016). Combining these characteristics with appropriate nutritional management could be an opportunity to increase milk production in tropical regions. However, data on the performance of F1 crossbreed (1/2 Holstein \times 1/2 Zebu) lactating cows grazing on paddocks supplemented with different degradable protein sources is still limited and contradictory. This study aimed to evaluate the production and composition of milk from F1 crossbreed

cows (F1 H \times Z) grazing on Marandu grass (*Urochloa brizantha* cv. Marandu) or Tifton 85 grass (*Cynodon* spp. cv. Tifton 85), supplemented with high or low protein degradable concentrates.

MATERIALS AND METHODS

All the experimental procedures were approved following the ethics of the Institutional Animal Care and Use Committee in the Minas Gerais Agricultural Research Agency (EPAMIG). We confirm that this study was carried out in compliance with the Animal Research: Reporting of *in vivo* Experiments (ARRIVE) guidelines.

Study design

The research experiment was conducted at the Experimental Field of Felixlândia (FEFX) of the EPAMIG. The experimental period was from January to May (150 days), and the first 30 days of the study were used to adapt the cows, and the following days were used for evaluation and data collection.

A total of 36 crossbreed cows F1 H \times Z were used, with 65 ± 28.5 days in milk (DIM) and a body weight of 514 ± 38.8 kg at the beginning of the study. The cows were distributed in a completely randomized design, in a 2×3 factorial arrangement, with 6 cows per treatment: two types of grass: Marandu or Tifton-85 grass; two types of protein concentrate: 1) concentrate with low rumen degradable protein (LRDP; 10.25% RUP), 2) concentrate with high rumen degradable protein (HRDP; 6.94% RUP), or 3) no concentrate supply (NCS). The concentrate was provided in the amount of 2.0 kg per animal per day at milking time, 75% in the morning and 25% in the afternoon. Both concentrates were formulated according to the NASEM (2021) to be isoproteic (22% CP; Table 1). The concentrate contained 22% CP, 7.52% ash, 3.21% crude fat (CF), 0.89% calcium, and 0.52% phosphorus (data from previous analysis).

Grazing area and climate

The total grazing area was 7.44 ha divided into 32 paddocks measuring $2,324 \text{ m}^2$ (16 of *Urochloa brizantha* cv. Marandu and 16 of *Cynodon* spp cv. Tifton 85). The grasses and the sprinkler irrigation system were already established. The paddocks were only irrigated using 8 to 12 mm of rainfall equivalent. The soil water balance was calculated using the Thornthwaite and Mather method with a water retention capacity of 40 mm (Thornthwaite and Mather, 1955). Soil analysis of the area showed the following chemical characteristics in the 0 to 20 cm layer: pH in H₂O - 5.7; P and K - 9.8 and $70 \text{ mg}/\text{dm}^3$, respectively; Ca, Mg and Al (KCl 1 mol/L) - 1.7; 0.6 and $0.1 \text{ cmolc}/\text{dm}^3$, respectively; organic

matter - 2.0 dag/kg and base saturation of 39%. At the start of the research experiment, in January, the area was fertilized with 100 kg/ha of P₂O₅ and 55 kg/ha of K₂O. Top dressing was applied monthly from January to April at a dose of 52.8 kg/ha of N in the form of urea.

Each group of 18 animals was managed on a rotational grazing system in 16 paddocks with two days of occupation and 30 days of rest. Additional dry cows were used after the second day of grazing to lower the pasture to 10 to 15 cm of residue, if necessary. The cows had access to free water and mineral mix in the paddocks.

The rainfall and temperature data recorded during the experimental period were collected by the Vantage Pro2 (Davis Instruments Corp[®], Hayward, California, United States) weather station installed in the experimental area. Rainfall during the experimental period was 233, 0, 140, 47, and 22 mm for January, February, March, April and May, respectively.

Sampling and chemical composition

Before and after each grazing, the forage biomass was collected in four squares measuring 1.0 × 1.0 m, 10 cm from the ground for both grasses, in each paddock. The samples were weighed and divided into leaf blades, stems + sheath, and dead material. The sampled forage was packed in a paper bag, weighed, and placed in a forced circulation oven (MA035, Marconi, Piracicaba, São Paulo, Brazil) at 55 °C for 72 hours. The DM content (AOAC method 925.40), ashes (AOAC method 942.05), crude fat (CF; AOAC method 920.39), and, total nitrogen (AOAC method 984.13) was determined according to Association of Official Analytical Chemists (AOAC, 2012). The crude protein (CP) was calculated by multiplying the total nitrogen by 6.25. The neutral detergent fiber (NDF) and acid detergent fiber (ADF) were determined by the method described by Van Soest *et al.* (1991) in an Ankom 2000 fiber analyzer (Ankom Tech. Corp., Fairport, NY). Heat-stable α-amylase and sodium sulfite were included in the NDF analysis. The residues of NDF and ADF were further processed for their nitrogen content (AOAC method 984.13) to determine the neutral detergent insoluble nitrogen (NDIN) and acid detergent insoluble nitrogen (ADIN). The non-fiber carbohydrate (NFC) was estimated by equation: $NFC(\% DM) = 100 - (\% CP + \% EE + \% NDF + \% Ash)$.

The diet provided in this study was carefully monitored by the supplier company to ensure that aflatoxin levels were well below the established safety limits for animal feed. This precautionary measure was taken to safeguard the animals' health and welfare. By maintaining feed quality within safe limits, we aimed to minimize any potential influence of aflatoxins on the study results.

Milk quality analysis

Milk yield varies during the lactation process (Çilek and Tekin, 2005; Cilek, 2009). However, test day (daily milk) yields were used instead of lactation milk yields in this study. The cows were milked daily at 07 and 14h. Milk samples (morning and afternoon milking) were taken after homogenizing the milk, totaling 50 mL of milk, and placed in plastic vials containing 10 mg of 2-bromo 2-nitropropane 1,3-diol, and then cooled to 4 °C. The milk samples was analyzed by Fourier-transform infrared spectroscopy for nutritional composition analysis (Lefier *et al.* 1996) to determine fat, protein, lactose, and total solids (LABUFMG, Belo Horizonte, Brazil).

Statistical analysis

The data was tabulated and described statistically (mean+standard error of the mean (SEM)). Initially, the results were analyzed using the Shapiro-Wilk normality test and Levene's homoscedasticity test, and it were attended for both to $P > 0.05$. Furthermore, the results were analyzed using the z-test for outliers to identify possible discrepancies, which were excluded for $P < 0.05$. Following that, comparisons were made by GLM for repeated measures using the Statistical Package for Social Science software (SPSS, 2011). Interactions between factors were not observed in each case ($P < 0.05$). The significance was declared at $P < 0.05$.

RESULTS AND DISCUSSION

The paddock productivity showed no difference for either group of grasses (Table 2). The nutritional value of the Marandu and Tifton-85 grasses also showed no differences in the variables evaluated (Table 2).

The milk yield was not affected by the different grasses used for grazing (Table 3). Among the milk composition variables, only the lactose content was higher in cows grazing on marandu grass than in Tifton-85 grass ($P=0.032$, Table 3).

The cows that did not receive concentrate had lower daily milk yield ($P=0.010$) and a lower percentage of lactose ($P=0.011$) compared to the cows that received both types of concentrate (Table 3). The other variables were not influenced by the provision or not of concentrate (Table 3). No interaction between grass and concentrate was observed for the variables analyzed (Table 3).

This study aimed to evaluate the production and composition of milk from F1 crossbred cows (F1 H×Z) grazing on Marandu grass (*Urochloa brizantha* cv. Marandu) or Tifton 85 grass (*Cynodon* spp. cv. Tifton 85), supplemented with high or low protein degradable concentrates.

Table 1 Composition of ingredients expressed as a percentage of dry matter

Ingredient	Concentrate					
	HRDP			LRDP		
	%	CP	RUP	%	CP	RUP
Soybean meal	22.50	10.35	3.62	10.00	4.60	1.61
Cottonseed meal	-	-	-	23.90	9.08	4.54
Ground corn	55.80	5.02	2.76	38.00	3.42	1.88
Wheat bran	16.00	2.24	0.56	-	-	-
Promil	-	-	-	24.00	5.04	2.22
Urea	1.60	4.50	-	-	-	-
Molasses	0.80	0.02	-	0.80	0.02	-
Limestone	1.62	-	-	1.62	-	-
Premix	0.18	-	-	0.18	-	-
Phosphate	0.80	-	-	0.80	-	-
Mineral salt	0.70	-	-	0.70	-	-
Total	100.00	22.12	6.95	100.00	22.16	10.25

HRDP: concentrate with high rumen degradable protein; LRDP: concentrate with low rumen degradable protein; CP: crude protein and RUP: rumen undegradable protein.

Table 2 Productivity and nutritional composition (dry matter basis) of Marandu and Tifton-85 grass

Variables	Marandu grass ¹	Tifton-85 grass ²	SEM	P-value
Forage mass, kg.ha ⁻¹	9537.6	8822.7	634.47	0.620
Dry matter, %	24.2	26.3	2.00	0.134
Ash, %	7.9	7.7	0.20	0.473
Crude fat, %	0.95	1.4	0.10	0.089
Crude protein, %	10.95	13.2	1.01	0.153
Carbohydrates, %	80.2	77.7	0.90	0.721
Non-fiber carbohydrates, %	22.0	19.9	1.10	0.409
NDF, %	56.0	58.9	0.80	0.086
ADF, %	38.0	38.8	0.80	0.587
NDIN, %	0.73	1.02	0.10	0.130
ADIN, %	0.34	0.50	0.10	0.172

¹ *Urochloa brizantha* cv. Marandu.

² *Cynodon* spp.

NDF: neutral detergent fiber; ADF: acid detergent fiber; NDIN: neutral detergent-insoluble nitrogen and ADIN: acid detergent-insoluble nitrogen.

SEM: standard error of the means.

Table 3 The milk yield and composition of crossbred cows grazing on two types of tropical grass and receiving or not high or low rumen degradable protein supplementation

Variable	Marandu grass ¹			Tifton-85 grass ²			SEM	P-value		
	LRDP	HRDP	NCS	LRDP	HRDP	NCS		G	C	G × C
Milk yield, kg.d ⁻¹	13.30	12.97	11.04	12.68	13.74	11.93	0.628	0.630	0.010	0.401
Protein, %	3.26	3.19	3.15	3.24	3.31	3.14	0.112	0.699	0.535	0.750
Fat, %	3.69	3.91	3.63	3.83	3.69	3.45	0.301	0.677	0.551	0.743
Lactose, %	4.80	4.71	4.56	4.54	4.71	4.51	0.067	0.032	0.011	0.072
Total solids, %	12.67	12.72	12.21	12.50	12.61	12.01	0.372	0.538	0.200	0.988

¹ *Urochloa brizantha* cv. Marandu.

² *Cynodon* spp.

LRDP: low rumen degradable protein; HRDP: high rumen degradable protein; NCS: no concentrate supply; G: grass effect; C: concentrate supply effect and G × C: grass and concentrate supply interaction effect.

SEM: standard error of the means.

The chemical composition of the paddocks showed similar NDF and higher CP values compared to other studies with tropical grasses (Januszkiewicz *et al.* 2016; Serafim *et al.* 2021). However, the nutritional value of the forage was similar.

This similarity was probably due to the type of management provided to the pasture. As a result, there was no difference in milk production between cows grazing Marandu grass and Tifton 85 grass.

The only exception was the lactose content. The result was unexpected since lactose is the milk component least sensitive to dietary changes compared to fat (more sensitive) and protein (intermediary; Jenkins and McGuire, 2006; Walstra *et al.* 2006; Heck *et al.* 2009). According to Costa *et al.* (2020), the lower variation in lactose content may be related to physiological factors since lactose is the main component determining milk osmolality and is restricted to biological variations. As the nutritional value of

both pastures was the same, we could not hypothesize what other effect might have caused this result.

The daily milk yield is affected by a lot of factors (Bakir *et al.* 2009), in this study was not affected by the different grasses used for grazing. Protein supplementation can be much more effective in increasing milk production when compared to the type of grass provided to the cows. Even in Holstein cows, the results with RDP and RUP are still inconsistent. Kaufman *et al.* (2017) evaluated RDP:RUP ratios in diets to maintain milk production and improve the efficiency of N use in heat-stressed dairy cows and observed that reducing RDP decreased milk production by 10%. However, the nutritional support provided by the proteic concentrate made it possible for the cows to produce more milk than those not supplemented.

Teixeira *et al.* (2013) observed daily milk yield similar to that of the present study, with values of 12.7 liters/cow/day for H × Z crossbred cows that received concentrate supplementation under a rotational grazing system on irrigated Tifton grass pastures. Similar results in this study were reported by Alves *et al.* (2020) when evaluating three levels of CP (130, 160, or 180 g CP/kg DM) and three levels of RDP (80, 100, or 120 g RDP/kg DM) in diets with an average of 163 g CP/kg DM, using Holstein cows, the authors observed that cows receiving 180 g CP/kg DM produced 267 kg more milk than cows receiving 130 g CP/kg DM, the authors attributed the results to higher protein intake from the diet, which increased the availability of metabolizable proteins and amino acids to be used by the mammary gland. However, it is important to consider the rational use of concentrate feed, especially if using crossbred cows with lower genetic potential for milk production and lower digestive and metabolic capacity. The milk yield of the pure Holstein breed is quite high (Çilek and Tekin, 2005; Bakir *et al.* 2009). In addition, a possible loss in milk production resulting from crossbreeding can be compensated for by improved health, fertility, and longevity attributed to crossbred animals.

Supplementation with high and low RDP did not affect the cows' milk production at the levels provided. However, higher milk production was expected from cows supplemented with RUP, as consuming this type of protein improves the synchronism of energy and protein use, increasing the amount of metabolizable protein for the animals and leading to better production results (Martins *et al.* 2019).

Rehman *et al.* (2020), who evaluated the effect of RUP levels of 30, 40, 50, and 60% of CP in the diet of Holstein x Jersey crossbred cows and observed a linear increase in milk production with higher levels of RUP (14.06; 16.06; 16.68; 19.07 kg/day, for the different levels of RUP). Another aspect that needs to be addressed is the mechanism of endogenous N use and recycling in ruminants, which may

explain the efficiency of N use (Carmona *et al.* 2020), keeping yields similar between animals supplemented with different protein concentrates.

On the other hand, the results of this study also contrast with those reported by Savari *et al.* (2018), who observed that cows fed diets with higher degradability of CP produced more milk (+1.2 kg/d) than those fed lower degradability of CP. The authors suggested that the lower degradability of CP may have reduced microbial protein synthesis or that the source of RDP has low intestinal digestibility, reducing the availability of amino acids for milk production. Alves *et al.* (2020) observed that cows fed intermediate levels of RDP (100 g RDP/kg DM) produced 2.54 kg/day more milk than cows fed a higher level of RDP (120 g RDP/kg DM). According to the authors, increasing CP by increasing RDP should be done up to 100 g RDP/kg DM, thus avoiding N excretion and lower milk production. Therefore, there are still several inconsistencies in the results to determine which adequate levels of RDP and RUP are necessary to optimize animal performance since both types of protein have their specific importance (Martins *et al.* 2019; Rehman *et al.* 2020).

In this study, lactose showed the same response pattern as milk production, being higher in supplemented animals and lower in non-supplemented animals, 4.69 versus 4.54%. The lactose content probably increased as a result of the increase in milk production. It can be assumed that the grasses used provided adequate formation of volatile fatty acids (acetate) in the rumen, as the fat percentage in the milk was similar between the supplemented and non-supplemented animals. The absence of a significant difference in milk fat percentage was probably due to the adequate supply of lysine and methionine in the diet, as these play important roles in the synthesis of milk fat (Savari *et al.* 2018). The results are consistent with those reported by other researchers (Rehman *et al.* 2020), who also observed no differences in milk fat in crossbred cows.

Similarly, Rosendo Ponce *et al.* (2021), evaluating protein concentrate supplementation (22% CP) in animals grazing *Brachiaria mutica*, obtained values close to those reported in this work, which were 4.9; 3.4; 3.4 and 12.4% for lactose, protein, fat and total solids, respectively. The authors observed that supplementation was enough to increase milk production by 22% without altering its chemical composition.

Therefore, future studies should be conducted to find suitable RDP: RUP ratios that can maximize the production of mixed-breed cows on pasture to obtain a better protein level and amino acid profile in the supplement that adequately meets the animals' requirements. In future studies in this same area of research, we intend to investigate more extensively, including aspects that were not evaluated in

this study, such as intake, metabolism, ruminal parameters and ruminal microbiome.

CONCLUSION

The supplementation for F1 Holstein × Zebu cows providing 2 kg of protein concentrate daily on Tifton 85 grass or Marandu grass have similar milk production and quality. No significant effect was found on daily milk yield, and the effect on total lactation milk yield should be examined in future studies.

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