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Supplementation strategies for lactating F1 Holstein x Zebu cows on deferred pastures

Estratégias de suplementação para vacas F1 Holandês x Zebu em lactação em pasto diferido

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Highlights

Corn silage associated with the deferral of grazing does not improve yield of cows.
Cows on deferred pasture and supplemented with concentrate maintains milk yield.

Abstract

The objective of this study was to evaluate the effects of different concentrate supplementation strategies to lactating F1 Holstein x Zebu cows managed on deferred signal grass pasture on milk yield, composition and body weight gain. Thirty-six F1 Holstein x Zebu cows with average days in milk of 102 ± 10 and body weight of 501 ± 19 kg were allotted to a 4 x 5 completely randomized factorial design, with four feeding strategies and five weeks of evaluation. The treatments consisted of four nutritional strategies: deferred pasture as a source of roughage + 700 grams of protein supplement (PDPI); deferred pasture as a source of roughage + 1,200 grams of protein supplement (PDPII); deferred pasture + 15 kilograms of corn silage (natural basis) + 1,200 grams of protein supplement (PDSP) and corn silage (ad libitum) + 700 grams of protein supplement (CSS). There was no interaction (P = 0.99) between supplementation strategies and test days on milk yield and chemical composition. The mean milk yield of cows managed on PDPI, PDPII and PDSP was 11.50 kg/day (P > 0.05), which was 14.30% lower than that of cows managed on CSS. Fat content (P < 0.01), protein (P < 0.01), lactose (P < 0.01), defatted dry extract (DDE) (P< 0 .01), total solids (P < 0.01) and milk casein (P < 0.01) were affected by different supplementation strategies. F1 Holstein x Zebu cows

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on deferred Urochloa decumbens cv. Basilisk pasture and supplemented with concentrate maintains milk production at 11.50 kg with normal composition, maintaining satisfactory body weight and condition score.
Key words: Deferral of grazing. Urocloa decumbens. Crossbred cows. Milk yield. Casein.

Resumo

O objetivo deste estudo foi avaliar os efeitos de diferentes estratégias de suplementação com concentrado para vacas F1 Holandês x Zebu em lactação manejadas em pastagem de capim-braquiária diferida sobre a produção, composição e ganho de peso corporal do leite. Trinta e seis vacas F1 Holandês x Zebu com média de dias em leite de 102 ± 10 e peso corporal de 501 ± 19 kg foram distribuídas em um planejamento fatorial 4 x 5 inteiramente casualizado, com quatro estratégias de alimentação e cinco semanas de avaliação. Os tratamentos consistiram em quatro estratégias nutricionais: pasto diferido como fonte de volumoso + 700 gramas de suplemento protéico (PDPI); pastagem diferida como fonte de volumoso + 1.200 gramas de suplemento protéico (PDPII); pastagem diferida + 15 quilos de silagem de milho (base natural) + 1.200 gramas de suplemento protéico (PDSP) e silagem de milho (ad libitum) + 700 gramas de suplemento protéico (CSS). Não houve interação (P = 0,99) entre as estratégias de suplementação e os dias de teste na produção e composição química do leite. A produção média de leite das vacas manejadas com PDPI, PDPII e PDSP foi de 11,50 kg / dia (P > 0,05), que foi 14,30% menor que a das vacas manejadas com CSS. O teor de gordura (P < 0,01), proteína (P < 0,01), lactose (P < 0,01), extrato seco desengordurado (DDE) (P < 0,01), sólidos totais (P < 0,01) e caseína do leite (P < 0,01) foram afetados por diferentes estratégias de suplementação. Vacas F1 Holandês x Zebu em Urochloa decumbens diferida cv. O pasto de Basilisk e suplementada com concentrado mantém a produção de leite em 11,50 kg com composição normal, mantendo peso corporal e escore de condição satisfatórios.
Palavras-chave: Diferimento de pastejo. *Urocloa decumbens.* Vacas mestiças. Produção de leite. Caseína.

Introduction

Brazil is the world's fourth largest milk producer with approximately 34.84 billion liters produced annually, with a herd composed of 80% crossbred Holstein x Zebu cows (Belchior, Lopes, Gonçalves, & Leite, 2015; Daltro et al., 2019; Anuário Leite, 2021). Despite the high milk production, the average milk yield per animal ranges between 1400-1600 kg/lactation, which is lower than the world's average of 3500 kg/animal/lactation. This is explained by several factors, such as using non-specialized breeds and tropical forage plants as the primary source of nutrients (Santos et al., 2014; Santana et al., 2019a). Brazil has 167 million hectares of pasture, 80% of which belong to the genus Urochloa. However, the quantity and quality of forage throughout the year vary because of existing edaphoclimatic conditions, which affect the nutritional value of forage (Borges et al., 2019; Ribas et al., 2021). Dairy cows cannot express their genetic potential for milk yield under conditions of reduced nutritive value of the pasture, and supplementation with concentrate and/or roughage sources becomes necessary. Feedlot, which is based on the provision of complete diets in feed troughs using conserved forage (i.e., corn, sorghum, grass silages) as a source of roughage, is the main form of supplementation used by dairy farmers (Santos et al., 2012; Monção et al., 2020). However, this system is costly, requires more manpower and interferes with the life quality of dairy farmers.

Pasture deferment is a low-cost strategy that guarantees a continuous supply of forage throughout the year. However, the nutritional value of the pasture does not always meet the nutritional requirements of animals (Alessio et al., 2019). The strategic concentrate supplementation can correct nutrient deficiencies of the pasture (nitrogen and energy), besides supplying macro and micro minerals and improving animal performance. However, there are few studies on the effects of protein supplementation strategies for lactating F1 Holstein x Zebu cows on milk yield and composition. Thus, the objective of this study was to evaluate different concentrate supplementation strategies for lactating F1 Holstein x Zebu cows managed on deferred signal grass pasture on milk yield, composition and body weight gain.

Material and Methods

All animal care and handling procedures were approved by the Animal Care and Use Committee of the Universidade Estadual de Montes Claros, Brazil (protocol CEBEA-Unimontes 150/2017).

The experiment was conducted at EPAMIG Experimental Farm in Felixlândia, Minas Gerais, Brazil (18º04'04" South latitude and 44º58'48" West longitude, at an average altitude of 616 m). The climate of the region is classified as AW (humid tropical savanna, with dry winters and rainy summers), according to Köppen-Geiger's classification. The average annual rainfall is 1,126 mm. The experiment was carried out in the winter, which lasts from July to November in Central Brazil. Cows were allowed 14 days for adaptation to pasture, management and supplementation, followed by 35 days for data collection and sampling. The experimental area was formed with Urochloa (Syn. Brachiaria) decumbens cv. Basilisk (Signal grass) and provided with waterers and feed troughs for supplementation (1 linear meter per animal) to allow simultaneous access to the supplement. Before starting the experiment, the paddocks were deferred (not used for grazing) for 60 days. Nitrogen fertilization was performed at the beginning and end of the summer season to ensure adequate forage mass to animals. Nitrogen was applied as urea at 50 kg/ha of N per application.

Thirty-six F1 Holstein x Zebu cows, with average days in milk of 102 ± 10 days, an average body weight of 501 ± 19 kg and an average age of 7.4 years were used to evaluate milk performance and composition. The animals were individually identified by ear tags. A continuous stocking system was used. A criterion of mass offer and similar leaf offer was adopted between treatments. The amount of supplements offered was determined according to the characteristics of the deferred pasture and its representative use under tropical conditions. The animals received 1 of 4 nutritional strategies: deferred pasture as a source of roughage + 700 grams of protein supplement (PDPI); deferred pasture as a source of roughage + 1,200 grams of protein supplement (PDPII); deferred pasture + 15 kilograms of corn silage (natural basis) + 1,200 grams of protein supplement (PDSP) and corn silage (ad libitum) + 700 grams of protein supplement (CSS).

The cows were mechanically milked at 7 a.m. and 3 p.m. in the presence of their calves. During milking, all cows received concentrate supplementation to milk yield at 1: 3, where 1 kg of concentrate was provided for every 3 kg of milk produced above the initial 5 kg. This commercial concentrate supplement was different from that used in pasture and feedlot supplementation strategies (NUTRILAC®, Tecnutri Montes Claros, Minas Gerais, Brazil). The NUTRILAC® concentrate supplement was fed individually to the animals in wooden feed troughs (1 linear meter) coupled to the lateral piping of the milking line. After each milking, the animals were managed on paddocks equipped with waterers and feed troughs.

The animals that received corn silage as exclusive source of roughage associated with concentrate supplement were kept in a feedlot with feeders and waterers. Before feeding time, the leftovers from the previous day were collected. The animals receiving the PDSP strategy were first milked and, after the morning milking, were confined and offered corn silage (15 kg/animal/day of corn silage mixed with 700g/animal of protein supplement). After afternoon milking, PDSP animals received 500g/animal protein supplement to complete 1,200 grams/animal/day. The corn was grown on the experimental farm itself and ensiled in a bunker silo at the dough to dent stage.

Forage mass was estimated by cutting forage samples above ground level using 1.0 m² squares. Forage samples were collected every 15 days and weighed. The estimated forage mass available in each paddock averaged 5.22 t/ha of dry matter at the beginning of the experiment. During forage collection, pasture was sampled by the hand-plucking method. Whole-plant samples collected using squares and by the hand-plucking technique, concentrate supplements and corn silage were analyzed for dry matter (DM) (INCT - CA G-003/1), ash (INCT - CA M-001/1), crude protein (CP) (INCT - CA N-001/1), ether extract (EE) (INCT- CA G-004/1), neutral detergent fiber (NDF) (INCT - CA F -002/1). Acid detergent fiber (ADF) (INCT - CA F-004/1), lignin (INCT-CA F-005/1) and non-fibrous carbohydrates (NFC = 100 - ash - EE - NDFap - CP) were corrected for ash (INCT - CA M-002/1) and protein (INCT - CA N-004/1) according to the methods recommended by Detmann et al. (2012) (Table 1).

Milk yield (MY) was evaluated individually in two daily milkings at 7 a.m. and 3 p.m. once a week for five weeks, with the presence of the calf to stimulate milk release. After milking, the calves remained with their mothers and were allowed to suckle the residual milk. Milk yield was corrected to 3.5% fat content (FC) using the equation proposed by Sklan, Ashkenazi, Braun, Devorin, & Tabori (1992), MY 3.5% = MY × (0.432 + 0.163 × FC).

Table 1
Chemical composition of deferred pasture, corn silage and concentrate supplements used in the experiment

Item[1]	Ingredients (g/kg of DM)				
	Deferred pasture[2]	Deferred pasture[3]	Corn silage	Concentrate supplement	Nutrilac®[4]
Dry matter	644	663	316	788	861
Crude protein	33	47	70	1000	236
Ether extract	9	10	52	47	74.5
NDFap	697	624	424.8	159	318
ADF	456	400	241	27	41
Lignin	61	31	34	5	2
Total digestible nutrientes[1]	385	453	633	753	702
In vitro dry matter digestibility	524	552	596	920	931
In vitro crude protein digestibility	362	451	562	956	945
In vitro NDFap digestibility	456	576	488	920	900

[1]DM - Dry matter; NDFap - neutral detergent fiber corrected for ash and protein; ADF - acid detergent fiber. [2]Deferred *Urochloa* (*Syn. Brachiaria*) *decumbens* cv. Basilisk pasture - Whole plant. [3]Deferred *Urochloa* (*Syn. Brachiaria*) *decumbens* cv. Basilisk pasture - Simulated grazing. [4]Commercial ration for dairy cattle.

Milk samples of 50 mL were collected directly from the automatic meter, and weekly composite samples proportional to the morning and afternoon milk yields were made, always on the same day, as recommended by Broderick and Clayton (1997). Samples were preserved with Bronopol® (2-bromine, 2-nitropropane 1,3-diol) for determination of fat, protein, lactose, defatted dry extract (DDE), somatic cell count (SCC), total solids, milk urea nitrogen (MUN) and casein contents. The percentage of fat, protein content, lactose, DDE, MUN, SCC and casein were determined using flow cytometry. The costs with concentrates, roughage and total diet were calculated by multiplying the intake by the respective price of each component (which was calculated according to its composition and the price of each ingredient) according to Borges et al. (2019). The values per kilogram of feed ingredients were: corn silage - $0.05; concentrate - $0.46; and pasture rental rate - $0.37. The values are expressed in $ (dollars), considering the ratio of R$4.06 (real) for $1.

An electronic scale (Coima, São Paulo, Brazil) was used to evaluate the body weight of the animals (cows and calves). The animals were weighed at the beginning and end of the experiment. Body condition scores (BCS) were evaluated at the beginning and end of the experiment by a single technician. A 1 to 5-point scale with 0.10-point intervals was used to analyze the body condition score, in which 1 represents a very lean cow and 5 an obese cow (adapted from Mishra, Kumari, & Dubey, 2016).

Given the homogeneity of body weight, lactation stage, age and forage supply, a 4 x 5 completely factorial design with four supplementation strategies and five weeks of evaluation was used. Each animal was considered as an experimental unit (nine experimental units for each treatment). Data were subjected to analysis of variance using GLM procedure of SAS version 9.0. (Statistical Analysis System Institute [SAS Institute], 2008). Data normality (Shapiro-Wilk test at a significance level of 5%) was tested by the UNIVARIATE procedure of SAS. The statistical model was as follows: $\hat{Y}ijk = \mu + Ti + Wj$, Ti x Wj + εijk, in which $\hat{Y}ijk$ represents the observed response to treatment (Ti) (with i = 1, 2, 3 and 4) in the animal k (with k = 1, 2, 3 ..., 9), Wj represents the observed response to week (with J = 1, 2, 3, 4 and 5); Ti x Wj is the effect of interaction between treatment and week; μ is the overall mean and εijk experimental error associated with all observations, assumed to be independently and identically distributed in a normal distribution with mean zero and variance δ2.

Supplementation strategies (treatments) and interactions were compared by the Tukey's test when significant by the F-test. Test weeks were subjected to regression analysis using the REG procedure of SAS (SAS Institute, 2008). Differences were considered significant at α=0.05.

Results

There was no interaction (P=0.99) between supplementation strategies and test days on milk yield and chemical composition of F1 Holstein x Zebu cows (Table 2). The mean milk yield of cows on deferred pasture + protein, and in deferred pasture supplemented with more protein corn silage had an average production of 11.50 kg/day (P>0.05), which was 14.30% lower than feedlot cows receiving concentrate supplement and corn silage (CSS). The 3.5% fat-corrected milk yield was 21.86% lower in animals on deferred pasture + protein, and in deferred pasture supplemented with more protein corn silage, with an average yield of 11.08 kg/day (P>0.05) compared to cows receiving CSS. There was no effect of weeks on milk yield (P = 0.17) and 3.5% fat-corrected milk yield (P = 0.10). Fat content (P<0.01), protein (P<0.01), lactose (P<0.01), DDE (P<0.01), total solids (P<0.01), SCC (P = 0.04) and casein (P<0.01) of milk were affected by different supplementation strategies. Cows fed CSS had higher fat content and total solids in milk than cows subjected to other feeding strategies. Milk fat, protein, DDE and casein were reduced by 0.012, 0.0028, 0.0036 and 0.0037% for each test day, respectively.

Somatic cell count (SCC; P = 0.04) in the milk was higher for CSS- and PDPII-fed cows than for cows subjected to other supplementation strategies. The PDPI and CSS strategies resulted in higher DDE (P<0.01) and casein (P<0.01) content in milk. The lowest feeding costs were observed in animals receiving the PDPI, and PDPII strategies, respectively, compared to other strategies.

There was a significant interaction (P<0.01) between supplementation strategies and test days on milk urea nitrogen content (MUN; Table 3). In the first week of evaluation, the animals receiving the PDSP diet had a MUN 23.96% higher compared to cows receiving PDPI, and CSS strategies (mean of 16.55 mg/dL). In the other weeks of evaluation (7, 14, 28 and 35), there was no difference (P>0.05) between strategies for the MUN content, with a mean of 16.95 mg/dL.

The MUN content decreased linearly by 0.17 and 0.06 mg/dL for each test day in cows receiving the PDPII and CSS supplementation strategies, respectively. On the other hand, MUN content increased by 0.0025 mg/dL for each test day in PDPI cows. The means for the MUN content in cows receiving the PDSP strategy fitted the quadratic model, reaching a minimum point at 25 days.

Table 2
Milk yield and composition of F1 Holstein x Zebu cows under different concentrate supplementation strategies

Items[1]	Supplementation strategies[2]					p-value[4]		
	PDPI	PDPII	PDSP	CSS	SEM[3]	Treat	Days	Treat x Days
Milk yield, kg/day	11.42 b	10.99 b	12.09 b	13.42 a	0.33	<0.01	0.17	0.99
3.5% fat-corrected milk yield, kg/day	11.10 b	10.93 b	11.22 b	14.18 a	0.41	<0.01	0.10	0.99
Fat, %[5]	3.30 bc	3.45 b	3.04 c	3.84 a	0.10	<0.01	<0.01	0.92
Protein, %[5]	3.03 a	2.82 b	2.89 b	3.07 a	0.03	<0.01	0.04	0.25
Lactose, %	4.61 b	4.56 b	4.76 a	4.57 b	0.03	<0.01	0.41	0.47
DDE, %	8.59 a	8.34 b	8.60 a	8.58 a	0.05	<0.01	0.03	0.54
Total solids, %[5]	11.89 b	11.79 b	11.64 b	12.42 a	0.12	<0.01	0.41	0.69
SCC, thousand cells/mL[5]	92.93 b	132.71 a	64.93 b	129.68 a	23.20	0.04	0.36	0.97
Casein, %[5]	2.41 a	2.23 b	2.28 b	2.42 a	0.03	<0.01	<0.01	0.29
Feeding cost, $/day	0.70	0.96	1.67	1.81	0.12	-	-	-

[1]DDE: defatted dry extract; SCC: somatic cell count.
[2]PDPI: Deferred pasture as a source of roughage + 700 grams of protein supplement; PDPII: Deferred pasture as a source of roughage + 1,200 grams of protein supplement; PDSP: deferred pasture + 15 kg of corn silage (natural basis) + 1,200 grams of protein supplement; CSS: corn silage (ad libitum) + 700 grams of protein supplement. Means followed by equal letters do not differ by Tukey's test (α=0.05).
[3]SEM - standard error of the mean.
[4]P - Probability.
[5]Regression equation: $\hat{Y}$ fat = 3.61-0.012*X, R^2= 0.72; $\hat{Y}$ protein = 3.00 - 0.0028*X, R^2 = 0.60; $\hat{Y}$ DDE = 8.58 -0.0036*X, R^2=0.53; $\hat{Y}$ Casein= 2.39 - 0.0037X, R^2= 0.16.

Supplementation strategies did not change the final body weight (P = 0.11) and body condition score (P = 0.19) of cows and final body weight of calves (P = 0.95). The highest body weight gain (P = 0.03) was observed in cows receiving CSS compared to cows receiving PDPI, although similar to other supplementation strategies (Table 4). There was no difference for total weight gain in calves (mean of 13.85 of kg gain).

Table 3

Milk urea nitrogen content (mg/dL) of F1 Holstein x Zebu cows under different concentrate supplementation strategies

Supplementation strategies[1]	Test days					SEM[2]	p-value[3]		
	1	7	14	28	35		Treat	Days[4]	Treat x Days
PDPI	15.78 b	19.90 a	16.57 a	15.88 a	18.25 a	1.33	0.11	<0.01	<0.01
PDPII	19.90 ab	22.25 a	19.08 a	15.15 a	16.30 a				
PDSP	22.65 a	17.45 a	14.93 a	12.48 a	16.85 a				
CSS	17.32 b	19.48 a	14.79 a	14.95 a	16.93 a				

[1]PDPI: Deferred pasture as a source of roughage + 700 grams of protein supplement; PDPII: Deferred pasture as a source of roughage + 1,200 grams of protein supplement; PDSP: deferred pasture + 15 kg of corn silage (natural basis) + 1,200 grams of protein supplement; CSS: corn silage (ad libitum) + 700 grams of protein supplement. Means followed by equal letters do not differ by Tukey's test (α=0.05).
[2]SEM - standard error of the mean.
[3]P - Probability.
[4]Regression equation: $\hat{Y}PDPI = 17.23 + 0.0025X$, $R^2=0.0004$; $\hat{Y}PDPII = 21.43 - 0.17^*X$, $R^2=0.72$; $\hat{Y}PDSP = 23.68 - 1.00^*X + 0.02^*X2$, $R^2 = 0.96$; $\hat{Y}CSS = 17.68 - 0.06^*X$, $R^2 = 0.18$.

Table 4

Performance of cows and calves at the beginning and end of the experimental period under different concentrate supplementation strategies

Items[1]	Supplementation strategies[2]				SEM[3]	p-value[4]
	PDPI	PDPII	PDSP	CSS		
Cows						
Final body weight, kg	475.77	500.11	480.44	534.44	18.00	0.11
Total gain, kg	-10.66b	-8.33ab	-1.77ab	5.44a	3.94	0.03
BCS	3.85	3.87	3.88	3.92	0.02	0.19
Calves						
Initial body weight, kg	56.33	61.00	56.11	59.55	4.07	0.78
Final body weight, kg	70.00	72.11	72.75	73.55	4.49	0.95
Total gain, kg	13.66	11.11	16.63	14.00	1.93	0.27

[1]BCS: Body condition score.
[2]PDPI: Deferred pasture as a source of roughage + 700 grams of protein supplement; PDPII: Deferred pasture as a source of roughage + 1,200 grams of protein supplement; PDSP: deferred pasture + 15 kg of corn silage (natural basis) + 1,200 grams of protein supplement; CSS: corn silage (*ad libitum*) + 700 grams of protein supplement. Means followed by equal letters do not differ by Tukey's test (α=0.05).
[3]SEM - standard error of the mean.
[4]P - Probability.

Discussion

Deferment of pasture use is a fundamental strategy to conserve forage mass for use in times of food shortage and to ensure adequate nutrient supply to animals. In this study, the deferral of grazing in *Urochloa decumbens* cv. Basilisk allowed offering 5.22 t DM/ha of forage mass to the cows. However, the nutritional value of the deferred pasture was not sufficient to meet the nutritional requirements for milk production due to the advanced physiological maturity of plants, which were primarily limited by nitrogen (4.7% of DM; Table 1) and, later, by energy. Thus, the use of concentrate supplementation strategies was essential to correct forage-limiting nutrients and to ensure milk production in deferred pasture-based systems (mean of 11.50 kg/day). According to Peres et al. (2012), the use of tropical forage plants is a low-cost strategy in dairy systems, allowing a more competitive operation. The authors also state that Holstein x Zebu crossbred cows on pasture have the potential to produce from 11.6 to 12.4 kg of milk/day without concentrate supplementation in the rainy season. In this study, it is evident that the correct pasture management associated with the deferral of grazing may be advantageous to farmers since the average milk yield of cows on deferred pasture receiving concentrate supplement during the dry season was similar to that reported in the rainy season. When there is no potentially digestible dry matter from forage, the feedlot strategy based on providing corn silage and concentrate supplementation resulted in a milk yield increase of 14.30% compared to other strategies using deferred pasture with concentrate supplement. This difference is explained by the additional supply of protein and mainly energy provided by corn silage associated with the protein supplement.

Despite the low nutritional quality of tropical forages, the deferred *Urochloa decumbens* cv. Basilisk pasture was efficient in providing potentially degradable substrate for lactating cows since the difference in milk yield between pasture-based and CSS strategies were not discrepant. Oliveira et al. (2014) reported a milk yield of 10.93 kg/day in Holstein x Zebu cows on Tanzania grass pasture (*Panicum maximum* Jack.) with 13.43% crude protein in the summer season. These results allow us to infer that the correct use of deferred grazing associated with concentrate supplementation is a viable strategy in term of milk production in Holstein x Zebu cows during the winter since the production cost is lower than that of intensive and semi-intensive systems using corn silage as a source of roughage.

Therefore, the supply of 15 kg of corn silage (on natural basis/animal) to cows after milking was not feasible because it increased production costs without changing milk yield. Santana et al. (2019b) reported that mid-lactation Holstein x Zebu cows (111.5 days in milk) in feedlot receiving corn silage and concentrate supplement produced 13.03 kg of milk/day. In our study, the CSS strategy provided nutrients for the production of 13.42 kg/day and 14.18 kg/day (3.5% fat-corrected milk).

Milk fat content was higher in cows receiving CSS than in cows subjected to the other treatments. This occurs because corn silage digestibility contributes to greater availability of short-chain fatty acids (acetic and butyric) in the rumen, which are precursors of milk fat in the mammary gland

(Oliveira et al., 2014). According to the same authors, milk fat content is influenced by the roughage: concentrate ratio given the increase in pH and improvement of cellulolytic bacteria activity in roughage-rich diets, which leads to higher acetic acid production (Peres et al., 2012). The roughage: concentrate ratio in deferred grazing systems increased due to the amount of concentrate supplement consumed by the cows, which is theoretically enough to increase milk fat content. However, the low rate of fiber degradation in the rumen associated with pasture particle size and time spent ruminating possibly did not favor increased production of acetic acid in the rumen (Van Soest, 1994; Sousa et al., 2018; Santana et al., 2019ab). According to Peres et al. (2012), milk fat content can also be altered by genetic variations, which did not occur in this research because animals belong to the same genetic group. For Lucci (1997), the dietary nitrogen source may influence milk fat content, so that increases in dietary protein concentration from 12% to 18% of DM reduces fat concentration by up to 5 g/kg milk due to increased milk yield. There are small additions in protein content with increased dietary protein levels, which was observed in this study. Bachman (1992) reported that the dietary crude protein content must increase by approximately 100% and daily dry matter intake requirements must be met in order to increase milk protein content.

Higher levels of protein content in milk were observed for cows in the CSS and PDPI treatments (mean of 3.05%), which can be explained by the higher casein levels compared to the other diets. Casein is the most important milk protein, representing about 85% of milk proteins, and there are many types of casein with similar molecular structures. The low means for milk protein content for cows in the PDPII and PDSP treatments (mean of 2.85%) are not within the standards of the Normative Instruction 76 of November 26, 2018, determined by Ministry of Agriculture, Livestock and Food Supply Ministério de Agricultura, Pecuária e Abastecimento [MAPA], (2018), which establishes criteria for the improvement and modernization of the public health legislation regulating milk production. For the Brazilian legislation, milk must have a minimum of 2.9% of protein content to be classified as type C - cold raw milk. These changes in casein levels and, consequently, milk protein may be associated with lower alveolar cell efficiency, thus justifying the lower values observed in cows receiving PDPII and PDSP, with supplement intakes of 2.7 kg/day and 3.7 kg/day, respectively. According to Schingoethe et al. (1996); Peres et al. (2012), the energy content of the diet is one of the main factors influencing the protein content of milk. In our study, the supply of concentrate supplements favored an increase in dietary energy content by providing non-fibrous carbohydrates (NFC), which are rapidly fermentable in the rumen and may result in higher production of propionate in the rumen (Van Soest, 1994). In turn, it reduces the use of amino acids for gluconeogenesis, allowing their use for milk protein synthesis. Moreover, the synchronization of ruminal degradation between nitrogen compounds and carbohydrates also plays an important role (Schingoethe, 1996), which may increase the efficiency of microbial protein synthesis by making amino acids available in milk.

High lactose levels were found only in PDSP cows (4.76%), which can be explained by the adequate dietary energy supply, favoring the production of propionic acid in the rumen

(Van Soest, 1994). This short-chain fatty acid is subsequently converted into glucose in the liver and is the precursor of lactose in the mammary gland. Overall, variations in milk components may be associated with nutrient metabolism in the animal as some studies found that feeding does not influence lactose content in milk (Schingoethe, 1996; Peres et al., 2012). However, it seems to involve complex multiple nutritional factors since circulating glucose content in the bloodstream influences lactose synthesis in the Golgi apparatus of mammary gland cells, justifying the variations in lactose content observed in this study.

Cows receiving the PDPII and CSS treatments had higher SCC compared to animals subjected to other strategies. However, the SCC values are below the required (500,000 cells/mL) by the current instruction (76 and 77) (MAPA, 2018) and are indicative of good mammary gland health.

There was a significant difference between different supplementation strategies for MUN, in which the highest MUN concentration on the first day of evaluation was observed in PDSP cows (22.65 mg/dL) compared to other animals receiving other strategies. These variations are explained by the protein: energy ratio of diets. It indicates that carbon skeletons were not available for rumen microbial protein synthesis since the type of supplementation fed to animals increased the ammoniacal nitrogen content and the conversion of ammonia into urea in the liver. Therefore, adjusting the inclusion of fermentable carbohydrates or soluble protein levels in the supplement for cows on deferred pastures is essential. The lack of carbon skeletons for microbial protein synthesis in PDSP treatment may be associated with the low carbohydrate degradation rate in the cell wall of forage plants due to high lignin content at this stage.

Conclusion

F1 Holstein x Zebu cows on deferred *Urochloa decumbens* cv. Basilisk pasture and supplemented with concentrate maintains milk production at 11.50 kg with normal composition, maintaining satisfactory body weight and condition score.

Protein supplementation and corn silage supply associated with the deferral of grazing does not improve yield and milk composition of cows, but increases the feeding cost and considerably reduces the profit margin of the dairy activity.

The feedlot strategy based on corn silage as a roughage source associated with concentrate supplement improves milk yield but increases feeding costs and may lead to reduced profitability.

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