



## Effects of short-term feed restriction on the physiological parameters and metabolites of F1 Holstein x Zebu cows in different stages of lactation

[Efeitos da restrição alimentar de curto prazo sobre os parâmetros fisiológicos e metabólicos de vacas F1 Holstein x Zebu em diferentes estágios da lactação]

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### ABSTRACT

This study aimed to evaluate the effects of different nutritional plans on the productive, physiological and metabolic parameters of F1 ½ Holstein x ½ Zebu cows in different stages of lactation. Sixty lactating cows were allotted to a completely randomized 5 x 3 factorial design with five feed allowances and three lactation periods. The dry matter intake, milk yield and heart rate were reduced by 5.69kg, 2.41kg and 10.36 beats/min (morning) and 10.25 beats/min (afternoon) for each 1% feed restriction, respectively. There was no difference in the concentration of glucose, total protein, albumin, cholesterol and non-esterified fatty acids for cows subjected to different feed allowances, with means of 95.25, 7.98, 2.95, 121.68 and 0.45mg/dL, respectively. Feed restriction of up to 2.50% BW is a cost reduction strategy that does not alter milk yield, regardless of the stage of lactation.

Keywords: cholesterol, respiratory rate, glucose, NEFA

### RESUMO

Objetivou-se avaliar os efeitos de diferentes planos nutricionais sobre as características produtivas, fisiológicas e metabólicas de vacas F1 ½ Holandês x ½ Zebu. Foram utilizadas 60 vacas em lactação, seguindo-se o delineamento inteiramente ao acaso, em esquema fatorial 5 x 3, com cinco níveis de oferta de dieta e três períodos de lactação. À medida que se aumentou 1% na restrição da oferta da dieta, houve redução linear de 5,69kg no consumo de matéria seca pelos animais, 2,41kg na produção de leite, bem como de 10,36bat/min (manhã) e 10,25 bat/min (tarde) na frequência cardíaca dos animais. Não houve diferença para a concentração de glicose, proteínas totais, albumina, colesterol e NEFA com a restrição na oferta da dieta dos animais, sendo a média de 95,25, 7,98, 2,95, 121,68 e 0,45mg/dL, respectivamente. Recomenda-se a restrição de até 2,50% de peso corporal como estratégia de redução dos custos em todos os estágios em lactação, visando não alterar, economicamente, a produção de leite.

Palavras-chave: colesterol, frequência respiratória, glicose, NEFA

### INTRODUCTION

Milk is one of the most important foods in the human diet due to its high and complete nutritional value. Approximately 798 billion liters of milk are consumed annually by humans, either

fresh or processed. India is the world's largest milk producer with 179 billion liters produced per year, while Brazil ranks fourth in this ranking with 35.23 billion liters produced in 2018 (Anuário, 2018). Despite the high milk production in Brazil, the current productivity of 1.6 thousand liters per

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cow per year is lower than the world's average (3.5 thousand liters).

Most Brazilian farmers use Holstein x Zebu crossbred cows for overcoming the challenges posed by tropical climates to dairy production. Nowadays, about 80% of the milk in the country is produced by Holstein x Zebu crossbred cows (Salgado *et al.*, 2016). Crossbred cows reared under forage-based milk production systems are flexible in terms of milk production during the summer due to their adaptability and hardiness. On the other hand, in the winter, cows have to be supplemented in feed troughs due to the seasonal shortage of forage (Santos *et al.*, 2012; Borges *et al.*, 2019). Forage-based production can be more profitable than intensive dairy systems since feed has a significant contribution to the costs of milk production. Moreover, the profit depends on the lactation curve of cows, which represents the level of productivity throughout the lactation (Silva *et al.*, 2019).

Restricted feed allowance for cows in different stages of lactation kept under intensive systems has been used as an alternative to maximize nutrient use efficiency in several dairy breeds (Ferraretto *et al.*, 2014; Gabbi *et al.*, 2016; Monção *et al.*, 2019; Santana *et al.*, 2019). However, Ferraretto *et al.*, (2014) report that feed restriction may modify productive, reproductive and blood parameters in Holstein cows. However, information about physiological parameters of feed restricted F1 ½ Holstein x ½ Zebu crossbred cows in different stages of lactation in the tropical conditions of Brazil is scarce in the literature (Santos *et al.*, 2012, 2014). According to Keogh *et al.*, (2016), feed restriction can modify the size of the organs involved in nutrient metabolism by changing the nutritional requirements of the animals. Therefore, it is essential to determine the best feed allowance plan in order to maximize nutrient use efficiency.

Based on the above, this study aimed to evaluate the effects of different nutritional plans characterized by short-term feeding restriction on dry matter intake and productive, physiological and metabolic parameters of F1 Holstein/Zebu cows.

## 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 (CEBEA-Unimontes protocol n. 128/2016). The experiment was conducted at the experimental farm of EPAMIG in Felixlândia, Minas Gerais, Brazil (18°04'04" South latitude and 44°58'48" West longitude, 616 m above sea level). According to Köppen-Geiger classification, the region has a humid tropical savanna climate (AW) with dry winter and rainy summer.

Sixty F1 ½ Holstein x ½ Zebu cows with an average weight of  $482 \pm 35$ kg and mean parity of 4.5 were divided into three lactation stages (early lactation:  $42.6 \pm 12.77$  days in milk; mid-lactation:  $108.2 \pm 12.07$  days in milk, and late lactation:  $173.6 \pm 16.64$  days in milk). Each lactation stage consisted of five treatments with four animals per treatment. The 20 animals per lactating stage were managed under different feed allowances according to the body weight (2.00; 2.25; 2.50; 2.75% of body weight - BW) and a control group fed *ad libitum* (3.40% BW) for a target of 5% refusals (dry matter basis).

Each period lasted 21 days, divided into 16 days of adaptation to diets and five days for samplings. The cows were housed in individual pens of approximately 20 m<sup>2</sup> equipped with feeders and waterers. Milking was performed twice a day, at 7 a.m. and 2 p.m., using a mechanical milking machine, with the presence of the calf to stimulate milk release.

Feed intake was evaluated daily by weighing the feed provided and the refusals. Samples of diets and refusals were stored at -20 °C for further analysis. Diet samples, concentrate ingredients and refusals were analyzed for dry matter (DM; method 967.03), ash (method 942.05), crude protein (CP; method 981.10), and ether extract (EE; method 920.39) according to recommendations of the AOAC (1990). The contents of neutral detergent fiber corrected for ash and protein (using heat-stable alpha-amylase without sodium sulfite) (NDFap; Mertens, 2002; Licitra *et al.*, 1996) and acid detergent fiber (ADF) were determined as described by Van Soest *et al.*, (1991), and lignin was determined by treating the acid detergent fiber residue with

sulfuric acid at 72%. Non-fiber carbohydrate (NFC) contents were calculated as proposed by Detmann *et al.*, (2012):  $NFC (g/kg) = 100 - \text{ash} - EE - NDFap - CP$ . The total digestible nutrients (TDN) were estimated using the formula proposed by NRC (Nutrient..., 2001). A total mixed ration was fed twice a day after each milking, calculated individually based on the body weight of each animal and the leftovers. The diet consisted of 75% corn silage and 25%

commercial supplement containing 22% crude protein and 70% total digestible nutrients. The chemical composition of the dietary components is shown in Table 1.

The environmental conditions during the experimental period and the mean air temperature, relative humidity and black globe temperature and humidity index in the morning and afternoon are shown in Table 2.

Table 1. Chemical composition of ingredients and experimental diet

Item (g/kg as fed) <sup>1</sup>	Corn silage	Concentrate	Diet
Dry matter	502.74	925.92	608.54
Organic matter	962.02	922.31	952.09
Crude protein	68.37	218.25	105.84
NDIN	5.99	12.24	7.55
ADIN	0.79	0.75	0.78
Ether extract	27.77	28.28	27.9
Non-fiber carbohydrates	302.89	371.68	320.09
NDFap	562.98	304.1	498.26
Acid detergente fiber	300.5	72.21	243.43
Lignin	99.58	31.75	82.63
Total digestible nutrients*	561.99	734.16	605.03

<sup>1</sup> Dry basis (grams per kilogram) - DM – Dry matter; NDIN - neutral detergent insoluble nitrogen; ADIN – acid detergent insoluble nitrogen; NDFap – neutral detergent fiber corrected for ash and protein; \* National Research Council - NRC (Nutrient..., 2001).

Table 2. Environmental conditions during the experimental period in two shifts (morning and afternoon)

Period	Air temperature (°C)	RH (%)	BGTHI
Morning	27.0	39.9	75.2
Afternoon	31.7	45.8	80.0
CV (%)	6.6	8.1	4.3

RH - relative humidity; BGTHI - Blackglobe temperature and humidity index; CV - Coefficient of variation.

The individual milk yield (MY) was recorded daily during the experimental period. Milk quality was evaluated using composite milk samples from each cow during five consecutive days (17<sup>th</sup> to 21<sup>st</sup>) in each experimental period. Milk samples were collected during morning and afternoon milking, after weighing the milk, using the equipment Mark V® (DelavalLtda). A mechanical scale was used to measure the body weight of the cows (Valfran, Votuporanga, São Paulo, Brazil) at the beginning and end of the experiment. Body condition scores (BCS) were evaluated weekly by a single examiner at the beginning and end of each experimental period. A 1-5-point scale with 0.10-point intervals was used for BCS, in which 1 represents a very lean cow and 5 an obese cow (adapted from Mishra *et al.*, 2016). The initial BCS of the cows was 3.64±0.05.

The costs with concentrates, roughage and total diet was 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). The values per kilogram of feed ingredients were corn silage: \$0.05; concentrate: \$0.46. The values are expressed in US dollars, considering the ratio of R\$3.5 for \$1.

The blood of each animal was collected from the coccygeal vein in glass tubes containing anticoagulant (sodium fluoride and potassium oxalate), immediately after the afternoon milking (four animals per treatment and lactation stage were used as the experimental unit). Plasma was separated from blood cells by centrifugation at 4,000 rpm for 10 minutes and stored in Eppendorf

tubes frozen at  $-18^{\circ}\text{C}$  for further analysis. Blood metabolites (glucose, cholesterol, urea, total protein, albumin and non-esterified fatty acids - NEFA) were determined by the colorimetric method, using specific commercial kits. The physiological parameters (sweating rate, surface temperature, heart rate, respiratory rate and rectal temperature) were measured after the morning and afternoon milking.

The sweating rate was measured by the same examiner with the aid of a digital stopwatch, according to the methodology modified by Schleger and Turner (1965). Surface body temperature (skin temperature) was measured at the animal's forehead, back, hock and udder using a digital handheld infrared thermometer (four animals per treatment and lactation stage were used as the experimental unit). Heart rate was assessed by auscultation with the aid of a stethoscope placed on the left side of the animal, below the scapula, near the sternum. Respiratory rate was determined by visual assessment, observing flank movements. Rectal temperature was recorded using a digital clinical thermometer inserted directly into the animal's rectum.

A completely randomized 5 x 3 factorial design with five feed allowances and three lactation periods (early, mid and late lactation) was used. Data were analyzed using the SAS software version 9.0 (SAS, 2008). The PROC UNIVARIATE was used to detect outliers and test the normality of residuals. The data were analyzed according to the following model:  $Y_{k(ij)}$

$= \mu + NP_i + LS_j + NP_i \times LS_j + IBW + e_{k(ij)}$ ; in which:  $Y_{k(ij)}$  = observation of feed allowance "k", within the lactation stage "i" and animal "j";  $\mu$  = constant associated with all observations;  $LS_i$  = effect of lactation stage "i", with  $i = 1, 2, 3, 4$  and  $5$ ;  $A_j$  = effect of cow "j", with  $j = 1, 2, 3,$  and  $4$ ;  $NP_{k(ij)}$  = effect of feed allowance "k", with "k" =  $1, 2, 3, 4$  and  $5$ ;  $NP_i \times LS_j$  = interaction between feed allowance and lactation stages;  $IBW$  = initial body weight as a covariate;  $e_{k(ij)}$  = experimental error associated with all observations ( $Y_{k(ij)}$ ), assumed to be independently and identically distributed in a normal distribution with mean zero and variance  $\delta_2$ .

For all variables, the levels of feed allowance (3.40; 2.75; 2.50; 2.25 and 2.00% BW) were evaluated by polynomial regressions to test the linear and quadratic changes caused by increasing feed restriction. Lactation stages were compared by the Student Newman-Kells test (SNK). The reported values are least-square mean values  $\pm$  standard error of the mean (SEM) unless indicated otherwise. The mean values were considered different at  $\alpha < 0.05$ .

## RESULTS

There was no interaction ( $P > 0.05$ ) between feed allowance and lactation stage on any of the variables. i.e., the factors are independent. As feed restriction increased by 1%, there was a linear reduction of 5.69kg in dry matter intake (DMI) and 2.41kg in milk yield (Table 3).

Table 3. Dry matter intake and milk yield of F1  $\frac{1}{2}$  Holstein x  $\frac{1}{2}$  Zebu cows submitted to different feed allowances

Item	Feed allowance (% BW) <sup>1,2</sup>					SEM <sup>3</sup>	P-value <sup>4</sup>
	3.40	2.75	2.50	2.25	2.00		
Dry matter intake, kg/day	16.97	12.59	11.68	9.95	9.16	0.41	<0.01
Reduction in feed intake compared to the control group, % <sup>2</sup>	0	25.81	31.17	41.36	46.02	2.40	<0.01
Milk yield, kg/day <sup>3</sup>	13.47	12.12	11.14	11.13	9.97	0.29	<0.01
Reduction in milk yield compared to the control group, % <sup>4</sup>	0	10.02	17.29	17.37	25.98	2.12	<0.01
Diet cost, \$/day	2.59	1.92	1.78	1.52	1.40	-	-

<sup>1</sup> BW – Body weight. <sup>2</sup>  $\hat{Y} = -2.61 + 5.69 \times X$ ,  $R^2 = 0.98$ ; <sup>3</sup>  $\hat{Y} = 115.41 - 33.569 \times X$ ,  $R^2 = 0.98$ ; <sup>4</sup>  $\hat{Y} = 60.26 - 17.89 \times X$ ,  $R^2 = 0.96$ . \* Significant by the t-test ( $P < 0.05$ ); <sup>3</sup> SEM – standard error of the mean; <sup>4</sup> P – Probability.

The feed restriction at 2% BW reduced the DMI by 46.02% compared with cows fed *ad libitum* (3.40% BW; 16.97kg/day) Increasing feed

restriction decreased milk yield and diet cost by up to 25.98% and 27.08%, respectively. Milk yield was 27.07% higher in early lactation

compared to other lactation stages (mean of 10.29kg/day; Table 4). The body weight and BCS of cows were not affected by lactation stages, with means of 464.95kg and 3.97, respectively. Calves from late lactation cows had the highest body weight (P<0.01).

The heart rate, both in the morning and afternoon, was the only physiological parameter affected by feed restriction (Table 5). For each 1% feed restriction, the heart rate (morning and afternoon) decreased by 10.36 and 10.25 beats/min, respectively.

Table 4. Performance and body condition score of F1 ½ Holstein x ½ Zebu cows at different stages of lactation

Item <sup>1</sup>	Lactation stage <sup>2</sup>			SEM <sup>3</sup>	P-value <sup>4</sup>
	Early	Mid	Late		
Milk yield, kg/day	14.11a	10.85b	9.73b	3.65	0.01
Body weight, kg	469.35	471.1	454.4	2.19	NS
Body condition score	3.94	4.11	3.88	2.32	NS
Calf body weight, kg	56.30c	92.75b	127.75a	4.10	0.01

<sup>1</sup>Means within rows followed by different letters are different by the SNK test (P<0.05). <sup>2</sup>Early lactation (42.60 ± 12.77 days); Mid lactation (108.20 ± 12.07 days); Late lactation (173.60 ± 16.64 days). <sup>3</sup>SEM – standard error of the mean; <sup>4</sup>P – Probability; NS: not significant.

Table 5. Physiological parameters of F1 ½ Holstein x ½ Zebu cows submitted to different feed allowances

Item <sup>1</sup>	Feed allowance (%BW)					SEM <sup>4</sup>	P-value <sup>5</sup>
	3.40	2.75	2.50	2.25	2.00		
RTm (°C)	38.54	38.51	38.46	38.44	38.16	0.19	NS
RTa (°C)	39.08	38.91	38.91	39.05	38.98	0.18	NS
RRm (beats/min)	31.33	28.33	30.00	25.67	31.33	4.85	NS
RRa (beats/min)	33.33	32.67	33.00	34.67	32.17	4.08	NS
HRm (beats/min) <sup>2</sup>	73.33	66.67	65.00	60.00	59.67	3.36	0.01
HRa (beats/min) <sup>3</sup>	81.33	80.33	75.67	76.00	64.67	2.80	<0.01
SRm (g/m <sup>2</sup> /h)	49.97	53.33	57.80	49.95	50.91	8.47	NS
SRa (g/m <sup>2</sup> /h)	53.69	53.78	58.03	49.33	52.28	6.05	NS
STm (°C)	34.97	35.17	34.91	35.45	35.00	0.55	NS
STa (°C)	37.47	37.46	37.50	37.84	37.14	0.62	NS

<sup>1</sup>RTm - Rectal temperature of cows in the morning; RTa - Rectal temperature of cows in the afternoon; RRm - respiratory rate of cows in the morning; RRa - respiratory rate of cows in the afternoon; HRm - heart rate of cows in the morning; HRa - heart rate of cows in the afternoon; SRm - sweating rate of cows in the morning; SRa - sweating rate of cows in the afternoon; STm - surface temperature of cows in the morning; STa - surface temperature of cows in the afternoon. <sup>2</sup> $\hat{Y} = 38.22 + 10.36 * X$ , R<sup>2</sup>= 0.97; <sup>3</sup> $\hat{Y} = 49.17 + 10.25 * X$ , R<sup>2</sup>= 0.68. \*Significant by the t-test (P<0.05). <sup>4</sup>SEM – standard error of the mean; <sup>5</sup>P – Probability; NS: not significant.

The rectal temperature, heart rate and sweating rate in the morning and afternoon showed no significant differences between lactation stages (P>0.05; Table 6). Early and mid-lactation cows had higher respiratory rates in the morning than late lactation animals.

Only the plasma urea concentration was affected by feed allowance (Table 7). For each percentage unit of feed restriction, plasma urea concentration increased by 0.42mg/dL. There was no difference in the concentration of glucose, total protein, albumin, cholesterol and non-esterified fatty acids (NEFA) with feed restriction, with means of 95.25, 7.98, 2.95, 121.68 and 0.45mg/dL,

respectively. Glucose (P<0.01) and total protein (P=0.03) levels were higher in mid and late lactation cows than in early lactation cows (Table 8). Albumin concentration in mid-lactation cows was 0.54mg/dL higher than that found in late lactation cows. Cows at early lactation had higher cholesterol content (35.89%) compared to late lactation cows. There was no difference between lactation stages on NEFA concentration, with a mean of 0.44mg/dL. Urea concentration was 33.51% higher in late lactation cows compared to early and mid-lactation cows (31.86mg/dL).

Table 6. Physiological parameters of F1 ½ Holstein x ½ Zebu cows at different stages of lactation

Item <sup>1</sup>	Lactation stage <sup>2</sup>			SEM <sup>3</sup>	P-value <sup>4</sup>
	Early	Mid	Late		
RTm (°C)	38.33	38.44	38.49	0.19	NS
RTa (°C)	39.05	38.94	38.97	0.18	NS
RRm (beats/min)	33.40a	30.60a	24.00b	5.28	<0.01
RRa (beats/min)	27.90c	39.20a	32.40b	4.32	<0.01
HRm (beats/min)	64.00	64.60	66.20	3.45	NS
HRa (beats/min)	74.20	77.40	75.20	2.84	NS
SRm (g/m <sup>2</sup> /h)	806.35	613.87	820.11	9.95	NS
SRa (g/m <sup>2</sup> /h)	722.95	676.52	765.11	6.17	NS
STm (°C)	34.77b	34.82b	35.71a	0.58	<0.01
STa (°C)	37.37b	35.44c	39.64a	0.18	<0.01

<sup>1</sup>RTm - Rectal temperature of cows in the morning; RTa - Rectal temperature of cows in the afternoon; RRm - respiratory rate of cows in the morning; RRa - respiratory rate of cows in the afternoon; HRm - heart rate of cows in the morning; HRa - heart rate of cows in the afternoon; SRm - sweating rate of cows in the morning; SRA - sweating rate cows in the afternoon; STm - surface temperature of cows in the morning; STa - surface temperature of cows in the afternoon. Means within rows followed by different letters are different by the SNK test (P<0.05). <sup>2</sup>Early lactation (42.60 ± 12.77 days); Mid lactation (108.20 ± 12.07 days); Late lactation (173.60 ± 16.64 days). <sup>3</sup>SEM – standard error of the mean; <sup>4</sup>P – Probability; NS: not significant.

Table 7. Metabolic profile of ½ Holstein x ½ Zebu cows under different feed allowances

Item (mg/dL) <sup>1</sup>	Feed allowance (%BW) <sup>2</sup>					SEM <sup>3</sup>	P-value <sup>4</sup>
	3.40	2.75	2.50	2.25	2.00		
Glucose	93.78	94.27	97.28	97.11	93.82	5.97	NS
Total proteins	7.95	8.22	7.84	8.14	7.75	3.80	NS
Albumin	2.64	3.32	2.84	2.97	2.98	3.50	NS
Cholesterol	124.29	129.16	110.89	123.22	120.85	6.70	NS
NEFA	0.40	0.49	0.39	0.45	0.52	8.42	NS
Urea	34.88	35.82	35.23	41.61	43.94	2.78	<0.01

<sup>1</sup>NEFA - non-esterified fatty acids. Means within rows followed by different letters are different by the SNK test (P<0.05); <sup>2</sup> $\hat{Y} = 54.68 - 0.42 * X$ , R<sup>2</sup> = 0.67. \*Significant by the t-test (P<0.05); <sup>3</sup>SEM – standard error of the mean; <sup>4</sup>P – Probability; NS: not significant.

Table 8. Metabolic profile of F1 ½ Holstein x ½ Zebu cows at different stages of lactation

Item (mg/dL) <sup>1</sup>	Lactation stage <sup>2</sup>			SEM <sup>3</sup>	P-value <sup>4</sup>
	Early	Mid	Late		
Glucose	71.43b	105.16a	109.16a	6.47	<0.01
Total proteins	7.28b	8.26a	8.40a	4.04	0.03
Albumin	2.93ab	3.23a	2.69b	3.81	<0.01
Cholesterol	147.80a	122.50ab	94.75b	7.53	<0.01
NEFA	0.52	0.45	0.37	8.88	NS
Urea	33.03b	30.69b	47.92a	2.65	<0.01

<sup>1</sup>NEFA - non-esterified fatty acids. Means within rows followed by different letters are different by the SNK test (P<0.05). <sup>2</sup>Early lactation (42.60 ± 12.77 days); Mid lactation (108.20 ± 12.07 days); Late lactation (173.60 ± 16.64 days). <sup>3</sup>SEM – standard error of the mean; <sup>4</sup>P – Probability; NS: not significant.

## DISCUSSION

Dry matter intake is one of the main factors affecting the health and performance of ruminants (Nutrient..., 2001). According to the NRC (Nutrient..., 2001), the cow's metabolic weight, lactation week and milk fat content are determining factors of dry matter intake (DMI),

which would be estimated at 20.61g/kg BW (9.93kg/day) for crossbred cows used in this research. However, a voluntary dry matter intake of 34.84g/kg BW (mean of 16.97kg/day) was observed for cows in different stages of lactation, which is higher than the NRC (Nutrient..., 2001) estimate for maintenance and production in Holstein/Zebu crossbred cows. This difference

can be explained by the fact that NRC (Nutrient..., 2001) estimates are based on Holstein cows in temperate regions. Holstein x Zebu crossbred cows in early lactation (Monção *et al.*, 2019) and late lactation (Santana *et al.*, 2019) fed different diets without restriction had a dry matter intake of 33.0g/kg BW (15.70kg/day), which is similar to that found in this study (15.88kg/day). Borges *et al.*, (2019) reported a DMI of 19.43kg in ½ Holstein x ½ Zebu cows receiving sorghum silage and concentrate, producing an average of 13.62kg milk/day, presenting higher intake than animals obtained without feed restriction.

In this study, the dry matter intake was below the NRC (Nutrient..., 2001) estimate only for cows fed-restricted at 2.0% of body weight. It shows that F1 ½ Holstein x ½ Zebu cows subjected to feed restriction up to 2.25% BW were able to maintain milk production with up to 17.37% reduction in feed production and 41.36% in feed cost compared to non-restricted cows. The feed allowance of 2% of BW reduced the milk yield by 25.98%. Therefore, it is not an advantageous strategy, especially in late lactation, although the reduction in feed cost reached 46.02%. The feed allowance of 2.50% BW was the best strategy from the point of view of production and nutrition, especially for primiparous cows that are still growing muscle. In this study, only multiparous cows were used. F1 ½ Holstein x ½ Zebu cows can be subjected to feed restriction without losing production due to their dual-purpose traits. Therefore, cows' body reserves may have favored them under conditions of limited DMI supply. However, the body weight of cows was similar between lactation stages (mean of 464.95kg).

The lowest milk yields were observed for cows in mid and late lactation. The proximity of early lactation ( $42.6 \pm 12.77$  days in milk) to peak production explains this higher production. According to Daltro *et al.*, (2019) and Monção *et al.*, (2019), the peak production of F1 Holstein/Zebu cows occurs around the fourth lactation week, approximately 23 to 32 days after calving. According to Santana *et al.*, (2019), the lower milk yield for cows in mid and late lactation is associated with the accumulation of body fat, especially when fed corn silage and concentrate.

Feed restriction did not change the respiratory rate (RR) of cows (mean of 31.25 beats/min).

However, the RR in the morning was 25% higher for cows in early and mid-lactation than in late lactation cows (mean of 24 beats/min). It probably occurs because larger dairy animals produce more heat and thus need to lose heat through latent heat of evaporation. Changes in physiological parameters occur when heat transfer mechanisms (radiation, conduction and convection) are not sufficient to dissipate heat. As a result, mechanisms for dissipating heat are activated, including sweating and increased respiratory rate. At this point, the thermoregulatory center located in the hypothalamus initiates the thermolysis, especially by evaporation, thus increasing the respiratory rate (McDowell and Jones, 1975).

Heat stress is characterized by increased surface and rectal temperature of animals. Under this condition, the thermoregulation mechanisms controlled by the endocrine and nervous systems are triggered, and the primary responses include increased respiratory and heart rates (Arcaro Junior *et al.*, 2003; Martello *et al.*, 2004). According to Buffington *et al.*, (1981), THI values of up to 74 indicate a comfort situation for animals, while values from 74 to 78 indicate alert and values from 79 to 84 indicate danger. Values above 84 indicate an emergency and require intervention in the production system. In this study, the black globe temperature and humidity index (BGTHI) in the morning was 75.2, while it reached 80 in the afternoon. However, the rectal temperature of cows showed that they were efficient in maintaining the thermal balance.

The effects of heat stress on heart rate are variable. The increase or decrease in heart rate depends on the intensity of stress to which animals are subjected and their ability to adapt. At moderate thermal stress, the heart rate is reduced as a response to peripheral vessel dilation (Kadzerea *et al.*, 2002). The effects of heat stress are more pronounced in animals with high metabolic rate (West, 2003; Martello *et al.*, 2004), and it was observed by the increase of heart rate in cows with higher dry matter intake.

Feed restriction did not change the concentration of plasma glucose, total proteins, albumin, cholesterol and NEFA of F1 ½ Holstein x ½ Zebu crossbred cows. However, plasma glucose was lower in early lactation than in mid and late lactation cows, possibly because milk yield is different between lactation stages and also

because these cows were in negative energy balance. Plasma glucose concentrations in mid and late lactation cows were above the reference values suggested by Kaneko *et al.*, (2008), which range from 45 to 75mg/dL. It is explained by the lower milk yield, which would reduce the demand for glucose from the mammary gland. According to Payne and Payne (1987), glucose concentrations in ruminants show little variability due to the high capacity for hepatic gluconeogenesis. Moreover, adaptation mechanisms keep blood glucose levels within a narrow range because this component is essential to specific tissues such as the brain and mammary gland.

The concentration of total proteins (mean of 7.98mg/dL) and albumin (mean of 2.95mg/dL) were within the range suggested by Broderick and Clayton (1997) of 6.48 - 9.00mg/dL for total proteins and 2.95 - 3.48mg/dL for albumin. Only for urea concentration, in mid-lactation, differences were observed between animals subjected to restriction of 2.25 and 2.0% BW compared to animals without restriction. It demonstrates the highest excretion of urea by animals subjected to extreme feed restriction conditions.

According to Kaneko *et al.*, (2008), reference values for serum urea concentration range from 6.0 to 27mg/dL. In the present study, the values were above this range. According to Russell *et al.*, (1992), high amounts of amino acids resulting from proteolysis are determined and used as an energy source. As a result, ammonia production increases, and the excess is absorbed and lost through the urine as urea. Feed restriction may have led to increased urea levels due to reduced energy supply caused by the lower DMI.

### CONCLUSION

Feed restriction did not change physiological parameters and the concentration of nutrition-related metabolites of F1 Holstein x Zebu cows at different stages of lactation. Feed restriction of up to 2.50% BW is a cost reduction strategy that does not alter milk yield, regardless of stage of lactation.

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