



Feed restriction of F1 Holstein × Zebu cows in the final third of lactation modifies intake, nutrient digestibility, feeding behavior, and performance

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ABSTRACT - The objective of this study was to evaluate the effect of quantitative feed restriction level on nutrient intake and digestibility, nitrogen balance, efficiency and feeding behavior, and productive performance of F1 Holstein × Zebu cows in late lactation. Sixty F1 Holstein × Zebu cows were used at the stage of late lactation (183.0±17.5 days of lactation) and with initial body weight (BW) of 499±30 kg. The experimental arrangement adopted was the completely randomized design, with five feed restriction levels (3.39, 2.75, 2.50, 2.25, and 2.00% of BW) and twelve cows as replicates for each treatment. The roughage:concentrate ratio was of 75:25 in the total dry matter of the diet. The restriction of dietary supply from 3.39 to 2.0% of BW linearly reduced dry matter intake by 51.74%. The restriction in diet supply linearly increased dry matter digestibility in 13.76%. For each 1% BW restriction in the diet supply for the animals, there was a linear reduction of 3 h in feeding time. Restriction in diet supply of crossbred cows from 3.39 to 2.0% of BW reduced milk production by 24.84%. For each 1% restriction on supply, 2.17 L of milk were not produced. Milk production corrected to 3.5% fat was not altered with the feed restriction. Final BW decreased 41 kg for each percentage unit of diet restriction. Feed efficiency increased 28.72% with feed restriction. For F1 Holstein × Zebu cows in the late lactation, the restriction of dietary supply up to 2.0% of body weight decreases nutrient intake, nitrogen balance, rumination time, and milk production; however, it improves nutrient digestibility and feed efficiency of the animals.

Keywords: crossbred cows, feed efficiency, milk production, nitrogen balance

Introduction

The competitiveness of dairy products produced in Brazil with other commodities produced in the internal and/or external market is attributed, in part, to the use of forage plants as a source of nutrients for animals, which presents lower production costs in relation to concentrated inputs. However, forage production is not constant throughout the year in the country (Gabbi et al., 2016), especially in the semi-arid regions, which can impair the performance of animals.

The confinement of dairy animals has been a tool exploited by farmers, although the cost of feed increases considerably, and it is a major challenge to maintain production and competitiveness in the market. Therefore, Zhang et al. (2017) proposed that restriction in diet supply might be an important strategy in the management of confined cattle to reduce feed costs, which may represent more than 60% of the total production cost. However, the restriction in the diet supply can promote rapid mobilization

of body tissues for maintenance, as well as present direct reactions in the productive and reproductive activities of the animal (Schütz et al., 2013), which leads to metabolic and adaptive changes in the body (Gabbi et al., 2016). Burke et al. (2010) reported that, depending on the severity and time of feed restriction in lactating cows, there may be a reduction in milk production and, consequently, in the percentage of fat and protein and an increase in the somatic cell count (Fruscalso et al., 2013).

However, there are few studies about the restriction in diet supply (Ferreira et al., 2000) in crossbred Holstein × Zebu cows, mainly in specific phases of lactation. This crossbreed is responsible for most of the milk produced in Brazil (Santos et al., 2012) and is also recognized by its high potential of adaptation to various management conditions. Moreover, there are gaps in the knowledge of the best quantitative level of feed restriction for the cows and its impact on the nutritional and productive parameters. Thus, the hypothesis of this study is that there is an ideal level of restriction of dietary supply in the final stage of lactation of crossbred F1 Holstein × Zebu cows, which reduces feed costs and improves nutritional efficiency, without modifying production.

Based on the above, the objective was to evaluate nutrient intake and digestibility, nitrogen balance, feed efficiency, ingestive behavior, and productive performance of F1 Holstein × Zebu cows in late lactation and under different levels of dietary restriction.

Material and Methods

All procedures involving animals were approved by the institutional committee on animal use (case number 138/2017). The study was conducted at an experimental farm in Janaúba, MG, Brasil (15°52'38" S latitude, 43°20'05" W longitude).

Sixty F1 Holstein × Zebu cows in the final third of their lactation periods (183.0±17.5 days of lactation) with initial body weight (BW) of 454±80 kg (mean±SEM) and mean age of six years were used. The treatments were composed of five feed restriction levels (3.39, 2.75, 2.50, 2.25, and 2.00% of BW). The diet supply (kg dry matter/d), defined as percentage of body weight, were: *ad libitum* (3.39%), allowing 5% of refusals relative to the amount of dry matter (DM) provided, and diets provided with 2.75, 2.50, 2.25, and 2.00% of BW. Before the trial period, all cows received the experimental diet provided *ad libitum* for 14 days to allow animals to adapt to the diet and management.

The diet (Table 1) was given based on the BW of each cow and in accordance with each treatment, maintaining a roughage:concentrate ratio of 75:25 in the total DM of the diet. Diets were offered to animals twice a day, at 8:00 and 15:00 h, in a complete diet system. The roughage base for the diets was corn silage, which was weighed daily and then mixed into the concentrate.

Table 1 - Chemical composition of ingredients and diet used during experimental period

Item (g kg ⁻¹ as fed) ¹	Corn silage	Concentrated mixture	Diet composition
Dry matter	503.29	925.92	608.94
Organic matter	958.08	922.31	949.14
Crude protein	65.52	218.25	103.70
NDIN	6.39	12.24	7.85
ADIN	0.57	0.75	0.62
Ether extract	28.30	28.28	28.29
Non-fiber carbohydrates	340.83	371.68	348.54
NDFap	523.44	304.10	468.60
Acid detergent fiber	301.56	72.21	244.22
Lignin	131.75	31.75	106.75
Total digestible nutrients ²	529.04	734.16	580.32

NDIN - neutral detergent insoluble nitrogen; ADIN - acid detergent insoluble nitrogen; NDFap - neutral detergent fiber corrected for ash and protein.

¹ Nutrient on dry basis (g kg⁻¹).

² NRC (2001).

Cows were kept in individual pens with an area of approximately 26 m², equipped with troughs (1 linear meter) and drinkers (capacity of 200 L). Milking was performed mechanically twice a day, at 7:00 and 14:00 p.m., with the calf present to stimulate milk letdown. Immediately after milking, calves remained with cows to feed from the residual milk.

The experiment lasted for 63 days, being three periods of 21 days each, with the first 16 days for diet adaptation (diet supply levels) followed by five days for data and samples collection. Intake was evaluated daily by weighing the feed provided and refusals of the animals. Samples of the offered diets and refusals were stored at -20 °C for further analysis.

Samples of diets, concentrate ingredients, refusals, and feces were analyzed to evaluate feed intake and digestibility. The samples were analyzed for DM (method 967.03), ash (method 942.05), crude protein (CP; method 981.10), and ether extract (EE; method 920.39) according to the recommendations of the AOAC (1990). The contents of the neutral detergent fiber were corrected for ash and protein (NDFap) using heat-stable alpha-amylase without sodium sulfite (Mertens, 2002; Licitra et al., 1996), and acid detergent fiber (ADF) was determined as described by Van Soest et al. (1991). Lignin content was determined by treating the ADF residue with sulfuric acid at 72% (Detmann et al., 2012). Non-fiber carbohydrate (NFC) contents were calculated as described by Detmann et al. (2012): $NFC = 100 - \text{ash} - EE - NDFap - CP$.

Total digestible nutrients were estimated using the formula proposed by NRC (2001). To analyze the indigestible neutral detergent fiber (iNDF), feed samples were placed in nonwoven fabric bags (20 mg cm⁻²) and incubated in the rumen for 288 h (Detmann et al., 2012; method INCT-CA F-008/1). Two adult crossbred cattle cannulated in the rumen, weighing 480±30 kg, and with a mean age of eight years were used for sample incubation. The digestibility was evaluated during the last five days in each period trial in all animals. To determine the digestibility of each fraction, we used the following equation: $[(\text{ingested nutrient amount} - \text{amount nutrient excreted in the feces}) \times 100] / \text{ingested nutrient amount}$.

Milk quality was evaluated using composite samples constituted by samples collected from each cow, every five days (17th to 21th) in each trial period, during morning and afternoon milking, after weighing the milk, using the equipment Mark V® (Delaval Ltda). Samples were placed in plastic recipients (50.0 mL) containing preservative (bronopol®), without refrigeration, and sent to the laboratory. Levels of protein, fat, milk urea nitrogen (MUN), and total solids were determined in g/kg of milk by infrared absorption spectrophotometry in equipment Bentley 2000® (Bentley Instruments Inc.).

Blood samples were collected from the coccygeal vein into vacuum tubes containing sodium fluoride and potassium oxalate (Glistab® anticoagulant; Labtest Diagnóstica S.A., Lagoa Santa, Brazil) 4 h after the morning feeding on the last day of the experimental period. Samples were centrifuged at 4,000 rpm (rotation per min) for 20 min, and the serum obtained was conditioned in Eppendorf tubes and frozen at -18 °C for further analysis. Plasma urea concentrations were determined by a colorimetric enzymatic method using commercial kits (Ureia 500, Doles® Reagents; Panamá, Brazil).

Spot urine samples were obtained on the 18th day of each experimental period, 4 h after the first feed supply of the day, during spontaneous urination (Chizzotti et al., 2008). A single sample was collected per animal per period. An aliquot of 10 mL of the urine sample was filtered and immediately diluted in 40 mL of H₂SO₄, 0.036 N for later analysis of urea, and creatinine, as described by Chizzotti et al. (2008). Samples were then transferred to Eppendorf tubes and analyzed for urea content using the same method used for the blood samples. The end-point method determined the creatinine by means of picrate and acidifying with enzymatic methods. Quantification of the daily urinary volume of each animal was obtained by multiplying the respective body weight by the amount of creatinine excreted daily, and then dividing the product by the creatinine concentration (mg/L) in the spot sample. The average value of 24.04 (mg/kg BW) was used, according to Chizzotti et al. (2008), to obtain the total daily creatinine excretion.

To perform the nitrogen balance calculation, the ingested amount of nitrogen (N-ingested; g/d) and the amount excreted in the feces (N-feces; g/d), urine (N-urine; g/d), and milk (N-milk; g/d) were used.

The nitrogen utilization efficiency of the diet was calculated by dividing the concentration of the nitrogen retained in the milk by the nitrogen intake in kg/d.

Feed efficiency was calculated by dividing the average milk yield (kg/d) by the DM intake (kg/d). Costs with concentrates, roughage, and total diet were calculated by multiplying the intake by the respective value of each fraction, which was calculated according to its composition and the price of each ingredient (Rennó et al., 2008). The values per kilogram of the diet ingredients were as follows: corn silage was US\$ 0.05 and concentrate was US\$ 0.46, considering the ratio of R\$ 3.5 for every \$ 1.

Feeding behavior was assessed in the last two days of the trial period. For the evaluation of feeding behavior, all animals were observed visually for 24 h, and the observations were recorded at 5-min intervals. This parameter included eating, ruminating, and idle times (Mezzalira et al., 2011). On the same day, three observations were made for each animal: in the morning, at noontime, and at night. Trained observers using a digital timer collected the data. During the nocturnal observation, the environment was kept under artificial light. Feeding behavior variables (eating, ruminating, and idle times) were obtained according to Carvalho et al. (2011). The number of chews per ruminal bolus and time spent ruminating each bolus were recorded during the observation periods. The number of bolus ruminated daily was calculated by dividing the total rumination time (min) by the average time spent to ruminate one bolus.

During the trial period, milk production (MP) was recorded per cow. Milk production was corrected for fat content (FC) 3.5% using the equation proposed by Sklan et al. (1992): $MP_{3.5\%} = MP \times (0.432 + 0.163 \times FC)$.

To evaluate the body weight of animals, we used a mechanical scale. Animals were weighted (mechanical scale, Valfran, Votuporanga, São Paulo, Brazil) at the beginning and at the end of the experiment. Body condition scores (BCS) were evaluated weekly during the period, by a single technician. The BCS were also examined for three weeks further, after the end of the experimental period, to investigate the development of the animals. In the assessment of BCS, the 1 to 5-point scale with 0.10-point intervals was used, in which 1 represents a very lean cow and 5 a very fat cow (adapted from Mishra et al., 2016).

All statistical analyses were performed using MIXED procedure of SAS (Statistical Analysis System, version 9.0). The experimental design was completely randomized and had five feed restriction levels (3.39, 2.75, 2.50, 2.25, and 2.00% of BW) with twelve cows used for each treatment. The treatments were main factors. Each animal was considered as one experimental unit. The collected data were subjected to a polynomial regression analysis according to REG procedure of SAS software. Differences were considered significant when $P < 0.05$.

The statistical model used was:

$$y_{ij} = \mu + T_i + \varepsilon_{ij},$$

in which y_{ij} is the observation to treatment (T) i (five restriction levels; fixed effect) in the repeat j ; μ is general mean; and ε_{ij} represents the random error.

Results

The dietary supply restriction linearly reduced dry matter intake (DMI) by 51.74% ($P < 0.01$). Crude protein intake and total digestible nutrients decreased by 0.69 and 3.56 kg for each 1% of restriction ($P < 0.01$) (Table 2), respectively.

The dietary restriction linearly increased apparent digestibility coefficient of DM ($P = 0.01$) in 13.76%, with a mean of 0.66 g/kg for animals with a feed restriction of 2.0% BW. The highest apparent digestibility coefficient of CP was verified in the supply restriction in the order of 2.63% of BW ($P = 0.03$). There was an increase in the apparent digestibility coefficient of NDFap ($P = 0.01$) with restriction of the diet supply of the animals. For each 1% restriction, there was an increase of 6.21% in the apparent digestibility coefficient of NDFap.

The restriction in the diet supply of lactating dairy cows, from 3.39% to 2.0% of BW, reduced ingested nitrogen ($P < 0.01$), milk losses ($P < 0.01$), and feces ($P < 0.01$) and increased nitrogen excretion by 39.03% ($P = 0.02$), with 18.53 g for each percentage unit of restriction (Table 3). The nitrogen balance of the animals linearly reduced with dietary restriction ($P < 0.01$).

The efficiency of nitrogen use ($P = 0.18$) and concentrations of nitrogen in the urine ($P = 0.88$), plasma ($P = 0.14$), and milk ($P = 0.17$) were not altered with restriction of diet supply, with averages of 0.25, 6.94, 22.36, and 12.12 mg/dL, respectively.

For each 1% BW of restriction in diet supply of animals, there was a linear reduction of 3.0, 1.76, and 4.77 h for feeding time ($P < 0.01$), rumination ($P < 0.01$), and chewing ($P < 0.01$), respectively. The idle time increased 40.77% with the restriction of diet supply from 3.39 to 2.0% of BW ($P < 0.01$) (Table 4).

The number of feeding periods decreased by 7.21 units for each 1% restriction in diet supply ($P < 0.01$). The variation between the animals that received diet offered in 3.39 and 2.0% of BW was 70.17%. The time spent per feeding period increased from 28.68 (3.39% of BW) to 35.44 min (restriction of 2.0%) ($P < 0.01$). The highest time spent per period for leisure was observed when the diet supply was restricted to 2.67% of BW ($P < 0.01$) (Table 5).

Feed efficiency of DM ($P = 0.10$) and NDFap ($P = 0.08$) of the animals was not modified with feed restriction. Rumination efficiency of DM and NDFap linearly reduced 372.61 g DM/h and 162.60 g NDFap/h for each 1% restriction in diet supply.

The restriction in diet supply of crossbred cows from 3.39 to 2.0% of BW reduced milk production by 24.84% ($P < 0.01$). For each 1% restriction in supply, 2.17 L of milk were not produced. Milk production corrected to 3.5% fat was not altered with feed restriction ($P = 0.60$). Feed restriction increased 22.78% in fat content ($P = 0.03$) and reduced 17.5% protein content ($P = 0.02$) in milk. There was no difference

Table 2 - Nutrient intake and digestibility in F1 Holstein × Zebu cows under quantitative feed restriction in the final third of lactation

Item	Restriction level (% BW) ^a					SE	P-value			
	3.39 ^b	2.75	2.50	2.25	2.00		Treatment	Linear	Quadratic	
	Intake									
DM (kg/d) ¹	18.01	13.24	11.87	10.45	8.69	0.15	<0.01	<0.01	0.06	
CP (kg/d) ²	1.88	1.38	1.23	1.09	0.90	0.02	<0.01	<0.01	0.04	
EE (kg/d) ³	0.51	0.37	0.33	0.29	0.24	<0.01	<0.01	<0.01	0.02	
NFC (kg/d) ⁴	6.36	4.59	4.11	3.63	3.05	0.06	<0.01	<0.01	0.01	
NDFap (kg/d) ⁵	8.34	6.22	5.58	4.91	4.06	0.07	<0.01	<0.01	0.34	
TDN (kg/d) ⁶	10.84	8.66	7.81	7.09	5.72	0.22	<0.01	<0.01	0.33	
CP (% BW) ⁷	0.38	0.28	0.25	0.23	0.20	<0.01	<0.01	<0.01	<0.01	
EE (% BW) ⁸	0.10	0.07	0.07	0.06	0.05	<0.01	<0.01	<0.01	<0.01	
NFC (% BW) ⁹	1.30	0.96	0.87	0.78	0.70	0.01	<0.01	<0.01	<0.01	
NDFap (% BW) ¹⁰	1.69	1.29	1.17	1.05	0.94	0.01	<0.01	<0.01	0.03	
TDN (% BW) ¹¹	2.18	1.79	1.64	1.52	1.32	0.03	<0.01	<0.01	0.85	
	Nutrient digestibility (%)									
DM ¹²	57.32	63.46	63.82	65.91	66.47	1.67	0.01	<0.01	0.43	
CP ¹³	49.14	58.78	58.45	56.68	51.91	2.35	0.03	0.23	<0.01	
EE	77.93	82.82	84.86	87.60	84.40	2.15	0.06	0.01	0.27	
NFC	75.04	78.52	80.83	83.21	80.08	1.97	0.10	0.02	0.33	
NDFap ¹⁴	49.62	56.19	55.34	58.27	58.11	1.62	0.01	<0.01	0.33	

DM - dry matter; CP - crude protein; EE - ether extract; NFC - non-fiber carbohydrates; NDFap - neutral detergent fiber corrected for ash and protein; TDN - total digestible nutrients; BW - body weight; SE - standard error of the mean; P - probability.

^a Regression equation: ¹ $\hat{Y} = -4.60 + 6.61 * X$, $R^2 = 0.99$; ² $\hat{Y} = -0.499 + 0.696 * X$, $R^2 = 0.99$; ³ $\hat{Y} = -0.144 + 0.193 * X$, $R^2 = 0.99$; ⁴ $\hat{Y} = -1.74 + 2.36 * X$, $R^2 = 0.99$; ⁵ $\hat{Y} = -1.99 + 3.03 * X$, $R^2 = 0.99$; ⁶ $\hat{Y} = -1.16 + 3.56 * X$, $R^2 = 0.99$; ⁷ $\hat{Y} = -0.050 + 0.126 * X$, $R^2 = 0.99$; ⁸ $\hat{Y} = -0.013 + 0.0344 * X$, $R^2 = 0.96$; ⁹ $\hat{Y} = -0.190 + 0.4314 * X$, $R^2 = 0.98$; ¹⁰ $\hat{Y} = -0.16 + 0.539 * X$, $R^2 = 0.99$; ¹¹ $\hat{Y} = 0.13 + 0.605 * X$, $R^2 = 0.99$; ¹² $\hat{Y} = 80.52 - 6.64 * X$, $R^2 = 0.95$; ¹³ $\hat{Y} = -60.98 + 91.05 * X - 17.27 * X^2$, $R^2 = 0.99$; ¹⁴ $\hat{Y} = 71.51 - 6.21 * X$, $R^2 = 0.88$, in which X is the level of food restriction; R^2 is the coefficient of determination; and * is significant by the t test, at 1% probability.

^b Diet *ad libitum*, allowing 5% refusals regarding dry matter offer.

in total solids content ($P = 0.08$, mean 11.94 g/d) in milk with the restriction in diet supply. Final body weight decreased 41 kg for each percentage unit of diet restriction ($P < 0.01$). Feed efficiency increased 28.72% when the diet offer was reduced from 3.39 to 2.0% of BW (Table 6).

Discussion

Feed restriction is one of the factors that impair the productive performance of animals. This happens because, for the most part, the amount of nutrients present in the ingested DM, specially energy, is not sufficient to meet the nutritional requirements of animals for maintenance and production (NRC, 2001).

Table 3 - Nitrogen balance and efficiency use in crossbred F1 Holstein × Zebu cows under quantitative feed restriction in the final third of lactation

Item	Restriction level (% BW) ^a					SE	P-value		
	3.39 ^b	2.75	2.50	2.25	2.00		Treatment	Linear	Quadratic
N-ingested (g/d) ¹	288.7	207.8	182.6	164.4	153.6	8.15	<0.01	<0.01	0.03
N-milk (g/d) ²	65.19	55.21	44.95	44.86	40.61	3.19	<0.01	<0.01	0.70
N-fecal (g/d) ³	146.26	85.61	76.07	71.05	68.16	5.25	<0.01	<0.01	<0.01
N-urine (g/d) ⁴	47.36	48.46	50.58	57.96	77.69	6.27	0.02	0.01	0.03
Nitrogen balance (g/d) ⁵	29.91	18.55	11.02	-9.51	-32.90	7.15	<0.01	<0.01	0.02
NUE	0.23	0.27	0.24	0.27	0.26	0.01	0.18	0.06	0.46
UUN (mg/dL)	8.31	7.41	4.97	7.24	6.81	2.29	0.88	0.60	0.63
PUN (mg/dL)	22.07	22.64	20.76	22.26	24.09	0.84	0.14	0.27	0.12
MUN (mg/dL)	10.55	12.88	13.13	11.15	12.90	0.86	0.17	0.16	0.25

BW - body weight; NUE - nitrogen use efficiency; UUN - urine urea nitrogen; PUN - plasma urea nitrogen; MUN - milk urea nitrogen; SE - standard error of the mean; P - probability.

^aRegression equation: ¹ $\hat{Y} = 57.5 - 99.65 \times X$, $R^2 = 0.96$; ² $\hat{Y} = 3.12 + 18.24 \times X$, $R^2 = 0.95$; ³ $\hat{Y} = -57.65 + 57.05 \times X$, $R^2 = 0.87$; ⁴ $\hat{Y} = 104.2 - 18.53 \times X$, $R^2 = 0.61$; ⁵ $\hat{Y} = -107.1 + 42.9 \times X$, $R^2 = 0.84$, in which X is the level of food restriction; R^2 is the coefficient of determination; and * is significant by the t test, at 1% probability.

^bDiet *ad libitum*, allowing 5% refusals regarding dry matter offer.

Table 4 - Feeding behavior of F1 Holstein × Zebu cows under quantitative feed restriction in the final third of lactation

Item	Restriction level (% BW) ^a					SE	P-value		
	3.39 ^b	2.75	2.50	2.25	2.00		Treatment	Linear	Quadratic
Feeding									
h/d ¹	6.71	3.35	3.44	2.60	2.56	0.41	<0.01	<0.01	0.02
min/kg DM	23.38	16.18	18.66	15.87	16.34	2.05	0.10	0.02	0.28
min/kg NDFap	50.63	34.53	39.83	33.86	34.87	4.36	0.07	0.02	0.25
Rumination									
h/d ²	7.69	6.40	6.54	5.50	5.23	0.33	<0.01	<0.01	0.79
min/kg DM ³	26.63	30.90	35.68	33.25	33.99	1.52	<0.01	<0.01	0.14
min/kg NDFap ⁴	57.71	65.94	76.13	70.95	72.54	3.23	0.01	<0.01	0.17
Chewing									
number/bolus ⁵	62.20	65.96	63.41	67.90	67.62	1.81	<0.01	0.04	0.90
number/d ⁶	26705	21003	22271	1745	1677	10683	<0.01	<0.01	0.88
h/d ⁷	14.40	9.75	9.98	8.10	7.79	0.56	<0.01	<0.01	0.10
min/kg DM	50.01	47.08	54.34	49.11	50.33	2.41	0.34	0.78	0.98
min/kg NDFap	108.33	100.47	115.96	104.81	107.41	5.08	0.33	0.96	0.91
Idleness									
h/d ⁸	9.60	14.25	14.02	15.90	16.21	0.56	<0.01	<0.01	0.10

DM - dry matter; NDFap - neutral detergent fiber corrected for ash and protein; BW - body weight; SE - standard error of the mean; P - probability.
^aRegression equation: ¹ $\hat{Y} = -4.02 + 3.00 \times X$, $R^2 = 0.87$; ² $\hat{Y} = 1.72 + 1.76 \times X$, $R^2 = 0.94$; ³ $\hat{Y} = -942 + 372.4 \times X$, $R^2 = 0.77$; ⁴ $\hat{Y} = 468.7 + 162.6 \times X$, $R^2 = 0.76$; ⁵ $\hat{Y} = 5.09 + 16.80 \times X$, $R^2 = 0.74$; ⁶ $\hat{Y} = 2388 + 7158 \times X$, $R^2 = 0.90$; ⁷ $\hat{Y} = -2.31 + 4.77 \times X$, $R^2 = 0.92$; ⁸ $\hat{Y} = 26.30 - 4.77 \times X$, $R^2 = 0.93$, in which X is the level of food restriction; R^2 is the coefficient of determination; and * is significant by the t test, at 1% probability.

^bDiet *ad libitum*, allowing 5% refusals regarding dry matter offer.

In dairy cattle, the lactation phase as well as the genetic factor influence milk production (Santos et al., 2012). In this study with F1 cows in the late lactation, the reduction in milk production is mainly due to the reduction in nutrient, protein, and energy intake required for rumen microbial growth, as verified by Zhang et al. (2017). Through compilation, Gabbi et al. (2016) found that for Jersey and Holstein cows, 40 and 50% of dietary restriction reduced milk production by 14.2 and 40.9%, respectively. In this context, it is possible to highlight the sensitivity of animals of specialized breed for milk production in relation to crossbred animals. The presence of Zebu genetic base in F1 cows highlights the adaptability of these animals under conditions of feed restriction, a common fact in tropical regions (Garg et al., 2013), without major impact on production. For this reason, these animals, under feed restriction, readjust the size of the viscera and organs involved in metabolism in a shorter time, besides reducing

Table 5 - Number of periods and average time spent per period on the feeding, ruminating, and idle activities by F1 Holstein × Zebu cows under quantitative feed restriction in the final third of lactation

Item	Restriction level (% BW) ^a					SE	P-value		
	3.39 ^b	2.75	2.50	2.25	2.00		Treatment	Linear	Quadratic
Number of periods (n/d)									
Feeding ¹	14.25	4.50	4.75	4.00	4.25	0.74	<0.01	<0.01	<0.01
Ruminating	14.50	13.25	14.25	13.50	11.50	0.68	0.06	0.02	0.22
Idling	21.25	20.25	18.75	19.00	17.50	1.01	0.14	0.02	0.70
Time spent per period (min)									
Feeding ²	28.68	46.13	43.26	39.06	35.44	2.91	<0.01	0.10	<0.01
Ruminating	32.12	29.35	27.60	24.85	27.39	2.22	0.26	0.05	0.61
Idling ³	27.18	42.74	44.97	50.45	56.00	2.78	<0.01	<0.01	0.75
Feed efficiency									
g DM/h	2616	3809	3411	3849	3846	349	0.10	0.02	0.33
g NDFap/h	1206	1785	1598	1803	1802	163	0.08	0.02	0.31
Rumination efficiency									
Boluses/d	408.1	511.7	479.0	379.2	441.8	42.1	0.22	0.96	0.15
g DM/h ⁴	2260	1974	1691	1814	1772	91	<0.01	<0.01	0.13
g NDFap/h ⁵	1042	925	792	850	830	42	<0.01	<0.01	0.17

DM - dry matter; NDFap - neutral detergent fiber corrected for ash and protein; BW - body weight; SE - standard error of the mean; P - probability.
^aRegression equation: ¹ $\hat{Y} = 12.3 + 7.21 \cdot X$, $R^2 = 0.75$; ² $\hat{Y} = -138.2 + 139.3 \cdot X - 26.53 \cdot X^2$, $R^2 = 0.94$; ³ $\hat{Y} = 96.4 - 20.23 \cdot X$, $R^2 = 0.98$; ⁴ $\hat{Y} = 941.6 + 372.61 \cdot X$, $R^2 = 0.77$; ⁵ $\hat{Y} = 468.7 + 162.6 \cdot X$, $R^2 = 0.76$, in which X is the level of food restriction; R^2 is the coefficient of determination; and * is significant by the t test, at 1% probability.

^bDiet *ad libitum*, allowing 5% refusals regarding dry matter offer.

Table 6 - Performance, feed efficiency, and chemical composition of milk in F1 Holstein × Zebu cows under quantitative feed restriction in the final third of lactation

Item	Restriction level (% BW) ^a					SE	P-value		
	3.39 ^b	2.75	2.50	2.25	2.00		Treatment	Linear	Quadratic
Milk production (kg/d) ¹	11.55	10.45	8.83	9.13	8.68	0.54	<0.01	<0.01	0.63
Milk production corrected for 3.5 fat (kg/d)	10.38	8.58	9.16	8.96	9.04	0.81	0.60	0.25	0.34
Fat (g/kg milk) ²	2.88	3.19	3.32	3.30	3.73	0.30	0.03	0.03	0.18
Protein (g/kg milk) ³	3.60	3.38	3.22	3.15	2.97	0.11	0.02	<0.01	0.53
Total solids (g/kg milk)	12.00	11.16	12.48	11.83	12.25	0.31	0.08	0.47	0.21
Final body weight (kg) ⁴	519	502	494	483	457	6	<0.01	<0.01	0.05
Final body condition score	3.63	3.75	3.69	3.31	3.50	0.17	0.43	0.31	0.42
Feed efficiency (kg of milk/kg of DM) ⁵	0.67	0.84	0.80	0.92	0.94	0.05	0.01	<0.01	0.90

BW - body weight; SE - standard error of the mean; P - probability.

^aRegression equation: ¹ $\hat{Y} = 4.11 + 2.17 \cdot X$, $R^2 = 0.88$; ² $\hat{Y} = 4.67 - 0.53 \cdot X$, $R^2 = 0.87$; ³ $\hat{Y} = 2.12 + 0.44 \cdot X$, $R^2 = 0.97$; ⁴ $\hat{Y} = 385 + 41.05 \cdot X$, $R^2 = 0.89$; ⁵ $\hat{Y} = 1.33 - 0.19 \cdot X$, $R^2 = 0.90$, in which X is the level of food restriction; R^2 is the coefficient of determination; and * is significant by the t test, at 1% probability.

^bDiet *ad libitum*, allowing 5% refusals regarding dry matter offer.

their nutritional requirement (Casal et al., 2014). However, Gabbi et al. (2016) recommend caution in the severity and duration of feed restriction because cows, especially in late lactation, are also in the last trimester of pregnancy and feed restriction may compromise fetal development (Paradis et al., 2017).

Regarding the feeding of dairy cows, in addition to intake, the digestibility of diet determines the amount of available nutrients for maintenance and milk production (NRC, 2001). In this research, the increase of the digestibility of dry matter and fibrous fraction occurred due to the lower passage rate of the digesta, which is a result of the nutrient input behavior, through dry matter intake. Similar behavior was also verified by Souza et al. (2018); they observed that the increase of the digestibility of diets was related to the lower passage rate. This fact can be explained by the characteristics of the ingredients used in the formulation of diets, associated with the greater time intended for degradation in the rumen, therefore favoring the action of microorganisms, which increase the digestibility of the diet. Nevertheless, the higher digestibility was not enough to maintain or increase milk production. However, feed efficiency was better for cows with restricted diet, since milk production corrected to 3.5% fat did not change, with an average of 9.22 kg/d, which is explained by the increase in fat content of milk with the decrease of the supply levels of diet. On the other hand, the reduction of milk protein levels can be related to the lower availability of energy and degradable nitrogen in the rumen for the synthesis of microbial protein, with the feed restriction.

Feed restriction modified the ingestive behavior of animals. Cows under restriction spent 4.15 h less feeding when compared with animals without restriction (6.71 h). Nevertheless, animals under restriction had an increase of 5.42 chews per bolus ruminated. This behavior also justifies the greater digestibility of DM and fibrous fraction and the lower rumination time of these animals due to the reduction in particle size of the feed, which favors a higher performance of microorganisms in the substrate. Beauchemin (2018) reported that the physicochemical composition of the diet associated with particle size is the factor that most interferes with rumination time, justifying the findings of this study, since the diet was the same in all treatments. Welch and Hooper (1988) stated that time spent for rumination is highly correlated (0.96) with NDF intake in cattle. This would corroborate the behavior verified in this research for crossbred F1 cows in the late lactation. Consequently, the restriction in diet supply from 3.39 to 2.0% of BW increased the idleness time of animals.

In this research, the restriction of dietary supply increased the fat content by 22.78% and reduced the protein content in milk by 17.5% and milk production by 24.84%. In Holstein cows with 188±124 days in milk, Fruscalso et al. (2013) verified that the restriction diet in 50% of the quantity offered in the control diet reduced fat and protein content by 22.80 and 22.22%, respectively. The authors also verified decrease milk production by 40%, body condition score by 5% and lactose by 1.7.

As for BW of the animals, there was a decrease of 11.94% for cows under restriction. According to Fruscalso et al. (2013), underfeeding dairy cows generates a negative energy balance, and cows try to cope with the nutritional deficit by lowering milk production, mobilizing body reserves and BW. Gross et al. (2011) verified that the feed restriction of lactating cows for three weeks reduced their body weight. Ferreira et al. (2000) stated that the monitoring of postpartum BW of lactating cows is fundamental so that they have sufficient reserves for the manifestation of estrus and guarantee of pregnancy. The same authors evaluated Girolando cows under conditions of feed restriction and concluded that the weight loss of 15.2% in the early lactation did not affect reproduction. In addition, the authors mentioned that the BCS of 3.5 is ideal for cows to exhibit estrus in the 90 days postpartum. For this condition, it is necessary that these animals in the pre-partum, or late lactation, present a body score above 3. In this study, the mean value for BCS was 3.6 in the final third of lactation, even with the restriction of the diet supply, indicating that animals had sufficient energy reserves for the postpartum period. Ferreira et al. (2000) reported that the desirable BCS in the final third of lactation, close to the drying time of the cow, is close to 3.5, whereas at the time of delivery it is 3.5 to 4.0. This evaluation in the final third of lactation is important to prevent some cows from getting too thin in the drying period, so there is no time to recover the necessary reserves during the dry period, aiming to reach a good body condition (Smith et al., 2017).

The restriction in diet supply increased feed efficiency, because the variation in milk production was lower than the reduction in DMI, therefore, optimizing the ingested nutrients. According to Arndt et al. (2015), studies of this nature are relevant in the selection of efficient animals regarding the use of dietary nutrients. After all, efficiency also encompasses environmental and management factors that end up interfering with the efficiency with which feed is digested and the requirements of animals. In this sense, it is important to emphasize the adaptive capacity of F1 Holstein × Zebu cows to maintain body condition and milk production with less feed supply, improving feed efficiency. Another important factor is the reduction of feed costs and total production costs. The feed cost of the diet altered from \$12.14 (3.39% BW) to \$6.48 (2% BW). Feed restriction decreased the costs of feed by up to 46.6%. This was because, in the 2% supply restriction, for example, 0.94 kg of milk were produced for each unit of dry matter ingested, causing dilution of the nutritional maintenance requirements of the animals, mainly the metabolizable energy (NRC, 2001).

Conclusions

The restriction of dietary supply for F1 Holstein × Zebu cows in the final third of lactation up to 2.0% of body weight decreases nutrient intake, nitrogen balance, rumination time, and milk production, but improves nutrient digestibility and feed efficiency of the animals.

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