

## Feeding restriction impairs milk yield and physicochemical properties rendering it less suitable for sale

Vilmar Fruscalço<sup>§</sup>, Marcelo Tempel Stumpf, Concepta Margaret McManus, Vivian Fischer\*

UFRGS/Faculdade de Agronomia – Depto. de Zootecnia,  
Av. Bento Gonçalves, 7712 – 91540-000 – Porto Alegre,  
RS – Brasil.

\*Corresponding author <vfried@portoweb.com.br>

Edited by: Daniel Scherer de Moura

**ABSTRACT:** Feed shortages are relatively frequent in subtropical pasture-based dairy production systems. The effect of feed restriction on milk yield and physical-chemical traits was evaluated in this study. The experiment was carried out in Brazil's south region. Treatments consisted of control and restricted diet. Six multiparous and six primiparous cows, with  $499 \pm 47.20$  kg body weight (BW), at mid-lactation ( $188 \pm 124$  days in milk), producing  $19.35 \pm 4.10$  kg of milk were assigned to two groups, balanced for parity, each group receiving a different sequence of the dietary treatments for 56 days, in a crossover design. Diet nominated as control included 8 kg DM 100 kg BW<sup>-1</sup> of Bermuda grass var. Tifton pasture (*Cynodon dactylon* (L.) Pers.), 5.00 kg of concentrate and 2.50 kg of Tifton hay per day. The restriction diet consisted of 50 % of the quantity offered in the control diet. Milk production and physicochemical composition were evaluated. Feed restriction reduced milk production by 40 %, body condition score by 5 %, milk magnesium by 14.3 %, lactose by 1.7 %, titratable acidity by 10 % and stability to the ethanol test by 9 % and it tended to increase (7 %) milk potassium content. No changes were found for the remaining characteristics. Since feed restriction is quite frequent in Brazil's extensive dairy production systems, our concern is that besides decreased milk production, changes can occur in the physicochemical attributes of the milk, mainly a reduction in the stability to the ethanol test, which may increase the volume of milk rejected by the industry.

**Keywords:** alcohol test, grazing systems, milk stability, underfeeding

Received December 12, 2012

Accepted May 01, 2013

### Introduction

In the subtropics and tropics, cold and dry periods are unevenly distributed throughout the year, so that a considerable proportion of the dairy herd undergoes an inadequate nutritional supply, with reduced productivity, mainly due to a combination of low pasture allowance, high fiber content and inadequate feeding management (Verkerk, 2003; Njarui et al., 2011).

The effects of feed restriction may vary according to lactation stage, magnitude and length of the restriction. For cows with  $162 \pm 20$  days in milk, restriction of 30 % in the supply of dry matter (DM) during 21 days decrease milk, fat and protein yields in 12.0, 18.0 and 18.5 %, respectively (Guinard-Flament et al., 2007). Feed restriction of 44 % of DM during 14 days reduced milk, fat and protein production in 25.6, 31.6 and 33.3 %, respectively for cows with more than 28 days in milk (DIM) (Burke et al., 2010).

The effects of feed restriction on milk chemical attributes are highly variable, e.g. effects on protein content have been reported as negative (Guinard-Flament et al., 2007; Gross et al., 2011), without effect (Zanela et al., 2006) and positive (Auld et al., 2000; Lacy-Hulbert et al., 1999). The same effect variability was also reported for fat and lactose contents (Bjerre-Harpøth et al., 2012; Guinard-Flament et al., 2007; Gross et al., 2011). However, the effects of feed restriction on physi-

cal attributes are less well documented. Restriction of 40 % in DM intake increased the frequency of unstable milk to the ethanol test, but decreased titratable acidity without significant effects on the cryoscopy index and density (Zanela et al., 2006). These authors measured stability of milk at just one level of ethanol (76 °GL), which is above the official ethanol concentration required in Brazil – 72 °GL – (Brasil, 2011) and in several countries of Latin America, impairing its comparison with other studies.

Milk suitability for the dairy industry is tested on-farm and at industry platform and relies on milk stability to the ethanol test. In order to be accepted by the industry, milk should be stable when mixed with an alcoholic solution with at least 72 °GL (Brasil, 2011) as in Argentina (Chavez et al., 2004).

The present study aimed to evaluate the effects of severe feed restriction on milk production and its physical-chemical characteristics on mid-lactation Holstein cows.

### Materials and Methods

The experiment was carried out in the southwest region of the Rio Grande do Sul State, Brazil,  $27^{\circ}42'57''$  S,  $52^{\circ}37'39''$  W, altitude 583 m. Twelve Holstein cows were divided into two groups balanced for parity (six multiparous and six primiparous), with  $499 \pm 47.20$  kg of body weight (BW),  $188 \pm 124$  DIM and individual daily milk yield of  $19.35 \pm 4.10$  kg. All cows were milked twice daily at 05h30 and 17h30. The

<sup>§</sup>Present address: Emater/RS, R. Bartolomeu Dias, 03 – 99665-000 – Cruzaltense, RS – Brasil.

feeding trial was conducted during the hot season (Jan and Feb).

The whole experiment was a two-period crossover design and lasted 56 d. Each period lasted 28 d: 14 d for adaptation to the diet and 14 d for data collection (experimental period). Fourteen days before the start of the trial, all cows were fed the same standard diet. They were allowed to graze Tifton 85 (*Cynodon dactylon* (L.) Pers.) grass with mass availability of 6 to 8 kg DM 100 kg BW day<sup>-1</sup> and received 4 kg of concentrate; cows with daily milk production above 18 L received a further 1 kg high-protein supplement.

During the trial, two diets were used: control and restriction. Cows allocated to the control diet grazed Tifton 85 pasture with daily herbage mass availability of 8 kg DM 100 kg BW<sup>-1</sup> and received 2.5 kg of Tifton hay as well as 5 kg of concentrate. Cows in this group had an estimated daily dry matter intake of 16 kg, with 65 % of the DM derived from the pasture, which supplied approximately 15 % of crude protein, 67 % of TDN, 0.8 % calcium and 0.4 % phosphorus. Cows with daily milk production above 18 L received 2 kg of high-protein supplement per day. The cows assigned to the restriction diet grazed Tifton 85 pasture with daily availability of 4 kg DM 100 kg BW<sup>-1</sup> and received 50 % of the feed given to the non-restricted group, and we estimated that the nutrient supply was 50 % of the control diet.

The concentrate consisted of 676 g kg<sup>-1</sup> ground corn grain, 290 g kg<sup>-1</sup> ground barley grain, 20 g kg<sup>-1</sup> mineral salt and 10 g kg<sup>-1</sup> limestone. The high-protein supplement was composed of 750 g kg<sup>-1</sup> ground soybean and 250 g kg<sup>-1</sup> of soybean meal (Table 1). Concentrate, hay and protein supplement were supplied individually, twice a day and during the milking. The paddocks, enclosed by electric fences and with free access to water and shade, were located 150 m from the milking parlor.

During the trial, the two groups of cows grazed separately and remained for 24 h in each paddock, with a resting period of 20 d between pasture utilizations. The animals were transferred to a new paddock after the morning milking. Herbage mass was measured before and after grazing on each treatment, every day by cutting quadrats at ground level (Combellas and Hodgson,

1979). Pasture availability was calculated considering the weight of the animals' groups at the beginning of the experimental periods and adjustments were made by modifying the paddock area using the electric fence.

Samples of all individual ingredients (Table 1) were taken weekly and pooled monthly during the study and analyzed for DM content, crude protein (AOAC, 1995), acid detergent and neutral detergent fiber (Goering and Van Soest, 1970), potassium, calcium, sodium and magnesium content by atomic absorption spectrometry, and phosphorus levels by colorimetry (Fiske and Subbarow, 1925).

On days 14 and 1 prior to the beginning of treatments (d -14, d -1) and on days 14, 28 of each experimental period, at each milking, milk yield was recorded and composite milk samples were collected automatically. Daily milk yield was calculated and composite milk samples collected at each milking were analyzed for fat, protein and lactose using an infrared analyzer. Potassium, calcium, sodium and magnesium were measured by atomic absorption spectrometry. Phosphorus levels were obtained by colorimetry, and somatic cell count (SCC) was determined by flow cytometry. A subset of each composite milk sample was analyzed for titratable acidity (expressed as g of lactic acid per 100 mL), density, ethanol stability (by mixing 2 mL of milk with 2 mL of alcohol solution in a Petri dish, with ethanol concentrations starting at 68 °GL and raising until visual detection of clot formation; the result was considered as the minimal ethanol concentration that induced precipitation), thermal stability (by boiling milk samples and checking for clot formation; results were expressed as absence or presence of clots) and cryoscopy index (°H).

The data obtained at the end of experimental periods was subjected to analysis of variance, considering the crossover design, testing the effect of diet (control × restriction, n = 2), group (dietary treatment sequence, n = 2), period (n = 2) as fixed effects and cows within group as a random effect. The number of weeks in lactation was used as covariate, using the statistical program SAS®, MIXED procedure. The boiling test values were computed as zero for negative (normal milk) and one for positive milk (coagulated) and frequencies analyzed

Table 1 – Chemical composition of feeds supplied to cows receiving control (C) and restriction (R) diets.

	DM <sup>1</sup>	NDF <sup>2</sup>	ADF <sup>3</sup>	CP <sup>4</sup>	IVDMD <sup>5</sup>	Ca <sup>6</sup>	P <sup>7</sup>	Mg <sup>8</sup>	K <sup>9</sup>
Tifton 85 – pasture (C)	28.44	71.87	35.67	12.67	61.12	0.40	0.26	0.24	1.90
Tifton 85 – pasture (R)	38.90	77.22	40.79	9.29	57.13	0.40	0.20	0.21	2.44
Tifton hay	81.96	68.88	30.18	9.75	60.22	0.31	0.18	0.20	1.72
Corn, ground	88.12	13.54	3.99	9.25	-	0.04	0.26	0.10	0.37
Barley, ground	90.02	13.90	3.22	14.52	-	0.50	0.50	0.07	0.48
Soybean, ground	90.06	18.84	13.04	40.02	-	0.30	0.54	0.18	2.02
Soybean meal	88.00	15.09	8.98	46.40	-	0.36	0.61	0.31	1.87
Mineral salt <sup>10</sup>	100.0					23.0	9.00	2.00	-

<sup>1</sup>Dry matter (%); <sup>2</sup>Neutral detergent fiber (% of dry matter); <sup>3</sup>Acid detergent fiber (% of dry matter); <sup>4</sup>Crude protein (% of dry matter); <sup>5</sup>In vitro dry matter digestibility (%); <sup>6</sup>Calcium (% of dry matter); <sup>7</sup>Phosphorus (% of dry matter); <sup>8</sup>Magnesium (% of dry matter); <sup>9</sup>Potassium (% of dry matter); <sup>10</sup>Mineral salt (% of dry matter) 48 % Na, 23 % Ca, 15 % S, 9 % P, 2 % Mg, 100 mg kg<sup>-1</sup> Co, 700 mg kg<sup>-1</sup> Cu, 80 v l, 2000 mg kg<sup>-1</sup> Fe, 1250 mg kg<sup>-1</sup> Mn, 20 mg kg<sup>-1</sup> Se e 2700 mg kg<sup>-1</sup> Zn.

using NPAR1WAY procedure of SAS. Values of SCC were logarithmically converted to a linear score (0 to 9). The differences between the diets were detected by the Fisher test;  $p$ -values  $< 0.05$  were considered significant and trends towards significance are discussed at  $p < 0.10$ . Values for standard errors of the mean (SEM) are presented in the text.

## Results and Discussion

Feeding restriction tended to reduce body condition score (BCS): 2.77 (restriction group)  $\times$  2.92 (Control group) /SEM = 0.18;  $p = 0.0729$ ) while it did not affect BW: 489.20  $\times$  505.00 kg (SEM = 12.26 kg;  $p = 0.2165$ ) in the restriction and control groups, respectively. Although the reduction of food supply was severe, the absence of BW loss may be due to variation in the gut fill as cows were weighed once at the beginning of each experimental period and at the end of the trial. Cows belonged to commercial herd were not fasted prior to weighing. On the other hand, under restricted grazing time cows had lower dry matter intake and presented lower body weight than the unrestricted group (Pérez-Ramírez et al., 2009). Gross et al. (2011) imposed feed restriction during one week, and with more frequent weighings (without previous fasting) they detected differences in BW changes between treatment groups.

Cows submitted to feeding restriction showed decreased daily milk yield compared with cows in the control group (8.70  $\times$  14.37 kg; SEM = 0.92 kg;  $p = 0.0001$ ). Underfeeding generates a negative energy balance and cows try to cope with the nutritional deficit by lowering milk production and mobilizing body reserves. Their strategy depends upon genotype, DIM and magnitude of the nutritional deficit (Bjerre-Harpøth et al., 2012; NRC, 2001). In the present study, cows were in mid to late lactation and they compensated the nutritional deficit by lowering milk production. Bjerre-Harpøth et al. (2012) and Guinard-Flament et al. (2007) observed reduced milk yield and increased moderate lipid mobilization in feed-restricted cows.

Lactose (4.49  $\times$  4.56 g 100 g<sup>-1</sup>; SEM = 0.39 g 100 g<sup>-1</sup>;  $p = 0.0464$ ) and magnesium (0.12  $\times$  0.14 g L<sup>-1</sup>; SEM = 0.01 g 100 g<sup>-1</sup>;  $p = 0.0108$ ) contents and daily yields of 4 % fat corrected milk (11.47  $\times$  14.63 kg; SEM = 0.77 kg;  $p = 0.0003$ ), lactose (0.55  $\times$  0.70 kg; SEM = 0.04 kg;  $p = 0.0003$ ), fat (0.44  $\times$  0.57 kg; SEM = 0.03 kg;  $p = 0.0003$ ), crude protein (0.35  $\times$  0.45 kg; SEM = 0.03 kg;  $p = 0.0015$ ) and casein (0.18  $\times$  0.32 kg; SEM = 0.18 kg;  $p = 0.0001$ ) were lower for restricted than for control cows. Potassium content in milk tended to be lower for restricted than for control cows (1.41  $\times$  1.51 g L<sup>-1</sup>, SEM = 0.06 g L<sup>-1</sup>;  $p < 0.10$ ). No differences (Restricted  $\times$  Control;  $p > 0.05$ ) were found between the groups for concentrations in milk of crude protein (3.08  $\times$  3.14 g 100 g<sup>-1</sup>; SEM = 0.10 g 100 g<sup>-1</sup>), casein (2.24  $\times$  2.51 g 100 g<sup>-1</sup>; SEM = 0.19 g 100 g<sup>-1</sup>), fat (3.82  $\times$  4.00 g 100 g<sup>-1</sup>; SEM = 0.17 g 100 g<sup>-1</sup>), calcium (1.13  $\times$  1.05 g L<sup>-1</sup>; SEM = 0.07

g L<sup>-1</sup>), sodium (1.38  $\times$  1.50 g L<sup>-1</sup>; SEM = 0.06 g L<sup>-1</sup>) and phosphorus (0.81  $\times$  0.85 g L<sup>-1</sup>; SEM = 0.03 g L<sup>-1</sup>).

The reduction in milk production and synthesis of lactose, fat and protein is probably due to lower blood flow and mammary uptake of nutrients, although these were not measured. Because glucose is the primary precursor for lactose synthesis and lactose is the major osmotic agent in milk, reduced mammary glucose uptake has a major rate-limiting effect on milk synthesis as pointed out by Guinard-Flament et al. (2007). Although the milk volume was reduced by 40 %, lactose, protein and fat production were reduced to a lesser extent, respectively, 21, 22 and 23 %. While lactose content was lowered, milk protein and fat content were not, as also noted by Zanela et al. (2006). However, the effects of underfeeding upon milk solids content are highly variable, depending on the length and severity of the restriction and stage of lactation (Auld et al., 2000; Bjerre-Harpøth et al., 2012; Guinard-Flament et al., 2007; Gross et al., 2011; Zanela et al., 2006).

As 85 % of the SCC score values were below linear score 5 and as they did not vary among diets, this decrease in lactose content might be caused by either reduction in its synthesis, due to low blood flow and low glucose uptake (Guinard-Flament et al., 2007), or passage of lactose from alveoli lumen to blood, through increased permeability of tight junctions of the mammary epithelial cells following stress (Stelwagen et al., 2000) caused by feed restriction (Lacy-Hulbert et al., 1999).

The reduction in Mg content in milk from feed restricted cows can be partially related to the increased potassium content observed in the Tifton pasture consumed by the feed-restricted cows, when comparing with the Tifton pasture offered to the animals in the control group: 2.44 %  $\times$  1.90 %, respectively (Table 1). Potassium absorption is antagonist of Mg, and thus it may have impaired the absorption of Mg, lowering its concentration in milk (NRC, 2001). The probable larger K intake and its high permeability in the apical membrane of mammary epithelial cells could also explain the tendency of increase of the potassium in the milk of feed restricted cows. There were no differences in the concentrations of other minerals in the pasture, which might explain the absence of differences regarding other minerals in milk.

Milk stability to ethanol was lower in the restricted group than in the control (69.00  $\times$  75.80 °GL of ethanol in the alcoholic solution; SEM = 1.05 °GL,  $p < 0.001$ ). The reduction in the ethanol stability was partially related to the higher values for potassium content observed in the milk of feed restricted cows. In general, decrease in milk stability is related to several factors: acidity, mono (Na<sup>+</sup>, K<sup>+</sup>) and divalent cations (Ca<sup>++</sup> and Mg<sup>++</sup>) contents in milk, levels in milk of anions such as chlorine, citrate and phosphate, which affect the ionic strength and zeta potential, increasing the steric interactions between the casein micelles and inducing casein

aggregation and coagulation (Chavez et al., 2004). However, no difference (Restricted  $\times$  Control;  $p > 0.10$ ) was found for thermal stability evaluated by the frequency of clots formation in the boiling test ( $0.16 \times 0.00$ ), probably due to acidity values within the normal range (14 to 18 °D), which shows the lower sensitivity of the boiling test to detect changes in milk stability than the alcohol test (Fonteh et al., 2005; Lewis and Deeth, 2009).

Titrate acidity ( $15.17 \times 16.83$  °D; SEM = 0.32 °D;  $p = 0.0004$ ) was lower in the restricted than in the control group, while values of cryoscopy ( $-0.54 \times -0.55$  °H; SEM = 0.001 °H;  $p > 0.10$ ) and density of milk ( $1030.83 \times 1031.25$  g L<sup>-1</sup>; SEM = 0.48 g L<sup>-1</sup>;  $p > 0.10$ ) were not different between restricted and control groups, respectively. The reduction in titratable acidity was probably related to the smaller numerical values of phosphorus, total protein and casein in restriction cows compared to control cows. The absence of effects of feed restriction on density and cryoscopic index were probably due to the counteracted effects of decreased protein, lactose and fat contents, besides variation in the minerals contents.

There were no cases of clinical mastitis and the values of SCC were below 400,000 cells mL<sup>-1</sup> throughout the experiment. The logarithmic of SCC score (in which 1 is the lower and 9 the higher level) was not different between groups:  $3.42 \times 3.28$  (SEM = 0.37;  $p > 0.10$ ) for restriction and control diet, respectively.

The low values of SCC throughout the experiment resulted in minimal change in the production and milk composition due to the health of the mammary gland. Absence of differences for SCC between feeding regimens are in agreement with previous results of Zanela et al. (2006), but they are not in agreement with the increase in SCC noticed by Lacy-Hulbert et al. (1999) and O'Brien et al. (1999). In those latter studies, the increase in SCC in feed restricted cows could be due to the reduced milk volume, which tended to concentrate SCC values, probably not related to the decreased immune response. Moyes et al. (2009) did not find differences in the immune response of Holstein cows in mid lactation submitted or not to 40 % energy restriction for seven days and challenged with injection of *Streptococcus uberis* in one of the mammary glands.

Although feed restriction has altered some milk chemical and physical attributes of milk, most aspects of milk composition were adequate, except for stability in the alcohol test, concerning the Brazilian legislation for milk quality (Brasil, 2011), which requires 2.90, 3.00 and 4.30 g 100 g<sup>-1</sup> as minimum values for protein, fat and lactose contents, respectively, besides minimum ethanol stability of 72 °GL. However, as feed restriction reduced the minimum concentration of ethanol required to induce precipitation, it may lead to an increase in the number of positive samples in the test and, following the Brazilian national legislation on milk quality, increase the volume of milk rejected by the industry.

## Conclusions

A short but severe feed restriction in mid-lactation cows resulted in an immediate high decline in milk yield, accompanied by moderate declines in crude protein, casein, fat and lactose yields. Milk stability to the ethanol test was severely hindered and feed restricted cows produced more milk that may be rejected by the dairy industry.

## Acknowledgements

Authors gratefully acknowledge the material and financial support from the Federal University of Rio Grande do Sul (UFRGS), Sulina Association of Credit and Rural Assistance (NASCAR), the Cooperative of Small Ranchers at Campinas do Sul (COOPASUL) and CNPq scholarships.

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