Different levels of supplied energy for lactating cows affect physicochemical attributes of milk

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ABSTRACT. In the period from 2004 to 2011, seven trials on lactating cows were conducted to evaluate the relationship between the amounts of energy required, and milk production and composition. In total, 392 observations on 132 animals were selected due to different levels of energy intake. The observations were classified into three groups according to the amount of total digestible nutrients (TDN) provided by the diets, i.e. 67, 75 and 94% TDN. Such characteristics as proportion of energy provided in relation to cow requirements, experiment duration, daily milk production, and fat, protein and lactose contents, total solids percentage, somatic cell score, titratable acidity and ethanol stability test were considered. Levels of supplied energy were positively related with milk production, ethanol stability and lactose content. Seven variables were regarded as discriminant between levels of supplied energy, including proportion of provided energy related to cow requirement, duration of the experiments, ethanol stability test and milk production, and explained more than 70% of the variance among the observations. Milk production increased linearly with the proportion of the supplied energy from 14, 16 to 19 kg · d⁻¹ per cow, while lactose content increased from 4.37 to 4.56% as the level of TDN attained 93% of estimated requirements. Milk stability increased from 74.2 to 76.4% as the level of TDN attained 79% of estimated requirements. Increased level of the supplied energy related to requirements of lactating cows improved milk yield, milk stability and lactose content.

Introduction

Nutritional requirements for lactating cows can be calculated using different methodologies, such as those existing in the British (ARC), French (UFL) or North American (NRC) systems (Vermorel and Coulon, 1992). On the other hand, lactating cow energy requirements may be expressed as the amount of total digestible nutrients (TDN), which is still widely used since it can be easily determined by feed analysis (Weiss, 1998).

Decreasing levels of supplied energy imply changes in milk production (Reid et al., 2015), chemical composition (Sova et al., 2014), body weight and

body condition score (Weber et al., 2015). However, the effect of such reductions on the physical characteristics, in particular ethanol stability, has not been studied extensively. Moreover, the impact of restoring levels of supplied energy on milk chemical and physical attributes is also not precisely documented (Gross et al., 2011).

It is known that changes in the supplied energy intake affect yield and physicochemical composition of milk and may vary according to the productive potential of each cow, stage of lactation, and duration of the feed supply. The multivariate analysis (Macciotta et al., 2012) can be used to assess the relationship between these attributes and their effects on milk properties.

So, the aim of the study was to evaluate the changes in daily milk production and in physicochemical characteristics of milk from lactating cows fed diets with different levels of supplied energy.

Material and methods

Seven experiments to study the effects of energy intake on productive and milk traits were conducted in the south region of Brazil in Pelotas, RS (30–32°S latitude and 51–53°W longitude). The region is classified according to Cfa climate classification as hot summer and moderate cold winter with annual rainfall near 800 to 1200 mm and low altitude (below 100 msnm). The studies were performed from 2004 to 2011 by the researchers from the Centre for Research of Dairy Cattle and Animal Behaviour (NUPLAC), Federal University of Rio Grande do Sul (UFRGS), Federal University of Pelotas (UFPEL) and Brazilian Agricultural Research Corporation (Embrapa). In total, 132 cows in different lactation stages were used in the studies. The data analysis comprises 392 observations selected due to the different levels of energy intake, similar research protocols used and the same variables studied. Each feeding trial is described in detail in Table 1.

All animal procedures were approved by the respective Ethic Committee of each university or research centre under the following protocol numbers 11315, 520042/99-9 and 50405056.

Raw data from experiments were tabulated and included the original variables: levels of supplied energy (% TDN), period of feeding (number of days), breed (after transformation of qualitative data to numeric data: 1 – Jersey, 2 – Holstein × Jersey, 3 – Holstein), daily milk production, fat, protein and lactose contents, total solids percentage (TS), somatic cell score (SCS), titratable acidity and ethanol

test stability, measured as the lowest concentration of ethanol in alcoholic solution needed to promote the milk coagulation. The higher the ethanol concentration needed, the higher the milk stability.

Data obtained in the experiments were submitted to the multivariate statistical analysis. Observations were standardized for mean equal zero and standard deviation equal to 1 and submitted to the analysis of the principal factors (PROC FACTOR) to investigate the covariation between attributes considering three factors, those with eigenvalues equal or greater than 1, using alpha method as extraction factor. Data were grouped into clusters which were not ranked hierarchically (PROC CLUSTER) and further they were related to the original variables (PROC CANDISC). To determinate the most important variables in the clusterization PROC STEPDISC was used, with level of significance for average square of canonical correlation (ASCC) of $P < 0.0001$. The original variables with the greatest contribution in variance within each group with the same level of supplied energy were determined by PROC VARCLUS. Comparison of means among clusters was performed with multivariate analysis (MANOVA), using PROC GLM, which are compared with the Tukey's test with 5% significance. All statistical procedures were performed using the statistical software SAS 9.3 (SAS Institute Inc., Cary, NC, USA).

Results

The first principal factor explained 54.9% of the variance of the attributes and it was mainly related to the protein, fat and total solids contents, while the second principal factor, which explained 32.3% of the variance, was more related to the levels of supplied energy, milk production and milk stability to ethanol. A negative association between protein, fat and total solids contents with milk yield and proportion of Holstein alleles was observed. Whereas little or no association between these milk components with the levels of supplied energy, percentage of ethanol needed to coagulate the milk, lactose content and milk yield was found. On the other hand, levels of supplied energy were positively associated with milk yield, milk stability and lactose content. The duration of the experiments negatively affected the contents of fat, protein and total solids, but its positive association with milk production, lactose content and milk stability was noticed (Figure 1).

In total, 392 observations on 132 cows were grouped into three clusters, with 115, 135 and 142 observations. As the data were not previously

Table 1. Characteristics of feeding experiments

Trial	Feeding	Diet composition	Days of experimental period	Breed and number of animals	Live weight; body condition	Milk production; days in milk (DIM)
1	control diet: 66% roughage + 34% concentrate; restricted diet – only roughage	control diet, kg: concentrate 6, lucerne hay 3, maize silage 6 + temperate pasture; restricted diet, kg: lucerne hay 3, maize silage 6 + temperate pasture	two experimental periods each 14 d (reversible design)	Jersey 10 lactating cows	376 ± 54.3 kg; 2.6 ± 0.4	17.8 ± 3.2 kg· d^{-1} per cow; 75 ± 22.8 DIM
2	low levels of energy and protein (LEP), low level of energy and high level of protein (LEHP) and high levels of energy and protein (HEP)	LEP, kg: pasture + ground maize 1.62 + soyabean meal 1.26 + wheat meal 0.53 + salt + mineral and vitamin premix; LEHP, kg: pasture + lucerne hay 4 + ground maize 2.46 + soyabean meal 1.53 + urea 0.16 + salt + mineral and vitamin premix; HEP, kg: pasture + lucerne hay 5 + ground maize 4.4 + soyabean hulls 3.56 + salt + mineral and vitamin premix	pre-experimental: 8 d; adaptation to diet: 13 d; experimental: 28 d	Jersey 24 lactating cows	LEP: 365 ± 21.9 kg; 3 ± 0.2 LEHP: 398 ± 23.4 kg 3.3 ± 0.2 HEP: 391 ± 21.9 kg 3.2 ± 0.2	LEP: 10.9 ± 1.6 kg· d^{-1} per cow; 212 ± 28.1 DIM LEHP: 12.8 ± 1.68 kg· d^{-1} per cow; 244 ± 30.03 DIM HEP: 11.8 ± 1.57 kg· d^{-1} per cow; 196 ± 28.1 DIM
3	pasture and low supplementation (LS), and pasture and high supplementation (HS)	LS, kg: native pasture + commercial concentrate 2.6; HS, kg: native pasture + lucerne hay 5 + ground maize 4 + commercial concentrate 3.6	pre-experimental: 7 d; experimental: 49 d	Holstein 12 lactating cows	514 ± 58.5 kg; not measured	13.3 + 3.5 kg· d^{-1} per cow; 349 + 162 DIM
4	three different roughages: native pasture, millet and maize silage	respective roughage + commercial concentrate	experimental periods for native pasture: 14 d; millet: 42 d; maize silage: 32 d	Jersey 25 lactating cows	390 ± 43 kg; not measured	10.1 ± 2.2 kg· d^{-1} per cow; 457 ± 17 DIM
5	35:65, 45:55 and 55:45 (concentrate to forage ratio) ratios on a dry matter (DM) basis	maize silage, brewery residues, concentrate, shell meal, mineral salt	pre-experimental period: 15 d; experimental period: 56 d	Holstein 24 lactating cows	575 ± 68 kg; 3.1 ± 0.3	18.4 ± 3.0 kg· d^{-1} per cow; 121 ± 20.8 DIM
6	adjusted (100% NRC) and non-adjusted (80% energy and 88% protein) diets according NRC	adjusted group, kg: elephant grass pasture + fresh sorghum silage 28 + commercial concentrate 6.9 + soyabean meal; non-adjusted group, kg: elephant grass pasture + fresh sorghum silage 25 + commercial concentrate 4.5	pre-experimental period: 7 d; experimental period: 21 d	Jersey 16 lactating cows	adjusted group: 365 kg; 3.06 non-adjusted group: 358 kg; 3.4	adjusted group: 18 kg· d^{-1} per cow; 118 DIM non-adjusted group: 16.4 kg· d^{-1} per cow; 118 DIM
7	three different proportion of silage: hay (80:20, 60:40 and 40:60)	80:20, kg – maize silage 25.1 + Tifton hay 2.57 + commercial concentrate 6.6; 60:40, kg – maize silage 18 + Tifton hay 4.85 + commercial concentrate 6.6; 40:60, kg – maize silage 14.3 + Tifton hay 7.71 + commercial concentrate 6.6	pre-experimental period: 7 d; experimental period: 21 d	Jersey 21 lactating cows in feedlot	405 ± 47 kg; 2.9 ± 0.2	18.0 ± 4.8 kg· d^{-1} per cow; 120 ± 63 DIM
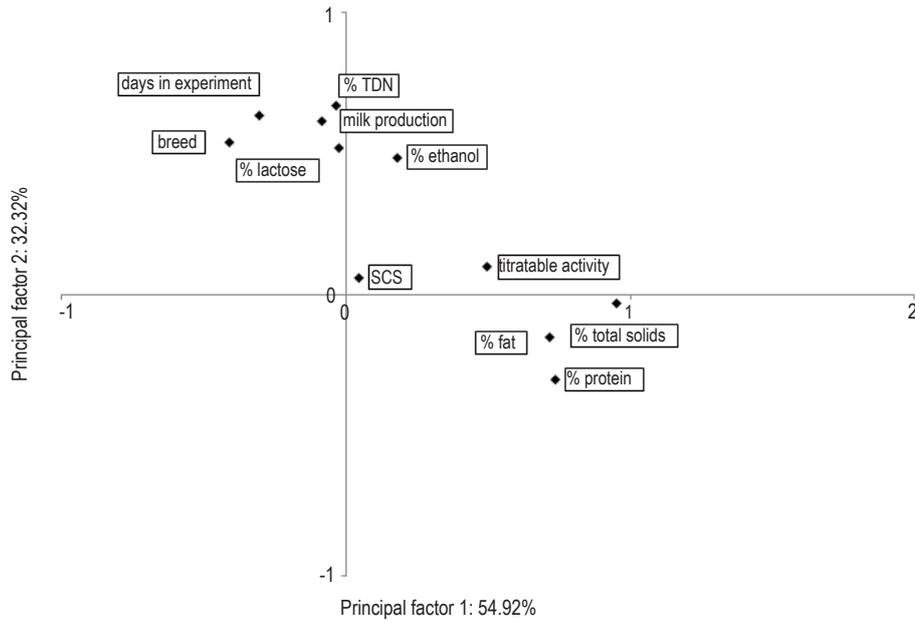
Figure 1. Variables (level of supplied energy, duration of the experiment, milk production and physicochemical characteristics) projected in principal factors 1 and 2

ranked, clustering of the observations was made by similarity between observations and three different levels of TDN found by cluster analysis. The values of canonical correlation (0.89 for canonical correlation 1 (Can 1) and 0.77 for canonical correlation 2 (Can 2)) showed a good separation of observations based on the level of supplied energy. In the canonical analysis it was found that such attributes as milk production, lactose content, ethanol stability and duration of the experiments were closely associated with observations of cluster 3, while such remaining variables as titratable acidity, SCS, protein and fat contents were more associated with the cluster 2 (Figure 2). Amongst 11 variables, seven were considered as discriminant to the formation of three clusters, being the most relevant, according the partial R^2 values, the level of energy supply, total solids content and titratable acidity of milk (Table 2). Distinct original variables explained the variance between observations within clusters.
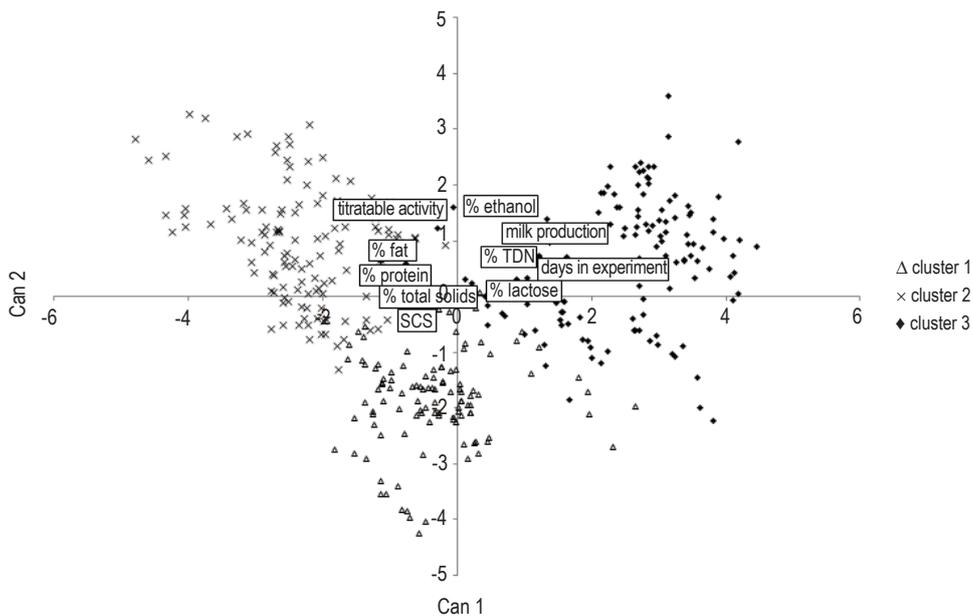
Figure 2. Standardized canonical means of productive attributes and physicochemical characteristics of milk from cows fed different levels of energy

Table 2. Discriminant analysis of variables selected for the classification of observations according to level of supplied energy

Indices	Partial-R^2	$P > F$	$P > ASCC$
TDN level	0.901	<0.0001	<0.0001
Total solids content	0.541	<0.0001	<0.0001
Titratable acidity	0.178	<0.0001	<0.0001
Milk ethanol stability	0.092	<0.0001	<0.0001
Protein content	0.086	0.0001	<0.0001
Milk production	0.045	0.0094	<0.0001
Duration of experiment	0.038	0.0201	<0.0001

Partial-R^2 – coefficient of partial determination; F – F statistic test; ASCC – average square of canonical correlation; TDN – total digestible nutrients

Table 3. Proportion of variance explained by the original variables within each level of supplied energy

Cluster	Original variable	Proportion of variance explained by variable, %
1	TDN level, ethanol stability, milk production and SCS	71.2
2	Fat, lactose and total solids content	65.9
3	Duration of trials, titratable acidity and protein content	55.9

SCS – somatic cells score, calculated as [$\log_2$(SCC / 100 000) + 3]; SCC – somatic cells count; TDN – total digestible nutrients

Table 4. Means of productive and milk traits of cows receiving different levels of energy

Indices	Cluster 1 (n = 115)	Cluster 2 (n = 135)	Cluster 3 (n = 142)	RMSE value	*P*-value
TDN level, %	66.9[a]	79.4[b]	93.9[c]	0.79	<0.0001
Breed of cows[1]	1.00[a]	1.03[a]	2.86[b]	0.97	<0.0001
Duration of experiment, days	28.6[a]	28.6[a]	48[b]	0.81	<0.0001
Days in milk	168[a]	241[b]	235[b]	0.73	0.0001
Milk production, kg · d⁻¹ per cow	14[a]	16.6[b]	19.8[c]	0.74	<0.0001
Milk stability, % ethanol	74.2[a]	76.4[b]	76.8[b]	0.90	0.0001
Titratable acidity, ºD	16.5[a]	19.1[c]	17.1[b]	0.94	<0.0001
SCS	3.37	3.70	3.35	0.96	0.1807
Milk composition, %					
fat	3.68[a]	5.03[b]	3.58[a]	0.87	<0.0001
protein	3.36[a]	3.94[b]	3.19[c]	0.90	<0.0001
lactose	4.44[a]	4.37[a]	4.56[b]	0.94	<0.0001
total solids	12.3[a]	14.2[b]	12.3[a]	0.90	<0.0001

[1]Jersey = 1, Jersey × Holstein crossbred = 2, Holstein = 3; RMSE – root mean square error; TDN – total digestible nutrients; SCS – somatic cells score, calculated as [$\log_2$(SCC / 100 000) + 3]; SCC – somatic cells count; [abc] – values with different superscripts within each row are significantly different (Tukey's test)

The most important attributes explaining the variance between observations were level of supplied energy, milk stability, milk yield and SCS; fat, lactose and total solids content; and duration of the trials, acidity and protein content for clusters 1, 2 and 3, respectively (Table 3).

The formation of three different groups according the level of supplied energy was possible due to the cluster analysis. Clusters 1, 2 and 3 showed overall means of 67, 75 and 94% provision of TDN requirements and they comprised observations within the range of 55–100, 60–100 and 80–105% provision of TDN requirements allowing to group observations into low, medium and high level of TDN supply, respectively. The daily milk production increased with the supplementation of TDN to the animals. Milk presented higher stability when TDN levels were supplied within a medium and high range. The means of the original variables for each of the three clusters are shown in Table 4.

Discussion

The increase in the nutrient supply results in higher nutrient availability to the mammary gland (Lemosquet et al., 2009a). The most noticeable is glucose, the precursor of lactose, which is the primary osmotic regulator of milk volume (Lemosquet et al., 2009b; Wall and McFadden, 2012). In the present study, higher level of supplied energy resulted in higher milk yield and lactose content.

Samples ranked as low level of supplied energy were taken from grazing cows or those fed silage with low to moderate addition of concentrates. Medium and high energy supply groups received at least roughage to concentrate proportion of approximately 60 to 40, with feeds of good quality. Energy supply is usually augmented by the inclusion of high quality forage with high digestibility or inclusion of concentrate into diet, resulting in higher concentrate to roughage ratio, which is a well-known and widespread tool to enhance milk yield (Whelan et al., 2012).

Feeding systems supplying similar energy levels as those described in the present study are characterised by an inverse association between stocking rate, milk yield and lactose content, but only under assumption that the low stocking rate allows to increase energy intake through selection of the highly digestible plants (McCarthy et al., 2013). However, the increase in the energy supply does not always affect milk physicochemical composition (Giallongo et al., 2015), and this relation is dependent on the original energy level, productive potential of cow, lactation stage and net increment of supplied energy (Benchaar et al., 2014; Boerman et al., 2015; Broderick et al., 2015).

The difference in milk protein content between clusters 1 and 2 has the same magnitude as the increase in milk production. Once both groups have similar proportion of animals of the same breed (Jersey), it can be speculated that this increment was possible due to the increase in the amount of metabolites necessary for protein synthesis, especially glucose precursors and amino-acids, probably because of larger amount of concentrate supplied. Increased amounts of rumen digestible carbohydrates, especially of starch, increased the protein content in milk (Lohrenz et al., 2010; Boerman et al., 2015).

High level of supplied energy supplemented as starch results in greater milk production and milk protein synthesis, which is attributed to increased secretion of insulin and insulin growth factor 1 (Rius et al., 2010). However, a reduction of milk protein content observed in the cluster 3 with higher energy supply can be partly explained by the dilution effect that increased the volume of milk by 40%, as well as by a higher proportion of Holstein cows in this cluster in comparison to the predominance of Jersey cows in groups 1 and 2.

The higher milk fat content observed in the cluster 2 than in the cluster 3, may have resulted from the greater use of roughage, which in turn increased the diet fibre content. In this cluster the predominance of Jersey cows was negligible. Xue et al. (2011), however observed that Jersey-Holstein crossbred cows fed either low (30%) or high (70%) contents of grass silage produced milk with high fat content. On the other hand, the low value of milk fat content observed in the cluster 1, albeit statistically similar to the cluster 3, was contradictory to our expectations. It was hypothesised that milk fat content in the group predominantly composed of Jersey cows, with an average of 168 days in milk and fed diets with about 65% of roughage should have been high.

The lack of relationship between the level of supplied energy with SCS is in agreement with results presented by Moyes et al. (2009) and Vance et al. (2012), who found no relation among energy deficit and characteristics of the immune system.

The improved milk stability in the ethanol test (with higher levels of nutritional supply) have been reported when diets with a low percentage of fibre (Barchiesi-Ferrari et al., 2007) or high overall nutrient supply (Fruscalso et al., 2013) were fed to animals, albeit in the latter just two diets (50% of feed restriction and with no restriction) were evaluated. The integrity of the tight junctions of mammary epithelial cells is disturbed by nutrient deprivation (Stumpf et al., 2013) and it is associated with lower lactose content but higher concentrations of sodium and chloride in milk (Chavez et al., 2004; Stumpf et al., 2013). In the present study, the measured attributes in milk do not allow to investigate the mechanism of action of the increasing energy supply on milk stability.

Conclusions

Increasing level of supplied energy to dairy cows improves milk yield, lactose content and milk stability in the ethanol test, but does not influence other milk traits or the health of the udder. The usage of the high level of supplied energy can be advantageous for farmers to whom dairy industry offers a better payment for better milk quality.

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