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Season of the year, the feeding and productive environment

effect on the content of casein in milk

Estación del año, alimentación y ambiente productivo

efecto sobre el contenido de caseína en leche

Estação do ano, alimentação e ambiente produtivo

efeito sobre o teor de caseína no leite

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Abstract

A study was carried out to determine the casein variation in milk along the year with season and herd feeding strategy as main source of variation. Monthly samples were taken from 30 dairy farms that sent milk to PILI SA (15 farms) and CLALDY SA (15 farms) during April 2012 - March 2013. A mixed model and a recursive partition of variance method were used. The protein content in milk was higher in autumn, and the casein content in milk was higher in autumn and winter probably due to the higher use of energy concentrated supplements with better synchronization with the protein contribution from pastures. It was possible to maintain high levels of casein content and a casein:true protein ratio in milk during spring with almost exclusively pastoral diets. Overall, the proportion of casein in relation to the total protein and the casein in relation to true protein in milk did not reach the values reported in the literature with more concentrated diets.

Keywords: casein, concentrated, nutrition, pastures, production systems

Resumen

Se realizó un estudio con el objetivo de estudiar el patrón de variación de caseína en leche a lo largo del año, y la estrategia de alimentación del rodeo lechero como principal fuente de variación. Se tomaron muestras mensuales en tambos remitentes a las empresas PILI SA y CLALDY SA (15 tambos por empresa) durante el período abril 2012 - marzo 2013. Se utilizó un modelo mixto y un método de partición recursiva de varianza. El contenido de proteína en leche fue mayor en otoño y el contenido de caseína en leche fue mayor en otoño e invierno, probablemente explicado por el mayor uso de suplementos concentrados que aportan energía con una mejor sincronización con el aporte de proteína por parte de las pasturas. Fue posible mantener niveles altos de contenido de caseína y relación caseína/proteína verdadera en leche en primavera, con dietas casi exclusivamente pastoriles. En general, la proporción de caseína en relación a la proteína total y la relación caseína/proteína verdadera en leche no alcanzó valores que reporta la bibliografía con dietas más concentradas.

Palabras clave: caseína, concentrado, nutrición, pasturas, sistemas de producción

Resumo

Foi realizado um estudo para determinar a variação da concentração de caseína no leite ao longo do ano com a estação do ano e a estratégia de alimentação do rebanho como principal fonte de variação. Amostras mensais foram retiradas de 30 fazendas leiteiras que enviaram o leite para PILI SA (15 fazendas) e CLALDY SA (15 fazendas) durante o período de abril de 2012 a março de 2013. Um modelo misto e um método de partição de variância recursiva foram usados. O teor de proteína no leite foi maior no outono, e o teor de caseína no leite foi maior no outono e inverno provavelmente explicado pelo maior uso de suplementos concentrados de energia com melhor sincronização com a contribuição proteica das pastagens. Foi possível manter altos níveis de conteúdo de caseína e uma relação caseína / proteína verdadeira no leite durante a primavera com dietas quase exclusivamente pastorais. De maneira geral, a proporção de caseína em relação à proteína total e a caseína em relação à proteína verdadeira do leite não atingiram os valores relatados na literatura com dietas mais concentradas.

Palavras-chave: caseína, concentrado, nutrição, pastagens, sistemas de produção

1. Introduction

Milk production in Uruguay has increased over the past decade to 2.3 billion liters in 2013. Only 8% of the total production in Uruguay is sold as pasteurized milk, the remaining volume is used to produce different products[1]. In recent years (2008 to 2012) between 35 and 38% of milk sent to Uruguayan industries has been destined for cheese processing, being one of the main export products together with milk powder[2].

Cheese yield influences the competitiveness of cheese industries[3]. Cheese billing for Uruguay's exporting dairy sector for 2019 was 106.6 (million FOB dollars), representing 16.4% of the total turnover of dairy exports for that year[4]. Therefore, improving cheese yield would directly impact the economic profit of cheese industries. Casein concentration in milk is known to be directly related to cheese yield[3][5]. In Uruguayan industrial plants, the amount of casein is assumed as a constant (80%) over total protein, based on bibliographic values, without local information on this indicator and its variation throughout the year.

Different factors affecting casein variation in milk in dairy systems have been studied, such as breed, season, productive environment, number and lactation stage, and feeding[6][7][8][9][10][11][12][13][14][15][16][17]. However, there is no history of national studies on seasonal variation of casein in milk, nor on the technological factors that can cause casein to vary within different seasons.

A restriction on pasture intake causes a decrease in the yield and content of protein and casein in milk due to decreases in energy intake. Variations in the protein content when providing different types of silage depend on the type of silage and its interaction with pasture, considering the total diet.

Reports of increases in the protein and casein content in milk with concentrates supply are consistent in bibliography[13][15][16][17][18]. An increase in energy supply in addition to nitrogen supply by pastures allow a growth in the supply of amino acids available in the mammary gland, increasing the synthesis of milk protein. An increase in the nitrogen level of the ration does not improve protein content in milk, unless it is limiting regarding animal requirements.

There are knowledge inconsistencies about factors that could affect casein:true protein and casein:total protein ratios.

In Uruguay, no records describe the variation pattern of casein in milk throughout the year. Based on this, a study was conducted aiming to determine the variation pattern of casein in milk throughout the year, and the effect of feeding and productive environment of dairy farms in the northwestern region of Uruguay.

2. Material and methods

Monthly samples were taken from 30 dairy farms which supplied the companies PILI SA and CLALDY SA (15 for each plant) for a year (April, 2012 to March, 2013). The number and size of farms per plant were determined according to the Neyman assignment, taking into account the size and variance of each stratum, considering a 95% confidence and an estimation error of 10%.

STRATUM	1			2			3		
TECHNOLOGY LEVEL	A	B	C	A	B	C	A	B	C
PILI (n° dairy farms)	3	4	1	0	1	2	0	0	4
CLALDY (n° dairy farms)	1	4	2	1	2	3	0	0	2

The sizes of the strata were established as follows: stratum 1. <50,000 monthly liters; stratum 2. 50,000 ≤ x < 150,000 monthly liters, and stratum 3. ≥ 150,000 monthly liters.

Once the sample size for each stratum was determined, the systems were classified according to technological level, considering feeding type and handling in three categories: extensive (A), medium (B), and intensive (C). The extensive system (A) was characterized by natural field-based pastures, which in some cases had some improvement, with very low levels of supplementation (less than 2 kg per milking cow per day), one milking lot a year and

with calving distributed throughout the year. Sown pastures, low to medium supplementation levels (2 to 4 kg per milking cow per day) and generally one milking lot characterized the intermediate system (B). The intensive system (C) was characterized by sown pastures with high-supplementation levels (more than 4 kg per cow per day), with strategic confinement and more than one milking lot. Once they were organized with the first variable (referral to plant), with plant technicians, they were selected by technological level and location (considering that they represent the totality of farms in the region).

The distribution of the technological levels of the production systems in the different strata could not be equitable due to the absence of some of these groups in a certain stratum. The extensive system (A) is found in stratum 1, and in some cases in stratum 2, but it was not possible to find this system in stratum 3. Similarly, it is more likely to find intensive systems (C) in stratum 2 and 3 than in stratum 1. Therefore, 5 extensive systems, 11 intermediate systems and 14 intensive systems were selected from both companies.

Samples were taken in tanks (if there were more than one, a mixture proportional to the volumes of each one was carried out), taking into account that the milk came from two or four milking. The sampling frequency was monthly, on Monday, Tuesday and Wednesday of the first and second week of each month. Then, samples were sent to the INIA laboratory, La Estanzuela, on Wednesdays each week, and analyzed on Thursdays (maximum three days of refrigeration with previous addition of preservatives).

Samples were analyzed in INIA La Estanzuela with the MilkoScan FT6000 FOSS equipment. The following determinations were made: protein (g/100g) by the Kjeldahl method IDF Standard 20B:1993 or after, casein (g/100g) by the reference method: IDF 29:1964, MUN (mg/dl) by the differential pH method. True protein was determined by subtracting milk urea nitrogen (MUN) from the total protein, and whey protein was calculated by the difference between true protein and casein.

Records were taken in each production system regarding: herd management, milk production, feeding routine of the milking cows, and productive environment when carrying out each sampling. Afterwards, such information referring to pasture was grouped and the following variables were determined:

V1 (h): Daily pasture access time (morning + afternoon)
V2 (cm): Average pasture height (morning and afternoon)
V3 (kg DM/ha): Average pasture availability (estimated based on average daily pasture height)
V4 (kg DM/VO/d): average daily assignment per cow
The productive environment variable was calculated based on an index of productive well-being, rating the factors that contribute to it during the twelve sampling months:

Variables	Rate					
Available water in the pasture	1: No	2: Yes				
Available water in nearby roads	1: No	2: Yes				
Available water in the farm	1: No	2: Yes				
Daily traveled distance	1: >5km	2: (4 to 5km)	3: (3 to 4km)	4: (2 to 3km)	5: (1 to 2km)	6: (<1km)
Road condition	1: Bad	2: Average	3: Good			
Waiting barn floor	1: Bad	2: Average	3: Good	4: Very good		
Waiting barn protection	1: Unprotected	2: Protected				
Milking barn floor exit	1: Bad	2: Average	3: Good	4: Very good		
Supplementation area	1: Floor	2: Feeders				
Feeding barn floor	1: Bad	2: Average	3: Good	4: Very good		
Feeding barn protection	1: Unprotected	2: Protected				
ITH*	0: D, E and F.	2: M, A, O and N	4: M, J, J, A and S			

* Correction by Temperature and Humidity Index.

The well-being index can vary between 11 and 37 (minimum and maximum, respectively), with this rating. The closer to 37, the better the system will be in terms of productive well-being; while closer to 11 means worse conditions of productive well-being for the animals in production.

To determine the pattern of seasonal variation of the variables of interest, a mixed model (ProcMixed SAS 2010) was used, with region, month, feeding variables and well-being index as fixed effect. Concentrate level, silage level, grazing time, pasture availability and forage allocation were the feeding variables included in this model. Differences between region and month were considered significant when P<0.05 (Tukey and Student's).

An analysis of variance (ANOVA) was performed for the variables, and the Tukey (P<0.05) was the method used to establish significant differences.

Pro Corr, SAS 2010 was used to establish correlations between variables. JMP Statistics and Graphics Guide version 1[19] was the statistical package used to determine the characteristics of the dairy farms that achieve the highest casein yields by time of year.

3. Results

Regarding dairy components, the fat content was high in March and April, being statistically different from December. Protein content was higher in March and April, with values above 3.30 g/100g, being statistically different from November, December and January. The rest of the months have an intermediate position.

The casein content (g/100g) was greater in March, April, May, June, July and August (autumn and winter), compared to November, December and January (lower values in summer), being statistically different.

The highest casein:true protein ratio values were observed in August, July and September, and the rest of the months presented a little lower values; except for January, that presented the lowest value, statistically different from the rest of the months.

Table 1. Individual production (average) in liters per cow per day (L/VO/d), fat content and protein content in milk according to the evaluated month

Month	L/VO/d	Fat (g/100 g)	Protein (g/100 g)
1	18,2abc*	3,51abc	3,16cde
2	15,1c	3,68abc	3,19bcd
3	16,6abc	3,85ab	3,35a
4	15,8bc	3,89a	3,33ab
5	17,9abc	3,69abc	3,28abc
6	18,3abc	3,74abc	3,27abc
7	19,1ab	3,65abc	3,26abc
8	19,7a	3,69abc	3,24abc
9	20,0a	3,72abc	3,22abcd
10	19,3ab	3,67abc	3,20bcd
11	18,1abc	3,50bc	3,09de
12	18,1abc	3,45c	3,05e

*Different letters are statistically different (Tukey P<0.05).

Table 2. Casein content and casein:true protein ratio in milk (average of all the evaluated systems) according to month of the year

Month	Casein (g/100g)	C/PV
1	2,37bc	0,772d
2	2,44ab	0,783bc
3	2,55a	0,783bc
4	2,52a	0,784abc
5	2,49a	0,781c
6	2,50a	0,786abc
7	2,49a	0,790ab
8	2,49a	0,791a
9	2,48ab	0,790ab
10	2,46ab	0,787abc
11	2,37bc	0,786abc
12	2,32c	0,784abc

*Different letters are statistically different (Tukey P<0.05).

Figure 1. Variation in milk production (L/VO/d), protein (g/100g) and fat (g/100g) in milk in the 30 evaluated farms during the 12 months of the year

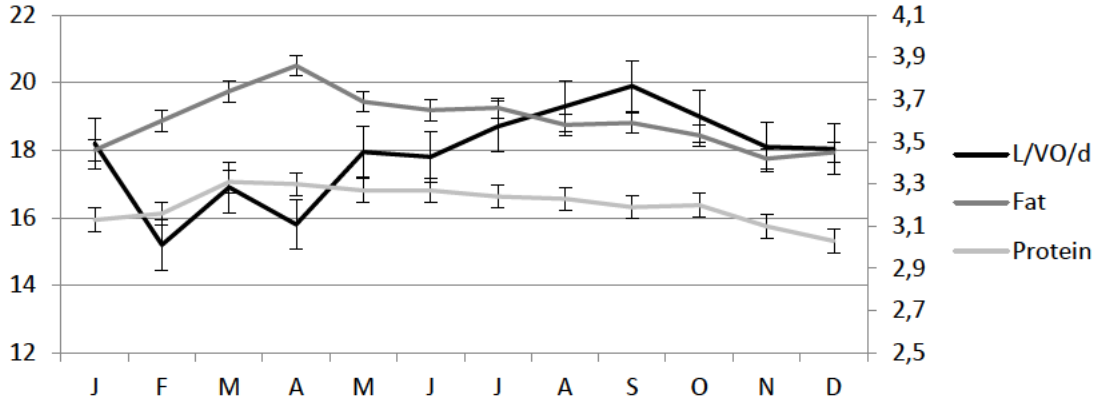
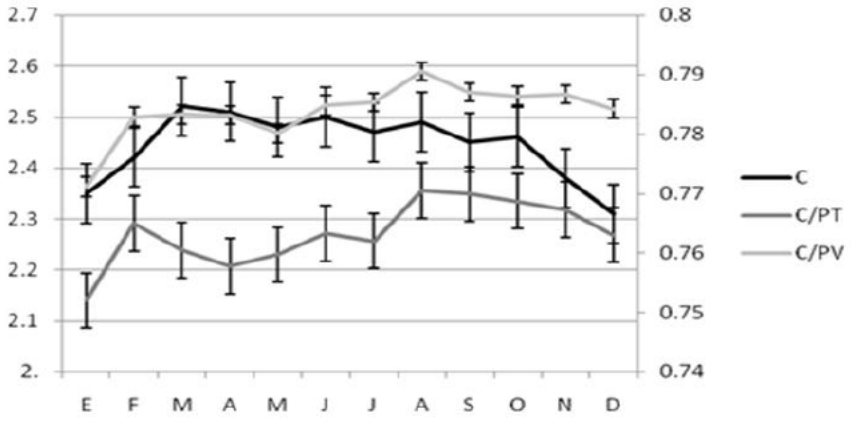


Figure 2. Variations in the casein content (C, g/100g), casein:true protein (C/PV) ratio and casein:total protein (C/PT) ratio during the year in the evaluated period



4. Discussion

Milk production per milking cow is higher in spring, probably associated with the autumn-winter calving season (in the evaluated dairy farms the highest concentration of calving happens in autumn and winter), which allows reaching the lactation peak in spring; and possibly due to the growth in grazing time, which increases the daily pasture intake[20] due to greater forage supply. It is possible to reach medium to high productions in autumn and winter due to high supplies of concentrate (6 to 7 kg of concentrate per milking cow per day in autumn-winter).

Table 3. Average amount of supplement used in the evaluated systems according to the season

Variable*	Autumn	Winter	Spring	Summer
Concentrate	7.4	6.5	5.9	6.0
Silage	12.8	6.2	6.4	5.7

*kg BF/VO/d average for all evaluated registries.

The highest fat concentration in March and April is possibly explained by lower milk production at this

time (Figure 1), which concentrates dairy components. Protein content was also higher in March and April compared to the rest of the year. These differences could be associated with the increased use of concentrates (Table 3), as mentioned by De Peters and Cant[13], explained by a greater energy supply[21]. Protein content presented medium values during winter and spring probably due to the high use of concentrates in winter and favorable characteristics to increase pasture intake in spring. Pasture becomes predominant in the diet during this time of the year, with a more efficient ruminal fermentation scheme, which agrees with Beever and others[22], Mayne and Thomas[23]; and with a predominance of legume species in pastures in the evaluated systems, as mentioned by Thomas and others[24], Thomson and others[24], and also Mayne and Thomas[23]. Protein content in milk is lower in summer, which could be due to thermal stress suffered by cows at this time in the evaluated region, as they decrease intake and increase energy losses.

Higher casein values in autumn and winter could be closely linked to the high supply of concentrates and silages, being higher during this time of the year if the average of all evaluated registries is considered (Table 3). These results coincide with what several authors stated: the supply of concentrates[13][17][25][26] and silages[27][28][29] increased the concentration of casein and protein in milk, which can be explained by an increase in energy intake[13][15][21][26][27][30][31]. The variables that largely determine the highest concentration of casein (g/100g) and protein in milk in this season are the energy provided by the concentrate and the silage, together with the protein contribution by pasture, since a protein deficit in the diet could limit milk protein[13][17][26][27][32]. Diet balance plays a fundamental role in controlling the casein content in milk. Supplements that provide energy are not enough, pasture proteins also have to be present (in the evaluated systems), so that there is sufficient availability of amino acids and glucose in the blood to be absorbed by the mammary gland and determine higher contents of casein in milk.

Casein content (g/100g) is numerically lower (without presenting statistical differences) in September and October than in autumn and winter, although it is considerably above December, being statistically different with this month. However, the supplement provides less energy in this season, the diet becomes more pastoral. Pasture in spring is predominant in the diet, with favorable characteristics to increase dry matter intake based on direct forage harvest, with a more efficient ruminal fermentation scheme, which coincides with what Beever and others[22], and Mayne and Thomas[23] stated, and with a predominance of legume species in the pasture in the evaluated systems, as mentioned by Mayne and Thomas[23], and Thomas and others[24][33].

In summer, the casein content (g/100g) drops sharply compared to the rest of the seasons. Supplement provision does not vary much from spring. Considering calving seasonality (autumn and winter) of most of these farms, late lactation would be in summer, and therefore the casein concentration would increase, as reported in bibliography[11][12][13][34][35]. However, casein and true protein (g/100g) values fall during this season. These results coincide with what González and others[6], and De Peters and Cant[13] observed. This could be due to the thermal stress suffered by cows in this season in the evaluated region, which determines lower energy intake and energy losses for preservation. Long walks, the lack of shade in the waiting, exit and feeding barns and the availability of drinking water in the field are some of the causes that can determine these results. During the summer, in this area of the country, the heat stress that animals suffer is very noticeable. The Temperature and Humidity Index (ITH by its Spanish acronym) that Thom[36] developed and Valtorta and Gallardo[37] modified exceeds 72 on average, even reaching extreme values, which would indicate danger and emergency for the cows. These ITH values could explain the low casein and true protein (g/100g) values observed in summer. On the other hand, the low quality of pastures in summer affects ruminal fermentation, and, consequently, the amino acids availability in the mammary gland, being a determining factor in the casein content in milk[22][23].

Regarding the casein:true protein ratio, these values are well below those observed by Balbinotti and others[7], who reported values of 0.88, 0.84 and 0.85 for the ES, SE systems and NE, respectively. They are also below those observed by Auldist and

others[38], 0.823 and 0.842 with ad libitum and restricted access to pastures, respectively, in early lactation, and 0.817 and 0.805 with ad libitum and restricted access to pastures, respectively, in late lactation.

It is interesting to observe this lower result in January. Perhaps the abovementioned heat stress[36][37], which has a significant impact that month in the region, could have a direct impact on this indicator. This could be due to the reduction in the synthesis, absorption and mobilization of metabolites (glucose, volatile fatty acids, lipids, amino acids) from the digestive tract, liver and adipose tissue and their utilization by the mammary gland, due to the high temperatures[39].

The casein:total protein ratio never reached values of 0.8, which shows that in our production systems casein does not represent 80% of total proteins. These values are well below those Balbinotti and others[7] observed, even lower than those from production systems with lower infrastructure and feeding conditions (0.81). Also inferior to those observed by Mackle and others[25], where values of 0.825 were observed in the worst condition (restricted access to pastures). On the other hand, Hurtaud and others[15] also observed higher values in this indicator, 0.811 and 0.831 for high and low energy levels, respectively.

The difference in the casein:total protein ratio between September (0.771) and January (0.75) was of 1.9%. Which can be decisive in maximizing the efficiency of converting milk to cheese, considering the volume that large companies industrialize.

To the extent that industries are interested in the amounts of casein in the milk produced, it would be more accurate to use casein as a reference variable. If they keep using total protein as reference, whey proteins and milk urea nitrogen (MUN) are being considered, which respond to different variation patterns and alter the real results of industrial interest. As an example, under the current payment system industries have, two suppliers can produce similar amounts of casein per liter of milk, but the one that contains higher levels of MUN and whey proteins, which will increase the total protein content (main contributor to the price of the current liter of milk), will get a better deal.

5. Implications

The level of concentrate and best forage supply associated with a calving season that is generally concentrated during autumn-winter would allow the highest milk productions to be achieved in August, September and October. Fat content in milk was higher in March and April, explained by lower individual production. Protein content was also higher in autumn, probably associated with the higher energy intake in the diet due to the significant use of concentrates in this season. Calving concentration and reserve mobilization could also contribute to high fat and protein concentrations.

Autumn and winter determined the highest values of casein content (g/100g), surely explained by the use of concentrated supplements (energy) to synchronize the protein contribution by pastures. However, in the evaluated production systems, it is possible to maintain high levels of casein content (g/100g) and casein:true protein ratio in spring with almost exclusively pastoral diets, possibly due to changes in the composition of the pasture that generate favorable conditions in the rumen to increase the availability of amino acids at mammary gland level. Energy losses due to high temperatures in summer, added to the low quality of the pasture supply in this season, produce very noticeable decreases in the casein content (g/100g) and the casein:true protein ratio, which questions the convenience of production in this season.

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Author contribution statement

All authors equally contributed to the content.

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