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ANIMAL PRODUCTION

Milk fat to protein ratio in the first test-day after calving on dairy cows

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ABSTRACT: *This study evaluated milk fat to protein ratio (FPR) in the first test-day after calving of dairy cows in Paraná State. Data from 257,847 first monthly test-days after calving of 114,162 cows were submitted to analysis after the data edition. Procedures MIXED, CORR and STEPWISE from SAS were used in the data analysis. In order to validate FPR, a herd with regular postpartum monitoring of blood β-hydroxybutyrate (BHB) was used to estimate the correlation with FPR. There was a strong positive correlation between FPR and fat content (r = 0.85; P < 0.01) and a weak negative correlation between FPR and protein content (r = - 0.23; P < 0.01). The regression equation that best fitted FPR was 1.1806 + 0.3304*%F - 0.3877*%P (R^2 = 0.98), where the variable with the greatest influence was milk fat content (partial R^2 = 0.72). Animals of 4th and 5th or more lactations had higher (P < 0.01) FPR, followed by animals of third, first, and second lactations. Jersey cows had higher (P < 0.01) FPR than Holsteins and Brown Swiss animals. Cows milked twice daily had higher (P < 0.01) FPR than animals milked three times daily. There were small positive correlations between milk FPR and blood BHB on days 4, 7 and 12 after calving (0.07, 0.13, and 0.14, respectively). In conclusion, milk fat content was reported to be the most important variable affecting FPR changes, but the milk FPR has limited value to evaluate hyperketonemia incidence during the transition period.*
Key words: *energy balance, hyperketonemia, ketosis, milk components, transition period.*

Relação gordura:proteína do leite no primeiro controle leiteiro pós-parto em vacas leiteiras

RESUMO: *Este estudo teve como objetivo avaliar a relação gordura:proteína (RGP) no primeiro controle leiteiro no pós-parto de vacas no estado do Paraná. Dados de 257.847 controles de 114.162 vacas leiteiras, realizados no primeiro controle mensal após o parto foram submetidos à análise estatística após edição dos dados. Os procedimentos MIXED, CORR e STEPWISE do programa estatístico SAS 9.4 foram utilizados. A fim de validar a RGP, um rebanho com monitoramento regular de β-hidroxibutirato (BHB) no sangue foi utilizado para estimar a correlação com RGP. Observou-se uma correlação forte e positiva entre a RGP e teor de gordura no leite (r = 0,85; P < 0,01) e uma correlação fraca e negativa entre a RGP e o teor de proteína (r = -0,23; P < 0,01). A equação de regressão que melhor se ajusta à RGP foi 1,1806 + 0,3304*%G - 0,3877*%P (R^2 = 0,98), na qual a variável com maior influência foi a porcentagem de gordura (R^2 = 0,72). Animais de 4ª e 5ª lactações ou mais, apresentaram maior (P < 0,01) RGP, seguidos dos animais de terceira, primeira e segunda lactações, com menores RGP. Vacas da raça Jersey apresentaram maior (P < 0,01) RGP que vacas da raça Holandesa e Pardo-Suíça. Animais com duas ordenhas diárias apresentaram maior (P < 0,01) RGP que os animais ordenhados três vezes ao dia. Observaram-se correlações positivas, mas de pequena magnitude, entre a RGP do leite e BHB no sangue no quarto, sétimo e 12 dias pós-parto (0,07, 0,13 e 0,14; respectivamente). Concluindo, o teor de gordura é o fator que mais impacta as variações da RGP do leite, mas a RGP tem valor limitado na avaliação da incidência de hipercetonemia no período de transição.*
Palavras-chave: *balanço de energia, cetose, componentes do leite, hipercetonemia, período de transição.*

INTRODUCTION

It is well known that the negative energy balance (NEB) is a physiological condition of dairy cows, especially the high-producing ones in early lactation. The drastic reduction in dry matter intake in the late pre-partum period, associated with large mobilization and partition nutrients to the mammary gland for meeting the demands of colostrum and milk synthesis, creates a negative difference between the energy required and energy consumed, known as NEB. This scenario results in the mobilization of adipose tissue, releasing non-esterified fatty acids (NEFA) in the blood (DRACKLEY, 2005).

The NEFA can be directed to the mammary gland, where they will contribute to milk fat yield, or to the liver where they can be completely oxidized to generate ATP, or converted into triglycerides

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(TG) and exported in the form of very low-density lipoproteins (VLDL) for the other tissues. A third possible pathway is incomplete oxidation, generating ketone bodies such as β-hydroxybutyrate (BHB). Finally, the fourth and last pathway is the accumulation of TG in the liver parenchyma, which gives rise to hepatic steatosis or fatty liver (LEBLANC, 2010). When this lipid mobilization is excessive, there is an increase in the amount of NEFA released and part of the produced ketone bodies are not metabolized, causing an accumulation of these metabolites, culminating in a clinical or subclinical ketosis (MÄNTYSAARI et al., 2019). In addition to ketone bodies accumulation, excessive lipidic mobilization can be detected by the high milk fat content, or more specifically by the high values for the relationship between milk fat and protein contents (FPR) (DENIS-ROBICHAUD et al., 2014).

If other factors that affect milk fat are controlled in the production process, the higher FPR in early lactation is due to the higher lipid mobilization, mainly fatty acids (FA) with more than 16 C, known as preformed FA. Because NEFA is particularly rich in long-chain FA such as C18:1 *cis*-9 (oleic acid), elevated concentration in milk fat of this FA was identified as a valuable early warning biomarker for subclinical ketosis (JORJONG et al., 2015).

Therefore, the assessment of this milk fat to protein ratio has become a useful tool for diagnosing metabolic disorders during the transition period, particularly ketosis and fatty liver. In review, GLATZ-HOPPE et al. (2020) concluded that evaluation of FPR could be used to verify the risk of ketosis, and when FPR values are above the maximum value of 1.4, the risk of disorders is increased.

This study identified the sources of variation in the milk fat to protein ratio collected in the first monthly test-day after calving, carried out in dairy herds of Paraná State under an official milk recording program. Our hypothesis is that the FPR can be used as an indicator of excessive lipidic mobilization, and factors such as parity, breed, milking number and calving month can change this ratio.

MATERIALS AND METHODS

The study was carried out using a database of monthly test-days between 2000 and 2013 by the Associação Paranaense de Criadores de Bovinos da Raça Holandesa (APCBRH), using only the first monthly test-days after calving, from 5 to 40 days in milk (DIM). After all editions, 257, 847 test-days, from 114,162 cows, remained in the database, with an average of 2.26 first test-days (or lactations) per cow.

All 526 dairy herds in the database are located in the Paraná State. The milk recording procedures were performed by APCBRH staff, where composite milk samples, representative of the entire milking of individual cows, were transferred into sample vials, each containing a tablet of bronopol preservative. When the herd test was complete, milk samples were refrigerated and transported to the Centralized Milk Quality Laboratory of APCBRH, in Curitiba, PR, Brazil. The samples were analyzed for fat and protein contents using optical and infrared systems in the Bentley 2000 equipment (Bentley Instruments®).

For the database edition, the age at calving was classified as follows: for first lactation animals, the minimum age was 20 months and the maximum age was 48 months; for second lactation cows, between 32 and 72 months; for the third lactation, between 44 and 96 months; for the fourth lactation, between 56 and 120 months; and for five or more lactations, between 68 and 240 months of age. Test-days with partial or incomplete data were excluded. The animals were classified according to the parity into five classes: 1st lactation (87,771 controls), 2nd lactation (68,638 controls), 3rd lactation (45,753 controls), 4th lactation (27,499 controls), and 5th or more lactations (28,186 controls). The test-days were also classified according to the cow's breed, consisting of Holstein (HOL; 242,685 controls), Jersey (JER; 9,207 controls), and Brown Swiss (BS; 5,955 controls). Herds with less than 10 test-days in the dataset were excluded.

To find the somatic cell count (SCC) value, the equipment uses the flow cytometry method; this variable was limited from 1 to 9,999 ($x10^3$). The milk solids contents were analyzed using the Fourier transform infrared (FTIR) spectroscopy method. The thresholds for fat percentage (%F) were from 1 to 9%, and for protein content (%P), between 2 to 5.5%. Test-day milk yield was limited between 5 and 66 kg/d. These values were established in order to provide data variability and to remove inconsistent information and errors in data collection or storage from the database. The same proportions of test-days below and above the average values were excluded. The milk fat and protein ratio (FPR) was calculated by dividing the fat value by the total protein value for each sample.

For statistical analysis, database was submitted to the SAS statistical program (Statistical Analysis System, version 9.4). The FPR adjusted means were generated using the MIXED procedure, where herd was considered to be a random effect and the fixed effects were breed, calving year, calving month, parity, and milking frequency. Due to computational limitations, the random effect of cow nested within herd was removed from the model. The statistical model is described below:

$y_{ijklmn} = \mu + H_i + CY_j + CM_k + P_l + B_m + MF_n + e_{ijklmn}$, where

y_{ijklmn} = response variable of milk fat to protein ratio (FPR); μ = general mean; H_i = fixed effect of herd (i = 1-526); CY_j = fixed effect of calving year (j = 2000, …, 2013); CM_k = fixed effect of calving month (k = 1, …, 12); P_l = fixed effect of parity (l = 1, …, 5); B_m = fixed effect of breed (m = HOL, JER, PAR); MF_n = fixed effect of milking frequency (n = 2x, 3x); e_{ijklmn} = random error.

The adjusted means were compared using the Tukey test. Pearson's correlations between the FPR and the variables age, parity, DIM, milk yield, fat content (%), protein content (%), and SCC were obtained by the CORR procedure. Finally, to assess which of the variables were more relevant for the statistical correlation model, data was submitted to regression analysis by the STEPWISE procedure.

To validate the FPR results, a large dairy herd from Palmeira County, Paraná State, was selected. This herd adopts an official milk recording scheme, and regularly monitors blood BHB on fresh cows, using reagent strips that detect BHB concentrations. Blood collections were performed on days 4 (d4), 7 (d7), and 12 (d12) after calving. A 1-mL whole blood sample was drawn from the coccygeal vessels of each cow and immediately analyzed for BHB concentration using the Precision Xtra meter, a cow-side electronic meter. The BHB result of 138 Holstein cows, which calved in 2013 was correlated with the milk FPR value on the day of the first test-day after calving. In 2013 (year of BHB data collection), the herd had an average of 38.7 kg/d on the first test-day and 9.670 kg per lactation. The mean DIM on the test-day was 24 d.

RESULTS

The general means, SD and minimum and maximum values of the variables evaluated are shown in table 1. Table 2 shows the Pearson's correlations between the milk FPR and the other productive variables.

Small positive correlations were observed between the animal's age (represented as calving age or parity; r = 0.05 for both; P < 0.01) and the milk FPR. Milk yield and DIM on the first test-day after calving had very small negative correlations (r = -0.05 and r = -0.03, respectively; P < 0.01) with FPR. The milk fat percentage showed a very high and positive correlation (r = 0.85; P < 0.01), and the milk protein percentage showed a weak negative correlation (r = -0.23; P < 0.01) with FPR.

The regression equation that best fitted FPR generated from the inclusion of the milk fat and protein contents was:

$FPR = 1.8058 + 0.3304^{*}\%F - 0.3877^{*}\%P; R^2 = 0.98$

For the regression equation, the variables that were most relevant were milk fat percentage (partial R^2 = 0.72) and milk protein percentage (partial R^2 = 0.26). The herd effect was included in the statistical model but the adjusted means will not be showed here.

Among the 14 years of this dataset, the lowest milk FPR was observed in 2001 and the highest in 2013; 1.13 and 1.19, respectively. Figure 1 shows the adjusted means of milk FPR, according to calving month. Warmer months from summer and fall seasons showed lower adjusted means for milk FPR than cooler months from winter and spring.

According to the parity (Figure 2), second lactation cows showed lower FPR (1.126), followed by first lactation (1.151) and third lactation cows (1.164). Fourth and fifth or more lactation cows had higher FPR; 1.183 and 1.185, respectively.

Figure 3 shows the adjusted means for FPR according to the animals' breed. Jerseys showed higher milk FPR compared to Holstein and Brown Swiss cows (1.185 vs. 1.149 and 1.152, respectively; P < 0.01). Cows milked twice daily showed higher FPR than animals milked three times daily (1.176 vs. 1.146; P < 0.01; Figure 4).

DISCUSSION

Although, the milk fat content varies widely among herds and even within herd, previous studies indicated that milk FPR values above 1.40 are worrisome and indicate energy deficiency (GLATZ-HOPPE et al., 2020). Assuming that the ideal FPR in the first test-day after calving should be lower than or equal 1.4, in the present database, 15.86% of the test-days were greater than 1.4. Other authors such as KLEIN et al. (2019) also suggested FPR as an indicator for ketosis and high lipolysis, but they chose FPR > 1.5 as a threshold value.

BUTTCHEREIT et al. (2011) evaluated lactations up to 180 DIM and observed a high positive correlation between the milk fat content and the FPR throughout the period. These authors observed

Table 1 - Average and standard deviation of age and performance of cows.

Item	Mean	SD	Minimum	Maximum
Calving age (m)	48.3	23.3	20	233
Parity number	2.46	1.56	1	14
Days in milk	20.9	9.2	5	40
Milk yield (kg)	31.56	9.94	5	66
% Fat	3.60	0.80	1.00	8.98
% Protein	3.05	0.36	2.00	5.48
FPR[1]	1.19	0.26	0.240	4.144
SCC ($x10^3$/ml)[2]	359	874	1	9999

[1]Fat to protein ratio; [2] Somatic cell count.

in the first 45 days of lactation a correlation very similar to the one reported in the present study. In the BUTTCHEREIT et al. (2011) study, the correlation of milk protein and FPR was also evaluated and the average of the first 45 DIM was higher than the one estimated here (-0.40 vs. -0.23), indicating greater relevance of the milk protein content over the FPR.

The current study indicated a much greater relevance of the milk fat content than the milk protein content for milk FPR. This was already expected, considering that fat is the milk component that has the highest variance. In fact, GLATZ-HOPPE et al. (2020) stated that energy status was more often correctly assigned by FPR than by milk protein content. Compared with healthy cows, cows with ketosis diagnosis had a higher first test-day FPR (KLEIN et al., 2019). Although, all correlations were significant because of the dataset size, only the milk fat and protein contents were relevant to better explain the milk FPR. Moreover, it is possible to observe that the higher the milk fat, the greater the FPR. This could be a reflect of the increased fat synthesis caused by elevated body fat mobilization during NEB (BELL, 1995). Conversely, but with lower impact, the higher the milk protein content, the lower the FPR. The same pattern was observed in the regression obtained by the stepwise method.

The results showed that the warmer months of the year (December to April) result in lower milk FPR. The heat stress can reduce dry matter (DM) intake, make cows more selective against forage intake, and promote other physiologic changes which lead to a reduced milk yield and lower milk fat content (RHOADS et al., 2009). As there is a strong positive correlation between milk fat and FPR, this would explain the drop in FPR in the warmer months. Recently, SALFER et al. (2019) showed that cows can have a seasonal pattern on the milk solids production, so during the warmer months of the year milk fat is typically reduced. Still according to SALFER et al. (2019), besides the heat stress factor, the natural seasonal rhythm of milk and solids production can also change the FPR data.

BUTTCHEREIT et al. (2010) compared primiparous (1 to 180 DIM) with multiparous (1 to 305 DIM) cows, and they reported that multiparous had a higher FPR, in agreement with the results of the present study. Data from these same authors

Table 2 - Pearson's correlations between milk fat to protein ratio and productive variables.

	Age	Parity	DIM[1]	MY[2]	%F[3]	%P[4]	SCC[5]
FPR	0.05**	0.05**	-0.03**	-0.05**	0.85**	-0.23**	0.02**

[1]Days in milk at the test-day; [2]Milk yield (kg) at the test-day; [3]Milk fat (%); [4]Milk total protein (%); [5]Somatic cell count (cells x 10^3/ml). **$P < 0.01$.

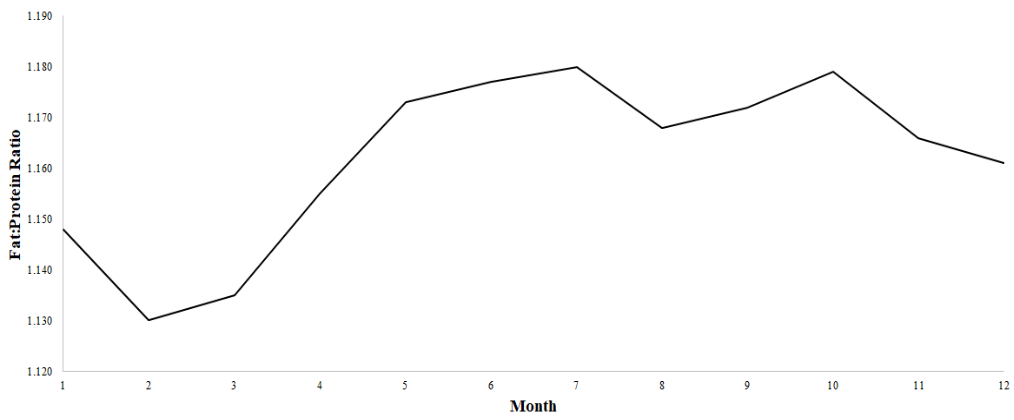

Figure 1 - Adjusted means for milk fat to protein ratio according to the calving month. SEM = 0.0049.

showed that second-lactation cows had lower NEB than cows with three or more lactations, which may explain the lower FPR. In the present study it is not possible to state this, as there is no additional information (such as body condition score data) that could help to explain the magnitude of the animals' NEB. In fact, PONCHEKI et al. (2015) estimated the absolute weight loss from calving to nadir BW and they found 48.9, 44.7, and 52.6 kg of weight loss for first, second, and third or more lactations, with no statistical differences among parities.

BERGK & SWALVE (2011) reported a negative correlation between FPR and milk yield. In this same study, it was observed that cows with

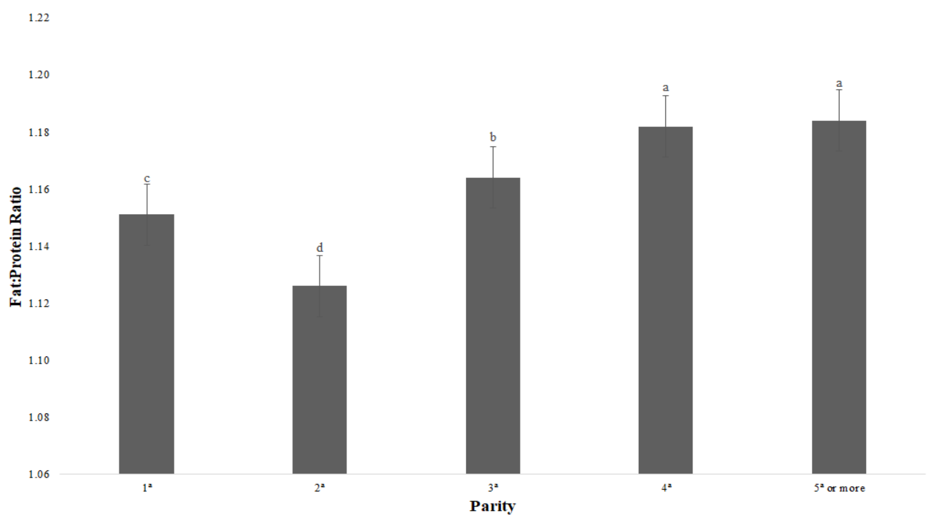

Figure 2 - Adjusted means for milk fat to protein ratio according to the parity number.
a, b, c, d Means followed by different letters differ from each other by the Tukey test (P < 0.01).
SEM = 0.0048.

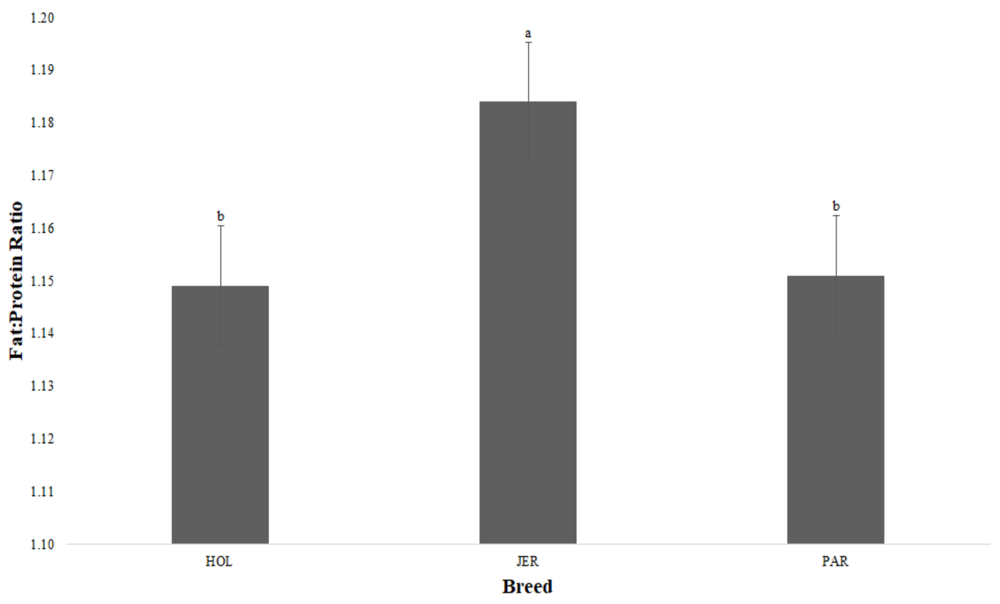

Figure 3 - Adjusted means for milk fat to protein ratio according to the breed.
a, b Means followed by different letters differ from each other by the Tukey test ($P < 0.01$).
SEM = 0.0055.

extreme milk FPR (below 1.1 and above 1.6) had greater culling rate and/or mortality. In our study, milk production on the first test-day had a negative and low-magnitude correlation with FPR, indicating that the higher the milk production, the lower the FPR. This can be explained by the already well-established negative correlation between milk yield and milk fat percentage (QUIST et al., 2008).

A negative correlation, but of small magnitude, was also observed between FPR and DIM, suggesting the closer to the calving the test-day occurs, the higher is the FPR. A plausible biological explanation is that the NEFA peak in the blood occurs around calving day. So, higher amounts of free fatty acids are available for the mammary gland and consequently higher milk fat contents are found just after calving. According to MÄNTYSAARI et al. (2019), plasma NEFA concentrations can be predicted with moderate accuracy based on the FPR and milk yield, and this accuracy can be improved if body weight and body condition score are also available.

TONI et al. (2011) indicated a weak correlation of FPR on the first test-day with the milk FPR collected on the 7^{th} day postpartum ($r = 0.20$ for primiparous and $r = 0.25$ for multiparous). This milk FPR behavior was also shown by NEGUSSIE et al. (2013), in which the highest FPR values were reported in the first days of lactation and a strong drop up to 100 DIM. RANARAJA et al. (2018) also found a downward trend in FPR as the DIM increased; however, this occurred with greater intensity up to the 6^{th} week of lactation. Therefore, the closer the calving the milk sample is collected, the better the reliability, and the result will be a more accurate reflection of management in the transition period. However, the sample should be collected only when regular milk yield begins, thus disregarding colostrum and transition milk collections.

As expected, the JER breed had higher FPR, since this genetic group has higher milk fat; studies show milk fat content averages above 4.5% (EDWARDS et al., 2019). Holstein and Brown Swiss breeds have similar milk fat contents (CECCHINATO et al., 2011) and probably for this reason there was no significant difference in milk FPR between them.

ERDMAN & VARNER (1995) showed in their review that cows milked three times a day have higher milk production and lower milk fat content. Although, the milk fat yield in kilograms is higher in cows milked three times a day, as the milk FPR uses

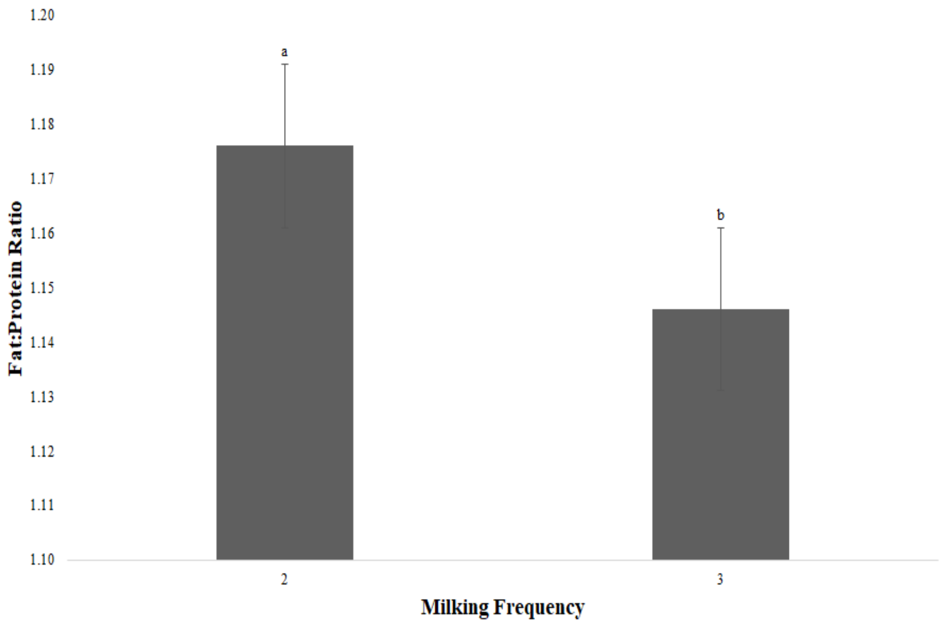

Figure 4 - Adjusted means for milk fat to protein ratio according to the milking number.
a, b Means followed by different letters differ from each other by the Tukey test (P < 0.01)
SEM = 0.0047.

the fat percentage, this explains the lower FPR found for cows milked three times daily.

For validation of FPR as a ketosis indicator, we reported positive but weak correlations between FPR evaluated on average at 21 DIM (from 5 to 40 DIM) and BHB blood levels evaluated at 4, 7, and 12 DIM. The greatest correlations were between the BHB value at 7 and 12 DIM and FPR, probably due to the proximity of the test-day and the blood sample date. According to KLEIN et al. (2019), phenotypically, an increasing ketosis incidence was associated with higher FPR, but only moderate genetic correlations were reported between them.

In a study conducted by VAN DER DRIFT et al. (2012), the plasma BHB concentrations between days 5 and 60 postpartum were evaluated. The average values were 0.76 mmol/L; however, the minimum value was 0.16 and the maximum value was 7.05 mmol/L, with a standard deviation of 0.56 mmol/L. Therefore, to generate better validation data, the ideal would be to measure BHB concentration on the milk test-day, which did not occur in the present study. However, this discrepancy reflects a more real situation, because typically BHB blood samples are collected until 10 DIM, while the first test-day (which will generate the milk FPR) can be done until 45-60 days after calving.

DENIS-ROBICHAUD et al. (2014) also estimated Pearson correlation coefficients between blood BHB and milk fat and milk FPR, and they also reported low estimates; 0.21 and 0.17, respectively. However, differently in our study, blood samples were collected within 4 h of milk samples for the DHI test. They also concluded that milk fat and FPR were poor predictors of hyperketonemia. By other hand, milk BHB and acetone values from flow-injections had excellent correlations with blood BHB values and they could potentially be used in herd-level surveillance programs for hyperketonemia.

CONCLUSION

The milk FPR has limited value to evaluate hyperketonemia incidence during the transition period. Because it is a non-expensive and a non-invasive method, milk FPR still can be used as a secondary tool for milk-recorded herds as an indicator of an adequate transition period. It is important to note that several factors can affect milk fat content and FPR results should be interpreted with caution. For proper interpretation of FPR, it is necessary to emphasize that FPR values obtained in a late lactation (> 40 DIM) no longer indicate body reserves mobilization, as the energy balance has already been restored.

For further studies, it is indicated to associate FPR with the fatty acid profile in the milk fat, as it is known that the preformed fat has different fatty acid profile from the fat synthetized in the mammary gland. Another suggestion would be to emphasize the milk BHB and acetone analysis, instead of milk FPR.

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DECLARATION OF CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHORS' CONTRIBUTIONS

All authors contributed equally for the conception and writing of the manuscript. All authors critically revised the manuscript and approved the final version.

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