

Influence of milk fat-to-protein ratio on the energetic status of Holstein-Friesian cows in early lactation

Original Article

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Abstract:

This study presents the results of an investigation into the organic components of milk in Holstein-Friesian cows during early lactation, with a particular emphasis on analyzing key biochemical parameters—specifically protein and fat—and their interrelationship. Special attention was given to evaluating the correlation between these components in order to identify cows experiencing a pronounced negative energy balance during early lactation, while simultaneously aiming to prevent metabolic disorders and reproductive complications. The research was conducted on 90 Holstein-Friesian dairy cows at an early stage of lactation, on a farm located in the municipality of Dimitrovgrad. Routine sampling for milk biochemical analysis was used for this purpose, focusing on fat and protein content and their ratio. The analysis was performed using a Milkoscan device. The results indicate that the energy status of high-yielding dairy cows has a significant influence on their production performance, reproductive efficiency, and overall health.

Key words:

early lactation, nutritional status, organic components, metabolism, energy balance

Apstrakt:

Uticaj odnosa masti i proteina u mleku Holštajn-Frizijskih krava na energetski balans u ranoj laktaciji

Ovaj rad prikazuje rezultate istraživanja organskih sastojaka mleka kod krava Holštajn-Frizijske rase tokom rane laktacije, s posebnim fokusom na analizu ključnih biohemijskih parametara (proteina i masti) i njihov međusobni odnos. Posebna pažnja posvećena je oceni korelacije ovih komponenti kako bi se identifikovale krave sa izraženim negativnim energetskim bilansom u fazi rane laktacije, uz istovremeno sprečavanje metaboličkih i reproduktivnih poremećaja. Istraživanje je sprovedeno na 90 mlečnih krava Holštajn-Frizijske rase u ranoj fazi laktacije, na farmi krava u opštini Dimitrovgrad. Za potrebe istraživanja iskorišćeno je rutinsko uzorkovanje za određivanje biohemijskog sastava mleka, a u ovom radu je analiziran sadržaj masti i proteina, kao i njihov međusobni odnos. Sadržaj masti i proteina određeni su pomoću uređaja Milkoskan. Rezultati ukazuju da energetski status visokoproduktivnih mlečnih krava značajno utiče na njihove proizvodne rezultate, reproduktivne performanse i zdravstveno stanje.

Ključne reči:

rana laktacija, nutritivni status, organski sastojci, metabolizam, energetski balans

Introduction

The most significant health disorders in high-yielding dairy cows arise as a result of disturbances in energy metabolism. It is well established that the most critical phase for the metabolic stability of high-producing cows is the transition from the dry period to early lactation. During this phase, the animal's energy and nutrient demands change drastically (Šamanc et al., 2006, 2011; Lin, 2024).

While energy balance is positive during late pregnancy, it becomes negative immediately after calving, when lactation begins and the organism's energy demands sharply increase. In conditions of negative energy balance, the body utilizes its internal energy reserves - first glycogen, then fat, and finally protein stores (Nogalski et al., 2012; Roche et al., 2013). Successful adaptation of the metabolic and endocrine systems during this period enables the cow to transition from a state of late gestation to one



of intensive lactation (Kirovski et al., 2011, 2012; Cabezas-Garcia et al., 2021; Barłowska et al., 2024).

The consequences of energy imbalance manifest as negative energy balance, during which cows may develop hepatic lipidosis (“fatty liver”) and other metabolic disorders (Šamanc et al., 2006, 2011, 2013). The quality of milk intended for human consumption is directly dependent on the maintenance of metabolic stability in dairy cows (Toscano et al., 2023). Increased lipomobilization, commonly observed during energy deficits, can elevate milk fat content while simultaneously reducing protein levels - changes that may adversely affect the technological properties of milk during processing (Van Knegsel et al., 2005; Kirovski et al., 2011, 2012; Knob et al., 2021).

It is evident that intensive cattle farming practices, aimed at maximizing the genetic potential for milk production and reproductive performance, may contribute to disturbances in energy metabolism. As previously noted, energy reserves in high-yielding cows are stored in adipose tissue during the late lactation phase and the dry period. Since cows in early lactation are typically unable to ingest sufficient quantities of food to meet their elevated energy requirements, the energy deficit is compensated by mobilizing body fat stores. Therefore, assessing energy reserves during the periparturient period is a crucial procedure for determining the animal’s energetic condition (Šamanc et al., 2006, 2011, 2013; Kirovski et al., 2011, 2012, 2013; Đoković et al., 2016; Bojković-Kovačević, 2016).

Given these considerations, maintaining metabolic stability in cows during the periparturient and early lactation periods is essential, not only to achieve high milk yields but also to ensure milk that meets the nutritional and hygienic standards of the modern market. Only through proper nutritional management, veterinary care, and rigorous control of raw milk quality can high-quality dairy products be consistently produced for human consumption, thereby supporting a sustainable and competitive dairy industry (Kirovski et al., 2011, 2012; Bojković-Kovačević, 2016; Đoković et al., 2016; Barłowska et al., 2024).

In the past decade, the determination of organic component concentrations in milk has been increasingly used as a method for assessing the energy status of dairy cows (Van Knegsel et al., 2005; Kampl, 2005; Šamanc et al., 2006; Kirovski et al., 2011, 2012; Knob et al., 2021). Such an assessment is highly reliable and easily applicable, as sample collection is non-stressful for the animal. Moreover, it is highly cost-effective when integrated into the routine analysis of raw milk components conducted for milk quality evaluation

(Kampl, 2005; Šamanc et al., 2006).

The energy supply status of animals can also be determined based on the ratio of fat to protein content in milk (Šamanc et al., 2006; Kirovski et al., 2011, 2012, 2013; Trifkovic et al., 2015).

Specifically, during intensive lipomobilization, the concentration of free fatty acids in the blood increases, leading to enhanced synthesis of milk fat and its concentration in milk (Nogalski et al., 2012). If the milk protein content exceeds 3.2% and the milk fat content remains below 4.5%, the energy supply is considered adequate. Conversely, if the fat content in milk increases while the protein content decreases simultaneously, this indicates an energy deficit within the herd (Kirovski et al., 2011, 2012; Lakić et al., 2018; Knob et al., 2021).

Materials and Methods

The study was conducted during July and August 2023 on a Holstein-Friesian dairy farm located in the municipality of Dimitrovgrad. The investigation included a total of 90 cows in early lactation (the first 60 days postpartum). The cows were divided into three groups (A, B, and C): Group A consisted of cows within the first 15 days of lactation, Group B included those within the first 30 days, and Group C included cows at 60 days of lactation. The cows were in their first to sixth lactation, with an average daily milk yield of 27.2 liters. Milking was performed twice daily, in the morning and evening.

For the purpose of this study, milk samples were collected during morning milking as part of the routine analysis of biochemical composition. The samples were transported to the laboratory in portable coolers at 8 °C and then stored at -20 °C until analysis.

The data used in this study pertained to the fat and protein content of milk. These parameters were analyzed using a Milkoscan device (series 130, model 10900, A/S, N. FOSS ELECTRIC) at the Eko-Lab d.o.o. laboratory in Belgrade (IDF, 2015; Lefier et al., 1996).

The fat-to-protein ratio was evaluated in order to reliably diagnose the energy status of the cows under investigation (Kirovski et al., 2011, 2013; Đoković et al., 2016).

The results were statistically processed and presented in tables using descriptive statistical parameters, including arithmetic means (X), standard deviations (SD), standard errors (SE), coefficients of variation (CV), and ranges (IV). Additionally, Pearson’s correlation coefficient was calculated for selected biochemical components using Microsoft Excel (Office 365).

Results and discussion

The protein and fat content of milk samples collected during morning milking were thoroughly analyzed and are presented in **Tab. 1**. These biochemical parameters in milk serve as key indicators of proper nutrition and metabolic function in cows and are sufficient for the rapid and efficient assessment of the energy status of the examined animals.

Table 1. Milk protein (%) and fat (%) content in Holstein-Friesian cows sampled during morning milking

| | | Milk fat (%) | Milk protein (%) |
|----------------------------|----|--------------|------------------|
| Group A (n=30) | X | 2.76 | 3.03 |
| | SD | 0.83 | 0.15 |
| | SE | 0.15 | 0.03 |
| | CV | 30.07 | 0.49 |
| | IV | 1.26-4.62 | 2.71-3.38 |
| Group B (n=30) | X | 3.15 | 3.16 |
| | SD | 1.03 | 0.25 |
| | SE | 0.18 | 0.05 |
| | CV | 32.69 | 0.79 |
| | IV | 0.95-4.88 | 2.71-3.87 |
| Group C (n=30) | X | 3.41 | 3.15 |
| | SD | 1.12 | 0.21 |
| | SE | 0.20 | 0.04 |
| | CV | 32.84 | 0.66 |
| | IV | 0.96-6.56 | 2.89-3.72 |
| A, B and C combined (n=90) | X | 3.11 | 3.11 |
| | SD | 1.02 | 0.21 |
| | SE | 0.11 | 0.02 |
| | CV | 32.79 | 0.67 |
| | IV | 0.95-6.56 | 2.71-3.87 |

Šamanc et al. (2006) found that the greatest deviations from physiological values in milk occur in fat content and urea concentration, while lactose and protein levels vary to a lesser extent.

In their study of four farms, they reported that milk fat content ranged from 1.07% to 3.89%. The results of our research showed that the average milk fat content on the studied farms was 2.76±0.83%, 3.15±1.03%, and 3.41±1.12%, respectively. The fat content in milk from cows on Farm A was significantly lower compared to that of Farm C ($p<0.05$). Compared to the data reported by those authors, only Farm C had fat content within the

optimal range, while the other two farms, particularly Farm A, exhibited lower values.

The decrease in milk fat content observed on two farms is most often attributed to changes in cow nutrition and energy status. Several hypotheses exist regarding the mechanisms responsible for the onset of milk fat depression syndrome. Most agree that it results from alterations in ruminal digestion processes. As a consequence, the supply of acetate decreases, along with the rate of milk fat synthesis, since acetate is known to be the primary precursor for milk fat synthesis in mammary epithelial cells (Grummer, 1993).

The fat content in the milk of cows in groups A, B, and C was individually measured at 2.76±0.83%, 3.15±1.03%, and 3.41±1.12%, respectively. The fat content in the milk of cows in Group A was significantly lower than that of Group C ($p<0.05$). The overall average fat content across all cows was 3.11±1.02%.

The investigation of milk organic components conducted by Šamanc et al. (2006) found that the fat content in milk from cows on Farms B and D was 1.66±0.75% and 1.07±0.26%, respectively. Based on these results, it can be concluded that these farms likely experienced milk fat depression syndrome. This diagnosis is made when milk fat content is at least 50% below the optimal level, typically in the range of 1.2% to 1.7%. On the other two farms (Farms A and C), the milk fat content was 3.89±0.77% and 3.38±0.93%, respectively - values considered normal for this breed (Šamanc et al., 2006).

The findings of this study are consistent with those reported by other authors, including Kirovski et al. (2012) and Đoković et al. (2016).

In comparison with the study by Đoković et al. (2016), which reported a milk fat content of 3.42±0.48% in Holstein cows, our results fall within a similar range, confirming a general trend in milk production across farms. However, compared to their findings on protein concentration, Holstein cows exhibited significantly higher levels at the beginning of lactation (4.67±2.72%), while our results indicate a lower protein content in the milk of the cows examined in this study. During intensive fat mobilization, the concentration of free fatty acids in the blood increases. This is reflected in the extent of fat synthesis within the mammary gland, leading to elevated levels of milk fat in such cases (Kirovski et al., 2011, 2012, 2013). Based on the obtained results, dietary adjustments and improvements in the health status of the cows can be recommended (Kirovski et al., 2012).

The examination of milk biochemical components - urea, protein, and fat - can contribute to a better

understanding of the energy status of high-yielding dairy cows and allow for the timely implementation of appropriate measures to prevent numerous health disorders that result from negative energy balance (Šamanc et al., 2006; Kirovski et al., 2011, 2012; Đoković et al., 2016).

Based on statistical analysis, Pearson's correlation coefficient between milk fat and protein content was determined to be approximately $r \approx 0.730$, indicating a moderately strong positive correlation between these variables. This correlation suggests that an increase in milk protein content is significantly associated with an increase in milk fat content, reflecting the interrelated synthesis processes of these components within the mammary gland.

Given that the levels of fat and protein in milk reflect not only the genetic potential of the cows but also the quality of their diet and metabolic status, these findings confirm that a balanced intake of protein and energy in the diet contributes to optimal milk quality (Kirovski et al., 2011; Toscano et al., 2023).

The testing of the null hypothesis (H_0), which posited that there is no statistically significant relationship between these parameters, resulted in its rejection, as the correlation was found to be statistically significant ($p < 0.05$). This finding is consistent with the results reported in the literature. These data further emphasize the importance of properly formulated nutrition in achieving optimal nutritional status and health in dairy cows, particularly during phases of high production demands (Roy et al., 2011).

From **Fig. 1**, it is observed that the majority of data points fall within the lower-left quadrant, indicating a significant energy deficit based on the ratio of milk fat content (below 4.5%) and protein content (below 3.2%) in the examined cows. A smaller proportion of cows, located in the lower-right quadrant, did not exhibit a negative energy balance, meaning they were fed an optimal amount of energy appropriate for the demands of early lactation (the first 60 days postpartum).

Based on the fat-to-protein ratio, Šamanc et al. (2006) determined that a significant energy supply deficit was present in the majority of cows. This observation is also confirmed by the results of our study. A large portion of the cows examined in the study by Kampl (2005) were likewise positioned in the lower-left quadrant, indicating a pronounced energy deficit, which aligns with the findings of our research. From the overall analysis of the fat-to-protein ratio, Šamanc et al. (2006) also concluded that the majority of cows across the four examined farms exhibited an energy deficit - an observation that closely aligns with the results of our investigation.

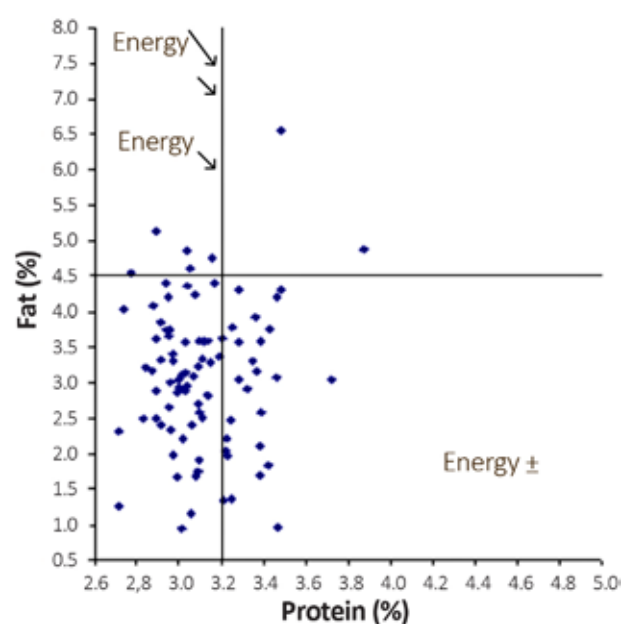


Fig. 1. Correlation between milk fat and protein content in morning milking, aggregated across large-scale farms A, B, and C, in Holstein-Friesian dairy cows.

The findings of this study are valuable for further research and for improving feeding management strategies to prevent metabolic and reproductive disorders, as observed in cow populations experiencing negative energy balance. These observations are consistent with the research outcomes of other authors as well (Roy et al., 2011; Schwab et al., 2017).

Conclusion

Based on the presented findings, it can be concluded that the analysis of biochemical milk components (urea, total protein and fat) can significantly contribute to a better understanding of the energy status of high-yielding dairy cows, as well as to the timely implementation of appropriate measures to prevent various health disorders arising from negative energy balance.

The use of milk organic components as indicators of cows' energy status has gained considerable interest among veterinary professionals due to its practical applicability in field conditions. This approach simplifies both the sampling of biological material and the required laboratory analyses. Compared to the long-standing metabolic profiling method, which involves testing a large number of parameters, this method represents a rational and financially feasible diagnostic approach for any producer, regardless of whether they operate a small-scale or large-scale cattle farm.

Our results support the widely accepted view that

the health condition and production performance of high-yielding dairy cows in our country are most often a reflection of inadequately applied modern feeding technologies.

The consequences of such practices include prolonged energy deficits and numerous metabolic disorders, accompanied by consistently reduced milk production and impaired reproductive performance. This, in turn, leads to significant financial losses and inefficient utilization of the cows' genetic potential for milk production. This issue was common in all former state-owned farms and is now increasingly evident on newly established small-scale farms with genetically superior Holstein and Simmental cattle.

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