

STUDY OF THE DYNAMICS OF THE MAIN QUALITY INDICATORS OF MILK PRODUCTION IN A HERD OF DAIRY COWS BELONGING TO THE ROMANIAN BLACK SPOTTED BREED

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Abstract

The aim of the study is to analyze the dynamic of the main quality indicators of milk production over the period 2023 - 2024 for a herd of dairy cows belonging to the breed Romanian Black Spotted exploited in condition of a farm from NE Romania. The data were obtained from the Official Production Control and were statistically processed using the computer programs SAVC and SPSS 16.00. The mean annual values of the SCC are 202.56 x 103/ml in 2023 respectively 188.09 x103 /ml in 2024. The milk components show variability with season and different THI thresholds so that, milk fat content is lower in 2023' summer being 3.89% and 3.65% in autumn 2024. The analysis was carried out done from influence of heat stress perspective because it is impacting the milk quality.

Key words: fat, protein, Romanian Black Spotted (BNR), temperature-humidity index (THI).

INTRODUCTION

The effects of heat stress (HS) on dairy cows used to be a problem for farmers in certain geographical areas but now, due to climate change, it has become a global problem. In the European Union alone, it was estimated that the milk production losses in 2015, compared to previous years, totaled between 70-550 kg of milk/day for a herd of 100 cows. (Herbut et al., 2018).

Average annual temperatures are increasing from year to year and in the summer months there are more and more days with maximum temperatures above 35°C, while the thermal comfort range of cows includes 21-25°C as maximum temperature values. (Maciuc, 2006). The effects of heat stress are evident especially in cows that have high milk production because they fail to dissipate internal heat and maintain their thermal homeostasis. The duration of the action of heat stress is also an important factor correlated with the resilience of the animals. If an acute episode of heat stress does not greatly decrease the productive level that returns in 5 days, in the case of long-term action (12 days and more) the effects become important and linger during the autumn months (Becker et al.,

2020; Souza et al., 2024). A vulnerable period for dairy cows is the dry period when they restore the structure of their mammary gland and the action of heat stress on this category of cows affects negatively the next milk production. Ambient temperatures above the comfort limit of dairy cows increase the risk of incidence of postpartum diseases, contribute to a decrease in fertility and conception rate by influencing hormone synthesis. Furthermore, heat stress experienced during the last period of gestation negatively influences fetal development and the immune function of calves, which negatively affects milk production during the productive life of calves, decreasing their productive performance. (De Rensis & Scaramuzzi, 2003; Hut et al., 2022). Under conditions of heat stress, food intake decreases (Morgado et al., 2023) which initially leads to a decrease of milk production and the manifestation of adaptive behavior from the animals. (Neves et al., 2022; Nzeyimana et al. 2023; Vidu et al., 2013; Mihai et al., 2020) Studies have shown that a decrease in dry matter intake has an impact on intestinal villi, which affects the intestinal barrier, its permissiveness being greater towards pathogens that enter the bloodstream through

the intestinal mucosa, which causes an immune response from the body (Cartwright et al, 2023).

In the case of cattle, an indicator of heat stress is the rectal temperature which it is correlated with the environmental temperature. The heat stress can be monitored and evaluated using meteorological variables such as temperature and relative humidity which, combined in calculation formulas, result in values of the temperature-humidity index (THI). It is considered that for ambient temperatures higher than 21°C cows begin to experience heat stress. (Brouček et al., 2006).

MATERIALS AND METHODS

The values of the qualitative parameters of the milk from the individual samples collected were obtained from the analysis bulletins carried out monthly within the Official Production Control (COP). In order to determine the influence of THI, data from the COP bulletins for the period 2023-2024 were used, which involved the processing of 6064 milk samples from dairy cows belonging to the Romanian black spotted breed (BNR), Holstein Friesian strain.

The cows are reared on a farm located in N-E of Romania, Iasi County and are exploited in an intensive system, in permanent free housing. The animals are fed from stock with feed administered in a mono-diet consisting of a basic (fibrous) and supplementary (concentrate) ration, the nutritional requirements being adjusted to the physiological state and the production they achieve. In the barn, the ventilation is achieved by natural ventilation and watering is done with constant-level drinkers (buffets), with water supplied from the distribution network. Cows are milked twice a day, with 12 hours between milkings.

The primary data were statistically processed with the computer programs S.A.V.C. used to determine the arithmetic mean (\bar{X}), the arithmetic mean error ($\pm S_{\bar{X}}$), the standard deviation (s), the coefficient of variation (V%), the Fisher test, the Tukey test and the Graph Prisma 9 program to test the significance of the differences.

The p-value of the test, given as a number between 0 and 1, represents the probability of

making an error if we reject the hypothesis H0. If p is less than the chosen significance threshold α – usually $\alpha = 0.05$ – we reject the hypothesis H0 and accept the hypothesis H1 as true.

The interpretation of p-values is done in most statistical tests as follows:

- $p < 0.05$, the statistical relationship is statistically significant (S, 95% confidence);
- $p < 0.01$, the statistical relationship is statistically significant (S, 99% confidence);
- $p < 0.001$, the statistical relationship is highly significant (HS, 99.9% confidence);
- $p > 0.05$, the statistical relationship is insignificant (NS).

The milk quality parameters for which the statistical processing was performed are: fat percentage (F %), protein percentage (P %), lactose percentage (Lact %), caseine content (Caz - g/l) and urea (mg/dl).

The temperature – humidity index (THI) is a value that represents the combined effects of air temperature and humidity and it is associated with the level of heat stress for using to evaluate the effects of heat stress (Bohmanova et al., 2007). To calculate the monthly THI values, the following formula was applied: $(1.8 \times T + 32) - (0.55 - 0.0055 \times RH) \times (1.8 \times T - 26)$ where T (°C) is the average monthly temperature and RH (%) is the average monthly value of relative humidity calculated based on meteorological local data accessible on www.wunderground.com.

RESULTS AND DISCUSSIONS

The influence of HS on milk production has been studied intensively, with a generous literature on this subject, but its influence on the dynamics of milk components can be supplemented even more, as there are not many studies on this subject for cow herds in Romania.

THI levels vary between authors, but in this study, the values obtained were grouped into 7 categories: THI1<30, THI2=30÷56, THI3=56÷60, THI4=60÷64, THI5=64÷68, THI6=68÷72, THI7≥72, as other authors have done.

If we were to analyze the evolution of temperatures during the period 2020-2024, we can see in Figure 1 that, from 2021 to 2024, the

average values of the annual maximums increase from 15.07°C to 17.66°C, which represents a difference of 2.59°C, i.e. 0.647°C per year. If we compare the maximum values of the temperatures in each month, the highest average is 24.42°C in 2024, which is 3.34°C higher than the average of 23.08°C in 2021.

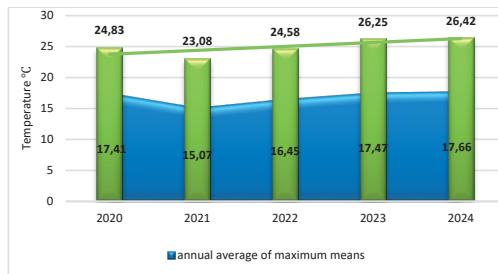


Figure 1. Annual averages of maximum monthly means and maximum monthly values (°C)

In 2023, the warmest months were July and August, for which the monthly average daily temperatures were 23.43°C and 24.83°C, respectively, and the absolute maximum temperatures were 36°C and 38°C, respectively. During 2024, the monthly daily averages were between -0.49°C in January and 25.17°C in August. In this year too, the average daily temperatures higher than 20°C (above the comfort limit) are recorded in the summer months (June, July, August), these being higher value than those of the previous year. During the day, temperatures tend to cause thermal discomfort starting from May to September, with the monthly average daily temperatures being between 22.48 and 24.60°C. The hottest month of the year was July followed by August, with absolute maximum temperatures of 39°C and 34°C respectively.

During 2023, THI values <39 are in December and January and the highest, above the threshold of 68, were recorded in July (69.54) and August (71.04). Compared to the previous year, in 2024 THI values > 68 are obtained for three months, all from the summer period: June - 70.03; July - 72.39 and August - 71.61.

Under these conditions, through the effects that heat stress has on the body of cattle, it is expected to find differences in terms of milk productivity and milk quality, especially during the summer months.

The decrease in feed intake due to heat stress explains the reduction in blood nutrient levels and the decrease in milk component synthesis. In addition, blood flow to the mammary gland is also reduced during heat stress and the body mobilizes available amino acids and energy sources to support adaptive physiological mechanisms.

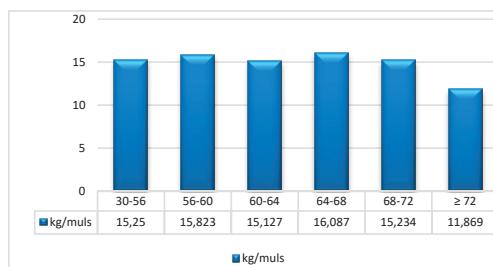


Figure 2. Influence of thermic stress on milk production

As is evident in all studies conducted, milk production decreases under heat stress conditions. For cows in the studied herd, the decrease in production starts at THI6, decreasing from 16.09 kg/milking to 15.234 kg/milking (5.32%) and is greatly reduced for THI \geq 72 values when the average is 11.87 kg/milking (Figure 2).

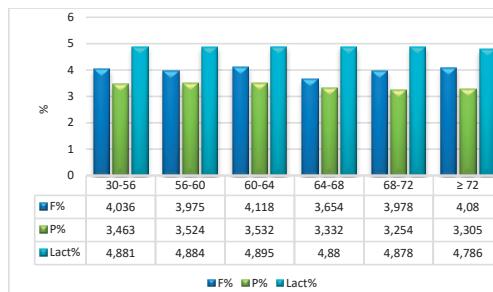


Figure 3. Dynamic of values of main quality parameters of milk composition

The lactose content of milk is correlated with milk production. As can be seen in the graphical representation of the values in Figure 3, the lactose content begins to decrease at THI6, so that at THI7 the lowest lactose content of 4.786% is recorded.

In the case of the fat percentage, it decreases by 12.7% at THI5 compared to the previous interval, being 3.654%, after which the fat percentage in milk increases as a result of the

fact that the cows mobilized body reserves to support metabolic processes.

In the same Figure 3 it can be seen that the percentage of milk protein starts to decrease from THI5 so that, under maximum heat stress, the percentage values increase to 3.305%. The processes behind the decrease in milk protein are not fully explained (Chen et al., 2024). The low level of amino acids in the blood that came from food and the fact that they are used in the synthesis of heat stress proteins and by the immune system for the synthesis of antibodies could be an explanation.

Higher values of the percentage of fat and protein in milk are recorded under heat stress conditions for $\text{THI} \geq 72$, the averages recorded being 4.08% and 3.305% respectively.

The increased level of protein that occurs in this case may be due to the increased level of synthesized stress proteins that reach the milk. The increases in the percentage of fat and protein in milk under stress conditions can also be explained from the perspective of phenotypic correlations. These two characters are strongly positively correlated with each other and each of them is negatively correlated with milk production. Thus, the decrease in milk production entails increases in the percentages of fat and protein in milk. (Promket et al., 2020).

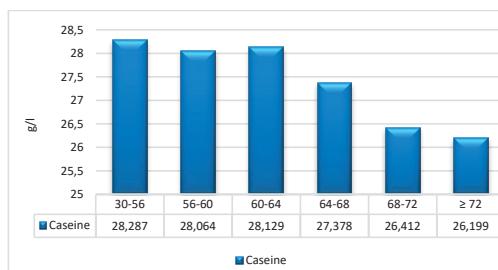


Figure 4. The influence of thermic stress on casein content in milk

The casein content in milk decreases starting from THI5 values so that, above the THI7 threshold, the lowest average of 26.199 g/l is recorded, 7.4% lower than the highest average of 28.287 g/l (Figure 4). Casein is the protein synthesized in the mammary gland. Considering that it represents the largest percentage of total milk proteins, its decrease in milk is due to the fact that the protein synthesis

process is affected at udder level. Amino acids are used in the body's defense mechanisms. (Cowley et al., 2015; Moore et al., 2024).

The results obtained in this study are similar to studies that showed that in the summer, when the temperature and humidity were high, from July to September, under the action of thermal stress, milk production decreases and at the same time the percentage content of the main components in milk decreases. (Moore et al., 2023). There are also works in which for high stress thresholds increasing percentages of milk fat were found as happened in this study. (Jo et al., 2022).

From the graphic representation of figure 5, it could be studied the evolution of the values of the amount of urea in milk according to the THI values and it can be observed that the maximum content is for THI4 after which it suddenly decreases for THI5 and after this interval the content increases again. Until the action of heat stress, the increased values are correlated with the energy content of the consumed food. The sudden decrease for THI5 is due to the reduction of food intake after which the values increase again, reaching 39.456 mg/dl urea content in THI7 milk under conditions in which the percentage of protein in milk is increasing under heat stress. Thus, the increasing level of urea nitrogen in plasma in heat-stressed cows indicates the modification of nitrogen metabolism in the body due to heat stress conditions. (Cowley, F.C. et al., 2015; Moore, S.S. et al., 2024; Ellett, M.D. et al., 2024).

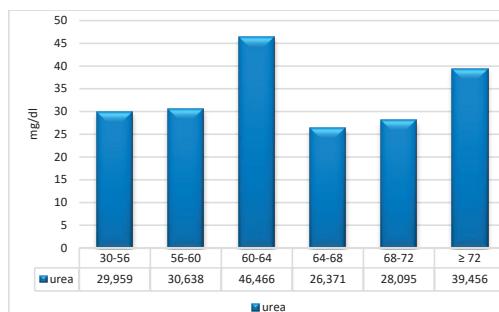


Figure 5. The influence of thermic stress on urea content in milk

In the next page, in Table 1 are presented the results of testing the significance of differences.

Table 1. Testing the significance of differences for the main parameters of milk production in the studied herd

Trait		Romanian black spotted				
	THI value	56-60	60-64	64-68	68-72	≥ 72
Fat	30-56	*	ns	****	*	ns
	56-60		**	****	ns	ns
	60-64			****	**	ns
	64-68				****	****
	68-72					ns
Protein	30-56	**	ns	****	****	****
	56-60		ns	****	****	****
	60-64			****	****	****
	64-68				**	ns
	68-72					ns
Caseine	30-56	ns	ns	****	****	****
	56-60		ns	***	****	****
	60-64			*	****	****
	64-68				****	****
	68-72					ns
Lactose	30-56	ns	ns	ns	ns	****
	56-60		ns	ns	ns	****
	60-64			ns	ns	****
	64-68				ns	****
	68-72					****

ns for $p>0,05$; * significant for $p<0,05$; ** significant - $p<0,01$; *** high significant for $p<0,001$; **** high significant for $p<0,000$

The cows in the studied herd belong to the BNR breed, which is a Holstein-Friesian strain, and whose milk production was in these two years on average 15.25 kg/milking. Based on testing the significance of the differences between the results, it can be said that:

- for P% and casein content, there are highly significant differences (for $p<0.001$ and $p<0.0001$) between most THI intervals up to the value of 72;
- as for the variability of the fat percentage, there are highly significant differences ($p<0.0001$) from THI = 64-68 and higher;
- in the case of the lactose percentage in milk, for $\text{THI} \geq 72$ there are statistically significant differences.

Thus, heat stress is a factor that influence milk production from a quantitative and qualitative point of view, also for cows raised in the climatic conditions of Romania. In the future, the influences of heat stress will have to be taken into account on farms in the country in order to take measures to minimize its effects, especially during the summer due to the economic implications mainly.

Elucidating the physiological response to heat stress that modifies the composition of milk

may form the basis for developing management strategies to mitigate losses as temperatures continue to rise (Ellett et al., 2024).

CONCLUSIONS

In the studied herd, due to heat stress, milk production and lactose percentage decrease from the THI value ≥ 68 , an effect that is amplified when the THI value exceeds the threshold of 72.

Regarding the dynamics of the other studied components, their content in milk changes from the $\text{THI} \geq 64$ thresholds, which means that they are more easily affected by thermal stress.

As noted, the milk fat percentage begins to decrease at $\text{THI} \geq 5$ when it is 3.654% and then, at higher THI values, increases again as a result of the mobilization of body reserves, reaching 4.08% at $\text{THI} \geq 7$.

The protein content decreases to 3.332% starting with THI values >64 and continues this trend until $\text{THI} \geq 7$ values when the milk protein percentage reaches an average of 3.305%. The increase in milk protein content at $\text{THI} \geq 7$ may be due to the increased blood levels of synthesized stress proteins that reach the milk, but also to the negative phenotypic correlation with milk production that decreases under heat stress conditions.

Caseine content in milk decreases starting with $\text{THI} > 60$ values because the reduction in intake causes a decrease in the level of amino acids which are used in the anabolism of other proteins necessary for body adaptation.

The percentage of lactose in milk varies correlated with production, its content decreases starting with $\text{THI} \geq 68$ so that at $\text{THI} \geq 7$ the lowest percentage content is recorded, of 4.786%.

After a maximum of the average content value of 46.46 mg/dl for the amount of urea in milk, it decreases greatly at $\text{THI} \geq 5$ to a value of 26.371 mg/dl as a result of the reduction in food intake. Then, its content in milk increases with the increase in thermal discomfort and reaches 39.456 mg/dl at $\text{THI} \geq 7$ because the nitrogen metabolism in the body is greatly affected by heat stress.

Because the performances of dairy cows are negatively influenced by heat stress and this immediately affects the economic efficiency of

animal husbandry, for this reason heat stress is not a factor to be neglected and must be taken into account in order to find and apply strategies to improve the management of dairy cow exploitation.

The recommendations refer to ensuring microclimatic conditions during the summer that prevent the effects of heat stress by achieving thermal comfort for the animals. Also, restructuring the feed rations to reduce the proportion of fibrous feed without omitting mineral balancing, which have low levels in the body affected by heat stress, influencing the electrolytic state of the cow.

REFERENCES

Becker, C.A., Collier, R.J., & Stone, A.E. (2020). Invited review: Physiological and behavioral effects of heat stress in dairy cows. *Journal of Dairy Science*, 103(8), 6751-6770. <https://doi.org/10.3168/jds.2019-17929>

Bohmanova, J., Misztal, I., & Cole, J.B. (2007). Temperature-Humidity Indices as Indicators of Milk Production Losses due to Heat Stress. *Journal of Dairy Science*, 90(4), 1947-1956. <https://doi.org/10.3168/jds.2006-513>

Brouček, J., Mihina, Š., Ryba, Š., Tonge, P., Kišac, P., Uhrinčat, M., & Hanus, A. (2006). Effects of high air temperatures on milk efficiency in dairy cows. *Czech J. Anim. Sci.*, 51(3), 93-101. DOI: 10.17221/3915-CJAS

Cartwright, S.L., Schmied, J., Karrow, N., & Mallard, B.A. (2023). Impact of heat stress on dairy cattle and selection strategies for thermotolerance: a review. *Frontiers in Veterinary Science*, 10. <https://doi.org/10.3389/fvets.2023.1198697>

Chen, L., Thorup, M., Kudahl, A.B., & Østergaard, S. (2024). Effects of heat stress on feed intake, milk yield, milk composition, and feed efficiency in dairy cows: A meta-analysis. *Journal of Dairy Science*, 107(5), 3207-3218. <https://doi.org/10.3168/jds.2023-24059>

Cowley, F.C., Barber, D.G., Houlihan, A.V., & Poppi, D.P. (2015). Immediate and residual effects of heat stress and restricted intake on milk protein and casein composition and energy metabolism. *J. Dairy Sci.*, 98, 2356-2368. <https://doi.org/10.3168/jds.2014-8442>

Cucu, I. G., Maciuc, V. et al. (2004). *Scientific research and elements of experimental technique in animal husbandry*. Iași, RO: Alfa Publishing House

De Rensis, F., & Scaramuzzi, R.J. (2003). Heat stress and seasonal effects on reproduction in the dairy cow - A review. *Theriogenology*, 60, 1139-1151. [https://doi.org/10.1016/S0093-691X\(03\)00126-2](https://doi.org/10.1016/S0093-691X(03)00126-2)

Ellett, M.D., Rhoads, R.P., Hanigan, M.D., Corl, B.A., Perez-Hernandez, G., Parsons, C.L.M., Baumgard, L.H., & Daniels, K.M. (2024). Relationships between gastrointestinal permeability, heat stress, and milk production in lactating dairy cows. *Journal of Dairy Science*, 107, 5190-5203. <https://doi.org/10.3168/jds.2023-24043>

Herbut, P., Ancreka, S. & Walczak, J. (2018). Environmental parameters to assessing of heat stress in dairy cattle - a review. *Int. J. Biometeorol.*, 62, 2089-2097. <https://doi.org/10.1007/s00484-018-1629-9>

Hut, P.R., Scheurwater, J., Nielsen, M., van den Broek, J., & Hostens, M.M. (2022). Heat stress in a temperate climate leads to adapted sensor-based behavioral patterns of dairy cows. *Journal of Dairy Science*, 105(8), 6909-6922. <https://doi.org/10.3168/jds.2021-21756>

Jo, J.H., Nejad, J.G., Lee, J.S., & Lee, H.G. (2022). Evaluation of Heat Stress Effects in Different Geographical Areas on Milk and Rumen Characteristics in Holstein Dairy Cows Using Robot Milking and Rumen Sensors: A Survey in South Korea. *Animals*, 12, 2398. <https://doi.org/10.3390/ani12182398>

Maciuc, V. (2006). *Cattle breeding management*. Iași, RO: Alfa Publishing House

Mihai, R., Mărginean, G.E., Marin, M.P., Hassan, A.A.M., Marin, I., Fintineru, G., & Vidu, L. (2020). Impact of Precision Livestock Farming on Welfare and Milk Production in Montbeliarde Dairy Cows. *Scientific Papers. Series D. Animal Science*, LXIII(2).

Moore, S.S., Costa, A., Penasa, M., Callegaro, S., & de Marchi, M. (2023). How heat stress conditions affect milk yield, composition, and price in Italian Holstein herds. *Journal of Dairy Science*, 106(6), 4042-4058. <https://doi.org/10.3168/jds.2022-22640>

Morgado, J.N., Lamonaca, E., Santeramo, F.G., Caroprese, M., Albenzio, M., & Ciliberti, M.G. (2023). Effects of management strategies on animal welfare and productivity under heat stress: A synthesis. *Front. Vet. Sci.*, 10. <https://doi.org/10.3389/fvets.2023.1145610>

Neves, S.F., Silva, M.C.F., Miranda, J.M., Stilwell, G., & Cortez, P.P. (2022). Predictive models of dairy cow thermal state: a review from a technological perspective. *Veterinary Sciences*, 9(8), 416. <https://doi.org/10.3390/vetsci9080416>

Nzeyimana, J. B., Fan, C., Zhuo, Z., Butore, J. & Cheng, J. (2023). Heat stress effects on the lactation performance, reproduction, and alleviating nutritional strategies in dairy cattle, a review. *Journal of Animal Behaviour and Biometeorology*, 11(3), 2023018. <https://doi.org/10.31893/jabb.23018>

Souza, V.C., Moraes, L.E., Baumgard, L.H., Santos, J.E.P., Mueller, N.D., Rhoads, R.P., & Kebreab, E. (2024). Modeling the effects of heat stress in animal performance and enteric methane emissions in lactating dairy cows. *Journal of Dairy Science*, 106, 4725-4737. <https://doi.org/10.3168/jds.2022-22658>

Promket, D., Kenchaiwong, W., & Ruangwittayanusorn, K. (2020). Effects of climate change on milk yield and milk composition in thai crossbred holstein cows. *International Journal of GEOMATE*, 18(67), 108–113. <https://doi.org/10.21660/2020.67.5807>

Sterup Moore, S.S., Costa, A., Penasa, M., & De Marchi, M. (2024). Effects of different temperature-humidity indexes on milk traits of Holstein cows: A 10-year retrospective study. *Journal of Dairy Science*, 107 (6), 3669–3687. <https://doi.org/10.3168/jds.2023-23723>.

Vidu, L., Băcilă, V., Călin, I., Udroiu, A., & Vladu, M. (2013). The importance of ancestral Grey Steppe breed in Romania for ensuring biodiversity cattle in South East Europe. *Annals of the University of Craiova, Agriculture, Montanology, Cadastre*, XLIII, 328–334. www.wunderground.com/history/monthly/ro/