The Effect of Milking Frequency, Heat Stress and Physiological State's Interactions on Daily Milk Yield of Holstein Dairy Cows in a Saharan Zone

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Abstract. This study focuses on enhancing daily milk yield (DMY) in the Saharan environment by exploring the relationships among milking frequency, heat stress, and lactation stage in 187 Holstein dairy cows in the Saharan Ghardaia region over a 12-year period. Key findings indicate that milking three times daily boosts DMY by about 22.67% compared with twice daily milking, with this increase being most significant (P < 0.0001) in cows during their second and third lactations. The temperaturehumidity index (THI) plays a pivotal role in DMY. The study shows that the best milk production occurs (P < 0.0001) at lower THI values (below 74), underscoring the importance of optimal environmental temperature and humidity for maximum milk yields. Cows exposed to these lower THI values and milked three times daily achieve the highest milk production (+25%). Thus, combining the right THI conditions with increased milking frequency can significantly (P < 0.0001) enhance milk production. Effective heat stress management is also crucial for optimal milk yields. The study recommends practical strategies like offering sufficient shade, ensuring good ventilation, and giving cows access to cool water. Adjusting their nutrition during high heat stress periods is also vital. Moreover, the timing of milking sessions, especially during the cooler parts of the day, is a key factor in milk production. In conclusion, the research highlights the intertwined roles of various factors, especially milking frequency and THI, in determining the DMY of dairy cows in Saharan areas. It supports the implementation of improved management practices to counter environmental challenges and maximize milk production in such demanding environments.

Introduction

The daily milk yield (DMY) of dairy cows during lactation is a crucial parameter used to assess milk production. The frequency of milking plays a significant role in determining milk yield and production efficiency. Dairy farmers often manipulate milking frequency as a management tool to enhance milk production. Increasing milking frequency has been shown to positively impact DMY and improve overall production efficiency (Erdman and Varner, 1995; Cabrera et al., 2010).

While increasing milking frequency has proven effective in boosting milk yield, it also raises operational costs and requires additional labor. Therefore, striking a balance between milking frequency and cost-effectiveness is essential. Nevertheless, studies have demonstrated that shortterm increases in milking frequency, particularly in early lactation, can improve milk yield and lactation persistency (Wall and McFadden, 2012). However,

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the time required for additional milkings at higher frequencies may potentially interfere with important cow behaviors such as feeding, rumination, and resting. These behaviors are crucial for maintaining energy balance, promoting digestive efficiency, ensuring cow health and welfare, and meeting the cow's production demands (Hart et al., 2013).

In addition to milking frequency, heat stress poses a significant challenge to dairy farmers, particularly in regions with hot climates. Heat stress negatively affects various aspects of dairy cow productivity, including milk yield, feed intake, reproductive performance, and overall well-being. The adverse effects of heat stress result in substantial economic losses for dairy farmers, especially in tropical countries. Researchers have focused on selecting dairy cattle with improved milk production traits, often resulting in increased feed intake and subsequently higher metabolic heat production, making animals more susceptible to heat stress (Kadzere et al., 2002).

The impact of heat stress on milk production is influenced by various factors, including breed, age, sex, and physiological stage of cows. The temperaturehumidity index (THI) has been established as a reliable indicator of the reduction in milk production caused by heat stress. THI considers the combined effects of

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ambient temperature and relative humidity, providing a quantifiable measure of heat stress (Ravagnolo and Misztal, 2000; Herbut and Angrecka, 2018). Furthermore, seasonal variations in dry matter intake and the activation of the negative feedback system of milk secretion contribute to the relationship between milk yield and the lactation stage (West et al., 2003; Silanikove et al., 2009; Cilek, 2009).

This study aims to enhance daily milk production in Holstein dairy cows in the Saharan region by examining the interplay between key factors such as milking frequency, heat stress, and lactation stage. The research focuses on understanding how these variables influence milk yield in the Ghardaia region of the Sahara, with a particular emphasis on optimizing conditions for maximum production. Specifically, the study investigates the effects of milking frequency and environmental parameters, including the temperature-humidity index (THI), on dairy output. Additionally, it aims to propose management strategies that mitigate the adverse impacts of heat stress, providing practical solutions for dairy farmers operating in this arid environment.

Materials and Methods

Animal data collection

The study was conducted on a dairy farm located in the Ghardaia region ($32^{\circ}41'06.7"$ N latitude and $4^{\circ}44'10.8"$ E longitude), focusing on daily milk records collected over a 12-year period from 2005 to 2016. A total of 18 178 daily milk yield (DMY) records were analyzed, obtained from 187 locallyborn purebred Holstein dairy cows with parity orders ranging from the first to the eighth lactation (with an average of 2.59 ± 1.61 of lactation rang), with lactation number frequencies distributed as follows (1 = 34.5%, 2 = 22.9%, 3 = 15.1%, 4 = 12.2%, 5 = 9.6%, 6 = 4%, 7 = 1.5% and 8 = 0.2%).

Throughout the study, the cows were milked

either twice daily (n = 177 cows) or thrice daily (n = 10 cows). The twice-daily milking sessions occurred at 06:00 and 18:00, while the thrice-daily milking sessions were conducted at 06:00, 14:00, and 21:00. These milking sessions took place in designated milking parlors equipped with automated milk meters, which precisely measured the total milk output over a 24-hour period for each cow.

The lactation stages of the cows were categorized into four classes: early lactation (< 120 days), middle lactation (120–179 days), late lactation (180–305 days), and prolonged lactation (> 305 days).

Environmental data collection

The data was acquired from the local meteorological station, representing the weather conditions at the farm. This data comprises the highest daily temperatures (AT °C) and the maximum relative humidity (%RH). These variables were employed to compute the temperature-humidity index (THI), following the formula proposed by (Mader et al., 2006):

 $THI = (0.8 \times T) + [(\%RH / 100) \times (T - 14.4)] + 46.4.$

The values of THI are divided into 4 classes, according to the classification by Nienaber et al. (2007), who evaluated the intensity of heat stress as follows: normal stress, moderate stress, severe stress, and very severe stress, corresponding to the following values: THI < 74, [75–78], [79–83], and THI > 84, respectively.

Feed and feeding

The dairy cows in the study were provided with a basic ration consisting of alfalfa hay and corn silage, supplemented with mixed concentrate feeds. The composition of the diets, including the dry matter percentage (DM %), dry matter intake (DMI) of the diet in kilograms (kg), and nutrient intake per cow per day, is presented in Table 1.

Table 1. Average daily ration composition of lactating cows during the study period (2005–2016)

Diet	% DM	DMI of diet (kg)	Nutrient intake/cow/day				
			UFL ¹	PDI ² (g)	Ca (g)	P (g)	
Fodder sorghum	21.36	5	3.4	330	16.5	17	
Alfalfa hay	18.80	4.4	3.21	365.2	70.84	11.88	
Wheat bran	10.68	2.5	2	187.5	3.5	24.25	
Flattened wheat grain	12.82	3	3,06	261	2.1	10.2	
Maize grain	17.09	4	4.24	336	1.6	10.4	
Date scraps	6.41	1.5	1.27	42.6	1.5	1.5	
Soybean meal	12.82	3	3.15	666	10.2	18.6	
Total	100	23.4	20.33	2188.3	106.24	93.83	
Rapport PDI/UFL: 107.6 g/UFL, Energy density of diet: 0.86 UFL/kg, Hay/Concentrate ratio: 59.83/40.17%,							

Dry matter (%) of ration: 38.84%, UEL³_{total}: 10.1. 1: Forage unit for milk production, per kg. 2: Digestible proteins in the intestine, in g/kg. 3: Encumbrance unit for

lactating females.

Statistical Analysis

The dataset was subjected to an extensive statistical evaluation using Minitab 18 to ascertain the influence of various factors on daily milk yield (DMY) in dairy cows. These factors included milking frequency (MF), lactation number (LN), lactation stage (LS), calving season (CS), and temperature humidity index (THI). To quantify these effects, a fixed-effect model was implemented, formulated as follows:

$$\begin{split} \boldsymbol{Y}_{_ijklm} &= \boldsymbol{\mu} + \boldsymbol{MF}_{_i} + \boldsymbol{LN}_{_j} + (\boldsymbol{MF} \ \boldsymbol{x} \ \boldsymbol{LN})_{_ij} + \boldsymbol{LS}_{_k} + (\boldsymbol{MF} \ \boldsymbol{x} \ \boldsymbol{LS})_{_ik} + \boldsymbol{CS}_{_l} + (\boldsymbol{MF} \ \boldsymbol{x} \ \boldsymbol{CS})_{_il} + \boldsymbol{THI}_{_m} + (\boldsymbol{MF} \ \boldsymbol{x} \ \boldsymbol{THI})_{_im} + \boldsymbol{e}_{_ijklm}. \end{split}$$

Here, $Y_{_{_{ijklm}}}$ signifies DMY, and μ denotes the population mean. This model facilitates a comprehensive understanding of the primary effects and interactions among these variables on milk production.

Results

According to the THI data shown in Fig. 1, thermal stress in the study area can be categorized as follows.

The months of April, May, and October experience mild to moderate thermal stress, with THI values being 72.94 \pm 4.49, 77.67 \pm 4.18, and 76.12 \pm 4.40, respectively. In contrast, June through September undergo severe thermal stress, indicated by THI values of 82.86 \pm 3.22, 85.26 \pm 2.59, 85.05 \pm 2.55, and 81.82 \pm 3.56, respectively. Meanwhile, from November to March, there is no thermal stress, with THI values ranging from 61.22 \pm 4.81, 62.63 \pm 5.00, 61.22 \pm 4.32, 64.23 \pm 4.81 and 68.35 \pm 4.81. These variations in thermal stress, especially in the Saharan regions, contribute to moderate to severe challenges that negatively affect the productivity of dairy cows.

The analysis of the results presented in Table 2 shows the effects of various factors on DMY in Holstein dairy cows.

Temperature-humidity index (THI) values significantly influence daily milk yield (DMY) (P <0.0001), with bovines exposed to lower THI values (< 74) demonstrating higher mean DMY (22.88 kg) compared with those in elevated THI ranges, and an inverse relationship observed between increasing THI values and mean DMY differences. Milking frequency (MF) also significantly impacts DMY (P <0.0001), with cows milked thrice daily (3X) producing a superior mean of 24.55 kg compared with 21.43 kg for twice-daily milking (2X). Calving season stress levels significantly affect DMY (P < 0.0001), with normal stress during parturition yielding the highest mean DMY (22.71 kg) and severe stress yielding the lowest mean DMY (20.28 kg), indicating an inverse correlation between calving stress intensity and DMY. Lactation number significantly influences DMY (P <0.0001), with third-lactation cows exhibiting peak mean DMY (22.49 kg), followed by a slight decline in the fourth or greater lactations (21.70 kg), while the first and the second lactations yield substantial mean DMYs of 21.76 kg and 21.22 kg, respectively. Lactation stage also significantly impacts DMY (P <0.0001), with early lactation cows demonstrating the maximum mean DMY (26.72 kg), followed by those in mid-lactation (22.75 kg), and late and extended lactation stages exhibiting progressively lower mean DMYs of 19.01 kg and 16.32 kg, respectively. The results presented in Fig. 2 demonstrate the interaction between MF and THI on the DMY of Holstein dairy cows.

The interaction between milking frequency (MF) and temperature-humidity index (THI) exhibits a significant influence on daily milk yield (DMY) (P < 0.0001). In twice-daily milking (2X) regimens, an inverse relationship is observed between THI and DMY. Bovines subjected to 2X milking demonstrate peak mean DMY (21.57 kg) at THI < 74, with



Fig. 1. Means of monthly distribution of THI values during the study period (2005–2016). Veterinarija ir Zootechnika 2024;82(1)

<i>Table 2</i> . The fixe	ed effects of th	e environmental	l, milking :	frequency a	and animal	factors o	n DMY
	(n =	18 178 records)) of Holste	in dairy co	WS		

Items	N	Mean	StDev	95% CI	P value	
THI values range	·					
< 74	7692	22.88	8.11	(22.70; 23.05)		
[75–78]	2769	22.18	7.83	(21.89; 22.46)	0.0001	
[79-83]	5020	21.03	7.70	(20.81; 21.24)	0.0001	
> 84	2697	19.33	6.31	(19.04; 19.62)		
Milking frequency						
2X	16 401	21.43	7.76	(21.31; 21.55)	0.0001	
3X	1777	24.55	7.75	(24.19; 24.91)		
Calving season						
Normal stress	9103	22.71	8.24	(22.54; 22.86)		
Moderate stress	2211	21.12	8.08	(20.87; 21.52)	0.0001	
Severe stress	3321	20.98	7.05	(20.72; 21.24)	0.0001	
Very severe stress	3543	20.28	6.07	(20.02; 20.53)		
Lactation number			·		·	
First lactation	6273	21.76	7.45	(21.57; 21.96)		
Second lactation	4171	21.22	7.99	(20.98; 21.46)	0.0001	
Third lactation	2736	22.49	8.16	(22.20; 22.78)	0.0001	
Fourth lactation or greater	4998	21.70	7.88	(21.49; 21.92)		
Lactation stage					·	
Early lactation	5050	26.72	7,94	(26.53; 26.91)		
Middle lactation	5170	22.75	6.34	(22.56; 22.93)	0.0001	
Later lactation	4700	19.01	5.99	(18.81; 19.20)	0.0001	
Prolonged lactation	3258	16.32	6.19	(16.09; 16.56)		

diminishing yields at higher THI ranges. Similarly, thrice-daily milking (3X) protocols display an inverse THI-DMY relationship. However, 3X milking yields superior mean DMY at both THI < 74 (24.95 kg) and THI 75–78 (26.42 kg). Comparative analysis reveals that 3X milking protocols result in substantial DMY improvements across various THI ranges: 15.71% (THI < 74), 28.97% (THI 75–78), 20.67% (THI 79–83), and 25.35% (THI > 84). In Fig. 3, the interaction between milking frequency (2X and 3X) and calving season on DMY of Holstein dairy cows is examined.

The findings suggest a significant difference in DMY between cows milked twice daily (2X) and those milked three times daily (3X), varying by calving season. Cows milked 3X consistently exhibit higher DMY across all levels of calving stress compared with cows milked 2X, with DMY increases ranging from 17.4% under normal stress to 29.6% under very severe stress. These results indicate that increasing milking frequency to 3X can lead to substantial gains in milk production, with more pronounced percentage increases observed under higher calving stress conditions. Fig. 4 shows the interaction between MF (2X and 3X) and lactation number on DMY Holstein dairy cows.

A significant increase in daily milk yield (DMY) was observed in cows subjected to a three-times-daily (3X) milking regimen compared with those milked twice daily (2X) across all lactations. The most pronounced difference in DMY between 3X and 2X milking was evident in the second lactation. While 3X milking consistently yielded higher DMY than 2X milking throughout the study, the magnitude of the difference between the two regimens gradually decreased with advancing lactation number. The relative increase in DMY achieved with 3X milking compared with 2X milking was 8.29%, 34.15%, 31.06%, and 16.95% for the first, second, third, and fourth or greater lactations, respectively. The results in Fig. 5 indicate the interaction between MF and lactation stage on the DMY of Holstein dairy cows.

A significant increase in daily milk yield (DMY) was observed in cows subjected to a three-times-daily (3X) milking regimen compared with those milked twice daily (2X) throughout all stages of lactation. The most pronounced effect of milking frequency (MF) on DMY was evident during early lactation. While 3X milking consistently outperformed 2X milking in terms of DMY, the magnitude of the difference between the two regimens gradually diminished as



Fig. 2. Means of DMY (kg) depending on (MF x THI) of Holstein dairy cows



Fig. 3. Means of DMY (kg) depending on (MF x Calving season) of Holstein dairy cows



Fig. 4. Means of DMY (kg) depending on (MF x Lactation number) of Holstein dairy cows Veterinarija ir Zootechnika 2024;82(1)



Fig. 5. Means of DMY (kg) depending on (MF x Lactation stage) of Holstein dairy cows

lactation progressed. Even in prolonged lactation, a significant disparity in DMY persisted between 3X and 2X milking, demonstrating the continued benefit of 3X milking. The relative increase in DMY achieved with 3X milking compared with 2X milking was 20.2%, 17.6%, 19.0%, and 29.2% for early, middle, later, and prolonged lactation stages, respectively.

Discussion

The interaction between milking frequency (MF) and lactation stage (LS) has yielded significant insights into daily milk yield (DMY). The findings demonstrate the critical influence of LS on milk production, showing a complex interplay with MF that shapes overall DMY. Notably, heat stress negatively impacts milk production at various LS, with the most pronounced reduction occurring during mid-lactation, as highlighted by Joksimović-Todorović et al. (2011). These results emphasize the need to account for LS when assessing the relationship between MF and milk yield.

Furthermore, the concept of peak milk yield, which represents the highest milk production during a lactation cycle, has been examined, revealing that cows milked three times daily surpass those milked twice daily during this peak period (Capuco et al., 2003). This underscores the positive correlation between increased MF and enhanced DMY, especially during peak lactation. The typical lactation curve, characterized by a gradual increase in milk yield after calving, reaching a peak, followed by a decline toward late lactation, aligns with the current study's findings, which observed a steady rise in milk yield up to midlactation, followed by a decline.

Additionally, responses to increased MF during early lactation vary between primiparous heifers and multiparous cows. Soberon et al. (2010) reported a stronger response in heifers, while later research by Soberon et al. (2011) found comparable responses in both heifers and multiparous cows at this stage. The positive impact of increased MF on DMY is wellestablished, with studies reporting increases of 7% to 15% when switching from twice-daily to three-timesdaily milking (Bogucki et al., 2009; Barlowska et al., 2012). However, this effect depends on factors such as LS and parity, with Bogucki et al. (2011) observing a diminishing trend in DMY with three-times-daily milking in cows beyond their first lactation.

Moreover, increasing MF during early lactation has been associated with improved milk yield and lactation persistency (Hale et al., 2003; Wall and McFadden, 2012), highlighting the long-term effects of MF manipulation during specific LS on milk production. Variations in udder anatomy, lactation persistency, and other factors may explain the differences in response to increased MF between primiparous and multiparous cows (De Vliegher et al., 2003). Therefore, a thorough investigation of the MF-LS interaction on milk yield must consider these complexities.

Conclusion

This study underscores the significant impact of the interaction between milking frequency and the temperature-humidity index on the daily milk yield of locally-bred Holstein dairy cows. The findings reveal that cows milked three times daily exhibit a substantially higher DMY (+22.67%) compared with those milked twice daily, particularly across varying THI levels. The lowest THI range (< 74) is associated with the highest DMY for both milking frequencies, highlighting the critical role of optimal THI conditions in maximizing milk production.

Furthermore, the combination of increased milking frequency and lower THI values shows great potential for enhancing milk production in Holstein dairy cows. Dairy farmers are encouraged to carefully manage milking frequency and maintain favorable environmental conditions, especially in terms of THI, to optimize production within their herds.

The study also confirms the significant influence of both milking frequency and lactation number on DMY. Cows milked three times daily generally produce more milk (25.11%), with this increase being most pronounced during the second and third lactations. These insights into the relationship between milking frequency, lactation number, and

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DMY can provide valuable guidance for dairy farmers seeking to optimize milk output in their herds.

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Competing interests

The authors declare that they have no potential conflict of interest.

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