Influence of Heat Stress and Some Related Physiological Indicators on the Content of Long-Chain Fatty Acids in the Milk of Holstein-Friesian Cows

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Keywords: *heat stress, temperature-humidity index, rectal temperature, long-chain fatty acids, Holstein-Friesian cows.*

Abstract. *The aim of the research was to study the effect of heat stress (HS) and associated changes in the rectal temperature and the respiratory rate on long-chain fatty acid (LCHFA) content in the milk of Holstein-Friesian cows. The study included 22 cows on different parities studied in two periods: under thermo-neutral environment conditions (May 2018) and under heat stress (August 2018). The fatty acid content of milk was determined using a chromatograph by the method of Rose-Gottlieb. It was found that HS in dairy cows leads to changes in the content of some LCHFAs (C17:0; C18:0; C18:2 and C18:3) in milk fat. Under conditions of moderate HS (temperature-humidity index over 79), a certain decrease in the content of C17:0 was reported, while in the other three LCHFAs, an increase in their content in milk fat was reported to varying degrees. The strongest effect of HS was reported on the content of C18:0, which was proportional to the levels of HS. With an increasing rectal temperature, an increase in the content of C18:0 was reported, the increase being most substantial at a rectal temperature above 39.5°C.*

Introduction

In the last few decades of the 19th century, we have witnessed dramatic changes in the Earth's climate associated with warming (Renna et al., 2010). Such trends are reported in different climatic zones, including the temperate continental. Milk cows are very sensitive to climate change and especially to elevated temperatures. Heat stress in dairy cows is usually associated with activation of certain thermoregulatory mechanisms to overcome the hyperthermia and maintain vital functions of the animal and, as a consequence, reduction of their productivity (Nardone et al., 2006). Exposure to heat stress can dramatically change both the quantity and composition of ruminant milk (Renna et al., 2010). Protein in milk has been found to decrease significantly under conditions of heat stress (Giustini et al., 2007; Kamiya et al., 2005). Studies by a number of authors have shown different trends in the milk fat content of cows' milk under heat stress conditions (Giustini et al., 2007; Bouraoui et al., 2002; Sevi et al., 2001; Lacetera et al., 1996). Some authors do not find changes in the amount of fat in cow's milk at HS (Roman-Ponce, 1977; Knapp and Grummer, 1991; Lacetera et al., 2003), while others report a significant decrease (Bouraoui et al., 2002; Hammami et al., 2015; Hill and Wall 2015). However, surveys that have studied the effect of HS on the fatty acid profile of milk are few, and the results between the authors are controversial (Lacetera et al., 2003). The fatty acid profile of milk has been found to be a potential indicator of energy balance in cows (Bastin et al., 2012). HS leads to certain physiological changes in dairy cows such as increased respiratory rate, increased heart rate and increased body temperature (Kadzere et al., 2002; Wheelock et al., 2010), but the most prominent change is the reduced intake of dry matter (West, 2003), which, together with the efforts for cooling, results in consumption of much energy and metabolic changes in the body of dairy cows (Bernabucci et al., 1999). According to Chilliard et al. (2000) and Parodi (2004), the content of LCHFAs after C16:0 in milk is a consequence of metabolism in the body and they are not synthesized de novo in the mammary gland of cows. According to the authors, all processes related to metabolism in the body under metabolic changes can cause a number of alterations in the percentage of LCHFAs. Most of the LCHFAs in milk have different effects on human health. Palmitic acid (C 16:0) together with some medium-chain saturated fatty acids (C12:0 and C14:0) are considered to be major factors in the development of cardiovascular diseases in humans (Kaylegian and Lindsay, 1995; Jensen, 2002). On the other hand, some unsaturated LCHFAs, such as oleic (C18:1), linoleic (C18:2) and linolenic (C18:3), have a positive effect on human health (Simopoulos, 2002). HS, leading to changes in the content of these fatty acids in milk, is of interest both in relation to the metabolic changes in the body of cows and respectively their welfare, and in relation to the qualities of produced milk as healthy food for humans.

The aim of the study was to investigate the effect of HS and associated changes in the rectal temperature and the respiratory rate on the percentage of

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LCHFAs – isomargaric (C17:ISO), margaric (C17:0), stearic (C18:0), oleic (C18:1), linoleic (C18:2) and linolenic (C18:3) – in milk fat in Holstein-Friesian cows.

Material and Methods

The study was conducted at a cattle farm with Holstein-Frisian cows near Karnobat, southeastern Bulgaria, in 2018. Cows were housed in a semi-open free stall dairy barn, fed year-round ad libitum with a total mixed ration at the following composition for a cow per day in kg: forage – 14 kg, concentrates – 14 kg, and vitamin and mineral supplements – 0.39 kg. The content of the forage expressed as a percentage of the total amount is as follows: corn silage – 77.15%, alfalfa hay – 12.14%, and straw – 10.71%. The content of concentrates expressed as a percentage of their total quantity is as follows: maize grain (ground) – 35.74%, wheat grain (ground) – 21.42%, barley grain (ground) – 7.14%, soybean meal – 11.42%, sunflower meal – 19.28%, and palm oil – 5%. The study included all cows calved between first April and tenth May 2018, thus excluding the effect of the lactation period on the fatty acid content of milk. The included cows were at first (9 cows), second (6 cows) and third to fifth (7 cows) parity or a total of 22 cows. To test for fatty acid content, individual milk samples with a volume of 100 mL from the morning and the evening milking were taken from each cow included in the study once in May and once in August. The daily milk yield as well as the content fat and protein percentage of the cows were taken from their official monthly milk performance records (for May and August).

Extraction of milk fat was performed by the Rose-Gottlieb method using diethyl ether and petroleum ether (Methodenbuch, Bd. VI VDLUFA-Verlag, Darmstadt, 1985). The solvents were then evaporated on a vacuum rotary evaporator. Sodium methylate (CH3ONa) was used to prepare the fatty acid methyl esters. The fatty acid content of milk fat was determined by a "Clarus 500" gas chromatograph with a flame-ionization detector and a "ThermoScientist" column (60 m, ID 0.25 mm, film: 0.25 μm).

To measure the heat stress, a temperature-humidity index (THI) was estimated using a "Kestrel" automatic measuring instrument. Reporting of THI was carried out in the cows' housing premises once a day, at 3 pm, with examination of the physiological parameters of each cow: the rectal temperature and the respiratory rate per minute. The rectal temperature was measured by a digital thermometer in degrees of Celsius. The respiratory rate was reported by visual observation and recording of the movement of the chest for a period of one minute according to the method of Zimbelman et al. (2009).

For better approximation factors object of the study were presented in classes as follows:

THI is presented in three classes according to the proposed THI scale by Armstrong (1994), respectively: Class 1 – THI up to 72; Class 2 – 72 to 79 (mid-moderate heat stress conditions); and Class 3 – THI above 79 (moderate heat stress conditions).

The rectal temperature of the cows, respectively: Class 1 – up to 38.5°C; Class 2 – from 38.5 to 39°C; Class 3 – from 39 to 39.5°C; and Class 4 – above 39.5°C.

Respiratory rate per minute: Class 1 – up to 40 per minute; Class 2 – 40 to 45 per minute; Class 3 – 45 to 55 per minute; and Class 4 – over 55 movements per minute.

For basic statistical processing of the data, a package MS Excel was used, and for obtaining the average values, errors, and analysis of variance, the corresponding modules of STATISTICA of StatSoft was employed (Copyright 1990–1995 Microsoft Corp.)

The following model was used to evaluate the influence of controlled factors on the content of long-chain fatty acids in milk:

$$Y_{ijkl} = \mu + THI_i + RT_j + B_k + e_{ijkl}$$

Where: Y_{ijkl} is the dependent variable (each of the fatty acids studied), μ is the mean effect, THI_i is the effect of THI (classes), RT_j is the effect of rectal temperature (classes), B_k is the effect of respiratory movements per minute (classes) and e_{ijkl} is the random residual effect.

By analysis of variance (ANOVA) for the model were obtained by classes of fixed factors the means of least squares (LSM).

Results

Table 1 presents the reported THI averages for the two calendar months, with a significant difference of 73.4 and 79.1, respectively. The variation in THI values for May corresponds to conditions from optimal to mid-moderate heat stress, THI 71.2 and THI 76.2, respectively. For August, the reported minimum-maximum values of THI were significantly higher corresponding to the conditions from mid-moderate heat stress (THI 77.1), to conditions of moderate heat stress (THI 81.5).

Table 1 also presents the average Test day records for milk yield, fat and protein percentage, and the average values of the reported physiological parameters of the examined cows for the two months of the study (May and August). The cows included in the study had a high average daily milk yield of 39.92 kg, a high fat content (4.13%) and a low protein content of 2.88%. A slight difference in the average TD performance values for the two months was observed. The average milk yield and fat percentage had lower averages for August compared with those for May but without a significant difference. There was a slight increase in the TD protein percentage, and although the difference was significant, it was very small – by only 0.1%.

Table 1. Average values and variation of productive traits for Test day, physiological indicators and temperature-humidity index by months of reporting

Indicator	May, n = 22			August, n = 19		
	x ± SE	min	max	x ± SE	min	max
Milk yield, kg	41.12 ± 2.52 n.s.	19.5	60.1	38.6 ± 2.19 n.s.	23.7	52.9
Fat %	4.31 ± 0.22 n.s.	2.70	5.87	3.94 ± 0.21 n.s.	2.28	5.56
Protein %	2.84 ± 0.02**	2.60	3.00	2.94 ± 0.02**	2.77	3.15
Rectal temperature, °C	38.55 ± 0.09***	37.3	39.2	39.27 ± 0.10***	38.2	39.9
Respiratory rate, number/min	39.27 ± 1.57***	24.0	56.0	54.95 ± 1.91***	36.0	682.0
Temperature-humidity index	73.4 ± 0.29***	71.2	76.2	79.1 ± 0.49***	77.1	81.5

n.s.– has no significant effect.
*Significance at P < 0.05. **Significance at P < 0.01. ***Significance at P < 0.001.

The average rectal temperature of cows increased from 38.55°C in May to 39.27°C in August, and the respiratory rate per minute increased from 39.27 to 54.95, respectively. Table 2 presents the average values and the variation in the content of long-chain fatty acids in milk fat. Under the moderate temperature conditions for May, the values of C18:0 and C18:3 were lower, 8.46% and 0.42%, respectively, and under conditions of low and moderate HS in August their values were increased to 9.38% and 0.54%. With LCHFA C17:0, the trend was opposite, i.e., the values decreased slightly under conditions of HS, from 3.46% to 3.21%.

Table 3 presents an analysis of variance for the influence of THI, rectal temperature and respiration rate on the contents of LCHFAs in the milk. The THI affected significantly four of the six studied fatty acids: C17:0; C18:0; C18:2 and C18:3. The rectal temperature had a significant effect only on the C18:0 content.

Table 2. Mean values (in %) and variation in the content of long-chain fatty acids in milk fat by months of reporting

LCHFAs	May, n = 22			August, n = 19		
	x ± SE	min	max	x ± SE	min	max
C17:ISO	0.55 ± 0.02 n.s.	0.26	0.72	0.58 ± 0.02 n.s.	0.37	0.76
C17:0	3.46 ± 0.09*	2.81	4.58	3.21 ± 0.06*	2.58	3.57
C18:0	8.49 ± 0.14***	7.10	9.65	9.38 ± 0.14***	8.16	10.33
C18:1	25.19 ± 0.56 n.s.	20.88	29.59	26.23 ± 0.34 n.s.	23.46	28.77
C18:2	2.60 ± 0.07 n.s.	2.04	3.18	2.60 ± 0.06 n.s.	2.01	2.95
C18:3	0.42 ± 0.03**	0.14	0.68	0.54 ± 0.02**	0.35	0.67
Total	40.33			42.54		

n.s.– has no significant effect.
*Significance at P < 0.05. **Significance at P < 0.01. ***Significance at P < 0.001.

Table 3. Analysis of variance for the influence of temperature-humidity index, rectal temperature and respiration rate on the contents of long chain fatty acids

Sources of variation	Degrees of freedom (n–1)	C17:ISO	C17:0	C18:0	C18:1	C 18:2	C18:3
		F P	F P	F P	F P	F P	F P
Total for the model	8	1.84 n.s.	1.64 n.s.	2.68 *	1.53 n.s.	1.85 n.s.	2.39 *
Temperature-humidity index	2	2.69 n.s.	4.27 *	6.61 **	2.35 n.s.	3.57 *	3.46 *
Rectal temperature	3	0.80 n.s.	1.31 n.s	3.30 *	1.23 n.s.	0.85 n.s.	2.00 n.s.
Respiratory rate	3	1.83 n.s.	1.18 n.s.	1.00 n.s.	1.73 n.s.	2.18 n.s.	1.01 n.s.
Error	32						

n.s.– has no significant effect.
*Significance at P < 0.05. **Significance at P < 0.01. ***Significance at P < 0.001.

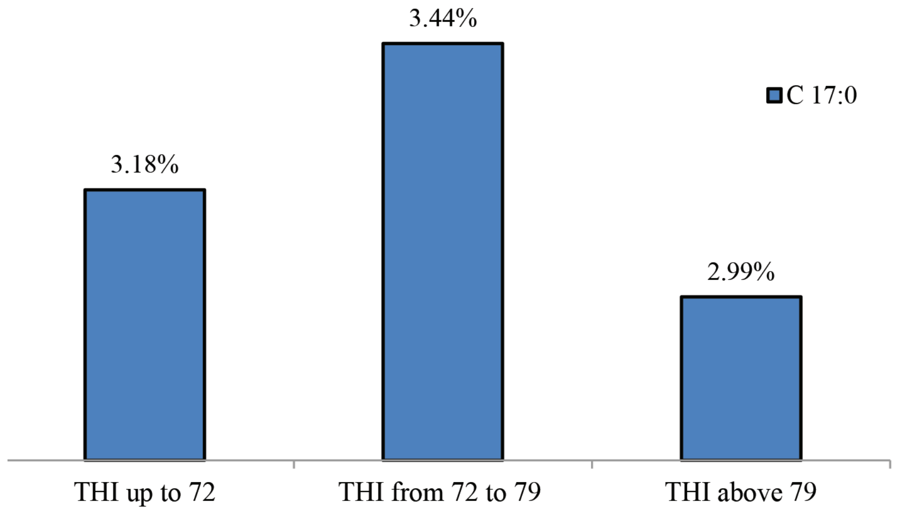

Fig.1. LS means for C17:0 content depending on the THI

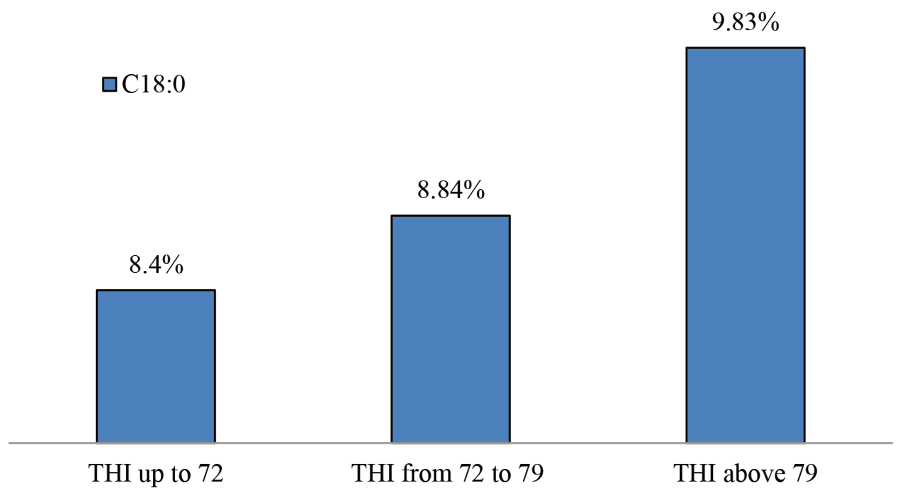

Fig. 2. LS means for C18:0 content depending on the THI

Fig. 1 shows the LS mean values for C17:0 content of milk fat, depending on the THI values. The highest percentage (3.44%) of C17:0 in milk fat was recorded at the THI values from 72 to 79 corresponding to mild heat stress. With an increase in the THI values above 79 (moderate heat stress), the percentage C17:0 content decreased to 2.99%.

Fig. 2 shows the LS mean values for the C18:0 content at different THI levels. At the THI up to 72, the content of C18:0 was 8.4%, at the THI from 72 to 79, it was 8.84, and at the THI above 79, it increased to 9.83%.

Fig. 3 presents the LS mean values of C18:0 depending on the rectal temperature. At values of the rectal temperature up to 38.5°C, the values of C18:0 were 8.58% and increased to 10% at a rectal temperature above 39.5°C.

Fig. 4 shows the LS means for the C18:2 content depending on the THI levels. With the increase in the THI values from mild (up to 72) to moderate HS (THI from 72 to 79), the increase in the content of this fatty acid was almost 0.4%.

Fig. 5 presents the LS mean values for the content of C18:3, depending on the THI. At the THI up to 72, the amount of linolenic acid (C18:3) was 0.36%, at the THI from 72 to 79, it was 0.47%, and at the THI over 79, it was 0.56%. The tendency of increasing content of C18:3 with the increase of the THI value was clearly evident here.

Discussion

Similarly to our data presented in Table 1, Dimov et al. (2017) also reported THI risk values for the region of southern Bulgaria. The authors indicate that, during the summer season, values determining conditions for mild to moderate heat stress in dairy cows were reported, namely, average daily values of the THI over 75. There was also a certain risk of such

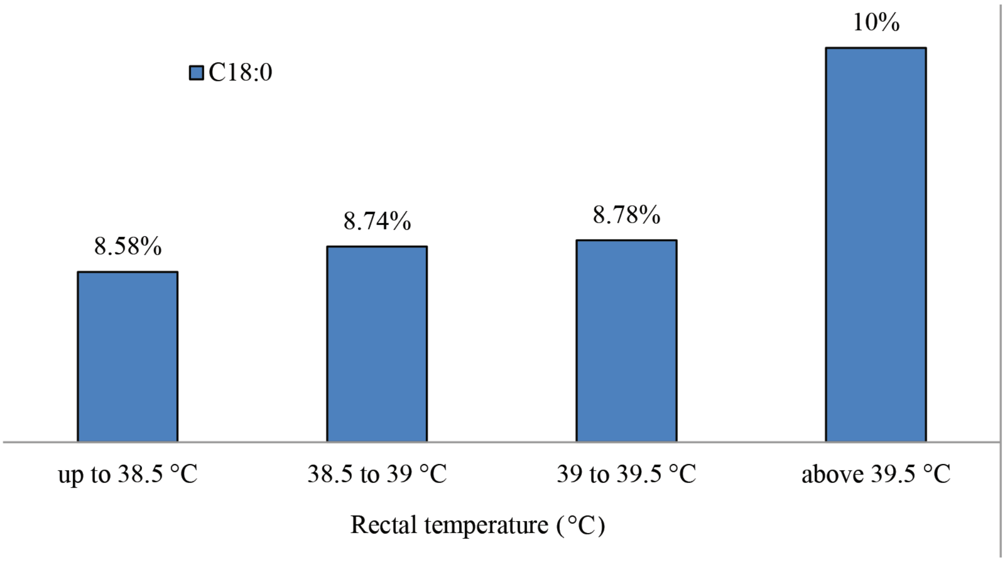

Fig. 3. LS means for C18:0 content depending on the rectal temperature

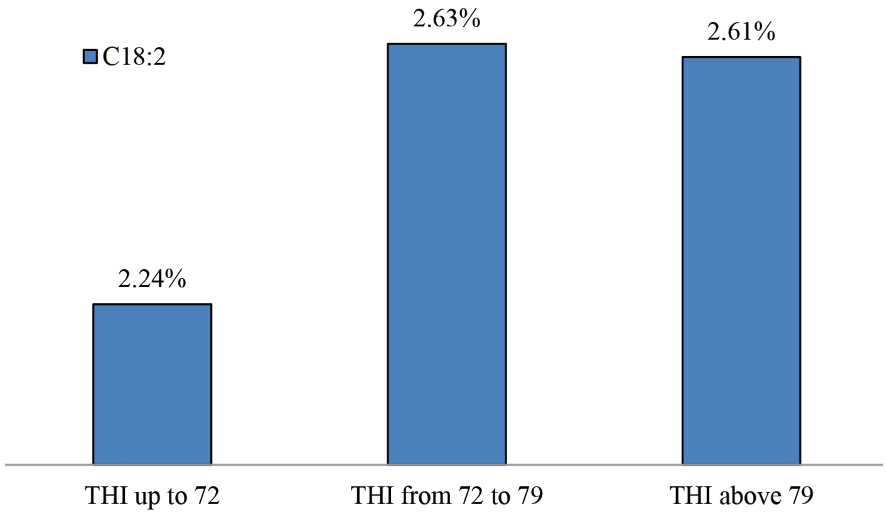

Fig. 4. LS means for C18:2 content depending on the THI

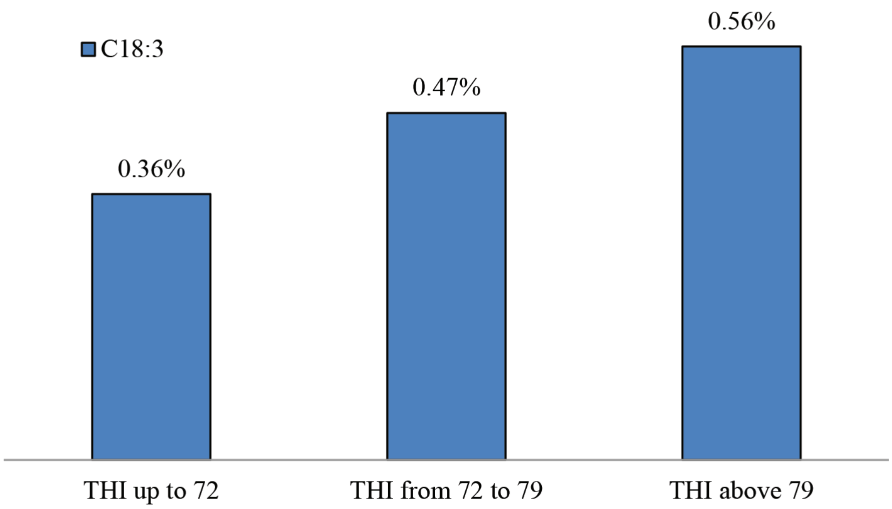

Fig. 5. LS means for C18:3 content depending on the THI

conditions in spring and autumn. It can be said that, with respect to the main productive traits of the cows included in the study, no significant differences were observed for the two calendar months and the reported THI values, although the trend showed a slight decrease in TD milk yield and TD fat percentage in milk and a slight increase in the TD protein percentage under mild to moderate HS in August (Table 1). There were no unidirectional data on the effect of HS on milk composition reported by other authors as well. Bouraoui et al. (2002) also found a decrease in milk yield and a fat percentage under the influence of HS, while Smith et al. (2013) found an increase in fat content in milk under mild HS (THI over 68). These differences may also be due to different levels of HS in the studies by different authors.

In respect of the two physiological indicators, i.e., rectal temperature and respiratory rate, the results in Table 1 show that in May at the optimal to slightly risky THI values significantly lower values for the rectal temperature and the respiratory rate per minute were reported. Similar changes in the rectal temperature values and the respiratory rate in cows under the influence of HS were also found by Bouraoui et al. (2002). According to Atkins et al. (2018), the respiratory rate increases by 1.95 breaths per minute at the THI below 70 and by 3.18 breaths per minute at the THI over 70. This explains the differences in the respiratory rate per minute in our study.

Significant differences in the content for the two months of studying were reported in three studied LCHFAs: C17:0, C18:0 and C18:3. Our results for C17:0 and C18:0 were consistent with the studies of Hammami et al. (2015). Under the conditions of HS, Liu et al. (2016) also found an increase in C18:3 content in milk fat. The other LCHFAs showed very small (C17:ISO and C18:1) or no difference (C18:2) under both optimal and HS conditions. Our results regarding the C18:1 greatly differed from the results of Liu et al. (2016). The authors found a significant increase in the content of this fatty acid under HS conditions lasting 4 days. According to Liu et al. (2016), under HS conditions, LCHFAs are increased of the occurred lipolysis of the adipose tissue.

The data presented in Table 2 show that the total amount of LCHFAs reported in August (conditions of mild to moderate HS) was higher by 2.21%, compared with their total in May, under optimal temperature conditions. Renna et al. (2010) also found an increase in the amount of LCHFAs under HS conditions.

The data in Table 3 show that the THI was the factor that significantly influenced four of the six studied fatty acids: C17:0; C18:0; C18:2 and C18:3. In regard of the two physiological indicators, the respiratory rate had no significant effect on the studied LCHFAs, and the rectal temperature had a significant effect only on the C18:0 content.

Similarly to our results in Figure 1, Hammami et al. (2015) also found a decrease in the content of margaric acid (C17:0) at the THI values above 62.

The THI had the highest significance effect ($P < 0.01$) on the C18:0 content (Figure 2). These data partly confirm the findings of Hammami et al. (2015), according to which C18:0 and C18:1 were the fatty acids affected by HS. Contrary to the results reported reported by Hammami et al. (2015), C18:1 was not significantly affected by HS levels reported in our conditions. This was probably due to the difference in the THI values. In our study, the reported THI values were around and above 79, while in the study of Hammami et al. (2015), the highest THI value was 75. A more detailed analysis of the data presented in Figure 2 shows that with the increase in THI values, the percentage of C18:0 in milk fat also increased. These results confirm those of Barber et al. (1997). According to the authors, when cows were subjected to a negative energy balance, as observed under HS, a higher ratio of C18:0 and C18:1 cis-9 was found. In other words, the presence of moderate heat stress led to lipolysis, which caused an increase in the C18:0 content. Based on our results, we consider that the most sensitive change in cows under HS conditions was reported in stearic acid (C18:0) compared with the other analyzed LCHFAs.

From the LS figures shown in Figure 2, it can be seen that the increase in C18:0 content in cow's milk at the THI up to 72 and from 72 to 79 is only 0.44%. When the THI increased above 79 (conditions of moderate HS), the content of this fatty acid increased by almost 1%. The increase in stearic acid values reflected the metabolic changes occurring in the body of the cows, which affected the fatty acid content of the milk they produce. Bandaranayaka and Holmes (1976) found that a high ambient temperature of 30°C was associated with a statistically significant increase ($P < 0.05$) of C18:0 content in milk fat.

In support of the above thesis that the amount of C18:0 in milk of dairy cows is an objective indicator of moderate heat stress presence, a significant effect ($P < 0.05$) of the rectal temperature on the content of this fatty acid was also reported (Table 3).

A clear trend for an increase in content of C18:0 with the increasing rectal temperature was observed. As the THI values increased, an increase in the cows' rectal temperature was also observed (Srikandakumar and Johnson, 2004). According to (Ammer et al., 2016), the normal body temperature of cows varied between 38°C and 39.2°C. Our study found that the increase in C18:0 content in milk fat in cows within the physiological norm for the rectal temperature (up to 39.5°C) was minimal, i.e., 0.2%. However, an increase in the rectal temperature of cows above 39.5°C was associated with a significant increase in the percentage of C18:0 in milk fat, possibly due to increased lipolysis in the body due to reduced dry matter intake (Barber et al., 1997; West, 2003; Wheelock et al., 2010; Bouraoui et al., 2002; Rhoads et al., 2009).

These results confirm the studies of Liu et al. (2016), who found an increase in the content of this fatty acid by 4.1 mg/g of milk fat under HS within the THI range between 72 and 84. In our study, it was found that increasing the THI values above 79 did not maintain the tendency of increasing the C18:2 content, as reported for C18:0. There was even a slight decrease in its content by 0.02% in moderate heat stress (THI over 79), compared with cows under mild HS (THI 72 to 79). In a study by Nantapo et al. (2014) with advancing of lactation, the content of linoleic acid (C18:2) in the milk decreased. The increase we found presented in Figure 4 shows that the influence of HS was very strong, overcoming the physiologically expected trend for lowering. In the studies of Liu et al., (2016), the C18:2 content increased in absolute values, but the authors did not consider how this fatty acid content changed at different levels of HS. Based on the results of our study it follows that lipolysis occurring in the body of cows under moderate HS most considerably affected the content of stearic acid. According to Hammami et al. (2015), the most substantial change in the fatty acid composition of milk under conditions of HS concerns mainly C18:1 cis-9. The differences between our results and those of other authors may also be due to the differences in the feeding strategy of the cows and their daily milk yield. In the study of Hammami et al. (2015), the average daily milk yield of cows reached 23.43 kg per day, while in our study it was significantly higher, i.e., – 39.92 kg per day. Cows with higher milk yield reacted with occurrence of symptoms of HS (increased respiratory rate and higher rectal temperature) at lower values of the THI due to their higher sensitivity to HS (Bernabucci et al., 2014). As cow productivity increases, the metabolic heat they produce increases (Purwanto et al., 1990), which increases their sensitivity to HS (Kadzere et al., 2002). The increase was 0.39% at the THI from 72 to 79, and 0.37% at the THI above 79, relative to thermo-neutral conditions (THI below 72).

According to Liu et al. (2016), HS induced an increase in the content of LCHFAs in milk. Our study on the content of C18:3 in milk confirmed the results obtained in studies of Liu et al., (2016), finding that the content of this fatty acid increased proportionally to the increase of heat stress. According to Nantapo et al. (2014), similarly to linoleic (C18:2), the content of linolenic acid (C18:3) should normally also decrease as lactation progresses. In our study in August, cows were in more advanced stages of lactation (third or fourth lactation month) compared with May (first or second) and a decrease should be reported in the content of these fatty acids. Higher values reported in August indicate that HS had a significant effect on the content of long-chain unsaturated fatty acids, which outweighs the physiological effect of the lactation stage.

Linolenic acid (C18:3) has a proven beneficial effect in the prevention of heart disease and improved immune response in humans (Muchenje et al., 2009; Palladino et al., 2010). The study found that heat stress caused an increase in the amount of long-chain fatty acids in cow's milk, especially stearic, oleic and linolenic acid. Increasing or maintaining the levels of unsaturated fatty acids in milk specific for early lactation, under the influence of HS, would be healthier for people who consume it. However, heat stress has a detrimental effect on the welfare and health of cows in many aspects, which, despite the above-mentioned positive effect on human health, requires that measures be taken to eliminate or mitigate it.

Conclusion

The heat stress in dairy cows results in changes in the content of some long-chain fatty acids (C17:0; C18:0; C18:2 and C18:3) in milk fat. Under conditions of moderate heat stress (THI over 79), a slight decrease in the content of C17:0 was reported, while in the other three LCHFAs, an increase of their content in the milk fat in varying degrees was found. The strongest influence of heat stress was reported on the C18:0 content, which was proportional to the levels of HS. As the rectal temperature increases, an increase in the C18:0 content is observed, with the increase being most substantial at a rectal temperature above 39.5°C.

References

1. Ammer S., Lambertz C., Gauly M. (2016). Comparison of different measuring methods for body temperature in lactating cows under different climatic conditions. J. Dairy. Res., 83: 165–172.
2. Armstrong D. V., 1994. SYMPOSIUM: Nutrition and heat stress. Heat Stress Interaction with Shade and Cooling. J Dairy Sci 77:2044-2050.
3. Atkins, I. K., N. B. Cook, M. R. Mondaca, C. Y. Choi, 2018. Continuous respiration rate measurement of heat-stressed dairy cows and relation to environment, body temperature, and lying time. Transactions of the ASABE Vol. 61(5): 1475-1485. https://doi.org/10.13031/trans.12451
4. Bandaranayaka D. D. and C. W. Holmes, 1976. Changes in the composition of milk and rumen contents in cows exposed to a high ambient temperature with controlled feeding. Trop. Anim. Filth Prod. (1976) 8, 38-46.
5. Barber MC, Clegg RA, Travers MT, Vernon RG, 1997. Lipid metabolism in the lactating mammary gland. Biochim Biophys Acta. 1997 Aug 16;1347(2-3):101-26
6. Bastin, C., D. P. Berry, H. Soyeurt, and N. Gengler. 2012. Genetic correlations of days open with production traits and contents in milk of major fatty acids predicted by mid-infrared spectrometry. J. Dairy Sci. 95:6113–6121.
7. Bernabucci U., Biffani S., Buggiotti L., Vitali A., Lacetera N., Nardone A. (2014). The effects of heat stress in Italian Holstein dairy cattle. J. Dairy. Sci., 97: 471–486.
8. Bernabucci, U., Bani, P., Ronchi, B., Lacetera, N. & Nardone, A. Influence of short- and long-term exposure to a hot environment on rumen passage rate and diet digestibility by Friesian heifers. J Dairy Sci 82, 967–73 (1999).
9. Bouraoui, R., Lahmar, M., Majdoub, A., Djemali, Mn. & Belyea, R. The relationship of temperature-humidity index with milk production of dairy cows in a Mediterranean climate. Anim. Res. 51, 479–491 (2002).
10. Chilliard, Y., Ferlay, A., Mansbridge, R. M. & Doreau, M. Ruminant milk fat plasticity: nutritional control of saturated, polyunsaturated, trans and conjugated fatty acids. Ann. Zootech. 49, 181–205 (2000).

11. German J.B. and Dillard C. J. (2006). Composition, structure and absorption of milk lipids: a source of energy, fat-soluble nutrients and bioactive molecules. Crit Rev Food Sci Nutr.,46:57–92.
12. Giustini, L., Acciaioli, A., Pianaccioli, L., Surace, R. and O. Franci (2007) Thermic stress effect on the main qualitative parameters of milk produced in the Mugello. Scienza e Tecnica Lattiero-Casearia 58(6): 383-402.
13. Dimov D., I. Marinov, T. Penev, Ch. Miteva, Zh. Gergovska. 2017. Influence of temperature-humidity index on comfort indices in dairy cows. SYLWAN., 161(6), 68 - 85.
14. Hammami H, Vandenplas J, Vanrobays ML, Rekik B, Bastin C, Gengler N, 2015. Genetic analysis of heat stress effects on yield traits, udder health, and fatty acids of Walloon Holstein cows. J Dairy Sci. 2015 Jul; 98(7):4956-4968.
15. Hill, D. L. & Wall, E. Dairy cattle in a temperate climate: the effects of weather on milk yield and composition depend on management. Animal 9, 138–49 (2015).
16. Jensen R. G., 2002. Invited Review: The Composition of Bovine Milk Lipids: January 1995 to December 2000. J. Dairy Sci. 85:295–350.
17. Kadzere C.T., Murphy M.R., Silanikove N., Maltz E. (2002). Heat stress in lactating dairy cows: a review. Livest. Prod. Sci., 77: 59–91.
18. Kamiya, M., Iwama, Y., Tanaka, M. and S. Shioya (2005) Effects of high ambient temperature and restricted feed intake on nitrogen utilization for milk production in lactating Holstein cows. Animal Science Journal 76(3): 217-223.
19. Kaylegian, K.E., R.C. Lindsay, 1995: In Handbook of milk fat Fractionation Technology and Applications AOCS Press, Champaign, III., 19-37
20. Knapp, D. M. & Grummer, R. R. Response of lactating dairy cows to fat supplementation during heat stress. J Dairy Sci 74, 2573–9 (1991).
21. Lacetera, N., Bernabucci, U., Ronchi, B. and A. Nardone (1996) Body condition score, metabolic status and milk production of early lactating dairy cows exposed to warm environment. Rivista di Agricoltura Tropicale e Subtropicale 90(1): 43-55.
22. Lacetera, N., Bernabucci, U., Ronchi, B. and A. Nardone (2003) Physiological and productive consequences of heat stress. The case of dairy ruminants. In: Lacetera, Bernabucci, Khalifa, Ronchi, Nardone (eds.) Interactions between climate and animal production. European Association Animal Production Technical Series No. 7, pp. 45-59.
23. Liu Z., V. Ezernieks, J. Wang, N. Wanni Arachchillage, J. B. Garner, W. J. Wales, B. G. Cocks & S. Rochfort, 2016. Heat Stress in Dairy Cattle Alters Lipid Composition of Milk. Scientific Reports | 7: 961 | DOI:10.1038/s41598-017-01120-9.
24. Mills, S., R.P.Ross, C.Hill, G.F.Fitzgerald, C.Stanton, 2011. Milk intelligence: Mining milk for bioactive substances associated with human health. International Dairy Journal (6): 377-401.
25. Muchenje, V., Dzama, K., Chimonyo, M., Strydom, P. E., Hugo, A., & Raats, J. G. (2009). Some biochemical aspects pertaining to beef eating quality and consumer health: A review. Food Chemistry, 112, 279–289.
26. Nantapo, C.T.W., V. Muchenje, A. Hugo, 2014. Atherogenicity index and health-related fatty acids in different stages of lactation from Friesian, Jersey and Friesian x Jersey cross cow milk under a pasture-based dairy system. Food Chemistry, 146: 127-133.
27. Nardone, A., Ronchi, B., Lacetera, N. and U. Bernabucci (2006) Climatic effects on productive traits in livestock. Veterinary Research Communications 30(Suppl.1): 75-81.
28. Parodi, P. W. Milk fat in human nutrition. Australian J. of Dairy Technol 59 (2004).
29. Purwanto B.P., Abo Y., Sakamoto R., Furumoto F., Yamamoto S. (1990). Diurnal patterns of heat production and heart rate under thermoneutral conditions in Holstein Friesian cows differing in milk production. J. Agric. Sci., 114: 139–142.
30. Renna Manuela, Carola Lussiana, Vanda Malfatto, Antonio Mimosi and Luca Maria Battaglini, 2010. Effect of exposure to heat stress conditions on milk yield and quality of dairy cows grazing on Alpine pasture. 9th European IFSA Symposium, 4-7 July 2010: 1338-1348.
31. Rhoads, M. L. et al. Effects of heat stress and plane of nutrition on lactating Holstein cows: I. Production, metabolism, and aspects of circulating somatotropin. J Dairy Sci 92, 1986–97 (2009).
32. Roman-Ponce, H., Thatcher, W. W., Buffington, D. E., Wilcox, C. J. & Van Horn, H. H. Physiological and Production Responses of Dairy Cattle to a Shade Structure in a Subtropical Environment. J Dairy Sci 60, 424–430 (1977).
33. Sevi, A., Annichiarico, G., Albenzio, M., Taibi, L., Muscio, A. and S. Dell'Aquila (2001) Effects of solar radiation and feeding time on behavior, immune response and production of lactating ewes under high ambient temperature. Journal of Dairy Science 84(3): 629-640.
34. Silanikove N. 2000. Effects of heat stress on the welfare of extensively managed domestic ruminants. Livestock Production Science, 67 , 1–18
35. Simopoulos, A. P., 2002. The importance of ratio of omega-6/omega-3 essential fatty acids. Biomedicine Pharmacotherapy, 56: 365-379.
36. Smith D. L., T. Smith, B. J. Rude, and S. H. Ward, 2013. Comparison of the effects of heat stress on milk and component yields and somatic cell score in Holstein and Jersey cows - Short communication. J. Dairy Sci. 96: 3028–3033
37. Srikandakumar, A., Johnson EH, 2004. Effect of heat stress on milk production, rectal temperature, respiratory rate and blood chemistry in Holstein, Jersey and Australian Milking Zebu cows. Trop Anim Health Prod. 2004 Oct;36(7):685-92.
38. West, J. W. Effects of heat-stress on production in dairy cattle. J Dairy Sci 86, 2131–44 (2003)
39. West, J. W. Effects of heat-stress on production in dairy cattle. J Dairy Sci 86, 2131–44 (2003).
40. Wheelock, J. B., Rhoads, R. P., Vanbaale, M. J., Sanders, S. R. & Baumgard, L. H. Effects of heat stress on energetic metabolism in lactating Holstein cows. J Dairy Sci 93, 644–55 (2010).
41. Zimbelman, R. B., Collier, R. J., Rhoads, R. P., Rhoads, M. L., Duff, G. C. & Baumgard, L. H. (2009). A re-evaluation of the impact of temperature humidity index (THI) and black globe humidity index (BGHI) on milk production in high-producing dairy cows. Proc. 24th Southwest Nutrition and Management Conf.

Received 16 June 2020
Accepted 17 July 2020