Influence of Heat Stress and Physiological Indicators Related to It on Health Lipid Indices in Milk of Holstein-Friesian Cows

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Abstract: The aim of the survey was to study the effect of heat stress (HS) on health lipid indices in milk of Holstein-Friesian cows. The study was conducted in a cattle farm with Holstein-Friesian cows in the region of Karnobat (Southeastern Bulgaria) in 2018. Cows were housed in semi-open free stall dairy barn, fed year-round ad libitum with a total mixed ration. The study included 22 cows on different parities studied in two periods - at thermo-neutral environment conditions and at heat stress, respectively, May and August. Extraction of milk fat was performed by the Rose-Gottlieb method. Conditions of HS lead to changes in the values of health lipid indices associated with a decrease in the values of Atherogenic index (AI), Thrombogenic index (TI), Lipid Preventive Score (LPS) and Desaturase (18) index (DI 18) and an increase in Health promoting Index (HPI), polyunsaturated fatty acids/saturated fatty acids (PUFA/SFA), unsaturated fatty acids/saturated fatty acids (UFA/SFA), mono unsaturated fatty acids (MUFA), Desaturase (16) index (DI 16) and hypcholesterolemic/hypercholesterolemic ratio (h/H). Increasing the Temperature-humidity index (THI) above 72, results in a decrease in the AI values and an increase in those of the PUFA/SFA. The values of health lipid indices showed a moderate positive correlation with those of THI (PUFA/SFA - 0.36) with rectal temperature (h/H, MUFA/SFA, UFA/SFA) r_p from 0.36 to 0.37, and with respiratory rate (h/H, PUFA/SFA), r_p of 0.33 and 0.31, respectively. Under the influence of heat stress, changes in the metabolic processes occur in the body of dairy cows leading to changes in the fatty acid content of milk related to the improvement of health lipid indices in terms of human health due to an increase in UFA and reduction in SFA.

Key words: health lipid indices, THI, respiratory rate

1 Introduction

In previous decades, cow’s milk was simply accepted as a source of food, despite its primary importance for the development of newborn babies. Despite many studies in recent decades, some of its biological functions are still unknown. Particular attention has been paid in recent times to the quantity and composition of milk fats. The fatty acid composition of cow’s milk includes approximately 400 fatty acids. In the last years, the effects of various fatty acids on human health have been studied and only a few have been found to have a negative impact on human health. Studies have shown that some saturated fatty acids (SFA) as lauric (C12:0), myristic (C14:0) and palmitic (C16:0), together with some trans fatty acids, are responsible for an increase in plasma cholesterol in the blood.

Only C16:0 (palmitic acid) has a clear relationship with increased risk of coronary heart disease. It is important from a health point of view to determine saturated fatty acids and some unsaturated fatty acids in the Atherogenic index recommended by Ulbricht and Southgate (1991). Unsaturated fatty acids are sometimes called “health acids”, especially when referring to their positive effect on blood cholesterol levels. Cow’s milk contains low concentrations of healthy unsaturated fatty acids. There are many studies focusing on the effect of ration composition, genotype, breed, physiology, and lactation stage on increasing health-promoting and disease-preventing groups of fatty acids in cow’s milk. However, few studies have investigated the effect of heat stress (HS) on the fatty acid profile of milk, and the results between the authors are...
controversial\textsuperscript{13}.

Cows under HS decrease feed intake reduced milk production and a change in milk composition during summer\textsuperscript{13}. In addition, high ambient temperature has a direct effect on reducing the secretory ability of udder cells\textsuperscript{13}. Heat stress negatively affects milk synthesis and impairs reproductive performance\textsuperscript{14}. The bioenergetic mechanism by which heat stress affects production and reproduction is partly explained by reduced feed intake, but also includes altered endocrine status, reduction in rumination and nutrient absorption, and increased maintenance requirements\textsuperscript{15}.

The aim of the study was to determine the impact of heat stress and related to it physiological indicators of animals on health lipid indices in milk fat of Holstein-Friesian cows under temperate continental climate.

\section{Materials and Methods}

\subsection{Housing system and ration composition}

The study was conducted in cattle farm with Holstein-Friesian cows near Karnobat, Southeastern Bulgaria in 2018. Cows were housed in semi-open free stall dairy barn. They were fed year-round ad libitum with a total mixed ration at the following composition for 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%; 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%; palm oil - 5%.

\subsection{Sample collection}

In the study were included all cows calved between 1.04 and 10.05.2018, thus excluding the effect of the lactation stage on the fatty acid content of milk. The included cows were at first (9), second (6), third and more parities (7) or a total of 22 cows.

Individual milk samples were taken of each of the cows included in the study to test for fatty acid content in May and August 2018, respectively. The daily milk yield, as well as the percentage content milk fat and protein, were taken from their official monthly milk performance records (May and August).

\subsection{Lipid extraction}

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 methyolate (\text{CH}_3\text{ONa}) 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.

\subsection{Measuring of a temperature-humidity index}

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 15 o'clock, with examination of the physiological parameters of each cow - rectal temperature and respiratory rate per minute. Rectal temperature was measured by a digital thermometer in degrees Celsius. 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.\textsuperscript{16}.

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\textsuperscript{17}, 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 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.

\subsection{Data analysis}

On the basis of the derived fatty acid profile of the milk following lipid indices were calculated:

\textbf{Atherogenic Index (AI)} = \left[ C_{12:0} + (4x\text{C}_{14:0} + \text{C}_{16:0})/\text{Σ\text{MUFA + PUFA}} \right], proposed by Ulbricht and Southgate\textsuperscript{18}.

\textbf{Thrombogenic index (TI)} = \left( \text{C}_{14:0} + \text{C}_{16:0} + \text{C}_{18:0} \right)/\left( (0.5\text{C}_{18:1}) + (0.5\text{Σ\text{MUFA}}) + (0.5\text{Σ\text{PUFA}}) \right), proposed by Ulbricht and Southgate\textsuperscript{18}.

\textbf{Health promoting Index (HPI)} = \left( \text{Σ\text{MUFA + Σ\text{PUFA}}} \right)/\left( \text{C}_{12:0} + 4\text{x}\text{C}_{14:0} + \text{C}_{16:0} \right), proposed by Chen et al.\textsuperscript{19} (2004).

\textbf{Lipid Preventive Score (LPS)} = TL + 2x\text{SFA-MUFA-0.5x\text{PUFA}}, Richard and Charbonniers\textsuperscript{20}.

\textbf{PUFA/SFA} = \left( \text{C}_{14:2} + \text{C}_{16:2} + \text{C}_{18:2} + \text{C}_{18:3} \right)/\left( \text{C}_{12:0} + \text{C}_{13:0} + \text{C}_{14:0} + \text{C}_{15:0} + \text{C}_{16:0} + \text{C}_{17} + \text{C}_{17:0} + \text{C}_{18:0} \right);

\textbf{UFA/SFA} = \left( \text{C}_{14:1} + \text{C}_{14:2} + \text{C}_{16:1} + \text{C}_{16:2} + \text{C}_{18:1} + \text{C}_{18:2} + \text{C}_{18:3} \right)/\left( \text{C}_{12:0} + \text{C}_{13:0} + \text{C}_{14:0} + \text{C}_{15:0} + \text{C}_{16:0} + \text{C}_{17} + \text{C}_{17:0} + \text{C}_{18:0} \right);

\textbf{MUFASFA} = \left( \text{C}_{14:1} + \text{C}_{16:1} + \text{C}_{18:1} \right)/\left( \text{C}_{12:0} + \text{C}_{13:0} + \text{C}_{14:0} + \text{C}_{15:0} + \text{C}_{16:0} + \text{C}_{17} + \text{C}_{17:0} + \text{C}_{18:0} \right);

\textbf{Desaturase (18)} index = 100x\left( \text{C}_{18:1}/(\text{C}_{18:1} + \text{C}_{18:0}) \right);
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Desaturase index \((16) = 100 \times (C16:1/(C16:1 + C16:0))\);

\(h/H = (C18:1 + PUFA)/(C14:0 + C16:0)\).

For a 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 (Copyright 1990-1995 Microsoft Corp.).

The following model was used to evaluate the influence of controlled factors on the values of health lipid indices of milk:

\[ Y_{ijkl} = \mu + \text{THI}_i + \text{RT}_j + B_k + e_{ijkl} \]

Where:

- \(Y_{ijkl}\) is the dependent variable (each of the health lipid indices studied),
- \(\mu\) is the mean effect,
- \(\text{THI}_i\) is the effect of THI (classes),
- \(\text{RT}_j\) is the effect of rectal temperature,
- \(B_k\) is the effect of respiratory rate 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).

3 Results

Table 1 presents the reported mean THI values for the two calendar months, with a significant difference of 73.4 and 79.1, respectively. For May, the reported values meet conditions from optimal - THI 71.2 to mid-moderate HS - THI 76.2. For August the reported THI values were significantly higher, meeting from conditions of mid-moderate HS - THI 77.1 to conditions of moderate HS - THI 81.5.

Table 1 also presents the average Test day (TD) records for milk yield, fat and protein percentage, and the average values of the reported physiological indicators 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%. There was a slight difference in the average TD performance values for the two months. The average milk yield and fat percentage had lower averages for August compared with those for May but without significant difference. There was a slight increase in the TD protein percentage, and although the difference was statistically significant, it was very small - by only 0.1%. 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 at 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.

Table 2 presents the average values of the studied fatty acids in the milk of Holstein-Frisian cows under conditions of thermal comfort (May) and heat stress (August). It should be noted that differences were observed in the values of the surveyed fatty acids for the two months, which were from insignificant to such of high statistical significance \((p<0.001)\), such as at C18:0 (stearic acid). Statistically differed also the percentage content of Myristoleic C14:1 and Linolenic acids C18:3 \((p<0.01)\) and Capric C10:0, Myristic C14:0 and Margaric acids C17:0 \((p<0.05)\) although of lower significance.

Our results showed an increase in the content of the three unsaturated fatty acids (C18:1, C18:2, C18:3), as in linolenic acid (C18:3) the increase under conditions of HS was statistically significant \((p<0.01)\).

Table 3 presents the average values of the studied lipid indices under conditions of temperature comfort (May) and heat stress (August). Of the lipid indices thus presented, only the desaturase index 18 showed a statistically significant change (decrease) under HS conditions against temperature comfort conditions. Although not exceeding the

| 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 |
| THI                             | 73.4 ± 0.29*** | 71.2, 76.2 | 79.1 ± 0.49*** | 77.1, 81.5 |

* - significance at \(p<0.05\); ** - significance at \(p<0.01\); *** - significance at \(p<0.001\); n.s.- has no significant effect
Table 2  Average values for content(%) of the surveyed fatty acids by months of reporting and significance of differences.

| Fatty acid               | May n=22 x ± SE | August n=19 x ± SE | P value |
|--------------------------|------------------|---------------------|---------|
| Butyric acid C4:0        | 1.76 ± 0.08      | 1.75 ± 0.04         | 0.89    |
| Caproic acid C6:0        | 1.13 ± 0.05      | 1.24 ± 0.04         | 0.15    |
| Caprylic acid C8:0       | 0.83 ± 0.03      | 0.76 ± 0.02         | 0.12    |
| Capric acid C10:0        | 2.65 ± 0.11      | 2.33 ± 0.07         | 0.02*   |
| Undecyl acid C11:0       | 0.19 ± 0.01      | 0.16 ± 0.01         | 0.09    |
| Lauric acid C12:0        | 3.18 ± 0.10      | 2.89 ± 0.09         | 0.06    |
| Tridecanoic acid C13:0   | 0.14 ± 0.01      | 0.14 ± 0.01         | 0.50    |
| Myristic acid C14:0      | 10.85 ± 0.21     | 10.24 ± 0.15        | 0.02*   |
| Myristoleic acid C14:1   | 0.90 ± 0.04      | 0.73 ± 0.02         | 0.004** |
| Tetradecadienoic acid C14:2 | 0.31 ± 0.01     | 0.33 ± 0.01         | 0.28    |
| Pentadecanoic acid C15:0 | 1.22 ± 0.05      | 1.24 ± 0.03         | 0.75    |
| Palmitic acid C16:0      | 33.83 ± 0.44     | 33.21 ± 0.37        | 0.29    |
| Palmitoleic acid C16:1   | 1.93 ± 0.09      | 2.00 ± 0.10         | 0.59    |
| Hexadecadienoic acid C16:2 | 0.38 ± 0.01      | 0.41 ± 0.01         | 0.18    |
| Iso margaric acid C17 iso| 0.55 ± 0.02      | 0.58 ± 0.01         | 0.28    |
| Margaric acid C17:0      | 3.46 ± 0.08      | 3.20 ± 0.06         | 0.03*   |
| Stearic acid C18:0       | 8.48 ± 0.14      | 9.38 ± 0.14         | 0.00008*** |
| Oleic acid C18:1         | 25.19 ± 0.55     | 26.22 ± 0.34        | 0.13    |
| Linoleic acid C18:2      | 2.6 ± 0.06       | 2.6 ± 0.05          | 0.95    |
| Linolenic acid C18:3     | 0.41 ± 0.03      | 0.53 ± 0.01         | 0.004** |

* - significance at p <0.05; ** - significance at p <0.01; *** - significance at p <0.001; n.s.- has no significant effect

Table 3  Average values of the studied Health Lipid Indices under conditions of temperature comfort and heat stress.

| Lipid Health Indices | Temperature comfort May, n=22 x ± SE | Heat stress August, n=19 x ± SE | P value |
|----------------------|--------------------------------------|---------------------------------|---------|
| AI                   | 2.57 ± 0.08                          | 2.36 ± 0.05                     | 0.05    |
| TI                   | 1.35 ± 0.29                          | 1.30 ± 0.29                     | 0.24    |
| LPS                  | 110.80 ± 1.98                        | 107.30 ± 1.24                   | 0.15    |
| HPI                  | 0.39 ± 0.013                         | 0.42 ± 0.008                    | 0.08    |
| PUFA/SFA             | 0.060 ± 0.001                        | 0.064 ± 0.001                   | 0.08    |
| UFA/SFA              | 0.51 ± 0.01                          | 0.54 ± 0.01                     | 0.22    |
| MUFA/SFA             | 0.45 ± 0.01                          | 0.47 ± 0.01                     | 0.26    |
| DI 18                | 74.71 ± 0.36                         | 73.63 ± 0.35                    | 0.04*   |
| DI 16                | 5.43 ± 0.30                          | 5.72 ± 0.30                     | 0.52    |
| h/H                  | 0.65 ± 0.02                          | 0.69 ± 0.01                     | 0.10    |

* - significance at p <0.05; ** - significance at p <0.01; *** - significance at p <0.001; n.s.- has no significant effect
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Table 4 Analysis of variance for the influence of THI, rectal temperature and respiratory rate on health lipid indices in milk of Holstein-Friesian cows.

| Source of variation | Degrees of freedom (n–1) | AI | TI | LPS | HPI | PUFA/SFA | UFA/SFA | MUFA/SFA | DI 18 | DI 16 | h/H |
|---------------------|--------------------------|----|----|-----|-----|----------|---------|----------|-------|-------|------|
| Total for the model  | 8                        |    |    |     |     |          |         |          |       |       |      |
| THI                 | 2                        | 3.46* | 2.53 n.s. | 2.38 n.s. | 1.10 n.s. | 5.76** | 0.59 n.s. | 0.32 n.s. | 1.22 n.s. | 0.21 n.s. | 1.14 |
| Rectal temperature  | 3                        | 0.97 n.s. | 0.79 n.s. | 1.01 n.s. | 0.25 n.s. | 1.66 n.s. | 1.36 n.s. | 1.30 n.s. | 0.81 n.s. | 0.84 n.s. | 1.27 |
| Respiratory rate    | 3                        | 1.87 n.s. | 1.80 n.s. | 1.64 n.s. | 0.19 n.s. | 3.94* | 3.12* | 2.77 n.s. | 1.09 n.s. | 1.53 | 3.35* |
| Error               | 32                       |      |     |     |     |          |         |          |       |       |      |

* - significance at $p < 0.05$; ** - significance at $p < 0.01$; *** - significance at $p < 0.001$; n.s. - has no significant effect

Table 5 Correlations between the studied health lipid indices and the indicators of heat stress in dairy cows.

| THI | Rectal temperature | Respiratory rate |
|-----|--------------------|------------------|
| h/H | 0.27               | 0.37             |
| DI 16 | 0.08               | 0.22             |
| DI 18 | -0.22              | -0.06            |
| MUFA/SFA | 0.18               | 0.36             |
| UFA/SFA | 0.21               | 0.36             |
| PUFA/SFA | 0.36               | 0.20             |
| HPI | 0.27               | 0.07             |
| AI | -0.29              | -0.23            |
| TI | -0.19              | -0.15            |
| LPS | -0.22              | -0.17            |

Values indicated with bold are significant at $p < 0.05$.

Statistical significance limit, HS has an effect on AI, leading to its decrease from 2.57 to 2.36 (Table 3). Our results were close to the AI values found by Pilarczyk et al. Generally speaking, during the period of HS (August), the values of the lipid indices TI, LPS and DI 18 decreased, while those of HPI, PUFA/SFA, UFA/SFA, MUFA/SFA, DI 16 and h/H increased.

Table 4 presents an analysis of variance for the influence of THI and physiological indicators of cows as rectal temperature and respiratory rate on the values of health lipid indices in cow’s milk. Only four of the indices studied were influenced by the HS and rectal temperature and respiratory rate as follows: AI by THI, PUFA/SFA by THI and respiratory rate, UFA/SFA and h/H by respiratory rate.

Table 5 presents the correlations between the studied health lipid indices and the heat stress indicators in dairy cows. The analysis of these data showed that only the health lipid index PUFA/SFA had a moderate positive correlation with the THI values ($p < 0.05$). Most lipid indices showed a positive moderate correlation with rectal temperature values (h/H, MUFA/SFA, UFA/SFA), and secondly with respiratory rate (h/H, PUFA/SFA).

Figure 1 shows the LS-mean values of AI, depending on the values of THI. It was found that as the values of THI increase, the values of AI decrease. At lack of heat stress (THI up to 72) AI values of 2.83 were reported and at moderate heat stress conditions (THI above 79) the AI values of 2.26, respectively.

Figure 2 shows the LS-means of PUFA/SFA, depending on the THI values. It was clear that when THI values increased from (up to 72) to (from 72 to 79), the PUFA/SFA increased from 0.053 to 0.063 and when THI values increased above 79, a slight decrease in the PUFA/SFA to 0.062 was observed.

Figure 3 shows the LS-means of PUFA/SFA depending on the respiratory rate that had a significant effect in the analysis of variance. Data showed that as respiratory rate per min increase, the PUFA/SFA becomes more favorable to human health. At respiratory rate up to 40 per minute, this ratio was 0.058. At respiratory rate from 40 to 45 the PUFA/SFA decreased to 0.054. In the next respiratory rate class from 45 to 55, the PUFA/SFA was 0.062. At the highest class of respiratory rate above 55 per minute, the PUFA/SFA was 0.061.

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Fig. 1  LS-means of AI depending on THI.

Fig. 2  LS-means of PUFA/SFA depending on THI.

Fig. 3  LS-means of PUFA/SFA depending on the respiratory rate (RR).
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4 Discussion

From the data presented on Table 1 it can be seen that the reported mean THI values for May meet conditions from optimal - THI 71.2 to mid-moderate HS - THI 76.2, and for August they were significantly higher, meeting from conditions of mid-moderate HS - THI 77.1 to conditions of moderate HS - THI 81.5. Other authors have also found risk values of THI for HS in dairy cows under the conditions of the temperate continental climate in Bulgaria. Dimov et al.\textsuperscript{21} reported risk values of THI for the regions of three dairy cattle farms in southern Bulgaria. The authors indicate that during the summer season, values determining the conditions for mild to mid-moderate HS in dairy cows were reported - average daily values of THI above 75. There was a certain risk of such conditions also in spring and autumn.

Table 1 also presents the average Test day (TD) records for milk yield, fat and protein percentage, and the average values of the reported physiological indicators. There was a slight difference in the average TD performance values for the two months. The average milk yield and fat percentage had lower averages for August compared with those for May but without significant difference. Bouraoui et al.\textsuperscript{22} also found a decrease in milk yield and a fat percentage under the influence of HS, while Smith et al.\textsuperscript{23} found an in-

\begin{figure}[h]
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\caption{LS-means of UFA/SFA depending on the respiratory rate (RR).}
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\begin{figure}[h]
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\includegraphics[width=\textwidth]{figures.png}
\caption{LS-means of h/H depending on respiratory rate (RR).}
\end{figure}
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. 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 at 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.

Table 2 presents the average values of the studied fatty acids in the milk of Holstein-Friesian cows under conditions of thermal comfort (May) and heat stress (August). In view of the set goal, particular attention should be given to lauric, myristic and palmitic acids, (C12:0; C14:0, C16:0) respectively, considered to be main risk factors for cardiovascular diseases in humans. The data for the content of these three fatty acids showed a reduction under the conditions of moderate heat stress, with only in C14:0 the lowering, being significant ($p<0.05$), in contrast to the lauric and palmitic acids. These results confirmed the study of Smith et al. that the content of these three fatty acids decreases in summer when the cows were under heat stress. However, it should be noted that in the study of Smith et al. cows were reared on pasture, and according to the authors the season, temperature and humidity affected both the chemical composition of the grass, and the content of fatty acids in the milk. Nevertheless, the researches should not focus only on the negative, i.e. the saturated fatty acids. Milk also contains low concentrations and of good for health unsaturated fatty acids, such as oleic (C18:1), linoleic (C18:2) and linolenic (C18:3). As part of the benefits to human health are for example: (C18:2) has a positive effect on human health associated with the reduction of total blood cholesterol, anti-carcinogenic, anti-diabetic and immune-modulatory effects. Linolenic acid (C18:3) has a proven beneficial effect in the prevention of heart disease and improved immune response in humans. Our results for the content of these three fatty acids differ from those obtained by Smith et al., who found a significant decrease in the same fatty acid content in summer under conditions of HS, which was probably due to the composition of grass during this period. In our view, at controlled year-round feeding with a total mixed ration, the heat stress was the factor that had influenced the increase of the content of these three fatty acids. In the study of Liu et al., the authors found a significant increase in C18:0; C18:1; C18:2 under conditions of HS in a premise with controlled climatic conditions (at THI 84 and above). In our study, a significant increase was observed only at C18:3 content under conditions of moderate HS.

From the presented on Table 3 average values of the studied lipid indices only the desaturase index 18 showed a statistically significant change (decrease) under HS conditions against temperature comfort conditions, as proven in the study of Liu et al. This was due to the significant increase in stearic acid content (C18:0) under the HS conditions, which takes part in the calculation of this index. Although not exceeding the statistical significance threshold, HS affected AI, leading to its decrease from 2.57 to 2.36 (Table 3). Our results were close to the AI values found by Pilarczyk et al.

Of the three factors influenced the health lipid indices studied by Analysis of variance shown in Table 4, only THI reflected the environment condition. The other two factors, rectal temperature and respiratory rate, are physiological indicators of cows and as such are strictly individual. These physiological indicators depend on many factors such as age, breed, stage of lactation, and especially cow productivity. High-performance cows have been found to be more sensitive to heat stress than less productive cows. The data on the correlations between the studied health lipid indices and the heat stress indicators showed that the condition, respectively, the metabolism of dairy cows and the fatty acid composition of milk under heat stress conditions depend not so much on the directly measured indicators of the environment, like THI, but rather on how the cows respond to these changes of environment. The authors found that after a decrease in THI values, the cows had a high body temperature and increased respiratory rate/min for several hours. All this explains why the lipid indices we studied were more influenced by physiological indicators such as the rectal temperature and respiratory rate than by THI values.

The values we have calculated for AI on Fig. 1 were considerably lower than those reported by Faustini et al. for Holstein-Friesian cows. The authors found no significant variation in AI depending on the lactation stage in their study in Northern Italy. In the research conducted by us in moderate continental climate and under HS conditions occurring in the summer, we consider that fatty acid profile expressed by AI is improving regarding health effect on humans. This positive effect of HS on human health was primarily due to the percentage increase in PUFA and MUFA, rather than the negligible decrease in the lauric, palmitic and myristic acids included in the calculation of AI.

With the increase of THI values from (up to 72) to (from 72 to 79) the PUFA/SFA ratio increased from 0.053 to 0.063 (Fig. 2). With an increase in THI values above 79, a slight decrease in the PUFA/SFA to 0.062 was observed. This slight decrease was probably due to the significant increase in stearic acid (C18:0) under HS. However, it can be assumed that HS in cows leads to an improvement in the PUFA/SFA, which is beneficial for human health. High values SFAs have been found to lead to the risk of chronic
illnesses such as atherosclerosis, heart problems and obesity. Therefore, as a general, the nutritional recommendations for humans are associated with a reduction in SFAs in the diet\(^{32}\). The authors believe that there is a misconception that dairy products, especially the whole-milk ones, contribute to the development of coronary heart disease. Our study showed that to improve the beneficial to human health indicators of milk the conditions under which cows were reared and in particular their temperature comfort are also important.

The data presented on Fig. 3 show that as respiratory rate per min increase, the PUFA/SFA ratio becomes more favorable to human health. According to Atkins et al.\(^{30}\), the respiratory rate is more precise criteria for the occurrence of HS in dairy cows than the various indices for its evaluation. The authors indicate that the number of respiratory rate/min is an indicator that can vary widely depending on the cow posture - upright or lying, productivity and other factors. According to the authors, it should be borne in mind that momentary reporting of this indicator could mislead the researchers, because cows may be stressed by the reporting itself and thus a change in this physiological indicator may occur.

Despite the decrease in the PUFA/SFA ratio at respiratory rate from 40 to 45, it can be accepted that this indicator can be important in determining health lipid status of cows’ milk. We should not forget the data in Table 4 in which there was a significant moderate correlation between PUFA/SFA and THI on the one hand and the respiratory rate on the other. Atkins et al.\(^{30}\) pointed that there was a relation between the duration of the period of reporting, the respiratory rate and THI. They indicated that the respiratory rate and rectal temperature remain high and even increase after THI values begin to decline.

From the data presented in Fig. 4 for the LS-means of UFA/SFA depending on the respiratory rate, the trend shown in Fig. 3 was observed - a slight decrease at RR from 40 to 45, followed by an increase in this ratio. According to Lacetera et al.\(^{11}\) and Hammami et al.\(^{35}\), under the influence of HS, there is a decrease in the content of short-chain fatty acids and an increase in the long-chain fatty acids content. In our study, generally an increase in long-chain fatty acids was observed, with the exception of C17:0 (margaric acid) that decrease under conditions of HS. Interesting is the fact that under HS, the content of linoleic acid (C18:2), which is part of the omega 6 fatty acids, does not change, and that of linolenic acid (C18:3), which is part of the omega 3 fatty acids, increases at HS. According to Simopoulos\(^{31}\), this trend in the omega 6/omega 3 fatty acids ratio makes it optimal for better cardiovascular health in humans.

From the data presented in Fig. 5, it is clear that with an increase in respiratory rate over 45/min, the cholesterolemic index h/H of milk in Holstein-Friesian cows increases. According to Ivanova and Hadzhinikolova\(^{34}\), the cholesterolemic index h/H is an indicator of the high functional and biological activity of food products, as its higher values are associated with a positive effect on human health. In other words, under conditions of HS and the resulting increased respiratory rate lead to an increase in the milk cholesterolemic index h/H and an increase in the positive effect on human health.

5 Conclusion

The heat stress in Holstein-Friesian cows causes a change in the fatty acid content of milk. This change leads to changes in the values of health lipid indices associated with a decrease in the values of AI, TI, LPS and DI 18 and an increase in the values of HPI, PUFA/SFA, UFA/SFA, MUFA/SFA, DI 16 and h/H. Most lipid indices showed a positive moderate correlation with rectal temperature values (h/H, MUFA/SFA, UFA/SFA) \(r_p\) from 0.36 to 0.37, and with respiratory rate (h/H, PUFA/SFA) \(r_p\) of 0.33 and 0.31, respectively. These data showed that the condition, respectively, the metabolism of dairy cows and the fatty acid composition of their milk under heat stress conditions depend not so much on the directly measured indicators of the environment, like THI, but rather on how cows respond to these changes of environment. From our survey it was found that under conditions of moderate heat stress, values of health lipid indices in milk improved in terms of their health effects on humans.

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