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MILK FATTY ACID COMPOSITION IN HOLSTEIN COWS UNDER CHRONIC HEAT STRESS CONDITIONS

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Abstract. This experiment was carried out to study the effect of temperature-humidity index (THI) and chronic heat stress on the intensity of fatty acid secretion by the mammary gland of Holstein cows. Twenty-five mature Holstein cows (130±25 days of lactation) with a daily milk yield of 30 kg were randomly assigned to one group. Morning milk was collected from these cows in spring, summer (high temperatures), and autumn, and the fatty acid profile of milk lipids was determined at comfortable temperatures of keeping and under conditions of chronic heat stress. Nineteen fatty acids were quantitatively determined in the milk of experimental cows by the gas chromatography method. Myristic (C14:0), palmitic (C16:0), stearic (C18:0), and oleic (C18:1n9c) fatty acids have the highest percentage content. These studies confirmed that long periods of heat can affect the quantitative composition of fatty acids in milk lipids. As a result of research, it was found that the THI can be used to predict the performance and milk fatty acid composition in lactating high-yielding cows of the Holstein breed. Under the values of THI 58 and 66, the intensity of lactation in cows and the fatty acid composition of the obtained milk did not differ significantly, although trends to changes were observed for individual fatty acids. An increase in the value of THI to 74 caused both a significant increase in the total content of unsaturated fatty acids (UFAs) in the milk of Holstein cows, as well as monounsaturated (MUFAs) and polyunsaturated (PUFAs), which was accompanied by a decrease in SFAs/UFAs ratio to the level of 1.87. This value was significantly lower than that of cows' milk collected in May and November. The increase in the relative content of milk fatty acids in Holstein cows at THI of 74 occurs mainly due to fatty acids with 18 carbon atoms in the chain. Thus, the relative content of C18:1n9c under conditions of chronic heat stress (THI 74) was 26.75±1.27, this value is 4.07% higher than in milk collected in May at THI of 66 and by 4.21% higher than in milk collected in November at THI of 58.

Keywords: milk, fatty acids, cows, temperature-humidity index, chronic heat stress.

Introduction. Formulation of the problem

Due to the strengthening of the greenhouse effect and global warming, the heat stress of productive animals has become one of the most important factors affecting the profit in dairy production. In recent years, scientists have conducted numerous studies on the metabolism of dairy cows under conditions of heat stress [1-3].

The studies revealed that when heat stress is accompanied by high ambient humidity, the effect of high temperature is more pronounced due to a decrease in heat transfer [4].

The change in climatic conditions of keeping cattle and other productive animals causes concern among many commodity producers. First of all, this is related to both a decrease in the general level of animal productivity and a change in the nutritional value of the obtained product. On the other hand, receiving a smaller amount of raw milk under the conditions of increasing heat load in lactating cows in the summer season is absolutely natural. The mammary gland cannot maintain a high level of synthetic processes of milk components due to a decrease in the ability to remove heat from this organ. Accordingly, less protein and other milk components are synthesized, and therefore, a lower total level of lactation. The processing of milk into dairy products imposes strict requirements on the characteristics of the obtained milk, and therefore there is a threat of reducing the nutritional value of the obtained dairy products [5].

Particularly interesting is how sensitive is the fatty acid composition of cows' milk to the level of heat load at the same performance parameters of the mammary gland. It may turn out that the conditional 30 liters per day from one cow in conditions of thermal comfort will differ in its parameters from the conditional 30 liters of milk from another cow under conditions of heat stress.

Analysis of recent research and publications

Changes in the milk lipid composition can be one of the markers of the intensity of heat stress in cows. Factors that cause changes in the amount of milk lipids and their fatty acid composition can be conditionally divided into two groups – internal and external. The first group of factors includes genetic factors, stage of lactation, rumen fermentation, state of health, primarily, infections of the mammary gland, and others. The second group of factors includes the composition and structure of the main diet (the amount and composition of feed fat, concentrates/roughage ratio, protein content, energy value), and the influence of the season and the temperature of keeping cows are also important [6-8].

As evidenced by studies of milk obtained from cows under conditions of increased heat load, the content of polar lipids such as phosphatidylethanolamine, phosphatidylserine, phosphatidylcholine, lysophosphatidylcholine, and glucosylceramide in the secretion of their mammary gland significantly decreased. The researchers also found that the level of short-chain fatty acids decreased under the experimental conditions [9].

The study of milk fat composition in cows kept at the temperature of 32.2 °C showed a decrease in the proportion of the following milk fatty acids: lauric (C12:0), myristoleic (C14:1), pentadecanoic (15:0), oleic (C18:1) and linoleic (C18 :2) with a simultaneous increase in content of palmitic (C16:0) and stearic (C18:0) [10].

Chronic heat stress affects both lipid metabolism in general and the intensity of their oxidation in particular. Heat stress progressively reduced dry matter intake and milk yield, with milk production reaching a nadir (33%) in the third week of heat load [11].

Researchers emphasize that both the sensitivity to temperature changes and the parameters of the obtained milk can be separate genotypic features of breeds and breed lines of productive animals [12].

Research conducted on Holstein-Friesian cows (south-eastern Bulgaria) showed that changes in metabolic processes occur in the body of dairy cows under the influence of heat stress, which leads to changes in milk fatty acids. At the same time, lipid indices improve from the point of view of human health due to an increase in unsaturated fatty acids (UFAs) and a decrease in saturated fatty acids (SFAs) [13].

In our previous studies, we found that the increase in the concentration of free fatty acids, including SFAs and polyunsaturated fatty acids (PUFAs) in the blood serum of cows under conditions of long-term heat stress was associated with the influx of fatty acids (FAs) into the blood from the adipose tissue as a result of negative energy balance. An increase in the ratio of PUFAs n-6/n-3 may indicate a violation of the function of biomembranes, which may have negative consequences for the body of dairy cows [14].

Therefore, we decided to investigate how the intensity of fatty acids entering the milk composition changes with the same level of secretion by the mammary gland of a lactating cow with changes in the intensity of the heat load on its body.

The purpose of the study was to determine the impact of chronic heat stress on the intensity of fatty acid secretion by the mammary gland of Holstein cows with a milk yield of 30 kg/day.

Research objectives:

1. To investigate the specifics of the performance in highly productive Holstein cows under changes in the temperature-humidity index (THI) in the conditions of the forest steppe of Ukraine.

2. To establish the peculiarities of fatty acid secretion by the mammary gland (milk performance of 30 kg/day) depending on the THI values.

Research materials and methods

Analysis of milk to determine the fatty acid composition was carried out in 2022 at the Ukrainian Laboratory of Quality and Product Safety of Agricultural Products of the National University of Life and Environmental Sciences of Ukraine, which is accredited in accordance with the requirements of the international standard DSTU ISO/IEC 17025:2019 (ISO/IEC 17025:2017) and confirmed by the Certificate of accreditation of the National Accreditation Agency of Ukraine No. 20724 dated November 1, 2022.

Animals. Milk was collected from breeding cows of the Holstein breed, aged 2–5 years, weighing 600–620 kg with a milk yield of 30 kg/day. Twenty-five lactating dairy cows on day 130±25 of lactation were randomly selected. Milk sampling from cows was preceded by a 26-day preparatory period, during which dairy cows were fed a standard diet. Fresh milk (excluding the first milk jets) was collected from cows during morning milking. Groups of cows were formed, from which milk was collected in spring, autumn, and summer under different natural and climatic conditions.

Weather conditions. Weather data from the nearest weather station were taken from the website of the Ukrainian Hydrometeorological Center, as we described earlier [15]. Data (temperature and relative humidity) were recorded every three hours a day. At the same time, the temperature-humidity index (THI), calculated according to Kibler [16], served as an indicator of heat stress in cows.

$$THI = 1.8 \times T - (1 - RH/100) \times (T-14.3) + 32,$$

where THI is the temperature-humidity index, T is the air temperature in °C, and RH is the relative humidity in %

The threshold THI value at which most dairy cows could experience heat stress was considered to be 72 units [17]. Weather data were taken into account during the day during the research, as well as during the week preceding the research.

Housing and feeding of cows. Milk was collected from cows kept at one of the commercial dairy farms in Ukraine (50°49′11″N, 31°49′22″E). The animals were kept untethered in a well-ventilated building all year round. The sand was used as litter in the stalls. The diet remained constant throughout all seasons and was balanced in terms of nutrients. It contained high-quality fodder: barley, oat, and corn grain, corn silage, alfalfa hay, cereal hay, and wheat straw. In addition, the animals were fed rapeseed, sunflower and soybean meal, dried pulp, and mineral and vitamin supplements. The diet ingredients were mixed with special mixers equipped with electronic scales. The frequency and rationing of the feed amount was carried out using a computer program. Free access to drinking water was provided by group drinkers.

Sample collection and preparation. The material for the research was the milk of dairy cows. The fatty acid composition of lipids was determined in the specified biological material. Samples for research were taken in spring (May) at temperatures of 15–18°C, in summer (June) at temperatures above 28 °C, and in autumn (November) at temperatures of 15–18°C.

From freshly collected milk, fat was extracted according to Folch's method [18]. The next stage of sample preparation was hydrolysis and methylation of fatty acids in lipids obtained from milk. For this purpose, 4 cm^3 of methyl solution of sodium hydroxide was added to 100 mg of the obtained fat, a reflux condenser was attached to a flask with a sample and boiled until the drops of fat disappeared, stirring the contents of the flask at intervals of 30–60 seconds. Five cm^3 of methyl solution of boron trifluoride was added to the flask, continuing boiling for 1 hour. Three cm^3 of hexane was added to the boiling mixture through the upper part of the reflux condenser and removed from the heating element. Twenty cm^3 of saturated sodium chloride solution was added to the still hot solution and stirred for 15 seconds. The upper (hexane) layer was selected for research and analyzed on the device according to DSTU ISO 5509-2002.

Chromatography equipment and conditions. The analysis of methyl esters of fatty acids was carried out on a gas chromatograph Trace GC Ultra (USA) with a flame ionization detector. The column temperature was 140–240ºC and the detector temperature – 260ºC. The sample was injected into the chromatograph using a TriPlus autosampler in a dose of 1 μl. The duration of the test was 65 minutes.

Identification of fatty acids was carried out using a standard sample of *Supelco 37 Component FAME Mix*. The quantitative assessment of the fatty acid spectrum of milk lipids was carried out by the method of internal normalization, determining their percentage content. The test was conducted in 3 replicates.

Statistical analysis. The STATISTICA 10 package of statistical programs (StatSoft, Inc., Tulsa, OK, USA) was used for statistical data processing. The distribution of almost all variational series was not subject to normality criteria, therefore, in the subsequent analysis, methods of non-parametric statistics were used. The significance of differences between groups was assessed by the nonparametric Mann-Whitney U test. Differences were considered statistically significant at $p<0.05$.

Results of the research and their discussion

The collecting of milk samples from cows in the summer was preceded by a 26-day hot period in June, during which the average value of the THI was 74 (the temperature on some days could reach 32 °C with THI max – 78.5). Such a long stay of experimental cows at THI 74 can be attributed to chronic heat stress. Regarding milk sampling in cows in May (at THI 66) and in November (at THI 58), the animals in these periods were in the thermal comfort zone (for cattle).

It should be noted that the milk performance of Holstein cows in this farm during chronic heat stress decreased by approximately 10–15%, compared to milk yields in the spring and autumn periods. Therefore, the composition of the mammary gland secretion was monitored exclusively according to the level of its milk performance (30 kg/day).

Using the method of highly sensitive gas chromatography, 19 fatty acids were detected and quantitatively identified in the milk of dairy cows. Among these the largest percentage fraction falls on the following four fatty acids: myristic (C14:0), palmitic (C16:0), stearic (C18:0), and oleic (C18:1n9c) (Figs. 1, 2). Including fatty acids with an odd number of carbon atoms, four were found in the milk samples (C11:0, C15:0, C17:0, C17:1), and their relative content varied within: max – 2.68% (spring), min – 2.65% (summer).

Under the secretion of the mammary gland of Holstein cows at the level of 30 kg of milk per day, the content of certain SFAs in the collected samples significantly depended on THI value (Fig. 1). Based on the results of the research, it was found that there were no significant differences between THI 58 (November) and THI 66 (May) in terms of the content of SFAs, although trends to changes were observed in individual fatty acids. In particular, the difference between THI 58 and THI 66 was for: C4:0 – 5%; C8:0 – 6%; C10:0 – 10%, C15:0 – 10%, C17:0 – 14%. A relatively stable position of fatty acids (C6:0, C11:0, C14:0, C16:0, C18:0, C22:0) was also revealed for the THI value within physiological limits.

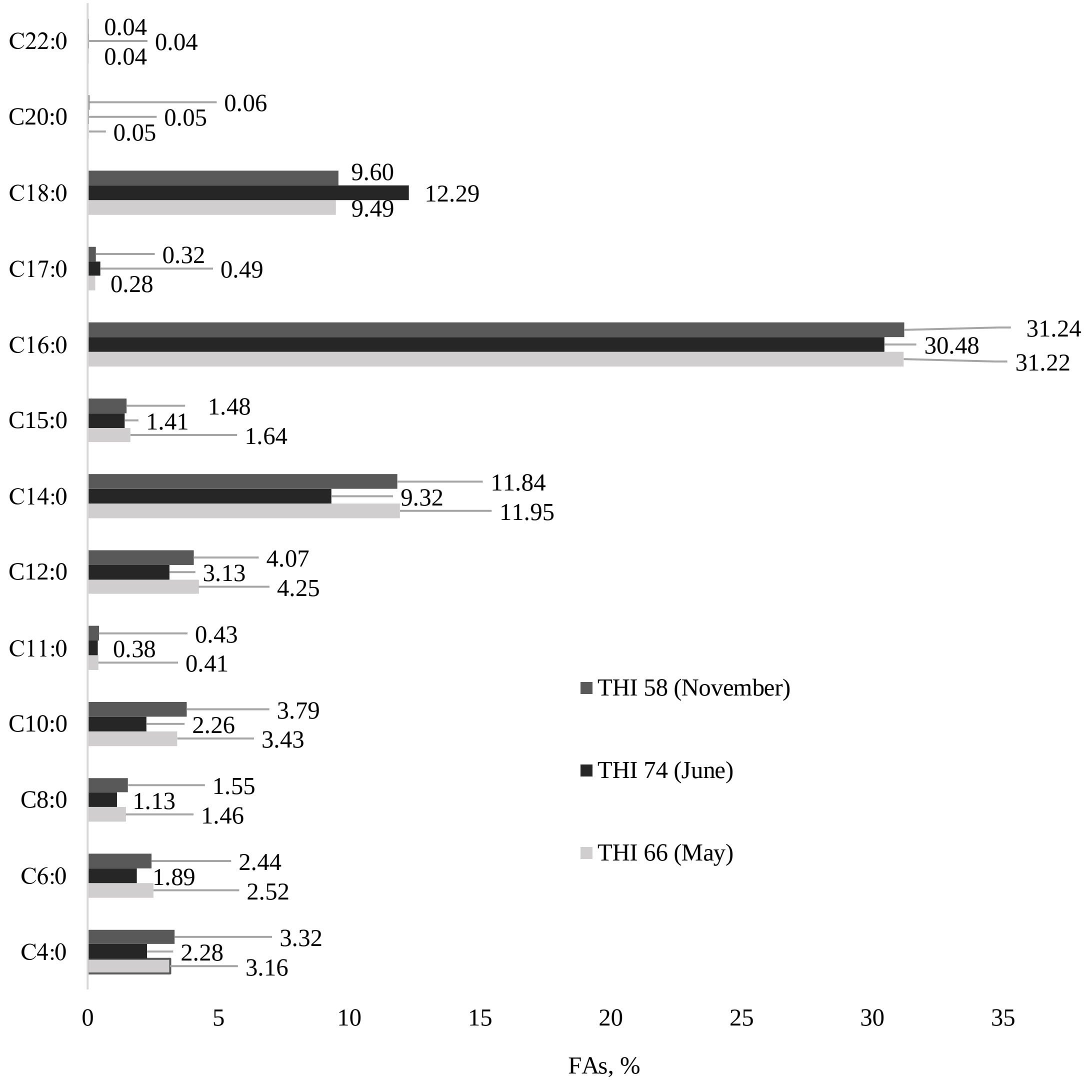

Fig. 1. Mass fraction of SFAs (%) in the secretion of the mammary gland in cows depending on THI value

Regarding THI value at the level of 74, it should be noted that the mammary gland of Holstein cows at the same level of milk performance (30 kg/day) and under conditions of chronic heat stress, secretes a much smaller amount of fatty acids with a chain length from C4:0 to C16:0, namely: 55.17±1.13% (THI 74), which is about 8.5% lower than during the autumn-spring lactation period with the same productive load on the mammary gland ($p<0.01$). Instead, the increase in the relative content of fatty acids in the milk of Holstein cows at THI of 74 occurs due to fatty acids with 18 carbon atoms in the chain, both saturated and unsaturated (Fig. 2).

So, as mentioned above, long-term heat stress in Holstein cows at THI of 74 results in an increase in the relative content of UFAs in the secretion of the mammary gland. Thus, the relative content of C18:1n9c (THI 74) was 26.75±1.27, which was 4.07% (THI 66) and 4.21% (THI 58) higher, respectively. The relative content of linoleic (C18:2n6c) and linolenic (C18:3n3) acids in cows' milk under conditions of long-term heat stress also increased by ≈1% and ≈0.1%, respectively, compared to comfortable conditions of keeping cows.

The mass fraction of SFAs and UFAs in milk under conditions of temperature comfort (THI 58 and 66) did not differ significantly and ranged within SFAs – about 70%, MUFAs – 26.5%, PUFAs – about 3.5% (Fig. 3).

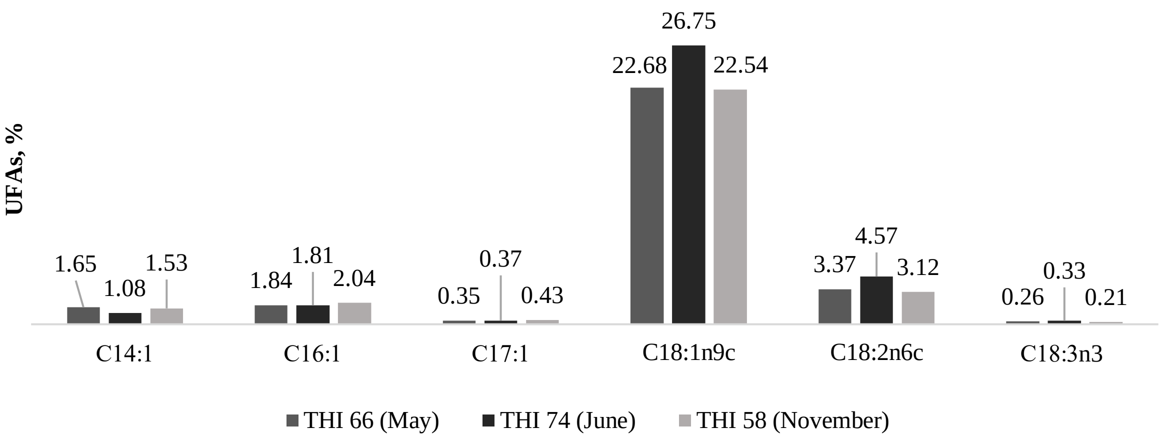

Fig. 2. Mass fraction of UFAs (%) in the secretion of the mammary gland in cows depending on THI value

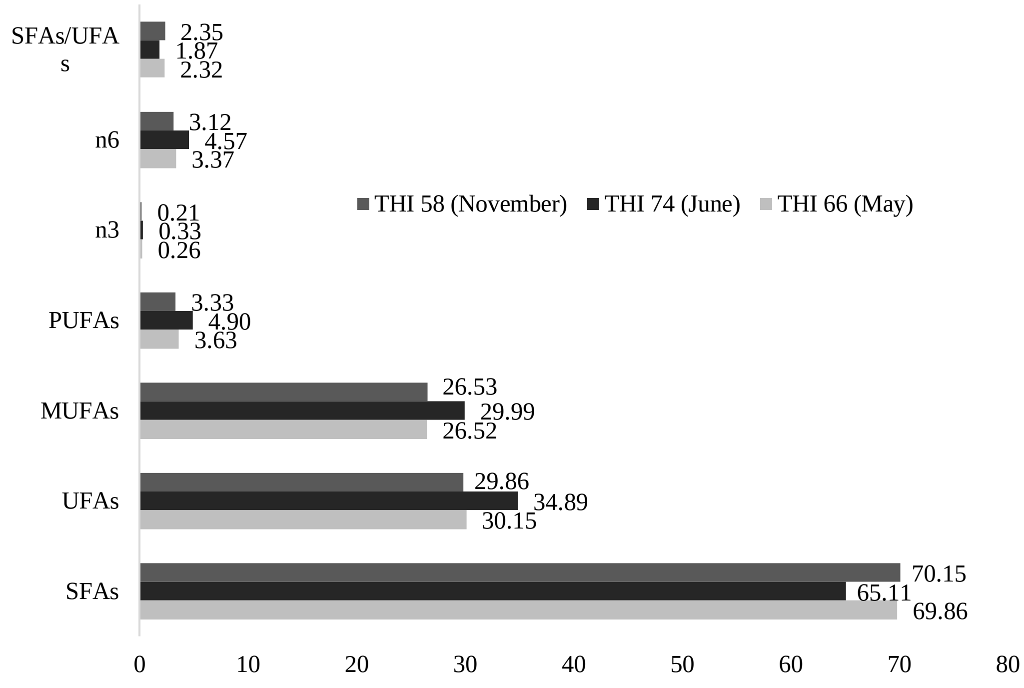

Fig. 3. Individual groups of fatty acids (%) and their ratio in cows' milk depending on THI value

Besides, the SFAs/UFAs ratio in these groups was approximately identical (≈2.3). Insignificant differences were observed only in the ratios of ω fatty acids in milk with the mentioned parameters of the THI value.

It should be noted that an increase in the THI value to 74 (Fig. 3) caused a significant increase in the total content of UFAs, MUFAs, and PUFAs in the milk of Holstein cows, which was accompanied by a decrease in the ratio of SFAs/UFAs to the level of 1.87. This value is significantly lower than in cows during the autumn-spring lactation period.

Global climate change on the planet has significantly affected both the livestock industry in general and the indicators of quality and safety of livestock products in particular. Dairy cattle are very sensitive to heat stress. The higher milk performance in cows, the more sensitive they are to the increase in ambient temperature. Reducing the threat of overheating of highly productive animals through using shade, fans, sprinkler, correction of feeding and grazing periods is widely used in industrial milk production. However, colossal economic losses due to a decrease in milk yield in cows as a result of chronic heat stress significantly affect the stability of production. The high level of formation and secretion of milk components in cattle involves the use of a significant amount of energy. Therefore, a significant part of the thermal energy generated as a result of metabolism must be diverted from the intensively working mammary gland to the environment.

According to the results of our previous studies, a significant decrease in the content of dry skimmed milk residue, mass fraction of fat and protein was observed in dairy cows during the period of long-term heat stress. The mass fraction of lactose and minerals tended to increase [15].

Fatty acids with carbon chain length from C4 to C10 are synthesized in the cytoplasm of secretory cells from acetate absorbed from the bloodstream and β-hydroxybutyrate, which are formed in the rumen wall [19]. On the other hand, β-oxidation of fatty acids is also one of the important mechanisms for the formation of short-chain fatty acids.

De novo synthesized fatty acids make up approximately 45% of the total amount of fatty acids and the remaining are derived from dietary fat [20].

From the results of our research, it turns out that a significant amount of fatty acids with a short carbon chain in the mammary gland of high-yielding Holstein cows under long-term heat stress (THI 74) simply stop being formed. Currently, it is quite difficult to claim whether this occurs due to a decrease in the intensity of metabolism in the rumen of ruminants during heat stress, or whether it occurs already in the mammary gland due to a decrease in the intensity of metabolite conversion in order to reduce heat generation in the organ.

This issue still needs additional research and verification. Under what conditions does THI begin to reduce the β-oxidation of long-chain acids in the mammary gland, or the synthesis of short-chain fatty acids in the rumen. A clear answer will still have to be given to these questions because they determine both the level of energy metabolism in lactating cows, and the level of metabolism and productivity of the mammary gland.

According to various authors, milk lipids contain up to 85% fatty acids, their ratio varies within very narrow limits, in particular, up to 70–75% SFAs, about 25% MUFAs, and 5% PUFAs [21].

According to our research, regardless of the THI value, 4 fatty acids dominated in milk: myristic (C14:0), palmitic (C16:0), stearic (C18:0), and oleic (C18:1n9c). This is consistent with the results of other researchers [22].

The authors of the article note that milk lipids (triacylglycerols and polar lipid profiles) undergo changes during heat stress. This leads to a decrease in the amount of short- and medium-chain fatty acids and an increase in the content of long-chain fatty acids [9].

Similar changes occurred in our experiment: with chronic heat stress in Holstein cows, with a THI index of 74, a decrease in the amount of fatty acids in the range from C4:0 to C16:0 is observed, while the regularity applies to fatty acids with an even number of atoms and with an odd one. And vice versa, starting with margaric acid (C17:0) and ending with behenic acid (C22:0) during chronic heat stress (THI 74), their fraction increases significantly.

Quite interesting is the fact that the relative content of UFAs in the milk of Holstein cows is increasing under chronic heat stress (THI 74) in comparison with the conditions of relatively comfortable keeping cows in the spring (THI 66) and autumn (THI 58) experimental periods. Undoubtedly, this occurs due to a decrease in the intensity of metabolism in the mammary gland. This process redirects the flow of fatty acids immediately into the secretion, which causes a change in milk composition under conditions of mammary gland overheating.

Regarding the quality of milk obtained from cows that are in a state of chronic heat stress, this issue is debatable. Of course, it can be argued that the accumulation of UFAs in milk is good, but short-chain fatty acids are a valuable metabolite for growing animals and therefore this question is open.

The fact that it is possible to use the THI value to predict not only the physiological state and performance of lactating cows but also the fatty acid composition of the mammary gland secretion turned out to be quite interesting. To what extent predicting the production of milk with a slightly higher level of UFAs will affect the stability of dairy industry production and its nutritional value remains an open question.

Conclusion

The temperature-humidity index can be used to predict milk performance and milk fatty acid composition in lactating high-yielding Holstein cows. Under the THI 58 and 66, the intensity of lactation in cows and the fatty acid composition of the collected milk did not differ significantly, although trends to changes were observed for individual fatty acids. When reaching the THI of 74, there is an increase in the relative content of unsaturated fatty acids due to a decrease in the level of fatty acids in a range from C4:0 to C16:0 by approximately 8.5% in comparison with the level of secretion in cows during the autumn-spring lactation period. The increase in the relative content of fatty acids in the milk of Holstein cows at THI of 74 is mainly due to fatty acids with 18 carbon atoms in the chain. Thus, the relative content of C18:1n9c under conditions of chronic heat stress (THI 74) was 26.75±1.27, this value is 4.07% higher than in milk collected in May at THI of 66 and by 4.21% higher than in milk collected in November at THI of 58.

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ЖИРНОКИСЛОТНИЙ СКЛАД МОЛОКА КОРІВ ГОЛШТИНСЬКОЇ ПОРОДИ ЗА УМОВ ХРОНІЧНОГО ТЕПЛОВОГО СТРЕСУ

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Анотація. Цей експеримент проведено з метою вивчення впливу температурно-вологісного індексу та хронічного теплового стресу на інтенсивність секреції жирних кислот молочною залозою корів голштинської породи. Двадцять п'ять повновікових голштинських корів (130±25 днів лактації) з добовими надоями 30 кг були випадковим чином віднесені до однієї групи. Від цих корів відбирали ранішнє молоко весною, літом (високі температури) та осінню і визначали жирнокислотний спектр складу ліпідів молока при комфортних температурах перебування та в умовах хронічного теплового стресу. У молоці дослідних корів методом газової хроматографії кількісно ідентифіковано 19 жирних кислот. Найбільший відсотковий вміст припадає на міристинову (C14:0), пальмітинову (C16:0), стеаринову (C18:0) та олеїнову (C18:1n9c) жирні кислоти. Цими дослідженнями підтверджено, що тривалі періоди спеки можуть впливати на кількісний склад жирних кислот ліпідів молока. У результаті досліджень встановлено, що показник індексу температури та вологості можна використовувати для прогнозування продуктивності та жирнокислотного складу молока лактуючих високопродуктивних корів голштинської породи. За показників температурно-вологісного індексу 58 (листопад) та 66 (травень) інтенсивність лактації корів та жирнокислотний склад одержаного молока вірогідно не відрізнявся, хоча за окремими жирними кислотами і спостерігалися тенденції до змін. Підвищення температурно-вологісного індексу (червень) до показника 74 викликало істотне зростання у молоці корів голштинської породи як загального вмісту ненасичених жирних кислот (ННЖК), так і мононенасичених (МНЖК) та поліненасичених (ПНЖК), що супроводжувалося зниженням показника співвідношення НЖК/ННЖК до рівня 1.87. Це значення було значно нижчим ніж у молоці корів, яке отримували у травні та листопаді. Зростання відносного вмісту жирних кислот у молоці корів голштинської породи за температурно-вологісного індексу 74 (червень) відбувається в основному за рахунок жирних кислот з 18 атомами Карбону в ланцюзі. Так, відносний вміст C18:1n9c за умов хронічного теплового стресу (температурно-вологісноий індекс 74) складав 26.75±1.27, це значення на 4.07% вище ніж у молоці отриманому у травні при ТНІ 66 і на 4.21% вище ніж у молоці отриманому у листопаді при температурно-вологісному індексі 58.

Ключові слова: молоко, жирні кислоти, корови, температурно-вологісний індекс, хронічний тепловий стрес.