UDC:637.12:636.2:616-001.16

QUALITY INDICATORS OF COW MILK UNDER CHRONIC HEAT STRESS

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https://doi.org/10.15673/fst.v19i3.3219

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Cite as Vancouver style citation

Quality indicators of cow milk under chronic heat stress / Danchuk O.et al. Food Science and Technology. 2025;19(3):46-53. https:// 10.15673/fst.v19i3.3219

Цитування згідно ДСТУ 8302:2015
Quality indicators of cow milk under chronic heat stress / Danchuk O.et al. // Food Science and Technology. 2025. Vol. 19, Issue 3. P. 46-53. https:// 10.15673/fst.v19i3.3219

Abstract. Heat stress during the maintenance of dairy cows, especially chronic, can change the metabolism of lipids in the body, as well as affect the intensity of their oxidation. This experiment was conducted to investigate the effect of heat stress on the milk productivity of dairy cows of the Ukrainian Black and White breed, to determine changes in the fatty acid composition and other quality indicators of the obtained milk. Physicochemical indicators of raw milk were determined by ultrasonic method on the Ekomilk device. To determine the fatty acid composition, methylation of fat obtained from milk was carried out by the Folch method. Identification and quantitative determination of fatty acids was carried out using a flame ionization detector using the gas chromatography method. Dairy processing enterprises can use the temperature and humidity index (THI) indicators of the agro-ecological zone to predict the supply of raw milk and its nutritional value. As the THI index increased from moderate ($72 < THI \le 80$) to severe ($80 < THI \le 85$), the daily milk yield on the farm decreased by 90 kg, an average of 0.459 kg per cow per day, i.e. 0.038 kg per unit of THI index. For each day of severe heat stress on the body of a lactating cow, the total yield on the herd decreased by an additional 24.28 kg of milk (an average of 0.124 kg per cow per day). The restoration of the level of milk supply from the farm can be predicted 7-10 days after the disappearance of symptoms of chronic severe heat stress. Raw milk from cows in a state of heat stress may contain less fat and protein, the density of the secretion may increase slightly. 17 fatty acids were found in the milk of cows. The fatty acid composition of milk shows a relatively high sensitivity to the intensity of the heat load on the body of a lactating cow. This especially concerns a dose-dependent decrease in the level of saturated short-chain fatty acids (C4:0-C6:0). At the same time, an increase in the level of saturated fatty acids C12:0 and C18:0 was noted, as well as an increase in the intensity of secretion by the mammary gland of the polyunsaturated fatty acid C18:2n6c.

Keywords: cow's milk, quality indicators, fatty acid composition, heat stress.

Introduction. Formulation of the problem

Global climate change creates significant problems in ensuring food security for the vast majority of countries in the world [1]. This fully applies to the production of milk and dairy products. The adaptive capacity of productive animals to changes in the climatic conditions of their maintenance (temperature, humidity, solar radiation, crowding of animals, ectoparasites, shading of the territory, ventilation, provision of drinking water, nutrition, pathologies) largely determines the quantity and quality of the milk produced as raw material [2, 3, 4]. To reduce the heat load on cattle, trees have already begun to be planted on pastures [5].

In the twenty-first century, milk production losses due to heat stress are projected to increase at a rate of over 170 kg/cow/decade [6]. A cow's heat balance is thought to be influenced by a number of factors, including age, genotype, diet, physiological state, performance, fat distribution and deposition, lactation period, health status, and adaptive capacity [7, 8]. The heat production in a cow's body to support metabolism is approximately 31% of the energy it consumes, calculated per 600 kg of body weight, with a milk yield of 40 kg of milk, 4% fat [9].

Analysis of recent research and publications

Modeling the impact of heat stress is one of the most promising ways to prevent it [10]. According to the study [11], it is possible to observe a loss of approximately 0.27 kg of milk for each subsequent unit increase in the temperature and humidity index (THI), which may be accompanied by apoptosis of epithelial cells in the mammary gland [12]. If we talk about acute and chronic heat stress in cows during the summer periods of lactation, then there is a fairly large amount of data on changes in the main parameters of the circulatory system, respiration, energy metabolism, and as a result, the animal tends to reduce the intensity of heat generation in the body and increase heat loss. All processes that are accompanied by the formation of a significant amount of chemical and thermal energy are slowed down. This applies not only to cicatricial digestion [13], but also to the synthesis of milk components and milk yield. It is known that heat stress is accompanied by a decrease in the secretory activity of the mammary gland. The mammary gland secretes less of individual components, and the fatty acid and protein composition of the secretion changes [14, 15, 16].

Therefore, the question arises of a clear understanding at what stage of heat load and what characteristics of milk begin to change. Ensuring the stability of the composition of raw milk is key for the dairy industry. An increase in the THI indicator above a certain level may be accompanied by a decrease not only in the secretory activity of the mammary gland, but also in the protein and fat composition of the secretion itself. This, in turn, may complicate the process of manufacturing high-quality dairy products.

Thus, summer samples of dairy cheeses may contain less protein and a higher level of dry matter, lipids, and mineral elements compared to cheeses made from dairy raw materials obtained in the spring [17]. Similar results were obtained with sheep cheese, where significant variations in the content of casein (from 15.33 to 23.07%), protein (from 16.0 to 23.93%), and fat (from 18.31 to 31.08%) were found [18]. The question arises of developing mechanisms for more detailed analysis of raw materials and predicting possible changes in its main characteristics [19, 20]. Since the identification of the stages of heat stress is to some extent subjective, researchers have created a system for assessing its level based on systems for assessing air temperature and relative humidity [21, 22]. At the same time, researchers also associate the possibility of fluctuations in mortality rates in dairy cows with the level of THI. Thus, THI values of 80 and 70 were the maximum and minimum THI in different farms, respectively, at which an increase in cow mortality was recorded and reached maximum values at THI of 87 (max) and 77 (min), respectively [23].

The use of publicly available weather station data to assess the possible impact of heat stress in cows on raw milk performance (milk yield, fat, and protein) could be very important for the dairy industry [24].

The aim of this work was to investigate the features of milk productivity of cattle during changes in the THI index from moderate to severe levels of chronic heat stress and to analyze the dietary (nutritional) value of cow milk.

Research objectives:

1. To determine the impact of heat stress on milk productivity of dairy cows of the Ukrainian Black and White breed.
2. To establish the dynamics of changes in quality indicators depending on the temperature-humidity index indicators when keeping dairy cows.

Materials and methods.

Experimental studies were conducted in the conditions of the farm of the village of Kalynia, Kamianets-Podilskyi district, Khmelnytskyi region. The farm keeps cattle of the Ukrainian black-and-white breed, free-range on deep litter. Access to feed and water is free. The average productivity on the farm is 7-8 thousand liters of milk per lactation. A group of full-grown cows of the Ukrainian black-and-white breed was randomly selected in the amount of 30 animals with a productivity of 20-25 liters of milk per day.

Chronic heat stress of lactating cows was studied in July after a long-term heat load on the animals (20 days). THI was determined daily, the indicator was constantly in the heat stress zone for dairy cows (min THI 71), the temperature on some days could reach 36°C with a max THI – 85 [25].

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

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

The level of heat stress during this study was classified according to THI levels as thermoneutral (TN, $60 < THI \leq 67$), mild ($67 < THI \leq 72$), moderate ($72 < THI \leq 80$), and severe ($80 < THI \leq 85$) [26]. Milk samples obtained from animals in the experimental groups served as the material for the studies.

Laboratory studies of milk were carried out on the basis of the laboratory of the Department of Normal and Pathological Morphology and Physiology of the Faculty of Veterinary Medicine and Technologies in Animal Husbandry of the Podolsk State University and the Ukrainian Laboratory of Quality and Safety of Agricultural Products of the National University of Life Resources and Environmental Management of Ukraine. Transportation of milk samples was carried out in thermal containers (+5°C). In milk, the following were determined: dry non-fat milk residue, %; mass fraction of fat, %; density, kg/m^3; mass fraction of protein, %; mass fraction of lactose, %; mass fraction of mineral substances, %; mass fraction of water, %; freezing point, (minus) °C; electrical conductivity, ms/cm; active acidity, units of pH and fatty acid composition (%). Physico-chemical indicators of raw milk were determined on the EKOMILK M MILK ANALYZER MILKANA KAM98-2A device. Fat from milk was isolated by the Folch method [27]. The analysis of fatty acid methyl esters was performed on a Trace GC Ultra gas chromatograph (USA) with a flame ionization detector according to DSTU ISO 5509-2002. Chromatography conditions: column temperature 140–240°C, detector temperature 260°C. THI indicators, herd milk productivity, physiological condition and animal behavior were determined daily. Control milkings were performed once every two weeks from May to September and, if necessary, when studying the clinical picture of heat stress. To compare milk quality indicators with standards, DSTU 3662:2018 Milk - raw cow. Technical conditions were used.

Results of the research and their discussion

The temperature and humidity index (THI) is commonly used to measure the potential level of heat stress and demonstrates good correlation with physiological parameters, including respiratory rate and body temperature of dairy cows [28]. Climate change in the forest-steppe conditions of Ukraine, and therefore THI parameters, significantly affects the summer milk production of cow herds (Fig. 1), especially when the THI index moves from a moderate level of heat stress (THI 73-80) to a severe level of heat stress (THI 81-85). During the increase in the THI index from level 73 (moderate heat stress) to level 85 (severe heat stress), the daily milk yield per farm decreased by 90 kg, an average of 0.459 kg per cow per day, i.e. 0.038 kg per unit of THI index.

However, when the THI was in the severe chronic heat stress zone for a relatively long time, the overall herd productivity continued to decline steadily. For each day of severe heat stress on the lactating cow, the total herd yield decreased by an additional 24.28 kg of milk (0.124 kg on average per cow per day).

It should be noted right away that these are generalized data on the dairy herd of cattle and they may differ significantly from the daily productivity indicators of individual cows (Fig. 2). The increase in daily productivity in individual cows with a decrease in the THI indicator from 81 to 76, in our opinion, is associated with the peculiarities of adaptation of different types of higher nervous activity to heat stress. There is a tendency that the higher the productivity, the more sensitive the animals are to temperature and humidity fluctuations. Which is quite natural from a biological point of view, because the formation of a sufficiently large amount of milk in the mammary gland requires an appropriate level of energy metabolism, which the animal cannot provide under heat stress. However, these data are extremely important for predicting the flow of raw milk to dairy processing enterprises. Based on the indicators of regional weather stations, dairy processing enterprises can predict the flow of raw milk for processing.

As shown by the results of the studies presented in Table 1, the physicochemical properties of milk under conditions of transition from moderate to severe heat stress undergo significant changes associated with an increase in milk density by 3 kg/m^3.

It goes without saying, the change in the physicochemical parameters of milk is a consequence of the deterioration of the physiological state of the animal under conditions of increased temperature load on its body and the loss of a significant amount of water due to increased heat transfer and increased need for drinking water. However, it should be noted that not all of the studied milk parameters clearly increased due to the increase in the intensity of chronic heat load on the cow's body. The mass fraction of lactose, freezing point, electrical conductivity, active acidity of milk under the conditions of the research did not change significantly.

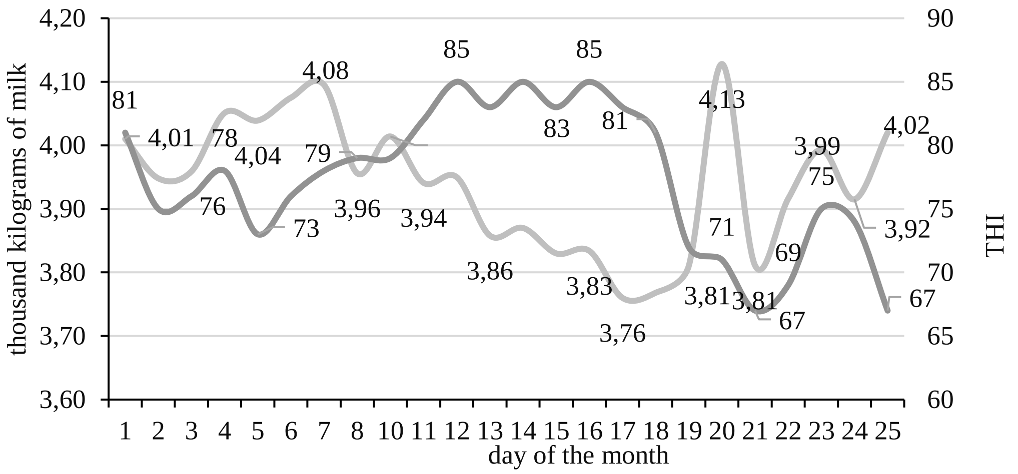

Figure 1. Dairy herd performance under chronic moderate and severe heat stress (n=196). Blue line – daily herd productivity; brown line – THI indicator.

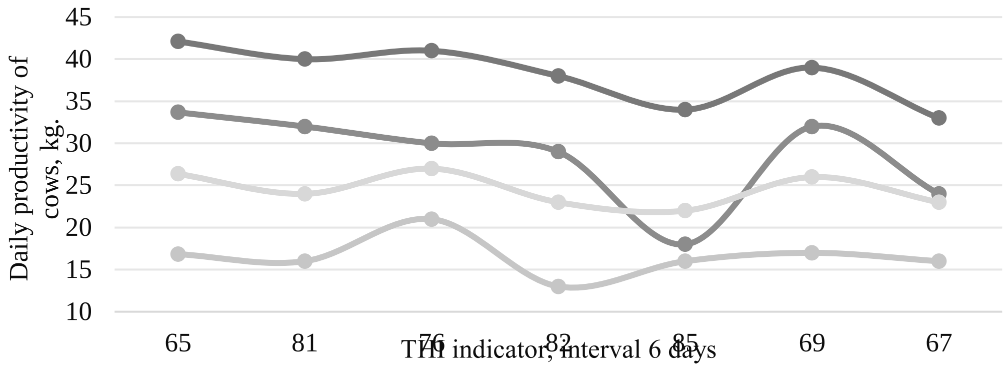

Figure 2. Individual daily milk yield of cows at different levels of THI (measurement interval 6 days)

Table 1 – Milk performance of cows with moderate and severe levels of chronic heat stress (M±m), n=10

Indicators	DSTU 3662:2018 Raw cow's milk	Moderate stress (THI 72-80)	Severe stress (THI 81-85)
Mass fraction of fat, %	3.4%	3.16±0.56	1.39±0.26**
Density, kg/m3	1028.0	1032.90±0.82	1035.90±0.24**
Mass fraction of protein, %	3.0	3.31±0.05	3.12±0.03*
Mass fraction of lactose, %	Not normalized	5.32±0.07	5.31±0.023
Mass fraction of minerals, %	Not normalized	0.79±0.01	0.822±0.006*
Freezing point, (minus) °C	not higher than 0.520	0.598±0.003	0.604±0.03
Conductivity, ms/см	Not normalized	5.16±0.03	5.24±0.05
Active acidity, pH units	From 6.6 to 6.7	6.61±0.01	6.60±0.03

Note: * – difference from the corresponding control variant is significant at $p < 0.05$; ** – $p < 0.01$. Source: compiled by the authors

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As for the fat content in the secretion of the mammary gland of a high-yielding cow, we noted a decrease in its concentration, both at moderate and severe heat stress on the animal's body. Thus, the fat content in the milk of Ukrainian black-and-white cows during the rapid transition of chronic heat stress from moderate to severe decreased by 2.27 times ($p<0.01$). This certainly indicates a high degree of sensitivity of the intensity of lipid metabolism in the mammary gland of high-yielding cows to the level of heat stress on her body. However, such a physiological reaction may not apply to animals with a lower level of productivity, because even a theoretical decrease in the intensity of cicatricial digestion and a decrease in the flow of short-chain fatty acids into the internal environment of the body may be quite sufficient to ensure a certain level of milk fat.

Using gas chromatography, we have detected and quantitatively identified 17 fatty acids in the milk of dairy cows.

The lipid composition of milk depends on many factors, among which temperature and humidity play an important role [29].

If we examine the intensity of secretion of individual fatty acids by the mammary gland, a similar pattern is observed regarding the decrease in the amount of individual fatty acids in the secretion of the mammary gland (Figure 3).

During chronic heat stress, the content of saturated fatty acids in cow's milk increases by ≈2% and, accordingly, the content of unsaturated fatty acids decreases.

However, it should be noted right away that the decrease was due to MUFA, and the total percentage of PUFA even increased slightly. Moreover, if we talk about short-chain fatty acids (C4:0 + C6:0), then their total content during chronic heat stress of moderate and severe levels decreased by 0.43% and 1.47%, respectively ($p<0.05$). Although other groups of fatty acids did not differ significantly, it should be noted a slightly higher level of C8:0-C12:0 acids in the milk of cows during chronic heat stress at THI 72-80 (by 1.83%) and at THI 81-85 (by 0.75%)

The relative content of individual fatty acids in milk of cows under chronic heat stress is shown in Table 2. Analysis of chromatograms of fatty acids in milk is a very complex and multi-vector matter, because their ratio in the composition of milk fat depends on many factors and, first of all, on the composition of the diet, the intensity of cicatricial digestion and lipid metabolism in the mammary gland. Therefore, in clinically healthy animals their ratio can fluctuate within quite wide limits.

However, if the diet remains unchanged and with an increase in the heat load on the body of a dairy cow, the intensity of metabolism in the rumen may decrease, and blood flow is redistributed to peripheral organs and tissues, the analysis of chromatograms can quite clearly indicate the state of lipid metabolism in the mammary gland of a cow in a state of chronic heat stress.

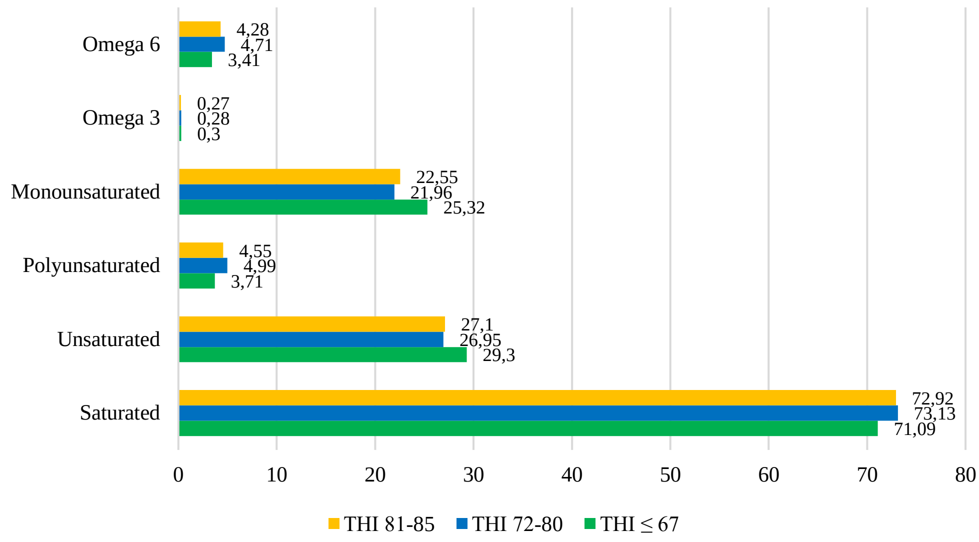

Figure 3. Ratio of fatty acids in milk of cows under chronic heat stress, %

Thus, the content of fatty acids C4:0, C14:1 and C18:1n9c in the mammary gland secretion decreased with an increase in the THI index, or had a tendency to decrease. As for C4:0, its percentage during chronic heat stress of moderate and severe levels decreased by 1.15 ($p<0.05$) and 1.79 ($p<0.001$) times, respectively, and the deterioration of the physiological state of the animal during the transition from moderate to severe levels of heat stress was accompanied by a decrease in the intensity of C4:0 secretion by the mammary gland, which can certainly be one of the markers of thermotolerance of lactating cows.

It should also be noted that chronic heat stress, regardless of the intensity of heat stress, was accompanied by a significant increase in the intensity of secretion by the mammary gland C18:2n6c ($p<0.05$), which in our opinion may be associated with both a decrease in the intensity of cicatricial digestion under excessive heat stress and the peculiarities of the secretory function of the mammary gland under heat stress. Highly productive dairy cattle are particularly sensitive to heat stress. With excessive heat stress on their body, diseases such as mastitis are most often found [30], milk yield in cows and milk composition decrease. This leads to a deterioration in the quality of dairy products and, accordingly, losses in the dairy industry [31, 32]. It has been established that even with moderate heat stress, significant changes occur in the body of dairy cows, which can significantly affect the physiological state, reproductive function and milk productivity of cows [33, 34].

Table 2 – Content of individual fatty acids (%) in milk of cows under chronic heat stress under moderate and severe load (M±m), n=10

Fatty acid	Temperature and humidity index value		
	THI ≤ 67	THI 72-80	THI 81-85
C4:0	4.10±0.18	3.55 ±0.12*	2.87±0.22**
C6:0	2.50±0.08	2.73±0.23	2.37±0.08
C8:0	1.55±0.04	1.87±0.10*	1.62±0.13
C10:0	3.57±0.61	3.82±0.44	3.27±0.18
C11:0	0.38±0.07	0.26±0.03	0.24±0.03
C12:0	4.05±0.43	5.34±0.48	5.08±0.12*
C14:0	10.93±1.34	12.09±0.45	11.82±0.80
C14:1	1.53±0.05	1.37±0.10	1.25±0.09
C15:0	1.63±0.31	1.51±0.21	1.49±0.15
C16:0	31.06±1.15	29.74±0.71	31.68±0.70
C16:1	1.64±0.13	1.38±0.08	1.36±0.07
C17:0	1.75±0.31	1.31±0.29	1.48±0.30
C18:0	9.83±1.29	10.53±0.33	10.67±0.60
C18:1n9c	22.13±1.50	19.21±0.52	19.03±0.44
C18:2n6c	3.41±0.27	4.71±0.49*	4.28±0.18*
C20:0	0.25±0.03	0.31±0.02	0.33±0.05
C18:3n3	0.30±0.01	0.28±0.05	0.27±0.04

Note: * – the difference from the corresponding control variant is significant at $p < 0.05$; ** – at $p < 0.01$. Source: compiled by the authors.

Studies have shown that regardless of the level of productivity of lactating cows, they are all sensitive to the THI index, the difference between animals is only in the level of reduction in the intensity of milk secretion by the mammary gland. However, highly productive dairy cows are still particularly sensitive to heat stress. During heat stress, milk production in highly productive dairy cows decreases by 0.335 kg/day compared to low-productive cattle, by only 0.158 kg/day [35].

The results obtained indicate that the total productivity of the dairy herd of Ukrainian black-and-white cows in forest-steppe conditions is very sensitive to the THI indicator, and with its increase, the total milk productivity of the herd of cows decreases. The mammary gland is one of the few places in the cow in which intensive heat removal to the surrounding environment is very difficult to combine with the synthesis of milk components. This pattern was noted quite a long time ago [11], but this issue requires more detailed study.

In our opinion, the THI indicator determined in a certain agro-ecological zone can indirectly indicate the predicted milk supply to milk processing enterprises. It is only necessary to investigate the general sensitivity of the technology of obtaining raw milk in the farm to the heat load on animals.

Based on the fact that climate change in our territories has been a long time coming, it is necessary to develop a system for forecasting the supply of raw milk to dairy processing enterprises. This is one of the main tasks in ensuring the food security of the state, because the role of dairy products in meeting the needs of the people is difficult to overestimate. And one of such marker indicators can be used as THI of agro-ecological zones.

The conducted studies show that the decrease in the intensity of lactation of dairy herds under heat stress is not a one-time process. With the increase in the THI index, the flow of raw milk to dairy processing enterprises continues to decrease, which may be one of the biological features of chronic heat stress. The increase in the THI index from level 73 (moderate heat stress) to level 85 (severe heat stress) was accompanied by a decrease in the total milk productivity of the herd of cows, which was an average of 0.459 kg per cow per day, or 0.038 kg per unit of the THI index. It should be noted that in addition to the THI value, it is necessary to pay attention to the duration of the intense heat stress on animals. Thus, the presence of the THI index in the zone of severe chronic heat stress was accompanied by a daily loss of 24.28 kg of milk per herd (0.124 kg on average per cow per day).

Studies have shown that not only the amount of raw milk entering dairy plants in the summer can change, but also the composition of milk, primarily the content of fats and individual fatty acids. Changes in the amount of fat in milk and its lipid profile can also be influenced by breast diseases [29, 36].

The change in the dietary value of milk in the summer season significantly affects the composition and nutritional value of dairy products, and in the milk of cows with severe chronic heat stress, the content of milk fats, individual fatty acids can significantly decrease, and the density of milk and the content of dry non-fat milk residue can increase. The latter indicators, in our opinion, are associated with the intensity of water exchange in the body under conditions of severe heat stress. Studies [37, 38] also showed that heat stress reduces the content of both fat and protein in milk, against the background of an increase in the number of somatic cells. These results are also consistent with previous studies [29], where a decrease in the intensity of lipid metabolism in the mammary gland was observed in Holstein dairy cows under prolonged moderate heat stress.

As for the fatty acid composition of milk from cows with chronic heat stress, in our opinion, special attention should be paid to the level of butyric acid (C4:0). After all, its fluctuations can indirectly indicate the intensity of cicatricial digestion and beta-oxidation of fatty acids in the mammary gland. Based on the fact that cicatricial metabolism during heat stress reduces its intensity, and the exchange of fatty acids in the mammary gland also slows down somewhat, the level of butyric acid in milk can be one of the biological markers of heat stress in lactating cows. However, this issue requires additional study.

Conclusion

Dairy processing enterprises can use the THI indicators of the agro-ecological zone to predict the supply of raw milk and its nutritional value. During the increase in the THI indicator from the moderate level ($72 < THI \leq 80$) to the severe level ($80 < THI \leq 85$), the daily milk yield per farm decreased by 90 kg, an average of 0.459 kg per cow per day, i.e. 0.038 kg per THI unit. For each day of severe heat stress on the body of a lactating cow, the total yield per herd decreased by an additional 24.28 kg of milk (an average of 0.124 kg per cow per day). The restoration of the level of milk supply from the farm can be predicted 7-10 days after the disappearance of symptoms of chronic severe heat stress.

Raw milk from cows under heat stress may contain less fat, the density of the secretion and the mass fraction of protein may increase slightly. The fatty acid composition of milk shows a relatively high sensitivity to the intensity of the heat load on the body of a lactating cow, especially this concerns a dose-dependent decrease in the level of C4:0 and an increase in the intensity of secretion of C18:2n6c acid by the mammary gland.

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ПОКАЗНИКИ ЯКОСТІ МОЛОКА КОРІВ ЗА ХРОНІЧНОГО ТЕПЛОВОГО СТРЕСУ

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Анотація. Тепловий стрес при утриманні дійних корів, особливо хронічний, здатний змінювати метаболізм ліпідів в організмі, а також впливати на інтенсивність їх окиснення. Цей експеримент був проведений з метою дослідити вплив теплового стресу на молочну продуктивність дійних корів Української чорно-рябої породи, визначити зміни жирнокислотного складу та інших показників якості отриманого молока. Фізико-хімічні показники молока-сировини визначали ультразвуковим методом на приладі Ekomilk. Для визначення жирнокислотного складу здійснювали метилювання жиру, отриманого з молока за методом Фолча. Ідентифікацію та кількісне визначення жирних кислот проводили застосувавши полум'яно-іонізаційний детектор з використанням методу газової хроматографії. Молокопереробні підприємства можуть використовувати показники індексу температури та вологості (THI) агроекологічної зони для прогнозування надходження молока-сировини та його поживної цінності. Під час зростання показника THI з рівня помірний ($72 < THI \leq 80$) до рівня важкий ($80 < THI \leq 85$), щодобовий надій по фермі знизився на 90 кг молока, в середньому на 0.459 кг одну корову за добу, тобто по 0.038 кг на одну одиницю показника THI. За кожен день важкого теплового навантаження на організм лактуючої корови, загальний надій по стаду знижувався додатково на 24.28 кг молока (по 0.124 кг в середньому на кожну корову щодобово). Відновлення рівня надходження молока з ферми можна прогнозувати через 7-10 днів після зникання симптомів хронічного теплового стресу важкого рівня. Молоко-сировина від корів у стані теплового стресу може містити менше жиру та білка, густина секрету може дещо зростати. У молоці корів виявлено 17 жирних кислот. Жирнокислотний склад молока проявляє відносно високу чутливість до інтенсивності теплового навантаження на організм лактуючої корови. Особливо це стосується дозозалежного зниження рівня насичених коротколанцюгових жирних кислот (C4:0-C6:0). У той же час відмічали підвищення рівня насичених жирних кислот C12:0 і C18:0, а також підвищення інтенсивності секреції молочною залозою поліненасиченої жирної кислоти C18:2n6c..

Ключові слова: молоко корів, показники якості, жирнокислотний склад, тепловий стрес.