Changes in the Qualitative Composition of the milk of Holstein Cows During Summer Chronic Heat Stress

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ABSTRACT


Organoleptic indicators of milk, electrical conductivity and active acidity of milk did not change in hot weather, their value indicated the naturalness of milk. The mass fraction of milk fat, which undergoes the greatest change under the influence of seasonal heat stress, is one of the most valuable components of milk, which has a direct effect on nutritional value and purchase price of raw milk. Therefore, a further deeper study of the fatty acid composition of milk using the method of chromato-mass spectrometry will provide valuable data necessary to search for possible herd management strategies to maintain high milk quality under conditions of seasonal heat stress.

**Key Words:** Dairy Cows, Organoleptic Indicators, Physico-Chemical Properties of Milk, Prolonged Hot Weather
INTRODUCTION

One of the gravest environmental problems facing humanity is global climate change. According to the forecasts of the leading world climate research centers, air temperature on the globe will be increasing by 2-5 degrees Celsius over the next century (National Ecological Center of Ukraine). Such a rate of global warming can cause severe climate change, so some plant and animal species will be in danger of extinction. It may well be argued that major climate change is happening now, which is a challenge for global agriculture.

Agriculture in general and livestock breeding in particular are primarily and most sensitive to all environmental changes (Abdela & Jilo 2016). Therefore, the sustainable development of livestock breeding remains an urgent problem under global climate change. It is with increasing annual temperatures and possible heat stress that most scientists associate the negative effects of these processes on dairy cattle raising (Herbut et al. 2018). According to Escarcha et al. (2018), short-term extreme weather events will have the greatest impact on feed production, animal health and product quality, and therefore food security is at threat today in many parts of the world, which needs to be addressed in long-term strategies for livestock adaptation to climate change (Wangui et al. 2018).

Despite some success in mitigating the heat stress impact on dairy farming, there has been a noticeable downward trend in production during hot periods in summer (Dunshea et al. 2013). Major losses in the dairy industry due to the manifestation of seasonal heat stress are associated with a decrease in cows’ milk yields and changes in milk components that lead to a dairy products deterioration (Maggiolino et al. 2020).

Heat stress not only reduces milk production, but also affects somatic cell count and milk composition, which are particularly susceptible to change even with slight increases in ambient temperatures beyond the physiological comfort of cows (Nasr MAF & El-Tarabany 2017; Bertocchi et al. 2020). For example, the fat and protein content of milk reduced by 0.012 kg and 0.009 kg, respectively, as a result of an increase in temperature and humidity index (THI) per unit increase in THI above 72 (Liu et al. 2019). A little earlier it was reported that when the average THI increased from 68 to 78 (from spring to summer), the protein and fat content of milk reduced from 2.96% and 3.58% to 2.88% and 3.24%, respectively, with a drop in milk yield by 21% (Herbut & Angrecka 2012). Studies by Maggiolino et al (2020) confirm that the above changes in the composition of milk under heat stress have a negative impact on the technological properties of milk and especially on cheese production. The objective of this study was to determine the changes in some qualitative parameters of milk in Holstein cows during chronic heat stress.

MATERIALS AND METHODS

Animals involved in the experiment

This experiment was conducted in accordance with the animal welfare requirements and approved by the Bioethics Commission of the Dnipro State Agrarian and Economic University. Ten multipara milking Holstein cows (160±14 days of lactation) were randomly assigned to one of the two groups. Some of the cows were assigned to the control group (n=5) in May (spring season) and the other part was referred to the experimental group (n=5) in August (summer). The average daily milk yield in cows of the control group was 30.4±0.17 kg/day, in cows of the experimental group - 31.0±0.26 kg/day, there was no significant difference in milk yield.

Weather conditions

Weather data from the nearest weather station was taken on the website of the Ukrainian Hydrometeorological Center, as described earlier (Mylostyyi & Chernenko 2019). The data (temperature and relative humidity) was recorded every three hours per day. At the same time, the temperature-humidity index (THI), calculated according to Kibler (1964), was used as the indicator of heat stress (HS) in cows. THI= 1.8 × T – (1 - RH/100) × (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 THI limit, at which most dairy cows could experience HS was considered to be 72 units (Herbut et al. 2018). Weather data was taken into account within 24 hours during the studies, as well as during the week preceding the studies.

Maintenance, feeding and milking of cows

The research was carried out on one of the large commercial dairy farms in Ukraine (50°49′14″ N, 31°49′23″ E). In short, milking Holstein cows were kept unattatched in a naturally ventilated barn (NVB). Rubber mats were used as bedding in the stalls. The cows had a general mixed diet including the own-produced fodder, which consisted of corn silage, lucerne haylage, cereal hay, wheat straw, barley grain, oats and corn. Rapeseed, sunflower and soybean meal, dried pulp, as well as mineral and vitamin supplements were also included in the diet. The fed rations were balanced in terms of essential nutrients according to the recommendations of the National Research Council (NRC 2001). The premises had a feeding table and freely accessible group drinking bowls. The fed ration was not changed during...
the year. The animals were kept permanently indoors, with no grazing. DeLaval equipment, a Cascade milking room for 72 cows with a production capacity of 300 heads per hour and computer identification of animals in the DairyComp 305 herd management system were used for milking cows. The milk is completely isolated from the external environment, which guarantees its high sanitary performance. The milking of cows is performed three times a day in all seasons.

Research facilities and indicators measured

The milk was studied in the Ukrainian Laboratory of Quality and Safety of Agricultural Products of NUBiP of Ukraine, accredited as per the quality system of DSTU EN ISO/IEC 17025:2019. Samples were taken from chilled bulk milk according to (DSTU ISO 707:2002 2002; DSTU 8553:2015 2015).

Organoleptic parameters of milk were identified: appearance, colour, taste, smell, and consistency. According to DSTU 3662:2018, which is in force in Ukraine, milk of all (three) grades must meet the following requirements in terms of organoleptic characteristics: consistency: homogeneous liquid without protein flakes and sediment; taste and smell: pure, typical of fresh milk, without foreign flavors and odors; color: from white to light cream.

According to DSTU 7057:2009, the density, mass fraction of fat, protein, and lactose were measured by the ultrasonic method on the Master Classis LM2PI milk analyzer. The same device, in addition, assessed: active acidity, dry skimmed milk residue, freezing point, electrical conductivity, mass fraction of minerals, mass fraction of water and freezing point.

Based on these indicators, according to DSTU 3662:2018, milk is divided into 3 grades: extra (density ≥1028.0 kg/m3, mass fraction of solids ≥12%; acidity: pH 6.6-6.7; freezing point (minus) ≥0.520°C), first grade (density ≥1027.0 kg/m3, mass fraction of solids ≥11.5%; acidity: pH 6.55-6.8; freezing point (minus) ≥0.520°C).

Statistical analysis

For statistical data processing, the statistical software package STATISTICA 12 (StatSoft, Inc., Tulsa, OK, USA) was used. The distribution of all the variation series was subject to the normality test. The data is represented as mean and standard error of mean (SE). The significance of the differences between the groups was assessed using Student’s t-test. The difference with values of P<0.05 was considered statistically significant.

RESULTS AND DISCUSSION

Weather conditions during the studies

Milk sampling in the cows of the experimental group was preceded by a 26-day period of continuous heat stress for the milk cows (THImax ≥72). At the time of research, the maximum daily value of THI was about 75 units (Table 1).

Considering the existing close relationship between the environment and the body, the direct influence of meteorological factors on the physiological state of productive animals, the assessment of the animals’ comfort using integral indicators or indices deserves special attention. The temperature-humidity index, which is based on air temperature and relative humidity measurements, has traditionally been used to quantify the degree of HS in animals. THI is easy to calculate and it is rather informative. In the reference group, milk sampling was carried out under thermal comfort (THI=67).

Table 1. Weather conditions on the day and during the week prior to milk sampling

<table>
<thead>
<tr>
<th>Indicator</th>
<th>Reference Group</th>
<th>Experimental Group</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Me</td>
<td>min</td>
</tr>
<tr>
<td>Weather conditions on the day of milk sampling</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Temperature, °C</td>
<td>17.5</td>
<td>14.0</td>
</tr>
<tr>
<td>Relative humidity, %</td>
<td>66.9</td>
<td>57.0</td>
</tr>
<tr>
<td>THI, units</td>
<td>62.4</td>
<td>57.3</td>
</tr>
<tr>
<td>Weather conditions during the week prior to milk sampling</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Temperature, °C</td>
<td>19.0</td>
<td>14.0</td>
</tr>
<tr>
<td>Relative humidity, %</td>
<td>73.0</td>
<td>56.0</td>
</tr>
<tr>
<td>THI, units</td>
<td>63.1</td>
<td>55.7</td>
</tr>
</tbody>
</table>

Parameters of the external environment were recorded according to the data from the nearest weather station.
### Table 2. Compliance of milk with procurement requirements by organoleptic characteristics

<table>
<thead>
<tr>
<th>Indicators</th>
<th>Reference Group</th>
<th>Experimental Group</th>
<th>Characteristics according to DSTU 3662</th>
</tr>
</thead>
<tbody>
<tr>
<td>Consistence</td>
<td>MS</td>
<td>MS</td>
<td>homogeneous liquid with no protein floccules and sediment</td>
</tr>
<tr>
<td>Taste and smell</td>
<td>MS</td>
<td>MS</td>
<td>inherent in fresh milk, with no extraneous flavours and odours</td>
</tr>
<tr>
<td>Color</td>
<td>MS</td>
<td>MS</td>
<td>from white to light-cream and a slight yellowish shade</td>
</tr>
</tbody>
</table>

Metts the standard (MS)

### Table 3. Results of measuring the quality of milk obtained under normal conditions and under chronic heat stress (Mean±SE, n=5)

<table>
<thead>
<tr>
<th>Indicators, units of measurement</th>
<th>Reference Group</th>
<th>Experimental Group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Milk Solids-Not-Fat, %</td>
<td>8.69±0.04</td>
<td>8.09±0.08*</td>
</tr>
<tr>
<td>Mass fraction of fat, %</td>
<td>3.73±0.01</td>
<td>3.50±0.05*</td>
</tr>
<tr>
<td>Density, kg/m³</td>
<td>1031±0.14</td>
<td>1029±0.14</td>
</tr>
<tr>
<td>Mass fraction of protein, %</td>
<td>3.24±0.02</td>
<td>3.03±0.06*</td>
</tr>
<tr>
<td>Mass fraction of lactose, %</td>
<td>5.16±0.02</td>
<td>5.34±0.08</td>
</tr>
<tr>
<td>Mass fraction of minerals, %</td>
<td>0.76±0.12</td>
<td>0.86±0.10</td>
</tr>
<tr>
<td>Mass fraction of water, %</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>Freezing point, (minus) °C</td>
<td>0.590±0.003</td>
<td>0.542±0.008</td>
</tr>
<tr>
<td>Electrical conductivity, mS/cm</td>
<td>5.6±0.01</td>
<td>5.6±0.03</td>
</tr>
<tr>
<td>Active acidity, pH units</td>
<td>6.63±0.02</td>
<td>6.63±0.03</td>
</tr>
</tbody>
</table>

*P<0.05

### Organoleptic parameters of milk

In terms of organoleptic indicators, the milk of the reference and experimental groups corresponded to the current regulations of Ukraine (DSTU 3662:2018) and had the following characteristics (Table 2). Thus, the data show that the basic organoleptic characteristics of the milk of the control and experimental groups were unchanged and fully complied with the current regulatory document in Ukraine.

### Milk solids-not-fat

The most variable proportion of milk solids is fat, therefore in dairy production, the indicator of the content of milk solids-not-fat (MSNF) is often used, the amount of which is obtained after subtracting the percentage of fat from the total amount of milk solids (Ilchuk et al. 2016).

This indicator is not standardized according to the regulatory documents of Ukraine, but in our studies we observed a probable MSNF reduction by 6.9% in the experimental group compared to the reference group (Table 3).

The amount of milk solids is associated with its chemical composition, in particular fat and protein content (Evers et al. 2021). The nature of seasonal changes in the content of milk solids is similar to the nature of seasonal changes in fat and protein: low content of milk solids is noted in the spring and summer period, while it is higher in autumn and winter. This is mainly traditionally associated with the changes in feeding and the consumption of juicy green fodder during grazing (Polieva et al. 2021). There is also additive genetic variability for productive traits in dairy cattle associated with the use of genetic breeding programs (Uribe & Gonzalez 2019).

But in our case, the diet of animals did not change. However, a decrease in the content of dry matter, milk fat and protein were observed. This can be explained by the direct effect of heat stress on the body of dairy cows. Since other researchers have also reported a decrease in these milk components under heat stress (Gao et al. 2017; Qin et al. 2018; Mavangira et al. 2018; Koshshavka et al. 2020).
**Mass fraction of fat**

The synthesis of lactic fat depends on fatty acids. Milk fat contains characteristic fatty acids. Under the influence of heat stress, significant changes in metabolic processes occur in the body of dairy cows (Mylostyyi et al. 2021b), which also leads to changes in milk fatty acids (Penev et al. 2021). For example, the study of milk fat composition when cows are kept at 32.2°C showed significant changes in the fatty acid composition of milk associated with a decrease in lauric (C12:0), myristoleic (C14:1), pentadecanoic (15:0), oleic (C18:1) and linoleic (18:2) fatty acids with a simultaneous increase in palmitic (C16:0) and stearic (C18:0) fatty acids compared to cows under isothermal conditions (Moody et al. 1971).

Milking cows suffering from heat stress often show a decrease in milk fat content, which can cause losses to producers in the difficult conditions of the today's dairy market (Liu et al. 2019).

The fat content in the milk of the experimental group of cows is reduced by 6.2% relative to the reference group. Our results are consistent with those of other authors (Qin et al. 2018; Mavangira et al. 2018), reporting a decrease in lactic fat content in cows due to heat stress.

**Mass fraction of protein**

This is the main indicator that characterizes the naturalness of milk. The naturalness of milk is very important for the Ukrainian market of milk consumers, as people want to consume natural milk. The level of falsification of the Ukrainian dairy market exceeds 50% (Karpenko 2020). It is also a decisive factor on which the cheese-making properties of milk and the yield of cheeses depend. The mass fraction of protein in milk corresponded to the baseline in the reference group. However, under conditions of chronic heat stress, it tended to decrease by 6.5% relative to the protein content in the milk of cows under isothermal conditions (Figure 1).

**Milk density**

This indicator is widely used to convert the amount of milk expressed in kilograms into liters and vice versa; to establish and control the naturalness of milk; to calculate dry matters, milk solids-not-fat (MSNF) and other components using relevant formulas.

Density refers to the ratio of the mass of a substance to its volume. The milk density is represented by the ratio of the milk mass at a temperature of 20°C to the mass of an equal volume of water (t = 4°C). The density of whole cow’s milk ranges from 1.027-1.033 (in individual animals from 1.026 to 1.031). These fluctuations depend significantly on the breed, feeding and housing conditions of the animals, mainly on the quantitative changes in the milk components, conditioning the milk density. The milk density in the milk of the experimental group of cows is reduced by 0.2 % compared to the reference group. In this study, the decrease in milk density is not an indication of milk falsification, but is a consequence of the effect of heat stress on the organism of dairy cows.

**Mass fraction of lactose**

Lactose in milk is the most stable component, the content of which hardly changes during lactation. This is a very important factor, since milk sugar plays a major role in maintaining a constant osmotic pressure in the
blood-milk system (Antoniuk 2016). Lactose is an osmotically active substance that conditions the volume of secretion of water with milk and, accordingly, is the main factor contributing to the level of milk yield, due to which the fluctuation of its content in milk is much lower than that of fat and protein (Tian et al. 2016). In our studies, the lactose content in the experimental group increased by 3.5% compared to the control group. Other researchers have also reported changes in the lactose content of milk from Brazilian Holstein cows during heat stress (Garcia et al. 2015).

**Mass fraction of minerals**

Milk contains about 1% of minerals, which include 80 elements of the Mendeleev's periodic table. The salt of milk constitutes a small portion of milk (8-9 g L⁻¹); this component contains calcium, magnesium, sodium and potassium for the main cations and inorganic phosphate, citrate and chloride for the main anions. In milk, these ions are more or less associated between themselves and with proteins. Depending on the type of ion, they are diffusible (cases of sodium, potassium and chloride) or partially associated with casein molecules (cases of calcium, magnesium, phosphate and citrate), to form large colloidal particles called casein micelles (Gaucheron 2005).

This indicator is not standardized by the regulations of Ukraine. In our experiment, the mass fraction of minerals in the milk of the experimental group of animals tends to increase by 13.2% compared to the reference group.

**Mass fraction of water and freezing point**

The water in milk indicates its falsification or violation of the processing technology. Therefore, there must be no water in the raw milk. This metric is related to the freezing point.

When milk is frozen and crystallized, energy is released in the form of structural heat, measured (by measuring the temperature) using direct cryoscopic methods (Tsekhmistrenko & Kononskyi 2014). The freezing point value is due to the number of truly soluble components of milk (lactose and mineral salts), the content of which in milk does not significantly change. When water is added to milk, the concentration of water-soluble substances changes, resulting in a change in the freezing point of milk (Rusko 2011).

Milk freezing temperature (point) is also currently controlled by Regulation (EC) No. 853/2004 within the range of 0.515. In DSTU 3662:2018, this indicator is not lower than 0.520. In the cows of the experimental group, there was a lowered freezing point by 8.1% compared to the reference group, remaining within the acceptable range.

**Conductivity**

An important indicator of milk quality is electrical conductivity. The conductivity of milk at 20°C is 3-6 mS/cm and varies depending on the concentration of ions in it. The addition of salts increases the concentration of ions and therefore increases the electrical conductivity of the milk. Adding water, proteins, sugar or insoluble salts reduces the concentration of ions and consequently reduces the conductivity of milk. This indicator is used to identify the milk of cows with mastitis. Electrical conductivity can also be used as an indicator of inflammation of the mammary gland (Fernando et al. 1982; Paudyal et al 2020; Bonestroo et al 2022).

In our studies, the electrical conductivity was the same in the milk of both groups of cows (5.6 mS/cm). In our study, the conditions of chronic heat stress did not affect this indicator in dairy cows.

**Active acidity**

Active acidity of milk is a value that shows the concentration of free hydrogen ions in milk, that is, the degree of its acidity and alkalinity. In fresh milk, the active acidity is within the range of 6.6-6.8, that is, the milk has a slightly acidic reaction. As milk sours, this indicator gradually decreases. If the value is higher than 6.8, it indicates milk falsification (when substances are added to prevent souring) or mastitis (Santoso 2020; Tomovska et al. 2016). In our studies, this rate was 6.63 in both groups.

Marketability of milk is the quantity of milk sold to a dairy plant as a percentage of the milk produced in a year (Ilchuk et al. 2016). According to our data, in spring (in the reference group), the marketability of milk was 96.3%, and in summer (in the experimental group) it decreased to 96.1%.

Thus, under conditions of moderate heat stress there was a significant decrease in the content of MSNF, mass fraction of fat and protein (P<0.05) in milk. The mass fraction of lactose and minerals tended to increase.

A number of researchers note that even with moderate heat stress (with THI ≥72-78), there are significant changes in the body of milking cows, which can significantly affect the physiological state (Wolfenson et al. 2019), fertility and dairy productivity of cows (Skliarov et al. 2022; López-Gatius et al. 2020). In particular, along with an increased respiratory rate, increased heart rate and increased rectal temperature (Koshchavka et al. 2020; Mylostyvyi et al. 2021a), there are also significant changes in blood biochemical parameters (Bernabucci et al. 2010); the number of red blood cells and the hematocrit levels are low, the content of hemoglobin and the number of white blood cells are reduced (Baumgard et al. 2015; Revskij et al. 2019). It is likely that possible changes in the physiological state of...
cows did not significantly affect the milk quality indicators studied and covered in this article. However, our previous studies (Mylostyvyi et al. 2021b) showed an increase in the concentration of free fatty acids, including saturated fatty acids (SFAs) and polyunsaturated fatty acids (PUFAs) in the serum of cows under prolonged heat stress. In the case of negative energy balance (NEB) during extensive lipolysis of adipose tissue and the entry of non-esterified fatty acids into the bloodstream, the fatty acid profile of milk changes in dairy cows (Hammami et al., 2015). For example, this is due to an increased concentration of oleic (C18:1) and linoleic acid (C18:2) (Lu et al. 2013). The same trend in the composition of milk fat was observed by other researchers (Tian et al., 2016), who reported an increase in the concentration of EPA and PUFAs in the milk of cows due to heat stress. Further in-depth study of the fatty acid composition of milk using the method of gas chromatography will provide valuable data necessary to find hered management strategies to maintain high milk quality under seasonal heat stress.

It should be noted that according to the regulatory document of Ukraine (DSTU 3662:2018 Cow’s Raw Milk. Specifications), the tested milk of both groups met the standards specified in this document. In our opinion, this result can be associated with the activation of compensatory mechanisms in the body of milking cows, due to which it is possible to minimize the deviation of milk indicators from the physiological standards. These processes can be embedded genetically, which ensures the continued existence of the species and the survival of offspring. These mechanisms may be the subject of further scientific research.

CONCLUSION

During the period of continuous moderate heat stress in milking cows, a significant decrease in the content of milk solids-not-fat, mass fraction of fat and protein was observed. The mass fraction of lactose and minerals tended to increase. In general, milk quality indicators were within the physiological standards. It follows that the body of animals used internal compensatory mechanisms to normalize the composition of milk.

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