UDC 631.147:316.644
636.2:591.543.1
636.2:591.128
Acta Agriculturae Serbica, 28 (56), 107–125, 2023 doi: 10.5937/AASer2356107C

Publisher: Faculty of Agronomy Čačak

www.afc.kg.ac.rs

Acta Agriculturae Serbica

FACULTY OF AGRONOMY • ČAČAK •

Heat stress of dairy cows in Serbia (Review)

Marko Cincović[1*], Mira Majkić[1], Jovan Spasojević[1], Slavča Hristov[2], Branislav Stanković[2], Dimitar Nakov[3], Sandra Nikolić[1], Jovan Stanojević[1]

[1] *Faculty of Agriculture-Department of Veterinary Medicine, University of Novi Sad, Trg Dositeja Obradovića 8, 21000 Novi Sad, Serbia*
[2] *Faculty of Agriculture, University of Belgrade, Nemanjina 6, 11080 Beograd-Zemun, Serbia*
[3] *Faculty of Agricultural science and Food, 16-ta Makedonska brigada, 1000 Skopje, N.Macedonia*

**Corresponding author: mcincovic@gmail.com*

Received 14 August 2023; Accepted 7 November 2023

A B S T R A C T

The climatic conditions in Serbia are suitable for the development of heat stress in cows. The values of the bioclimatic THI indicator point to a positive trend in increase in the number of days during which the conditions for the occurrence of heat stress are present. In cows, a change in body temperature is noted, activation of the cooling mechanisms, along with reduced milk production and reproductive efficiency. The behavioral changes of cows occur on farms, and the level of welfare decreases slightly when measured according to animal-based criteria. Changes in productivity are mainly related to metabolic, hematological and inflammatory adaptations. The metabolic adaptation that leads to a decrease in milk production is related to an increase in sensitivity to insulin with reduced lipolysis, an increase in TNF-α in cows, as well as to metabolic rearrangements of the mammary gland. Reproductive efficiency is reduced and is characterized by reduced expression of estrus and reduced response to estrus induction procedures. High ambient temperatures, in in vitro conditions, can affect the stability of laboratory parameters in the blood serum of cows. Cows exhibit different profiles of resilience to heat stress, and the study of resilience curves, for productive and blood parameters, represents the starting point for selecting cows that will tolerate heat stress better. For this purpose, the cumulative effects of heat stress expressed in the area under the resilience curve can be used. Certain precautionary measures such as providing shade, cooling by using fans and sprinklers or some other advanced technological methods, can reduce losses during heat stress. All of the above indicate that it is necessary to pay extra attention to all aspects of health care and production on cow farms in Serbia during late spring and summer when the animals are exposed to heat stress.

Keywords: temperature-humidity index, milk production, reproduction, prevention, resilience, metabolism.

И З В О Д

У Србији постоје климатски услови за развој топлотног стреса код крава. Вредности биоклиматског ТХИ индикатора указују на позитиван тренд раста броја дана у којима се стварају услови за настанак топлотног стреса. Код крава долази до промене телесне температуре, покретанај механизама расхлађивања уз смањену производњу млека и репродуктивну ефикасности. На фарама се дешавају промене у понашању крава, а незнатно опада и ниво добробити када се мери према анимал-басед критеријумима. Промене у продуктивности основи имају везе са метаболичким, хематолошким и инфламаторним адаптацијама. Метаболичка адаптација која доводи до опадања у производњи млека везана је за повећање сензитивности на инсулин уз смањену липолизу, као и пораст ТНФ-α код крава, а дешавају се и метаболичка престројавања у вимену. Репродуктивна ефикасност је смањена и одликује се смањеним испољавање меструса и лошијим одговором на поступке индукције еструса. Високе амбијенталне температуре у ин витро условима могу утицати на стабилност лабораторијских параметара у узорку крвног серума крава. Краве показују различите профиле резилијентности на топлотни стрес, а проучавање кривих резилијенције за продуктивне и крвне параметре представља основу за одабир крава које ће боље толерисати топлотни стрес. У ту сврху се могу користити кумулативни ефекти топлотног стреса исказани кроз површину испод криве резилијенције. Одређене превентивне мере као што је обезбеђивање сенке, расхлађивање вентилаторима и прскалицама или коришћење других напредних технологија може умањити губитке током топлотног стреса. Све наведено указује да је потребно посветити додатну пажњу у свим аспектима здравствене заштите и производње на фармама крава у Србији током касног пролећа и лета када су животиње изложене топлотном стресу.

Кључне речи: индекс температуре и влажности, производња млека, репродукција, превенција, резилијенција, метаболизам.

1. Introduction

In the last two decades, and especially after 2006, we have witnessed a significant change in the climate, which is characterized by elevated ambient temperatures during the summer, an increase in minimum daily temperatures, and the occurrence of a fair number of days with extremely high temperatures. On July 24, 2007, the highest temperature was measured in Serbia, which was 44.9°C in Smederevska Palanka and 43.6°C in Belgrade, while in other, lower altitude regions, the temperature was mostly over

42°C, and in the mountainous regions over 30°C was measured (https://www.vremeradar.rs/). It is the year during which farmers encountered a significant decline in milk production for the first time. Our research on heat stress began a year earlier, in 2006, when we first demonstrated milk losses due to heat stress on a cow farm in South Banat, and described the possibilities of modifying the microclimate in order to prevent the negative impact of heat stress (Cincović, 2006). This was followed by a large number of projects of scientific and scientific-technological importance that had research tasks related to heat stress in cows, and were supported by the Ministry of Education, Science and Technological Development of Serbia (2011-2019) as well as the Provincial Secretariat for Higher Education and Scientific Research Activity of AP Vojvodina (2011-2012, 2016-2017 and currently active 2023-2024). A certain number of projects were indirectly related to the study of heat stress and welfare determination on farms, such as the bilateral project Serbia-Slovenia, financed by the Ministry of Education, Science and Technological Development of Serbia, as well as the Science Agency of Slovenia (2016-2017). In addition, we have tried to introduce the measurement of stress and welfare on farms through student activities and courses that we have prepared for veterinarians. The need to study and understand this topic has arisen from the fact that even during the ongoing year 2023 we have encountered extreme climate changes, characterized by high temperatures during July, so for the first time in many countries over 49°C has been measured (Mediterranean, some locations in China and Canada). Unfortunately, after such climate changes, supercell storms occurred that caused significant damage in the territory of Serbia, so a new issue of protecting animals and animal housing facilities from natural disasters caused by wide temperature variations arose. In addition, the decreasing content of humus in the soil appears to be a problem, and it is considered that by the end of this century the soil in Vojvodina will become a desert-like soil if the number of animals and the amount of manure originating from animals are not increased or some new biotechnical solution to these problems is not introduced. The poor quality of the soil also means the poor quality of cereals and coarse feed, which are the basis of the nutrition of cows for the production of quality milk.

Our research activities have contributed to the fact that, over time, we have obtained a large number of scientific results characteristic of the territory of Serbia, that is, for the region of Vojvodina, where the farms the research was carried out on were located. The aim of this paper is to critically present our results.

2. Homeothermy of cows, thermoneztral zone and heat stress

Cattle are homeothermic animals (Collier and Gebremedhin, 2015), which means that their body temperature is constant. The body temperature of cows measured per recti is 38.5-39.5°C. Body temperature is maintained by the hypothalamus center. The experiment proved that the preoptic region of the hypothalamus contains a large number of neurons sensitive to heat, while in other parts of the hypothalamus, as well as in the septum and reticular formation of the brain, there are neurons sensitive to cold. Cold-sensitive neurons are few in number. The CNS has a more developed system for feeling heat, and the periphery of the body has a more developed sense of cold (skin receptors). In the corpora mammillary of the posterior hypothalamus, signals arrive from the preoptic region and the periphery of the body, so they together stimulate reactions to generate or radiate heat. This system is called the hypothalamic thermostat. The formula that shows the body temperature of animals looks like this: Body Temp. = Metabol. heat ± Conduction ± Convection ± Radiation + Evaporation (Hristov, 2002).

Heat stress could be defined as a condition in which an organism's body is exposed to excessive heat that exceeds its ability to dissipate heat and maintain normal regulatory functions, and during which a whole series of biochemical, hematological, molecular and genetic adaptations are triggered (McManus et al., 2020). The concept of stress was created and developed by Claude Bernard, Walter Cannon, Hans Selye and John Mason (Hristov, 1991; Cincović, 2010). Subsequent research, which has been carried out to this day, represents an upgrade on the foundations laid by these scientists. In 1878, Claude Bernard described the animal's efforts to overcome unfavorable environmental factors. Walter Cannon, a physiologist at Harvard University, was the first to link the emotional state to the activity of the adrenal gland. He was the first to use the term stress for the process of overcoming a certain effort, although the term distress with a similar meaning existed five centuries earlier, and was described as a state of endurance, pain, anguish, etc. According to his theory, various negative factors (hypothermia, bleeding, trauma) activate their own mechanisms to preserve homeostasis. This theory of activating different mechanisms in maintaining homeostasis was valid until Hans Selye discovered the universal stress reaction of the organism. Research has shown that stress is a sum of different stimuli that damage or can cause damage to the body, and are able to stimulate the secretion of adrenocorticotropic hormone - ACTH, with a consequent effect on all physiological aspects of the body. Physical stressors were considered significantly more potent in causing stress and inducing GAS compared to psychological stressors, until in the sixties, John Mason pointed out the role of psychological factors in the occurrence of stress. His research, in which the concept of the psycho-endocrine mechanism was established, proposes the response of the hypothalamic-pituitary axis as a specific reaction to psychological stress, and a non-specific reaction to all other stressors. Mason had determined in his experiment that a sudden and unexpected increase in the external temperature from 20 to 30°C led to an emotional response in Rhesus monkeys and to an increase in the concentration of urinary cortisol. A gradual increase in the enclosure temperature over a period of 15 days resulted in a decrease in urinary cortisol concentration. A similar situation has been described in cattle. Another important conclusion of this researcher was that stress had activated multiple and competing neuroendocrine systems. Considering that, he monitored the changes of many hormones after the stressors had been applied. At that point, the ambient temperature had become a very interesting stressor, which gained importance with global warming and climate change.

The body needs energy for every activity, including cooling and heating processes. The biggest issue concerning thermal stress is that part of the productive energy (energy that should be used for production) is spent on cold thermoregulation when the animals are outside the thermoneutral zone. If the animals are situated in an area of increased temperature, a decrease in thermal energy production will occur, the animals will exhibit lower food intake, and cooling mechanisms such as conduction will appear. Considering a reduced intake and increased consumption of energy, changes in productivity and health of the cows could be inevitable (Cincović and Belić, 2020). According to the previous research, the temperature range for cattle in which there was a thermoneutral zone, i.e. in which there was no thermal stress and energy consumption for cooling or heating, varied from -25°C and even -30°C to 25°C (Hristov, 2002). The maximum temperature that we encounter in everyday life is much higher than 25°C, so the necessary prerequisites for the development of heat stress in cows are present.

3. Environmental factors, bioclimatic temperature humidity index and heat stress in cows

To understand the heat load of cows in Serbia, it is necessary to consider the annual reports of the Republic Hydrometeorological Institute of Serbia. This respectable institution has been monitoring the climate of Serbia since the end of the 19th century. Temperature data originates from publicly available reports from the website https://www.hidmet.gov.rs/ (accessed in July 2023). According to the Institute's measurements, thirteen of the fifteen warmest years in Sebia were registered after 2000 (observed for the period 1951-2019), while in Belgrade fourteen of the warmest years were registered after 2000 (observed for a much wider period from 1888-2019). The above data shows that after the year 2000, certain climatic deviations occurred that created conditions for heat stress. Vranić and Milutinović (2016) concluded that Serbia was most likely going to experience an increase in average temperature of up to 4°C and a decrease in summer precipitation of up to 50%. Linear changes and deviations indicate that these changes might be more dramatic in the future.

Table 1.
Temperature in Serbia from 2017 to 2022 (according to the official data of the Hydrometeorological Institute of Serbia)

Year	Average temperature	Maximum temperature, tropical days and nights
2017	On the territory of Serbia, the year 2017, with an average air temperature of 11.5°C, is the twelfth warmest year in the period from 1951 to the present day, in Loznica and Ćuprija the fourth warmest, in Negotin the fifth and in Belgrade the seventh warmest since the beginning of the meteorological service stations (1888). The average annual air temperature ranged from 10.4°C in Požega to 13.9°C in Belgrade, while in mountainous areas from 4.4°C in Kopaonik to 8.6°C in Zlatibor. The deviation of the mean annual air temperature relative to the reference period 1981-2010. ranged from 0.5°C in Sjenica and Zaječar to 1.3°C in Zrenjanin, Loznica, Belgrade and Cuprija. According to the percentile 1 distribution, the year 2017 was in a warm category in most parts of Serbia, very warm in the northwestern and northeastern regions, normal in Sjenica, and extremely warm in Ćuprija.	The highest daily air temperature in 2017 was measured on August 6 in Ćuprija and was 41.6ºC. Most of the country recorded 17 to 25 tropical days more than the average number for the reference period 1981-2010. The highest number of tropical days was recorded in Negotin and was 68 days, which is 24 days more than the average number of tropical days for that station. 63 tropical days were recorded in Belgrade, which is 26 days more than the average values for Belgrade. 39 tropical nights were registered in Belgrade, which is 22 nights more than the average, then 25 tropical nights were recorded in Zrenjanin, 19 more than the 1981-2010 average. A greater number of tropical nights were observed in the north, part of eastern, western and central Serbia.
2018	The year 2018 is a year of climatic records in Serbia: - the warmest in the history of meteorological measurements, - the warmest spring, - the warmest April, - the warmest summer according to the minimum temperature. On the territory of Serbia, the year 2018, with an average air temperature of 12.0°C, is the warmest in the period from 1951 to the present day, and in Belgrade, with 14.5°C, it is the warmest year since the beginning of the meteorological station's operation (in 1888). The average annual air temperature ranged from 10.9°C in Požega to 14.5°C in Belgrade, and in mountainous regions from 5.2°C in Kopaonik to 9.1°C in Zlatibor. Deviation of the mean annual air temperature in relation to the reference period 1981-2010. ranged from 0.6°C in Zaječar to 2.0°C in Belgrade. According to the distribution of percentile 1, the year 2018 was in the extremely warm category in most parts of Serbia.	The highest daily air temperature in 2018 was measured on June 12 in Kraljevo and was 35.4°C. In most of Serbia, 2 to 17 tropical days more than the average numbers for the reference period 1981-2010 were recorded. The highest number of tropical days was recorded in Negotin and was 70 days, which is 26 days more than the average number of tropical days for that station. 49 tropical days were recorded in Belgrade, which is 12 days more than the average number. 42 tropical nights were registered in Belgrade, 25 nights more than the average. 15 tropical nights were recorded in Negotin, 12 in Palić, and less than eight in the rest of Serbia.
2019	2019 - THE YEAR OF CLIMATE RECORDS IN SERBIA: -The warmest in Serbia since 1951, and the warmest in Belgrade since 1888, -The average air temperature in 2019 was 12.3°S in Serbia, 0.3°S higher than the previously warmest 2018, - Average air temperature in Belgrade in 2019 was 14.7°S, 0.2°S higher than the previously warmest year 2018, - The third warmest June 2019 in Serbia according to the minimum temperature, - The warmest October 2019 in Serbia according to the maximum air temperature, - The warmest November 2019 in Serbia, - The warmest autumn 2019 in Serbia. In Serbia, the year 2019, with an average air temperature of 12.3°C, was the warmest in the period from 1951 to the	The highest daily air temperature in 2019 was measured on August 12 in Leskovac and was 38.1°C. The highest number of tropical days was recorded in Negotin and Leskovac and was 71 days. In most of Serbia, three to 29 tropical days more than the average numbers for the reference period 1981-2010 were recorded. 59 tropical days were recorded in Belgrade, which is 22 days more than the average number. 48 tropical nights were registered in Belgrade, 31 nights more than the average. 14 tropical nights were recorded in Negotin and Zrenjanin, 11 in Palić and Loznica, and less than eight in the rest of Serbia.

	present day, and in Belgrade, 14.7°C, the warmest since the beginning of the meteorological station's operation (in 1888). The average annual air temperature ranged from 10.9°C in Požega to 14.7°C in Belgrade, and in mountainous regions from 5.2°C in Kopaonik to 9.4°C in Zlatibor. Deviation of the mean annual air temperature in relation to the reference period 1981-2010. ranged from 1.2°C in Zaječar and Požega to 2.2°C in Belgrade. According to the distribution of percentile 1, the year 2019 was in the category of extremely warm throughout Serbia, except in Zaječar, where it was in the category of very warm.	
2020	The year 2020 in Serbia, with an average air temperature of 11.7°C, was the seventh warmest year in the period from 1951 up to the present day, and in Belgrade, with 13.9°C, was the ninth warmest since the beginning of the meteorological station's operation (1888.). The average annual air temperature ranged from 10.6°C in Požega to 13.9°C in Belgrade, and in mountainous regions from 5.0°C in Kopaonik to 8.8°C in Zlatibor. Deviation of the mean annual air temperature in relation to the reference period 1981-2010. ranged from 0.9°C in Zaječar, Kruševac, Sjenica and Požega to 1.8°C in Negotin, and 1.4°C in Belgrade. According to the percentile distribution in 2020, the year was in the very warm category in most of Serbia, while it was in the extremely warm category in Negotin, Kuršumlija, Ćuprija, Dimitrovgrad and Kopaonik. The year was average in precipitation in most of Serbia, and very rainy and extremely rainy in the south, southwest and southeast parts. Snow was not noted for the first time in Kikinda; the lowest number of snow days was registered in Loznica, Negotin and Zaječar; the latest date of the snowfall recorded in Belgrade. The summer of 2020 was the second wettest summer in Serbia in the last 70 years. The driest April in Veliko Gradište and at Crni Vrh; the rainiest August in Sjenica; the driest September in Zlatibor; the driest November in Kuršumlija; the hottest December in Banatski Karlovac and on Kopaonik.	The highest daily air temperature in 2020 was measured on August 31 in Cuprija and was 36.9°C. The highest number of tropical days was recorded in Negotin and it was 63 days. In most of Serbia, three to 19 more tropical days were recorded from the average number for the reference period 1981-2010. 48 tropical days were recorded in Belgrade, which is 11 days more than the average number. 32 tropical nights3 were registered in Belgrade, 15 nights more than the average. Negotin recorded 14 tropical nights, Zrenjanin 11, and the rest of Serbia less than seven. Tropical nights were not recorded in the southwest and southeast of Serbia.
2021	On the territory of Serbia, the year 2021, with an average air temperature of 11.4°C, was the sixteenth warmest year in the period from 1951 to the present day, and in Belgrade, with 13.7°C, it was the twelfth warmest since the beginning of the meteorological station's operation (1888). The average annual air temperature ranged from 10.3°C in Požega to 13.7°C in Belgrade, and in mountainous regions from 4.3°C in Kopaonik to 8.7°C in Zlatibor. Deviation of the mean annual air temperature in relation to the reference period 1981-2010. ranged from 0.5°C in Sombor to 1.2°C in Belgrade, Negotin and Cuprija. According to the percentile1 distribution, the year 2021 was in the warm and very warm category.	The highest daily air temperature in 2021 was measured on June 30 in Smederevska Palanka and was 40.7°C. The highest number of tropical days2 was recorded in Leskovac and was 71 days. In most of Serbia, 49 to 69 tropical days were recorded, which is 13 to 32 tropical days more than the average number for the reference period 1981-2010. 60 tropical days were recorded in Belgrade, which is 23 days more than the average number for the reference period 1981-2010. 45 tropical nights were registered in Belgrade, 28 nights more than the average. 22 tropical nights were recorded in Negotin, 18 in Zrenjanin and Novi Sad, 17 in Valjevo, 16 in Loznica, and less than 15 in the rest of Serbia. Tropical nights were not recorded in the southeast and parts of western and central Serbia.
2022	In Serbia, the year 2022, with an average air temperature of 12.1°C, was the second warmest year (Figure 1) in the period from 1951 to the present day, and in Belgrade, with 14.5°C, it was the second warmest since the beginning of the meteorological station's operation (1888). In Negotin and Sombor, 2022 was the warmest year since the beginning of measurements at those stations. The average annual air temperature ranged from 10.9°C in Požega to 14.5°C in Belgrade, and in mountainous regions from 4.9°C in Kopaonik to 9.2°C in Zlatibor. Deviation of the mean annual air temperature concerning the reference period 1991-2020. ranged from 0.6°C in Leskovac to 1.4°C in Negotin and Novi Sad. According to the distribution of percentile 1, the year 2022 was in the category of very warm and extremely warm in most of Serbia.	The highest daily air temperature in 2022 was measured on July 23 in Smederevska Palanka and was 40.6°C. The highest number of tropical days2 was recorded in Veliko Gradište and Leskovac and counted 72 days. In most of Serbia, 40 to 70 tropical days were recorded, which is five to 26 tropical days more than the average number for the reference period 1991-2020. 65 tropical days were recorded in Belgrade, which is 25 days more than the average number for the reference period 1991-2020. 40 tropical nights3 were registered in Belgrade, 14 nights more than the average. 20 tropical nights were recorded in Negotin, 14 in Zrenjanin, 13 in Palić, 12 in Novi Sad, 11 in Sombor, and less than nine in the rest of Serbia. Tropical nights were not recorded in the southeast and parts of western and central Serbia.

In addition to the increase in average temperatures, the maximum measured temperature and the increase in temperature deviations indicate a significant problem with heat stress, a very important problem that causes heat stress is the development of heat waves. Heat waves represent large anomalies in the ambient temperature so that the temperature deviates by more than 5 as opposed to the multi-decade average (the standard was defined by each country for itself according to geographical characteristics). These heat waves occur in the hottest part of the year (July and August), although deviation waves can also occur in other seasons. During heat waves, there is high humidity, days and nights become tropical, and overall conditions are created for the development of major heat stress in animals and humans. The number and duration of heat waves are increasing with global warming (Guo et al., 2017). If environmental protection measures are not performed and warming is not reduced, we will have about 4 more heat waves than to this day, which will be significantly longer, but also certain extreme years may become one almost uninterrupted heat wave. In this case, we will have summers during which the maximum daily temperatures will not drop below 35°C. High temperature anomaly was recorded in June 2023 (Figure 1, left). July 2023, which is currently ending at the moment of writing of this paper, represents the hottest July since the pre-industrial era and will likely be declared the world's hottest month ever. The Earth was on average 1.5°C warmer (statement for the Guardian, by António Guterres from the United Nations, reported in the Serbian daily newspaper on 07/27/2023). According to the weekly bulletin of the Hydrometeorological Institute of Serbia (bulletin for the week from July 17 to 23, 2023; source: https://www.hidmet.gov.rs/latin/meteorologija/klimatologija_produkti.php#) during the previous week, the situation was the following: the deviation of the maximum daily air temperature in relation to the reference period 1991-2020 was from +2.0 to +6.0°C; the deviation of the minimum daily air temperature in relation to the reference period 1991-2020. was from +2.1 to +5.0°C; the highest daily air temperature was 38.2°C, and was measured on July 17 in Ćuprija and Sombor; five to seven tropical days were registered in most of the country; a heat wave was recorded in the period from July 15 to 19 in Palić, Kopaonik, Sremska Mitrovica and Dimitrovgrad; at several stations, on July 17, the daily air temperature maximums were exceeded or equaled, i.e. the highest temperatures were measured on July 17, since measurements had been performed at those stations, namely Palić, Kikinda, Crni Vrh, Zlatibor, Kopaonik and Dimitrovgrad. Temperature trends and deviations in the period 1979-2023 can be seen in Figure 1 (Right). In previous measurements, 2022 was the globally warmest year, while previous measurements confirmed that 2023 would be the warmest year so far.

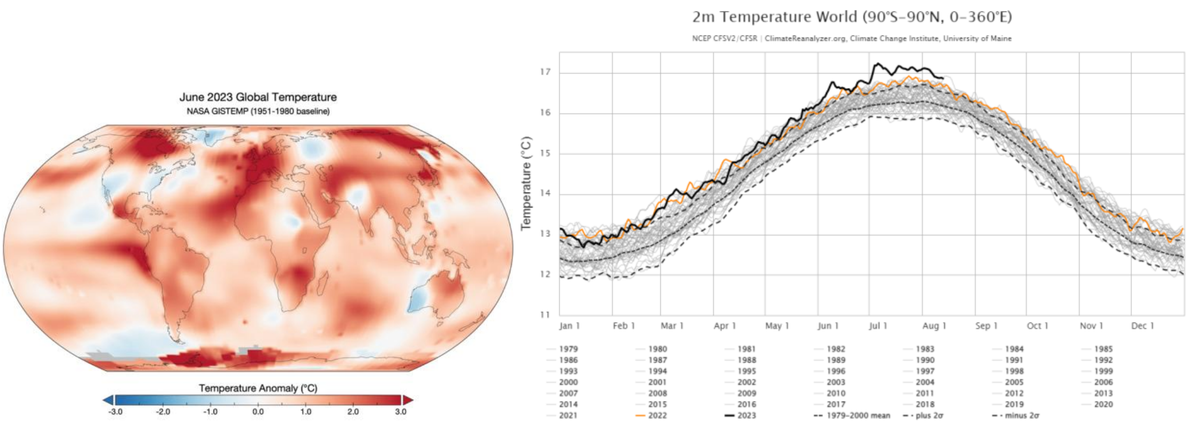

Figure 1. Temperature anomaly in june 2023 (left) and temperatures from 1979 to 2023 (right) (source: https://climatereanalyzer.org/clim/t2_daily/). Bold black line represents data in actual year 2023.

Temperatures significantly exceed the biological optimum in cows. In addition to temperature, air humidity must also be taken into account in order to assess the stressogenic index of the high ambient temperatures. That is why wet bulb temperature is introduced as a parameter. Namely, we are talking about the temperature of a wet thermometer, which is an ordinary mercury thermometer around which a cotton cloth moistened with water is wrapped. The water from this cloth evaporates and cools the surrounding air, as a result of which the wet thermometer shows a slightly lower temperature than an ordinary, dry thermometer, which shows the ordinary air temperature. The greater the temperature difference between these two thermometers, the drier the air is. The amount of water vapor air can contain depends on its temperature. The higher the temperature, the more water vapor the air can contain. When the air is saturated with water vapor, i.e. when the maximum amount of water vapor for a given temperature is reached in the air, all evaporation stops. Similar values of the wet bulb and the dry bulb temperatures indicate increased relative humidity. When these temperatures are equalized, the air is saturated with water vapor, so the evaporation of water from the cloth, and also the evaporation of liquids that cool the body stops. Warm and moist air is more dangerous than warm and dry air. It is considered that a safe wet bulb temperature for humans is up to 35°C, although according to recent research, this temperature is lower and is proposed to be up to 31°C (Sherwood et al., 2010; Raymond et al., 2020). In our region, the optimal WBT range from 16 to 28°C/30°C (source: https://news.climate.columbia.edu/wp-content/themes/sotp-foundation/dataviz/heat-humidity-map), so it can be considered safe. The number of deadly days for humans is 3, and by the year 2100, it will increase to almost 10 days. However, in the European countries of our region, which are closer to the Adriatic and Mediterranean seas, the increase in fatal days will be significantly higher after 2030, so that in 2100 it may reach a level of over 40 fatal days (https://geoxc-apps.bd.esri.com/MoraLabs/GlobalRiskOfDeadlyHeat/index.html#) .Due to heat waves and rapid climate transfers from this region to our country, it is necessary to know the above-mentioned data. In cattle, the adaptation to heat stress which implies changes in body temperature, food and water intake, changes in heart rate, and acid-base status occurs when the WBT is higher than 28 or 30°C (Beatty et al., 2006).

Temperature-humidity index (THI) is used as a much more sensitive indicator of heat load in cows all over the world. The THI index is calculated in several ways, using dry bulb temperature, wet bulb temperature, relative humidity or dew point temperature using different formulas (Behera et al., 2020). Different formulas have been made in order to obtain the best possible correlation of the THI index with different productive parameters in cows. Many of them exhibit better predictive effects on the health and productivity of cows in certain parts of the world, while in others these correlations are less significant. The dynamics of the change in the value of the THI index on the territory of Vojvodina is identical regardless of the formula that was used (Spasojević et al., 2023), as shown in Figure 2a. Practically, it is best to use the THI index, which uses dry bulb temperature and relative humidity, because these data are easily available by measuring in the animal housing facility or from the official data by meteorological stations. The trend of THI values calculated using 10 different formulas is shown in Figure 2a. The threshold value of the THI index which causes a medium-strength response to heat stress in cows is 72. This index value will already be reached at a temperature of 26°C degrees if the air humidity is 40%. Severe heat stress will already appear at a temperature higher than 35°C (THI is above 80). Considering the temperature oscillations in Serbia in the past decade, it is clear that very favorable conditions are present for the development of heat stress in cows. THI values of 68 to 72 indicate mild heat stress from the point of milk production, which depends on the lactation period of the cows (Ouellet et al., 2021; Yan et al., 2021).

In our research, we have investigated the values of the THI index during the ten-year period from 2005 to 2016, the values of THI obtained from different measuring posts in Vojvodina and examined the trend of changes in the values of the THI index (Cincović et al., 2017; Majkić et al., 2017c, 2020). The results indicated that the THI value ranged from 40.3 in January to 77.5 in July. The average maximum value of THI in the period from 2005 to 2016 indicated the existence of heat stress during May, June, July and August, and September reached the critical value of 72 during a certain number of days. In the period from 2005 to 2016, a positive linear trend of THI values was noted, measured in the warmest part of the day. There was a statistically significant positive linear trend of increasing THI values during every month, except for January, October and November. The linear regression equation demonstrated that from 2005 to 2016 a trend of increase in the average maximum THI index existed during summer months: June an increase of about 0.17 per year, July an increase of about 0.29 per year and August an increase of about 0.51 per year (Figure 2b). The number of days during which the THI index was outside the cow's thermal comfort area for more than 12 hours a day has been increasing since 2005. In 2007 and 2012, extremely negative conditions persisted with the THI index above the optimum for more than 12 hours a day, which also occurred in 2017, 2018, 2022 and 2023. In another study, we investigated THI values based on temperature and humidity data obtained from seven measuring posts on the territory of Vojvodina. The lowest values of the THI index were measured in Palić (THI 75-86), and the highest values were measured in Zrenjanin (THI 77-93). A positive correlation between the measured values of the THI index from all seven measuring places on the territory of AP Vojvodina was determined, and the correlation was at the level of r=0.662 to r=0.858 (P <0.001). The deviation of the measured THI values from the general average for Vojvodina, for each measuring site, was in the range of 0-4%, and a small number of individual measurements exhibited a deviation of more than 5%. The possibility of predicting the maximum value of THI during June, July and August based on the value from May was possible because a statistically significant linear relationship existed.

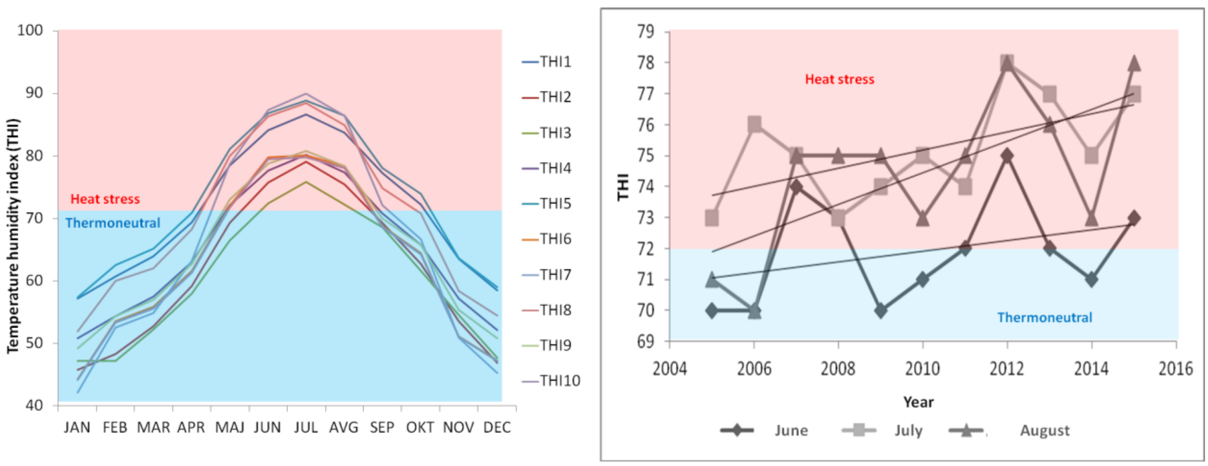

Figure 2. THI value in Serbia (Vojvodina) - a) Average value of THI index in 2022 calculated using ten types of formulas for THI (Spasojević et al., 2023) b) Trend of change in THI value during summer in the period 2005-2015 (Majkić et al. al., 2017c)

Several authors (Broucek et al., 2007; Könyves et al., 2017; Menegassi et al., 2016) calculated that the average value of the THI index was 78.4 in the period from May to August. The researchers concluded that 96% of the days during the study period had critical values of the THI index. In addition to temperature and relative humidity, THI index values were also influenced by the direct effect of solar radiation and air movement. The value of insolation positively correlated to the maximum measured values of the THI index (Majkić et al., 2019). The greatest insolation was present during the summer months when the ventilation systems were not sufficient enough to correct the high ambient temperatures and high air humidity. Data on insolation, average day and night temperature, and average day and night air humidity for May, June, July and August in the past decade were included in the research. The data were obtained from the Hydrometeorological Institute of Serbia report and calculated at the level of monthly averages. The linear regression and correlation between insolation and air temperature and humidity, as well as the maximum calculated values of the THI index during the day, were examined. The test results demonstrated a positive correlation between insolation and day and night temperature and a negative correlation with air humidity measured during the day and night. The value of insolation positively correlated with the maximum calculated values of the THI index. The next important factor that affected the value of the THI index was wind speed. Herbut (2018) explained that a higher air flow speed led to cooling by convection, which could affect the reduction of the THI index value during the summer months. Mader et al., (2006) proved that with every increase in wind speed by 1 m s^{-1}, the THI index decreases by 1.99 units. Ventilation as a way of protecting cows from seasonal variations in milk production was proposed by Cincović (2006), even a year before the development of the more extensive climate changes we see today. Therefore, the introduction of ventilators is one of the precautionary measures on farms. The results from neighboring Croatia also confirmed that during elevated values of the THI index, a decrease in milk production in cows had occurred, so THI had been confirmed as a good index of the stress load of cows (Gantner et al., 2011). The upper critical level of THI was also determined in calves and was 88, while in cows deaths were possible at THI=84 (Kovács et al., 2020).

Changes in THI values during the year, reaching critical values and the tendency to increase maximum THI values with significant climatic changes and deviations indicate that heat stress in cows is a significant problem, which we will be facing more often in the future.

4. The impact of heat stress on physical adaptation, behavioral characteristics and welfare of cows

Heat stress triggers numerous adaptation mechanisms of the organism. Primarily an increase in body temperature is noted, which causes activation of the mechanisms for reducing thermogenesis and increasing thermolysis, including reduced food intake, increased sweating, increased breathing frequency, searching for an adequate place to stand and lie down, etc. All of the above can affect the level of welfare on the farm.

Heat stress in dairy cows occurred during July and August (THI>72). The physical response in cows included an increase in rectal temperature and more pronounced diurnal variations during the summer (38.4:39, P <0.01), as well as a significant increase in respirations per minute (46.5:65, P <0.01) and a decrease in ruminal contraction (10.75:5.5, P <0.01) (Belić et al., 2010). In the following study, the association of THI with physical response in cows was examined. The study aimed to examine the relationship between rectal temperature and respiration frequency and milk production during heat stress (Cincović et al., 2011). It was determined that an increase in the THI value causes the breathing frequency to increase, so for each THI unit above 64, the frequency increases by 0.6. The correlation coefficient between breathing frequency and THI was at the level of 0.55 (P <0.05). The rectal temperature in cows increased by 0.09°C for each THI unit above 64. The correlation between THI values and average rectal temperature was highly significant and reached up to 0.92 (P <0.01). The respiration rate and THI exhibited a slight linear dependence, probably because there was a mild to moderate thermal load on the cows during the experiment. The skin and respiratory system of cattle have a great capacity to dissipate heat through evaporation, sweating and panting. Increased respiratory rate is the result of the body's tendency to give off excess heat by evaporating through the lungs (Gaughan et al., 2000).

The respiratory dynamic and panting score (PS from 0 to 4) of cattle are in a close relationship (Islam et al., 2020): 0-Normal breathing, no forward–backward heaving. Respiration rate <60 breaths min^{-1}; 1-Forward–backward heaving, mouth closed, no drool or foam, easy to see chest movement. Respiration rate between 60 and 100 breaths min^{-1}; 2-Forward–backward heaving, mouth closed, but drool or foam present. Respiration rate between 100 and 120 breaths min^{-1}; 3-Forward–backward heaving, mouth open or intermittent mouth open, excessive drooling, tongue not extended, neck extended, and head held up. Respiration rate between 120 and 160 breaths min^{-1}; 4-Forward–backward heaving, open mouth with tongue protruding either occasionally or for prolonged periods, excessive drooling, neck extended, head held up or down. Respiration rate >160 breaths min^{-1} and may be variable due to phase shift in respiration.

Sweating in dairy cows occurs in two forms, insensible sweating (perspiration, which occurs constantly), and visible sweating, when the ambient temperature rises significantly. The temperature required to turn water into water vapor is called the latent vaporization temperature, and the energy is consumed during this process. Vasodilatation increases blood flow to the periphery of the body, to the subcutaneous tissue and sweat glands, when sweating increases significantly. In dairy cows, the maximum evaporation from the skin surface in the amount of 150g m^2 h^{-1} occurs if the ambient temperature is 40ºC, while the evaporation through the respiratory system accounts for about 30% compared to the skin. Quantifying sweating is difficult to perform, so for stress assessment, body temperature is determined by different methods: rectal measurement, tympanic temperature measurement, carotid blood temperature measurement, infrared thermography or subcutaneous implantation of telemeters connected to the software. Any object at a temperature above absolute zero (-273°C) emits thermal energy in the infrared region. A thermal imaging camera is a device for non-contact recording of heat emission, i.e. infrared radiation, so they are called infrared cameras. The result of recording with a thermal imaging camera is a photograph - a thermogram. Thermal cameras contain quantum detectors that collect quanta of a certain amount of thermal energy, and for this purpose, cadmium mercury telluride is most often used. Changes in the electrical resistance of the detectors occur when a quantum of heat energy is received, and these changes, detected by the sensors and processed by an electronic unit form a thermogram (Corsi, 2010).

In one study, the authors concluded that the following technological, biological and environmental factors affected the temperature values that would be displayed on the thermogram (Montanholi et al., 2008): there was a high reproducibility of the thermogram values if they were made within 10 seconds, the measured temperature decreased with the distance between the camera and the cow, the results were consistent even when recorded by more different people, wind and debris (various debris) on the skin reduced the temperature of the body surface, exposing the body to direct sunlight increased the surface body temperature but the values returned to the initial level after a few minutes spent in the shade, the physical activity of the animal increased the body temperature, the administration of sedatives and anti-sedatives affected the temperature of the body surface, and the imprint of the fetus caused higher temperatures of the body surface of pregnant cows in the thermogram. In our experiment, the significant impact of the season and exposing the body to direct sunlight on the characteristics of the thermogram in cows was confirmed, and the temperatures measured in almost all anatomical regions exhibited a positive correlation with the THI index (Cincović et al., 2017; Belić et al., 2017; Spasojević et al., 2023). The thermogram of the cow's body during the heat stress when the cows were exposed to direct sunlight is shown in Figure 3. The temperature of the body surface is almost 10 degrees higher compared to the temperatures in the shade and the thermoneutral period and exceeds the upper physiological value for the temperature measured rectally. Physical and metabolic responses are interrelated. Our latest results show that in heat-stressed sheep there is a significant correlation between body surface temperature measured by infrared thermometry and THI index values and numerous blood metabolic parameters (Čukić et al., 2023). In a future experiments, we will examine these associations in dairy cows.

Increased ambient temperature leads to certain behavioral changes in dairy cows. The basic behavioral changes during heat stress in dairy cows are reduced food intake, increased water intake, and also a disturbed ratio between standing and lying down during the day. When exposed to high temperatures, cows tend to lie down more. However, if the substrate is not of sufficient quality, the cows will stand for a long time, which additionally burdens the hooves and enhances the development of lameness. If a large number of cows are standing or lying near natural openings on the farm, or in the shade of a drain or a tree (if they were let out to graze), and they are also huddled together, we can assume that the ambient conditions are not in the thermoneutral zone. Other interesting signs that can be noticed during the inspection are the increased humidity of the nasal mirror and the leakage of saliva from the half-opened mouth, and in extreme situations, dryness and peeling of the mentioned parts. Cows that are situated in the paddocks or loose at the pasture usually take a position towards the sun, with their head opposite to the sun, and their body positioned longitudinally, to reduce the impact of direct solar radiation and the possibility of hyperthermia (Silanikove, 2000).

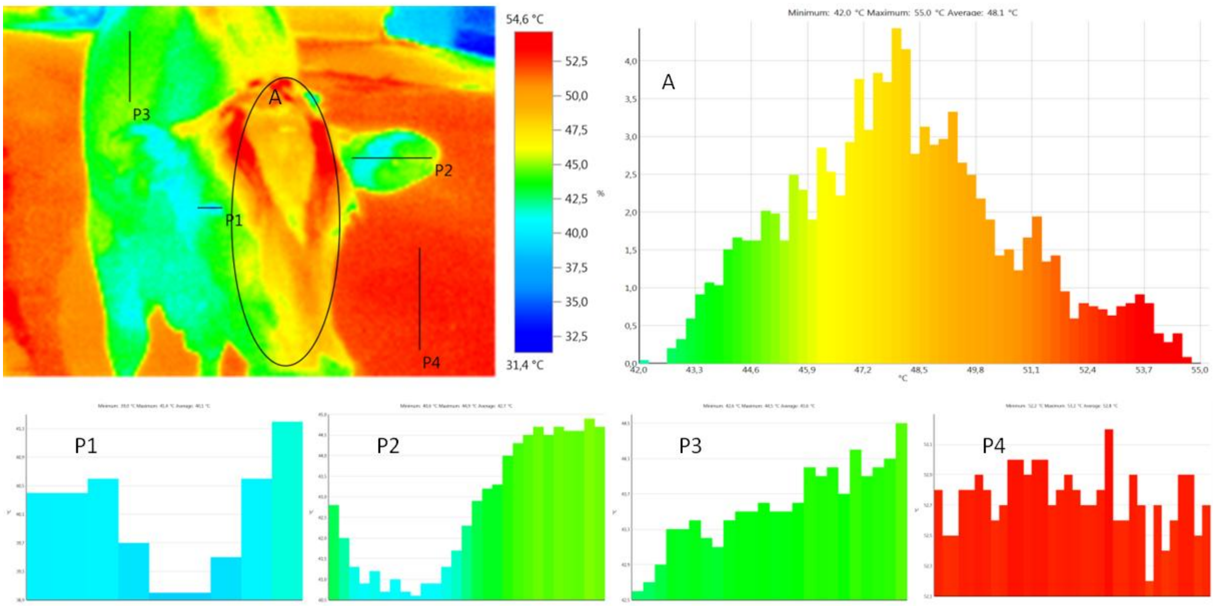

Figure 3. Infrared thermogram of cows in heat stress exposed to direct sunlight (original photo). A-head area 42-55°C; P1-eye area 39-41.4°C; P2-ear area 40-44.9°C; P3-abdomen area 42.6-44.5°C; P4-floor area 52.2-53.2°C.

In our previous research (Cincović et al., 2011a), which had been carried out for several days on a farm of 200 cows, we determined that during heat stress the feeders were more filled after a meal (due to reduced consumption), which correlated with the THI index. During heat stress, food consumption dropped significantly, which was supported by a higher score of feeders filling. The feeder occupancy score in thermoneutral conditions averaged 2.2. This score suggested a normal diet because the amount of food consumed counted for 90-95% of the offered food. During heat stress, the feeder filling score was slightly above 3. This score indicated that the cows did not eat about 25% of the offered meal (Heinrich, 2004).

High ambient temperatures are a significant stressor that negatively affects the welfare of cows

(Polsky and von Keyserlingk, 2017). Knowledge of rearing conditions, animal need index and welfare principles is a significant prerequisite for the protection of animals from various stressogenic factors (Hristov et al., 2008, 2014, Hristov and Stanković 2009; Ostojić-Andrić et al., 2022, 2022a). Measurement of welfare on the cow farm was performed according to the Welfare Quality® scoring system protocol in the thermoneutral period and during heat stress. This system is an animal-based system for welfare assessment and is based on 4 principles, which are derived from 12 criteria, and the criteria are formed based on a large number of measurable indicators originating from animals.

In cows in heat stress, compared to the thermoneutral period, a higher score for the criterion of good nutrition (48:41; due to the reduced participation of extremely thin cows) and good housing was noted (67:75; due to the reduced participation of cows with skin injuries and soiled ventral parts of the body and limbs) and a lower score for the criterion of good health (36:45; due to an increased % of cows with difficulty breathing, nasal discharge, diarrhea, dystocia, etc.) and adequate behavior (49:62; due to agonistic behavior and the allowed distance test) in cows (Majkić et al., 2017a). The same authors determined that deviations of scores for certain criteria did not lead to significant changes in the overall welfare assessment on the farm, and did not affect the classification of the farms according to the welfare assessment. Additional research is needed to examine the effect of season on farm welfare scores.

5. Effect of heat stress on milk production and reproduction in cows

Milk yield is a quantitative trait, defined by several minor genes, and therefore milk yield is significantly influenced by environmental factors. Experimental studies during the eighties described that cows subjected to heat stress in experimental chambers consumed less food (13.6 vs. 18.4 kg day^{-1}; P<0.01), more water (86.0 vs. 81.9 L day^{-1}; P<0.01), and produced less milk (16.5 vs. 20.0 kg day^{-1}; P<0.01), compared to cows in a thermally neutral environment (Schneider et al., 1988). The correlation between the physical adaptation of cows and the amount of milk produced is shown in the results obtained by Cincović et al., (2011). The results of our research exhibited a very high negative correlation between respiration rate and milk production (-0.82; *P* <0.01), as well as between rectal temperature and milk production (-0.92, *P* <0.01). For each respiration unit (breath/min) over 45, the amount of milk decreased by approximately 0.3 L, while for each degree higher concerning the rectal temperature, milk production decreased by approximately 3.5 L (0.35L/0.1°C). Therefore, apart from the increase in the THI index in heat stress, homoeothermic discrepancies were significant for the change in milk production in cows.

The impact of the season on milk production was described by Cincović (2006), and the first review papers on the effect of heat stress on milk production and reproduction were published by Hristov et al., (2007a). Cincović (2006) determined that the correlation between temperature and milk production was at the level of 40% during the year, but when daily temperatures exceeded 25°C the correlation was significantly higher. For every 1°C above 25°C, the amount of milk on a farm of 200 cows decreased by 271.5 liters (1.3 liters/individual). In percentage terms, the total milk production decreased (in the period from July 15. -August 15, 2006) by 25-30% compared to the thermoneutral period. The same paper determined that: - the amount of milk fat decreased in the first warm days, but after the body had gotten used to the heat stress, the amount of milk fat stabilized, partly at a higher level.

The changes in the content of protein and dry matter of milk in the observed period were almost identical; - the number of somatic cells increased from April to June (up to 250,000 somatic cells, without the effect on the amount of milk) and then from the beginning of August to September (up to 500,000 somatic cells, possibly led to a decrease in milk production); - the number of bacteria in milk was related to the hygienic conditions of milk production, and since the hygienic criteria on the farm were satisfactory, the temperature did not impact the number of bacteria in milk. Hristov et al., (2006) determined that a large number of factors could influence the number of somatic cells, and one of the most significant factors was the season. Disinfection and good hygiene practice reduce the number of somatic cells in milk and other aspects of milk quality (Hristov et al., 2007, 2023, 2023a).

Cincović and Belić (2009) noticed a decrease in milk production and milk fat and protein in cows during the summer months. Cincović et al., (2010) confirmed that during heat stress, the amount of milk had decreased, but also that the number of somatic cells had increased and the reproductive efficiency of cows had decreased. In the following years, research teams in Serbia had been dealing with the quantity and quality of milk, but also the correlation between the THI stress index and the quantity of milk. Joksimović-Todorović et al. (2011) determined the following: -total average milk production per cow was significantly higher in spring period (42.74±4.98L) than in summer (39.60±5.09L; *P*<0.05); -a higher rate of milk fat was noticed in spring compared to the summer period, the level of significance being (*P* <0.01); - the content of protein in milk in spring period was 13% higher than in the summer (*P* <0.001). Although the authors found that the amount of milk fat and protein had decreased during the summer, further results of Cincović and Belić (2011a) showed that in cows in acute heat stress when exposed to direct sunlight, an increase in milk fat persisted and a decrease in protein, which was the result of sudden exposure to the negative ambient conditions as well as due to the development of a negative energy balance due to poor adaptation.

Exposure of cows to acute heat stress led to a negative energy balance that could be determined through the composition of milk (Cincović and Belić 2011a). Thirty-five Holstein cows during the second and third lactation were included in the experiment. Within 7 days the cows were exposed to direct sunlight for a period from 9am to 6pm. Collective and individual samples of milk from these cows were used for the determination of milk fat and protein after each day. The results showed that milk production decreased significantly during the first days of exposure to heat stress. The average concentration of milk fat was more than 45 g L^{-1}, while the average protein concentration was below 32 g L^{-1}. The duration of exposure to heat stress significantly affected milk production (L) and milk protein concentration (g L^{-1}). The largest number of cows (65.71%) developed a negative energy balance three days after the beginning of exposure to heat stress. Milk can be a useful indicator of energy balance, but only as an individual sample, because of variations in milk fat. These results show that heat stress in cows has a significant impact on energy metabolism, which will be discussed in the next chapter.

The decrease in milk production depended on the period of lactation, and the highest negative correlation between THI and milk production was noticed in early and middle lactation, while no significant correlation was noticed for the last third of lactation (Cincović, 2010). In the following experiment, a longitudinal study was performed at the level of the entire lactation period and the lactation curve. It was established that the highest decrease in milk production occurred in mid-lactation (Cincović et al., 2010). The obtained results are explained by the fact that in mid-lactation milk production is high, and depends on the amount of food ingested, which decreases during heat stress. In addition to the lactation period, it had been shown that cows producing more than 30 L of milk per day were much more sensitive to heat stress compared to those that produced less milk.

In addition to the reduced food intake, the disturbances in the digestion process are another reason for the reduced production and quality of milk. Namely, during thermal stress rumination is reduced (Aganga et al., 1990), as well as the blood flow through the ruminal epithelium (Hales et al., 1984). Decreased rumination may be related to a reduced concentration of somatotropic and thyroid hormones, which leads to a decrease in the level of metabolism (Beede and Collier, 1986), or it is related to a decrease in the pH value in the rumen (due to an increase in the concentration of lactic acid) during thermal stress (Yadav et al., 2013). One of the reasons for reduced food intake and disturbed digestion may be the slowed passage of chyme through the digestive organs during thermal stress (Silanikove, 1992). Reduced quantity and quality of milk during thermal stress can be directly related to the udder. It was found that during thermal stress, the secretory capacity of the udder is reduced (Silanikovie, 1992), the blood flow through the udder is reduced (Lough et al,, 1990), and the permeability of the pores between the cells of the mammary epithelium is disturbed, especially due to the action of cortisol as a stress mediator (Stelwagen et al,, 1998), which can be reasons for qualitative and quantitative changes in milk production. Finally, the occurrence of inflammatory processes in the udder can be a significant factor in reducing milk production (Hristov and Relić, 2003).

The reproductive efficiency of cows decreases during exposure to high ambient temperatures (De Rensis and Scaramuzzi, 2003; Dovolou et al., 2023). The results of the research obtained by Cincović (2010) indicated that the occurrence of the first visible postpartum estrus is prolonged during summer. 6.67% of cows calved in winter did not exhibit visible signs of estrus until 60 days postpartum, compared to 20% that calved in summer (P <0.01). The rate of conception after the first insemination decreased significantly during the summer months. By analyzing the regression of THI and the rate of conception, we concluded that the decrease in the rate of conception with the increase of THI was statistically significant (P <0.01). During thermal stress, a greater number of inseminations are required until fertile insemination (P <0.05), and the service period is slightly longer; although the stated finding is not statistically significant. THI outside the thermoneutral zone as well as an elevated rectal temperature over 39.5ºC at the time of insemination represent statistically significant risk factors (relative risk>1) for the occurrence of early pregnancy loss and a reduced rate of conception after insemination. Concerning the sensitivity of embryos, it can be concluded that the early embryos until the moment of nidation were the most sensitive. Irregular return occurs most often, and signs of estrus are present on the 25th-35th day after insemination-before nidation (in 50.37% of cows), followed by the regular return-when fertilization of the ovum did not occur or the fertilized ovum was quickly lost (in 34.99% of cows). Estrus induction by prostaglandin F2-alpha indicates that estrus is less pronounced in summer than in winter (P<0.01) and spring (P<0.05). During the summer, there is also a postponement of the peak of estrus induced by prostaglandin (Cincović, 2007). The causes of postponement of the ovarian activity after calving, reduced fertility and weaker expression of estrus after induction are various: decrease in estradiol concentration (Wolfenson et al., 1995), shorter duration of estrus (Abilay et al., 1975), alterations of the follicle and disruption of its dominance (Ginther et al., 2003), a decrease in progesterone concentration in the summer period (Wolfenson et al., 1988), the release of old oocytes from the follicles that breached the ovarian surface too early in the cycle and entered the prolonged follicular phase (Mihm et al., 1994), faster movement of the fertilized ovum towards the uterus during heat stress and the reluctance of the uterus to accept the prematurely arrived fertilized ovum is one of the reasons for regular return during the summer period. Most often, regular type of return occurs immediately before embryo nidation. Research shows that heat stress damages oocytes at an earlier stage of development and that antral follicles are most at risk (Roth et al., 2001). Exposure of a two-cell embryo extracted 28 hours after artificial insemination to temperatures of 38, 41 and 43ºC leads to changes at the ultrastructural level of the cells, namely: all organelles move to the center of the blastomere, the number of mitochondria decreases and their structure is disrupted, while the nuclei and cell membrane exhibit signs of protein denaturation (Rivera et al., 2003).

Two-cell embryos are much more sensitive to temperature shock than 4- or 8-cell embryos. The reason is that the early embryo is unable to form the protective protein HSP70, which is induced by thermal stress (Edwards et al., 1997). Also, the increased activity of free radicals has an important role in the deterioration of the embryo. Namely, during stress a decrease in the activity and concentration of glutathione in the embryo occurs, which is an important antioxidant (Arechiga et al., 1995). Increased levels of embryonic apoptosis and necrosis also occur during temperature stress (Paula-Lopes and Hansen 2002). In the later period of pregnancy, the main problem is overheating of the pregnant uterus, which leads to disruption of DNA transcription and protein production (Malayer et al., 1990), as well as reduced blood flow through the placenta, which compromises the nutrition of the fetus (Alexander et al., 1987). About

2/3 of animals exhibit signs of estrus between 48. and 76. hour after the prostaglandin compounds were applied. In this paper, it was demonstrated that in the summer period, this peak was at 72-96 hours after stopping the prostaglandin treatment, which could be explained by the reduced concentration of sex hormones and the prolonged follicular phase during stress (Roth et al., 2001a). This indicates that artificial insemination in the summer period can be performed only after the detection and diagnosis of estrus, and not at a predetermined time, as the manufacturers of hormonal drugs most often recommend. It is necessary to implement various auxiliary and precautionary activities, such as vitamin application, that positively affect the manifestation of estrus and the service period (Cincović, 2007a).

6. The effect of heat stress on blood metabolic, hematological and immunological parameters

The etiopathogenesis of the decreased milk production and reproductive efficiency, as functional and health disorders in cows during exposure to heat stress is based on changes in the animal's metabolic, hematological and immune systems. The study of metabolic, hematological and endocrinological changes in cows is of great importance for understanding the biology of their adaptation (Cincović, 2016). In the first few days of heat stress investigation on cow farms, it was concluded that certain metabolic parameters exhibit a significant diurnal deviation, as their values change in the warmer part of the day compared to the morning or evening hours. The concentration of NEFA and urea increased significantly during the stressful period of the day, while the concentration of glucose and total cholesterol decreased, and the concentration of protein increased during the warmer part of the day, but not significantly (Cincović et al., 2010b). The obtained results could be associated with reduced food intake during the warmer period of the day, negative energy balance, and changes in hydration and protein metabolism.

Metabolic adaptation to heat stress depends on other factors that define cow metabolism, such as the lactation period and body condition. The results of Cincović et al., (2011b) indicated significantly higher concentrations of BHB (P <0.01), NEFA (P <0.05) and AST (p <0.05) in cows that calved during the summer in the first week after calving. Cholesterol and NEFA were significantly higher in the fourth week after birth in winter-calving cows (P <0.05). Other parameters did not demonstrate significant differences during various measurement periods in both groups of cows. A significantly higher concentration of NEFA and BHB in cows exposed to heat stress might be a consequence of the joint effect of parturition and heat stress. Lower NEFA concentrations in the fourth week postpartum were consistent with adaptation to prolonged heat stress. In a separate experiment, cows in early lactation and cows in mid-lactation were observed, and both groups were exposed to heat stress (Cincović and Belić, 2011): - in both groups of cows a reduced food intake was noticed in the examined period, - in cows in the peripartum period, the concentration of non-esterified fatty acids (NEFA) (P <0.01) and betahydroxybutyrate (BHB) (P <0.05) increased significantly, while the concentration of glucose decreased (P <0.01), in cows in mid-lactation the concentration of NEFA significantly decreased (P <0.01) as well as glucose concentration (P <0.05), while the concentration of BHB exhibited a decreasing tendency (P <0.1). It is obvious that the homeoresis and metabolic stress of early lactation, which result in lipolysis and ketogenesis, are additionally activated by heat stress, while in mid-lactation heat stress inhibits lipolysis and ketogenesis, even though reduced food intake and a decrease in glycemia occur. Obesity is a significant risk factor for quality adaptation to heat stress. Our authors investigated the effect of high body condition score on adaptation to heat stress, with special emphasis on metabolic adaptation (Cincović et al., 2011c). Obese cows (BCS>4) had a lower ability to adjust to heat stress compared to normal and lean cows (significantly lower milk production and quality, and significantly higher rectal temperature and respiration rate compared to other groups of cows). Obese cows exhibited significantly higher NEFA concentration and significantly lower glucose concentration during exposure to heat stress. The constant increase in NEFA concentration and the decrease in the amount of tallow suggested that fat was used as an energy source, which significantly increased the heat balance and led to poor adaptation to heat stress in obese cows. Cows with high body condition were at higher risk of developing liver failure and lipidosis (reduced cholesterol, increased bilirubin concentration) during heat stress, which could be related to increased blood fatty acid concentration. An increased concentration of urea was noticed during exposure to heat stress, with a significantly higher concentration in obese cows. The utilization of fat as an energy source depended on insulin sensitivity, which increased during heat stress. Obese cows are naturally less sensitive to insulin and more prone to lipolysis.

Lipolysis and insulin resistance are important mechanisms that maintain lactation in cows. They also change due to the effect of heat stress. The intravenous glucose tolerance test indicated that in cows with preserved lipid mobilization, a higher level of insulin resistance occurred, which was reflected in higher glucose values and NEFA values (Cincović et al., 2012):
- The mean value of glycemia was significantly higher at the 30th, 60th and 90th minute (P <0.01; P <0.05 and P <0.05) after glucose application in the group of cows with preserved lipomobilization, - NEFA concentrations tended to be higher in cows with preserved lipid mobilization compared to cows with reduced lipomobilization at the 20th and 30th minute after glucose application (P <0.1). It was concluded that cows with preserved lipomobilization tolerate glucose less than cows with reduced lipomobilization during heat stress.

It is clear that in cows exposed to heat stress a change in sensitivity to insulin occurs, which regulates the concentration of glucose and NEFA. Thus, the following study was conducted, which determined the changes in insulin, glucose, NEFA concentrations and insulin resistance index values in cows during heat stress and thermoneutral period (Cincović et al., 2014). 30 cows of the Holstein-Friesian breed were included in the experiment: 10 cows in heat stress (THI>70) (5-7 days after calving), 10 cows in metabolic stress (5-7 days after calving), and 10 cows as a negative control group (cows in the thermoneutral zone and in the middle of lactation). The nutrition and care conditions were the same for all the cows. The results indicated that in cows exposed to heat stress, an increase in the concentration of insulin and a decrease in the concentration of glucose and NEFA occurred, while in cows in early lactation the concentration of insulin and glucose decreased, and the concentration of NEFA increased. By comparison of the two groups of cows during heat and metabolic stress, it was concluded that in cows in metabolic stress a significantly lower concentration of insulin and a higher concentration of NEFA persisted. The RQUICKI index of insulin resistance was the lowest in cows in metabolic stress (the most resistant to insulin), followed by cows in the control group and the highest in cows in heat stress (RQUICKI= 0.45:0.52:0.60). The RQUICKI correlated the most with the values of the insulin: glucose and insulin: NEFA indices, and there was a positive correlation between them. The increase in insulin concentration during heat stress and the existence of a higher concentration of insulin per unit of glucose and NEFA, reduced insulin resistance, that is, increased insulin sensitivity. The different rate of insulin resistance was the main distinction in the adaptation of cows to metabolic and heat stress. The increased sensitivity to insulin in heat stress was related to the reduced concentration of NEFA in these cows because insulin had an antilipolytic effect.

Changes in milk production are related to the metabolic adaptation of cows to heat stress. In cows exposed to heat stress, there was a statistically significant reduction in plasma glucose concentration (2.97:3.58 mmol L^{-1}, *P* <0.001) and cholesterol (5.97:6.46 mmol L^{-1}, *P* <0.01). The concentration of total proteins in the blood increased significantly during heat stress (70.41:73.59 g L^{-1}, *P* <0.001). The percentage of milk fat (3.59:3.228, *P* >0.05), milk proteins (3.305:3.004%, *P* <0.001) and dry matter (8.612:8.157%, *P* <0.001) decreased during the warm period. Blood and milk parameters correlated significantly (except milk fat and blood cholesterol) (Cincović et al., 2010c). In addition to the correlation analyses, it was necessary to examine the cause-and-effect relationship between metabolic adaptation and the decrease in milk production in cows exposed to heat stress. The aim of a study (Belić et al., 2011) was to determine the postabsorptive use of glucose, non-esterified fatty acids (NEFA), beta hydroxybutyrate (BHB) and urea in the process of milk production, by determining the postprandial concentration of metabolites and the degree of metabolite extraction by the mammary gland. Glucose was actively used as an energy source during heat stress, so a smaller amount of glucose reached the mammary gland. Thus, the concentration of lactose in milk decreased.

The mammary gland adapts to the reduced glucose supply and increases the use of NEFA and BHB for its needs, which negatively affects milk fat and proteins. Urea, which is increased in concentration during heat stress, easily passes through the mammary gland and exhibits a negative effect on milk proteins. All of the above reduces the amount of milk produced. Reduced glucose supply to the mammary gland, increased utilization of NEFA and BHB for milk production and increased urea concentration during heat stress directly affect milk production and quality. In addition to these studies, it was determined that dynamic changes in the values of metabolites and insulin sensitivity index significantly affected milk production in dairy cows during exposure to heat stress (Majkić et al., 2017b). With approximately 90% specificity, we could detect cows with a high decline in milk production, so those cows had the following relative changes in metabolite values during heat stress compared to the thermoneutral period: an increase in insulin values by ≥12.5%, a decrease in NEFA and glucose values by ≤14.1% or ≤21.5% and an increase in RQUICKI index, insulin: NEFA and insulin: glucose ratios by ≥9.6%, ≥20.1% and ≥20.3%. All of the above confirms the concept that during heat stress, the body attempts to use glucose to a greater extent for energy needs when the insulin sensitivity of tissues increases. The reason is that less energy (heat) is released from a certain amount of metabolized glucose compared to the same amount of fat. Due to the increased insulin sensitivity, the uddermammary gland no longer has a dominant role in the use of glucose, and lactose decreases. Lactose is a significant regulator of the volume of milk produced, so a reduced concentration of lactose reduces the volume of milk produced. On the other hand, we found that lipids could be used by the mammary gland, which additionally burdened the homeothermy of the udder, and contributed to a further decrease in milk production. Finally, the reduced food intake further emphasized all these reasons that led to a decrease in milk production during heat stress. The mentioned paradigms have been described and experimentally confirmed by several authors (Wheelock et al., 2010; O'Brien et al., 2010; Baumgard et al., 2013).

Changes in hematological and inflammatory parameters were found in cows exposed to heat stress. Our results indicated that certain hematological changes occurred during heat stress, namely: a decrease in the number of red blood cells (7.38:6.51; *P* <0.01), a decrease in MCV (53.25:47.31, *P* >0.05), a decrease in hematocrit (39.3:30.8, *P* <0.01), a decrease in the number of white blood cells (9.18:7.75, *P* <0.01) and platelets (425:351, *P* <0.05), the hemoglobin concentration also decreased (111.5:107, *P* >0.05), and a correlation between physical and hematological response persisted (Belić et al., 2010). The obtained results were consistent with the findings of other researchers, who explained that the obtained changes had been caused by hemodilution, altered inflammatory response or direct effects of heat stress or cortisol to the bone marrow (Koubkova et al., 2002; Casella et al., 2013; Mazzulo et al., 2014; Bagath et al., 2019; Sinha et al., 2019).

The inflammatory response in cows exposed to heat stress plays a very important part in the regulation of metabolism and its relation to milk production. In one experiment, we predicted milk production using THI values, as well as by using a model where the impact of glucose-related metabolic indicators and the inflammatory parameter TNF, whose concentration in the blood increased during heat stress, were added to the THI value (Majkić et al., 2018). THI could explain 37% of the variance in milk production. The percentage of explained variance and the correct prediction of milk production increased by 11-28% in models that combined THI and metabolic parameters compared to the independent use of THI as a predictor of milk production. By including individual metabolic parameters, milk production will be correctly determined in 51% (THI+NEFA and THI+insulin models), 52% (THI+RQUICKI and THI+G:I models), 56% (THI+TNF-α model) and 58% (THI+glucose model) of cases. Models based on mechanisms of adaptation to heat stress give a higher percentage of the explained variance of milk production compared to the control model where only THI is included.

The values of the explained variance were: 48% for the carbohydrate and lipid adaptation model (THI+glucose+insulin+ NEFA), 54% for the inflammation model (THI+insulin+TNF-α), 57% for the insulin sensitivity model (THI+RQUICKI+ G:I), 58% for the overall model (THI+all metabolic parameters), 65% for the model including glycemia and inflammation (THI+glucose+TNF-α). All models were statistically significant at the P <0.001 level. Glucose and TNF-α play a dominant role in the prediction of milk production during heat stress, since the participation of these two metabolites in the models significantly reduces the importance of other metabolic variables, including THI itself. By excluding glucose as a control variable, the association between THI and milk production, as well as other metabolic parameters and milk production, decreases to a level below statistical significance. Glucose did not moderate the correlation between TNF-α and milk production, which remained significant after excluding glucose. The importance of TNF confirms that the inflammatory response in cows is very important in metabolic adaptation and milk production during heat stress. TNF-a is a very significant indicator that connects tissue inflammation, response to inflammatory processes, food intake, central regulation via the hypothalamus and productivity in cows (Bradford and Swartz, 2020; Kuhla, 2020).

Heat shock proteins (Hsp) are chaperones necessary for the proper formation of the polypeptide chain and are responsible for its translocation in the cell. These proteins were found during exposure to heat stress, when their concentration and expression in cells increased, thus explaining their name (Kiang and Tsokos, 1998). Depending on its localization, Hsp70 has the ability to exert completely opposite effects. Extracellular (eHsp70) reaches the bloodstream from living cells exposed to stress through vesicular secretion, exosomes or lysosomes, through an intact lipid membrane, but also passively from damaged and necrotic cells. Intracellular (iHsp70) helps to restore the natural conformation of denatured proteins under the influence of various stressors, preventing their aggregation and protecting cells from apoptosis and exhibiting an anti-inflammatory effect. Extracellular Hsp70 has a cytokine role, an immunostimulatory role (helps in the synthesis of pro-inflammatory cytokines) and improves antitumor control. The balance between intracellular and extracellular chaperones of Hsp70 indicates its dominant role (Krause et al., 2015; Costa-Beber et al., 2022). Investigations by Petrović et al. (2020) indicated that in the examined cows the Hsp70 concentration had the following values: 4.75±1.1 ng mL^{-1} in March; 6.05±1.4 ng mL^{-1} in May and 8.9±2.3 ng mL^{-1} in July. The heat stress load index (THI) of cows during the examined season ranged from 55.6 in March, 66.9 in May to 75.6 in July. Hsp70 concentration and THI index were significantly higher from spring to summer. The test results revealed an increase in the concentration of Hsp70 with an increase in the THI index. A positive correlation was found between serum Hsp70 and the value of the THI index. Our author's team (Petrović et al., 2022) determined that extracellular eHsp70 had a pro-inflammatory role and affected homeoresis in early lactating cows due to its association with numerous metabolites and pro-inflammatory cytokines. The above results indicate that a significant link persists between metabolic adaptation, immune and inflammatory response and productive characteristics in cows exposed to heat stress.

7. The effect of ambient temperatures in vitro on the value of laboratory parameters in cows

Preanalytical errors are the biggest source of variability in laboratory practice, and this includes all the factors from the moment of blood collection to the separation of laboratory aliquots for analysis (Cincović and Belić, 2020). Ensuring a cold chain during sample transport is a significant priority in order to obtain a high-quality and stable sample that will be processed in the laboratory. In one study, we examined the effects of various preanalytical factors on the stability of laboratory parameters in the blood of cows (Kovačević et al., 2021). The largest number of biochemical analytes in bovine blood serum exhibited preserved stability in the first 6 days at + 4 ° C or for 6 months at -20 ° C if transported to the laboratory within 8 hours after sampling, avoiding the causes of preanalytical errors. Prolonged transport, inadequate transport at room temperature, hemolysis or small sample volume shortens the stability of the samples in both temperature regimes. Variations in parameters such as BHB, NEFA, TBIL, AST, and Ca have shown potential clinical significance. Ambient temperatures and the absence of a cold chain significantly disturb the stability of the sample in in vitro conditions, so the possibility of heat stress must also be considered during sampling. Metabolic changes due to heat stress with the additive effect of changes occurring in vitro due to transport at ambient temperatures can lead to false laboratory measurements and inadequate interpretation of the obtained results.

Our latest research (Cincović et al., in press) indicates that temperature affects the value of the hematological parameters in vitro. In the experiment, three temperature regimes were used: the temperature regime at room temperature for 6 days (18-22°C), the temperature regime of the refrigerator (3-5°C) and the temperature regime of the freezer where the samples were exposed to (-18 to -22°C) for 21 days. Examination of the hematological parameters of the samples at room temperature indicated that during the six-day storage of the samples at room temperature, no significant change in the value of hemoglobin, hematocrit and MCH existed, as they exhibited a very high stability during the storage of the samples. However, in these samples, there was a decrease in the red blood cell count, an increase in the MCV value, a decrease in the MCHC value, and a decrease in the number of leukocytes, accompanied by a decrease in the neutrophil count, but an increase in the lymphocyte count. Platelet count decreased significantly over time. Storage of the blood at refrigerator temperatures stabilized the mentioned processes until the fifth day of the experiment so that the parameters varied up to the level of permitted stability. Freezing significantly affected the value of the examined hematological parameters, which, in addition to easily exceeding the threshold of permissible instability also demonstrated a significant statistical deviation of the mean values. The red blood cell count decreased with the duration of freezing, and it is inversely proportional to the intensity of hemolysis. The level of hemolysis increases linearly with the number of days since freezing. Short-term freezing during one day did not affect the stability of the number of erythrocytes. Hematological parameters were to a lesser extent subjected to changes due to the effect of ambient temperatures compared to biochemical parameters, but due to the sensitivity of erythrocyte and erythrocyte indices, the temperature regime of sample storage and transport must be taken into account. This can be particularly important when blood is sampled during exposure to heat stress in cows. In addition, an increasing number of studies has been conducted that resulted in the determination of plasma thermograms using differential scanning calorimetry (DSC), and that concluded that blood plasma, depending on its composition, exhibited a specific thermogram fingerprint (Garbett et al., 2015).

8. Prevention of the effect of heat stress and resilience of cows

There are a number of ways to prevent heat stress (West, 1999; Negrón-Pérez et al., 2019; Levit et al., 2021). Noordhuizen and Bonnefoy (2015) describe major prevention and control measures in situations of heat stress in dairy cattle in area of nutrition, drinking water, barn climate, management of reproduction and calves, etc. Nutrition: increase the frequency of feeding to 4-6 times a day; give the highest proportion at late evening or during the night, reduce the effects of a negative energy balance around calving, maintain feed intake at a normal level during close-up and fresh cow period. Take care of optimal claw health. Drinking water: increase the number of drinking places, total width of drinking places must be 600 to 900 cm for 100 cows, provide water of low temperature (< 15°C) and clean troughs every 2 days, check water quality beforehand (chlorates, sulfates, microbes) regularly. Barn climate: create shadow over feed bunks (at 4-5 m^2 per cow) at 4 m height, install and use appropriate fans, install and use showers, water spray producing devices, or sprinklers, Increase the number of sprinkling and drying cycles per day. Management of reproduction and other: Do not use a natural service bull (spermatogenesis will most probably be disturbed), provide cooling to dry cows from 4 weeks before calving onwards (sprinkling, drying, shadow, fanning), create shadow in the pasture, and make sure that wind can pass freely over the pasture plots (no trees or bushes), reduce the walking distance from pasture (or pen) to milking parlor and back, if possible, reduce the time the cows spend outside; reduce the waiting time before milking; reduce the number of cows per group for milking, check more frequently the vitamins and mineral status of the feed (rations): K, Na, Cl, P. If needed, increase K, Na, Mg in the ration, adjust ration composition (≤ 2-3% fat in DM; protein level < 18%; rumen degradable protein < 61 % of total protein; salt; Lysine 1% of DM; water addition (4-5 liter per 20 kg DM), eliminate citrus pulp from the ration because it contributes to increase heat production. Calves: check IgG in colostrums (colostrometer) and in serum of all calves born in heat stress periods (refractometer; laboratory). Other measures: reduce cattle density in the barn (alternate groups inside and groups outside); reduce group size, check behavioral preference of cows for cubicles and resting areas, if deviations occur, take measures, avoid cattle handling/processing/treatment because this increases body temperature with some degrees, if handling is needed, let the cow be confined for less than 30 min and do it at night or early morning (before 06.00 hrs), handling areas should preferably be provided with a fan and sprinklers/sprayers, maintain a program for controlling (biting) flies.

In our study, the effect of cooling cows with fans (Cincović et al., 2013) and the possibility of using electromagnetic stimulation (EMS) (Andjušić et al., 2022) to reduce the decrease in milk production and improve metabolic adaptation in cows was examined. During July and August, cows were exposed to fans as needed, mostly in the period after morning feeding and before evening milking. Heat stress was detected when the calculated value of THI the in the building was over 72. Exposure of cows to fans led to an increase in milk production and an increase in feed consumption, but the values that existed during the thermoneutral period were not reached. The metabolic characteristics of cows after exposure to fans were the following: lower blood pH value, higher glucose concentration, a tendency of non-esterified fatty acids (NEFA) concentration increase and reduced sensitivity to insulin (RQUICKI), while the concentration of T3 and T4, cortisol and growth hormone tended to increase. The parameters indicating the degree of hydration of the cows (hematocrit, total proteins and creatinine) did not differ significantly. The resulting metabolic adaptations indicated reduced heat emission through the respiratory organs (decrease in blood pH), a tendency towards increased thermogenesis that antagonized the cooling effect of the fan (T3, T4, cortisol tended to increase) and the use of sugar for milk production (insulin resistance of tissues, higher glycemia), as well as the improvement of the anabolic status in general (STH tendency towards increase). Hydration parameters did not differ significantly, probably because the cows had enough fresh water. The effect of EMS on milk production demonstrated that milk production was significantly higher ($p<0.05$) in the experimental group of cows compared to the control group of cows in the first, second, third and the week after completion of EMS. Dynamic changes in the concentration of OT and milk produced during the experiment exhibited a positive correlation ($R2=0.31$; $P<0.01$), which was striving towards zero in the control group. EMS showed a tendency ($R2=0.08$; $P<0.1$) to increase milk production despite the rise in the temperature humidity index (stress load) in experimental, but not in control dairy cows. EMS could reduce the loss in milk production in cows during heat stress by stimulation of OT production.

Recently, in addition to prevention, the resilience of cows to heat stress has become increasingly important, that is, the selection of cows with the ability to react as little as possible to heat stress and to adapt very quickly and preserve their production and metabolism during heat stress. According to the definition, resilience is the ability of a system to react to some external or internal factor and the ability to withstand and recover after exposure to those factors, and it is described as a change that occurred over time during which the performance of the examined system has been improved (Linkov and Tramp, 2019). The principles of measuring resilience in dairy cows are presented in a recent review by Kašná et al., (2022). Resilience can be determined on a genetic and molecular basis, so as such it represents an important tool for obtaining more resilient individuals (Berghof et al., 2019; Cheruiyot et al., 2021). We tested the resilience of cows to heat stress by determining the resilience curve, which is the standard for analyzing the resilience of a system (Poulin and Kane, 2021). Concerning milk production, Majkić et al. (in press) determined the three types of resilience curves for milk production. The first type implies a sudden decrease in milk production and a gradual recovery. The second type of curve is a symmetrical curve that illustrates a slight decrease in milk production so that its lowest point is in the middle of the examined period, after which a slight recovery occurs. The third type of curve is a curve that represents a slight decline in milk production, with the lowest production occurring later and with an accelerated recovery after the cessation of heat stress. These curves occur at all stages of lactation. The above-mentioned three types of curves are very important in the assessment of milk production or milk loss during heat stress.

Cumulative milk loss (the area under the curve indicating milk loss) depended on the type of curve: with the first type curve, the weekly loss with an average production of 30L of milk was 37-43L, with the second type curve it was from 45 to 49L, while for the third type curve it was from 49 to 55L. The longer the period from the beginning of the stress to the period of lowest milk production, the greater the cumulative loss of milk, so the acute response to heat stress is more favorable for production. By investigating early lactation, different results were obtained. Cows with the first and third type curve exhibited a smaller increase in milk production (from 20-30L per week, if the beginning of lactation was 15L and the peak was 30L) compared to cows with the second type curve. The obtained results indicated that in the first few weeks of lactation and then in the period of several weeks before the expected peak of lactation, cows must be additionally protected from heat stress. Metabolic parameters in cows established 4 prototypes of resilience curves according to shape, with the curves in a positive or negative direction depending on whether the value of the metabolite increases or decreases. The first type of curve is the one in which there is a sudden increase in one direction, then there is a rapid return to the initial value and the departure of the metabolite value in the opposite direction (if it had increased, it then decreased, and if it had decreased, it then increased). Cortisol, insulin, T3, T4, NEFA, glucose, urea and neutrophil count exhibit this type of curve. The second type of curve is a curve where the value rises/falls sharply and then is maintained at that level during the heat stress and then suddenly returns to the initial position. This is the case with BHB. The third type of curve is a curve where there is a slight decrease/increase in metabolites, with the highest deviation near the middle of the experimental period, and then there is a slight return to the initial level. Cholesterol, the number of erythrocytes, leukocytes and platelets, MCV, HGB, MPV, AST and GGT demonstrated such a curve. The fourth type of curve is a curve in which there is a slight decrease/increase in the value of the blood parameter over time, and this includes the lymphocytes, total proteins, albumin and triglycerides. The first and second types of curves have the greatest cumulative effect on metabolite values, while the third and fourth types of curves show less cumulative effects on metabolite values during the experimental period. Cumulative change in metabolic parameters and cumulative loss of produced milk expressed in the area under the resilience curve represents a good model for examining the impact of metabolic adaptation on milk production, as very high correlations are achieved between the examined parameters. In addition to this, the results of Amamou et al., (2019) indicated that cows resistant to heat stress with no drastic decrease in milk production exhibited a higher respiration rate, skin and rectal temperature. The same authors concluded that respiration rate could be used as a reliable indicator for thermotolerance.

Precautionary measures, along with the knowledge of resilience to heat stress in cows can significantly reduce losses in milk production or poor metabolic and physical adaptation of cows.

9. Conclusions

Heat stress in cows in Serbia is a significant factor that affects all aspects of productivity and cow health. Heat stress is determined by the elevated values of the THI index, which have been increasing in Serbia almost since the beginning of this century. Physical and behavioral changes occur, resulting in altered metabolic adaptation and productivity in cows. Significant climatic deviations along with their cumulative effect on adaptation and production threaten all aspects of dairy cattle breeding in our country. Due to the current trends in climate change and the very complex adaptation in cows that result in health, productive and economic losses, it is necessary to take a serious approach to alleviate the cause of heat stress and the consequences of heat stress in cows, with the constant selection of individuals that are more resilient to high ambient temperatures.

Acknowledgments

This work is the result of project number 142-451-3035/2023-01/01, financed by the Provincial Secretariat for Higher Education and Scientific Research of Vojvodina.

Declaration of competing interests

Authors disclose any personal and/or financial relationships with other people or organizations that could inappropriately influence (bias, non-compliance with the academic code) their work.

References

Abilay, T.A., Johnson, H.D., Madan, M. (1975). Influence of environmental heat on peripheral plasma progesterone and cortisol during the bovine estrous cycle. *J. Dairy Sci.*, 58, 1836-1840.

Aganga, A. A., Umunna, N.N., Oyedipe, E.O., Okoh, P.N., Aduku, A.O. (1990). Response to water deprivation by Yankasa ewes under different physiological states. *Small Ruminant Research*, 3(2), 109-115.

Alexander, G., Hales, J.R.S., Stevens, D., Donnelly, J. B. (1987). Effects of acute and prolonged exposure to heat on

regional blood flows in pregnant sheep. *J. Dev. Physiol.*, 9,1-15.
Amamou, H., Beckers, Y., Mahouachi, M., & Hammami, H. (2019). Thermotolerance indicators related to production and physiological responses to heat stress of holstein cows. *Journal of thermal biology*, 82, 90-98.
Andjušić, L., Milankov, Z., Marić, D., Milošević, B., Djoković, R., Milanović, V., Savić, Ž., Savović, M., Cincovic, M. (2022). Influence of electromagnetic stimulation on secreting oxytocin and milk production in dairy cows under the heat stress. *Large Animal Review*, 28(5), 221-226.
Arechiga, C.F., Ealy, A.D., Hansen, P.J. (1995). Evidence that glutathione is involved in thermotolerance of preimplantation mouse embryos. *Biol. Reprod.*, 52, 1296–1301.
Bagath, M., Krishnan, G., Devaraj, C., Rashamol, V.P., Pragna, P., Lees A.M., Sejian, V. (2019). The impact of heat stress on the immune system in dairy cattle: A review. *Research in veterinary science*, 126, 94-102.
Baumgard, L.H., Rhoads Jr, R.P. (2013). Effects of heat stress on postabsorptive metabolism and energetics. *Annu. Rev. Anim. Biosci.*, 1(1), 311-337.
Beatty, D.T., Barnes, A., Taylor, E., Pethick, D., McCarthy, M., Maloney, S.K. (2006). Physiological responses of Bos taurus and Bos indicus cattle to prolonged, continuous heat and humidity. *Journal of Animal science*, 84(4), 972-985.
Beede D.K., Colier R.J. (1986). Pootencial nutritional strategies for intensively managed cattle during thermal stress. *J.Anim.Sci.*, 62, 543-554.
Behera, R., Mandal, A., Rai, S., Karunakaran, M., Mondal, M. (2020). Temperature Humidity Index and Its Relationship with Production Traits of Dairy Cattle and Buffaloes - A Review. *International Journal of Livestock Research*, 10(3), 38-48.
Belić B., Cincović M. (2020). Referentne vrednosti laboratorijskih parametara u krvi životinja. Monografija. Poljoptivredni fakultet, Departman za veterinarsku medicinu, Novi Sad.
Belić, B., Cincović, M.R., Stojanović, D., Kovačević, Z., Medić, S., Simić, V. (2010). Haematology parametars and phisical response to heat stress in dairy cows. *Contemporary agriculture*, 59(1-2), 161-166.
Belić, B., Cincović, M., Majkić M., Plavša, N., Lakić, I., Petrović M., Krnjaić, S. (2017). Upotreba termovizijske kamere u proceni toplotne opterećenosti krava na farmama. Zbornik radova 28. Savetovanje veterinara Srbije, Zlatibor 7-10.9., pp.168-171.
Belić, B.,Cincović, R.M.,Popović-Vranješ, A., Pejanović, R., Krajinović, M. (2011). Metaboličke promjene i iskorištavanje metabolita u proizvodnji mlijeka kod krava u toplinskom stresu. *Mljekarstvo*, 61(4), 309-318.
Berghof, T.V.L., Poppe, M., Mulder, H.A. (2019). Opportunities to improve resilience in animal breeding programs. *Front Genet.*, 9, 15 p.
Bradford, B.J., Swartz, T.H. (2020). Following the smoke signals: inflammatory signaling in metabolic homeostasis and homeorhesis in dairy cattle. *Animal*, 14(S1), s144-s154.
Broucek, J., Ryba, S., Mihina, S., Uhrincat, M., Kisac, P. (2007). Impact of thermal-humidity index on milk yield under conditions of different dairy management. *Journal of Animal and Feed Sciences*, 16(3), 329-344.
Casella, S., Scianò, S., Zumbo, A., Monteverde, V., Fazio, F., Piccione, G. (2013). Effect of seasonal variations in Mediterranean area on haematological profile in dairy cow. *Comp. Clin. Pathol.*, 22, 691–695.
Cheruiyot, E.K., Haile-Mariam, M., Cocks, B. G., MacLeod, I. M., Xiang, R., Pryce, J. E. (2021). New loci and neuronal pathways for resilience to heat stress in cattle. *Scientific reports*, 11(1), 16619.
Cincović, M., Majkić, M., Spasojević, J., Jožef, I., Mitrović, J., Kovačević D., Kovačević D. (2023). Stabilnost hematoloških parametara kod krava u uzorcima čuvanim pod različitim temperaturnim režimima. Letopis naučnih radova, in press.
Cincović, M. (2007a). Uticaj preporođajne aplikacije preparata AD3E vitamina na uspostavljanje ovarijalne aktivnosti i ispoljavanje estrusa posle porođaja kod mlečnih krava. Zbornik devetog savetovanja iz kliničke patologije i terapije životinja Clinica veterinaria, Palić , 18-22 jun.
Cincović, M., Belić, B. (2011). Metabolička adaptacija na peripartalni i toplotni stres kod mlečnih krava.*Veterinarski žurnal Republike Srpske*, 11(2), 155-159.
Cincović M., Belić B. (2020). Opšta patološka fiziologija. Udžbenik. Poljoprivredni fakultet, Departman za veterinarsku medicinu, Novi Sad.
Cincović, M., Belić, B., Stevančević, M., Toholj, B., Đoković, R. (2013). Produktivne i metaboličke karakteristike krava izloženih ventilatorima tokom letnjeg perioda. *Letopis naučnih radova*, 37(1), 75-82.
Cincović, M., Belić, B., Stevančević, M.,Toholj, B., Potkonjak, A., Stojanović, D., Kovačević, Z. (2014). Ispitivanje insulinske rezistencije kod krava u toplotnom i metaboličkom stresu.25. Savetovanje veterinara Srbije, Zlatibor, Srpsko veterinarsko drustvo 11.-14.septembar, pp.91-96
Cincović, M., Belić, B., Toholj, B., Stevančević, M., Potkonjak, A., Lako, B. (2011). Influence of respiration rate and rectal temperature in holstein cows to milk production during heat stress. *Contemporary agriculture,* 60(1-2), 183-189.
Cincović, M., Belić, B. (2011a). Determination of negative energy balance in acute heat stressed cows using milk fat and protein concentration. 22nd International Symposium Food safety production, Trebinje , 19-25 June, pp.28-30.
Cincović, M.R. (2010). Toplotni stres krava – fiziologija i patofiziologija. Zadužbina Andrejević, Beograd.
Cincović, M.R., Belić, B., Stevančević, M., Toholj, B., Lako, B., Potkonjak, A. (2012). Uticaj stepena lipomobilizacije na rezultate testa opterećenja glukozom kod mlečnih krava u toplotnom stresu. *Veterinarski glasnik*, 66 (1-2), 3-11.
Cincović, M.R., Belić, B., Toholj, B., Stevančević, M. (2011b). Postpartum metabolic profile in heat-stressed holstein cows. 19th International congress of mediterannean federation of health and production of ruminants, Beograd, 25-28. maj, pp 285-289.
Cincović, M.R., Belić, B., Stojanović, D.,Kovačević, Z.,Medić, S., Simić, V. (2010c). Metabolic profile of blood and milk in dairy cows during heat stress. *Contemporary agriculture*, 59(1-2), 167-172.
Cincović, M.R., Belić, B., Toholj, B., Stevančević, M. (2011a). On-farm measurement of heat stress load in dairy cows. *Veterinarska Stanica*, 42 (Supplement 1), 285-289.
Cincović, M.R. (2006). Uticaj temperaturnog stresa na proizvodnju mleka u intenzivnom govedarstvu i mogućnost kondicioniranja mikroklime; Zbornik, 30. Međunarodna smotra naučnih radova studenata Poljoprivrede, pp. 257-260.
Cincović, M.R. (2007). Uticaj temperaturnog stresa na ispoljavanje estrusa indukovanog prostaglandinom F2α u mlečnih krava; Zbornik, 31. Međunarodna smotra naučnih radova studenata Poljoprivrede, pp. 25-30.
Cincović, M.R., Belić, B., Toholj, B., Potkonjak, A., Stevančević, M., Lako, B., Radović, I. (2011c). Metabolic acclimation toheat stress in farm housed Holstein cows with different body condition scores. *African Journal of Biotechnology*, 10(50), 293-303.
Cincović, M.R., Belić, B. (2009). Uticaj termalnog stresa krava na količinu i kvalitet proizvedenog mleka. *Veterinarski žurnal Republike Srpske*, IX (1), 53-56.
Cincović, M. (2016) Metabolički stres krava. Novi Sad: Poljoprivredni fakultet, Departman za veterinarsku medicinu.
Cincović, M.R., Belić, B.M., Toholj, B.D., Radović, I.V., Vidović, B.R. (2010). The influence of THI values at different periods of lactation on milk quality and characteristics of lactation curve. *Journal of Agricultural Sciences (Belgrade)*, 55(3), 235-241.
Cincović, M.R., Majkić, M., Belić, B., Plavša, N., Lakić, I., Radinović, M. (2017). Thermal comfort of cows and temperature humidity index in period of 2005-2016 in

Vojvodina region (Serbia). *Acta Agriculturae Serbica*, 22(44), 133-145.
Cincović, M.R., Belić, B., Stevančević, M., Lako, B., Toholj, B., Potkonjak, A. (2010b). Diurnal variation of blood metabolite in dairy cows during heat stress. *Savremena poljoprivreda*, 59, 3-4, 300-305.
Čukić, A., Rakonjac, S., Đoković, R., Cincović, M., Bogosavljević Bošković, S., Petrović, M., Savić, Ž., Anđušić, Lj., Amđelić, B. (2023). Influence of heat stress on body temperatures measured by in-frared thermography, blood metabolic parameters and its correlation in sheep. *Metabolites*, 13. https://doi.org/10.3390/metabo13080957.
Collier, R.J., Gebremedhin, K.G. (2015). Thermal biology of domestic animals. *Annual Review of Animal Biosciences*, 3, 513–532.
Corsi, C. (2010). History highlights and future trends of infrared sensors. *Journal of Modern Optics*, 57, 18, 1663–1686.
Costa-Beber, L.C., Hirsch, G.E., Heck, T.G., Ludwig, M.S. (2022). Chaperone duality: the role of extracellular and intracellular HSP70 as a biomarker of endothelial dysfunction in the development of atherosclerosis. *Archives of Physiology and Biochemistry*, 128(4), 1016-1023.
De Rensis, F., Scaramuzzi, R.J. (2003). Heat stress and seasonal effects on reproduction in the dairy cow—a review. *Theriogenology*, 60(6), 1139-1151.
Dovolou, E., Giannoulis, T., Nanas, I., Amiridis, G.S. (2023). Heat Stress: A Serious Disruptor of the Reproductive Physiology of Dairy Cows. *Animals*, 13(11), 1846.
Edwards, J.L., Ealy, A.D., Monterroso, V.H., Hansen P.J. (1997). Ontogeny of temperature-regulated heat shock protein 70 synthesis in preimplantation bovine embryos. *Mol. Reprod. Dev*., 48, 25-33.
Gantner, V., Mijić, P., Kuterovac, K., Solić, D., Gantner, R. (2011). Temperature-humidity index values and their significance on the daily production of dairy cattle. *Mljekarstvo*, 61(1), 56-63.
Garbett, N.C., Mekmaysy, C.S., DeLeeuw, L., Chaires, J.B. (2015). Clinical application of plasma thermograms. Utility, practical approaches and considerations. *Methods*, 76, 41-50
Gaughan, J. B., Holt, S., Hahn, G. L., Mader, T. L., Eigenberg, R. (2000). Respiration rate: Is it a good measure of heat stress in cattle?. *Asian-Australasian J Anim Sci*, 13(Supplement Vol C), 329-332.
Ginther, O.J., Beg, M.A., Donadeu, F.X., Bergfelt, D.R. (2003). Mechanism of follicle deviation in monovular farm species. *Anim. Reprod. Sci.*, 78, 239-257.
Guo, X., Huang, J., Luo, Y., Zhao, Z., Xu, Y. (2017). Projection of heat waves over China for eight different global warming targets using 12 CMIP5 models. *Theoretical and applied climatology*, 128, 507-522.
Hales, J.R.S., Bell, A.W., Fawcett, A.A., King, R.B. (1984). Redistribution of cardiac output and skin AVA activity in sheep during exercise and heat stress. *J. Therm. Biol.*, 9, 113–116.
Heinrich, J. (2004). Feed bunk scoring for dairy cows, Pen State, Cattle nutrition workshop.
Herbut, P., Angrecka, S., Walczak, J. (2018). Environmental parameters to assessing of heat stress in dairy cattle—a review. *International journal of biometeorology*, 62(12), 2089-2097.
Hristov, S. (2002). Zoohigijena. Poljoprivredni fakultet, Beograd.
Hristov, S., Bešlin, R. (1991). Stress domaćih životinja. Poljoprivredni fakultet Zemun, Beograd.
Hristov, S., Relić, R. (2003): Učestalost pojave i ekonomski značaj subkliničkih oblika mastitisa. *Mlekarstvo*, 22, 761-765.
Hristov, S. V., Mihajlović, L.S., Cincović, M.R., Dimitar, N.D., Stanković, B.M. (2023). The improvement of the body and udder hygiene influence on the milk safety and composition on small dairy farms in Serbia. *Journal of Agricultural Sciences (Belgrade)*, 68(1), 105-119.
Hristov, S., Stanković, B. (2009). Welfare and biosecurity indicators evaluation in dairy production. *Biotechnology in Animal Husbandry*, 25, 623-630.
Hristov, S., Joksimović-Todorović, M., Relić, R., Stojanović, B., Stanković, B., Vuković, D., Davidović, V. (2007). The influence of udder disinfections, period of lactation and season on cow mastitis occurrence. *Savremena poljoprivreda*, 56(1-2), 138-143.
Hristov, S., Relić, R., Stanković, B., Vuković, D. (2006). Influence of some factors on somatic cell counts in cow milk. *Zbornik naučnih radova Instituta PKB Agroekonomik*, 12(3-4), 47-57.
Hristov, S., Stanković, B., Joksimović-Todorović, M., Bojkovski, J., Davidović, V. (2007a). Influence of heat stress on dairy cows production. *Zbornik naučnih radova Instituta PKB Agroekonomik*, 13(3-4), 47-54.
Hristov, S., Stanković, B., Samolovac, L., Ostojić-Andrić, D., Nakov, D. (2023a). Hygiene procedures before, during and after cow milking. *Archives of Veterinary Medicine*, 16(1), 5-15.
Hristov, S., Stanković, B., Zlatanović, Z., Joksimović-Todorović, M., Davidović, V. (2008). Rearing conditions, health and welfare of dairy cows. *Biotechnology in Animal Husbandry*, 24(1-2), 25-35.
Hristov, S., Zlatanović, Z., Stanković, B., Dokmanović, M., Ostojić-Andrić, D., Mekić, C. (2014). The relationship between rearing system, animal needs index and dairy cows milk traits. *Mljekarstvo*, 64(3), 186-194.
Islam, M.A., Lomax, S., Doughty, A.K., Islam, M.R., Clark, C.E.F. (2020). Automated Monitoring of Panting for Feedlot Cattle: Sensor System Accuracy and Individual Variability. Animals, 10(9), 1518.
Joksimović-Todorović, M., Davidović, V., Hristov, S., Stanković, B. (2011). Effect of heat stress on milk production in dairy cows. *Biotechnology in Animal Husbandry*, 27(3), 1017-1023.
Kašná, E., Zavadilová, L., Vařeka, J., Kyselová, J. (2022). General resilience in dairy cows: A review. *Czech Journal of Animal Science*, 67(12), 475-482.
Kiang, J.G., Tsokos, G.C. (1998). Heat shock protein 70 kDa: molecular biology, biochemistry, and physiology. *Pharmacology & therapeutics*, 80(2), 183-201.
Könyves, T., Zlatkovic, N., Memisi, N., Lukac, D., Puvaca, N., Stojsin, M., Miscevic, B. (2017). Relationship of temperature-humidity index with milk production and feed intake of holstein-frisian cows in different year seasons. *The Thai Journal of Veterinary Medicine*, 47(1), 15.
Koubkova, M., Knizkova L., Kunc P., Hartlova H., Flusser,J., Dolezal, O. (2002). Influence of high environmental temperatures and evaporative cooling on some physiological, hematological and biochemical parameters in high-yielding dairy cows. *Czech Journal of Animal Science*, 47(8), 309-318.
Kovačević, D., Cincović, M., Belić, B., Đoković, R., Majkić, M. (2021). Blood Serum Stability Limit and Maximum Storage Time of Bovine Samples. *Acta Scientiae Veterinariae*, 49.
Kovács, L., Kézér, F. L., Póti, P., Boros, N., Nagy, K. (2020). Upper critical temperature-humidity index for dairy calves based on physiological stress variables. *Journal of Dairy Science*, 103(3), 2707-2710.
Krause, M., Heck, T.G., Bittencourt, A., Scomazzon, S.P., Newsholme, P., Curi, R., Homem de Bittencourt, P.I. (2015). The chaperone balance hypothesis: the importance of the extracellular to intracellular HSP70 ratio to inflammation-driven type 2 diabetes, the effect of exercise, and the implications for clinical management. *Mediators of inflammation*, https://doi.org/10.1155/2015/249205
Kuhla, B. (2020). Pro-inflammatory cytokines and hypothalamic inflammation: implications for insufficient feed intake of transition dairy cows. *Animal*, 14(S1), 65-77.
Levit, H., Pinto, S., Amon, T., Gershon, E., Kleinjan-Elazary, A., Bloch, V., Ben Meir, Y.A., Portnik, Y., Jacoby, S., Arnin, A., Miron, J., Halachmi, I. (2021). Dynamic cooling strategy

based on individual animal response mitigated heat stress in dairy cows. *Animal*, 15(2), 100093.

Linkov, I., Trump, B.D. (2019). The science and practice of resilience. New York, Springer.

Lough, D.S., Beede, D.K., Wilcox, C.J. (1990). Effects of feed intake and thermal stress on mammary blood flow and other physiological measurements in lactating dairy cows. *J. Dairy Sci.*, 73, 325–332.

Mader, T.L., Davis, M.S., Brown-Brandl, T. (2006). Environmental factors influencing heat stress in feedlot cattle. *Journal of Animal Science*, 84(3), 712-719.

Majkić, M., Cincović, M., Spasojević, J., Jožef, I., Blond, B., Kovačević, D. 2023. Kriva rezilijentnosti i kumulativni odgovor krava na toplotni stres. Letopis naučnih radova, in press.

Majkić, M., Belić, B., Cincović, M.R., Plavša, N. (2019). Povezanost insolacije sa ambijentalnim pokazateljima toplotnog stresa kod krava. Zbornik XXX Savetovanje veterinara Srbije, Zlatibor 12-15. septembar, pp. 117-121.

Majkić, M., Cincović, M.R., Belić, B., Plavša, N., Lakić, I., Đoković, R., Krnjaić, S. (2017a). Uticaj toplotnog stresa tokom letnje sezone na ocenu dobrobiti na farmi krava. Zbornik radova 28.Savetovanje veterinara Srbije, Zlatibor 7-10.9., pp.158-162.

Majkić, M., Cincović, M.R., Belić, B., Plavša, N., Lakić, I., Radinović, M. (2017b). Relationship between milk production and metabolic adaptation in dairy cows during heat stress. *Acta Agriculturae Serbica*, XXII, 44, 123-131.

Majkić, M., Cincović, M.R., Belić, B., Plavša, N. (2017c). Indeks termalnog komfora (THI) krava u letnjim mesecima od 2005 do 2016 godine na teritoriji AP Vojvodine. Zbornik radova XXII Savetovanje o biotehnilogiji sa međunarodnim učešćem, Čačak 10-11.03., pp.737-742.

Majkić, M., Plavša, N., Cincović, M.R., Belić, B., Lakić, I. (2020). Vrednosti indeksa temperature i vlažnosti vazduha (THI) u sedam mernih stanica i procena toplotnog stresa krava na teritoriji Vojvodine. *Veterinarski pregled*, 1, 83-89.

Majkić, M., Cincović, M.R., Belić, B., Plavša, N., Hristov, S., Stanković, B., Popović Vranješ, A. (2018). Variations in milk production based on the temperature-humidity index and blood metabolic parameters in cows during exposure to heat stress. *Animal Science Papers and Reports*, 36, 4, 359-369.

Malayer J.R., Hansen P.J. (1990). Differences between Brahman and Holstein cows in heat-shock induced alterations of protein secretion by oviducts and uterine endometrium. *J.Anim. Sci.*, 68, 266–280.

Mazzullo, G., Rifici, C., Lombardo, S.F., Agricola, S., Rizzo, M., Piccione, G. (2014). Seasonal variations of some blood parameters in cow. *Large Animal Review*, 20, 2, 81-84.

McManus, C.M., Faria, D.A., de Bem, A., Maranhão, A.Q., Paiva, S.R. (2020). Physiology and genetics of heat stress in cattle. *CABI Reviews*, https://doi.org/10.1079/PAVSNNR202015018.

Menegassi, S.R.O., Pereira, G.R., Bremm, C., Koetz, C., Lopes, F.G., Fiorentini, E.C., Barcellos, J.O.J. (2016). Effects of ambient air temperature, humidity, and wind speed on seminal traits in Braford and Nellore bulls at the Brazilian Pantanal. *International journal of biometeorology*, 60(11), 1787-1794.

Mihm, M., Curran, N., Hyttel, P., Boland, M.P., Boland, J.F. (1994). Resumptionof meioses in cattle oocytes from preovulatory follicles with a short and long duration, *J. Reprod. Fertil.*, 13, 14.

Montanholi, Y.R., Odongo, N.E., Swanson, K.C., Schenkel, F.S., McBride, B.W., Miller, S.P. (2008). Application of infrared thermography as an indicator of heat and methane production and its use in the study of skin temperature in response to physiological events in dairy cattle (Bos taurus). *J Therm Biol*., 33, 468-475.

Negrón-Pérez, V.M., Fausnacht, D.W., Rhoads, M.L. (2019). Invited review: Management strategies capable of improving the reproductive performance of heat-stressed dairy cattle. *Journal of Dairy Science*, 102(12), 10695-10710.

Noordhuizen, J., Bonnefoy, J.M. (2015). Heat stress in dairy cattle: major effects and practical management measures for prevention and control. *SOJ Vet. Sci*, 1(1), 103.

O'Brien, M.D., Rhoads R.P., Sanders, S.R., Duff G.C., Baumgard, L.H. (2010). Metabolic adaptations to heat stress in growing cattle. *Domestic animal endocrinology*, 38(2), 86-94.

Ostojić Andrić, D., Hristov, S., Stanković, B., Nikšić, D., Stanojković, A., Samolovac, L., Marinković, M. (2022a). The Effect Of Herd Size On Dairy Cows' Welfare Quality–Provision Of Good Feeding And Housing. *Biotechnology in Animal Husbandry*, 38(1), 1-16.

Ostojić Andrić, D., Hristov, S., Zlatanović, Z., Nikšić, D., Pajić, M., Petrović Caro, V., Stanojković, A. (2022). Dairy Cows' Welfare Quality in Relation to Housing System. *Large Animal Review*, 28, 265-272.

Ouellet, V., Toledo, I.M., Dado-Senn, B., Dahl, G.E., Laporta, J. (2021). Critical temperature-humidity index thresholds for dry cows in a subtropical climate. *Frontiers in Animal Science*, 2, 706636.

Paula-Lopes, F.F., Hansen, P.J. (2002). Heat-induced apoptosis in preimplantation bovine embryos is a developmentally-regulated process. *Biol. Reprod*., 66.

Petrović, M.Ž., Đoković, R., Ilić, Z.Ž., Rakonjac, S., Bogosavljević Bošković, S., Kurćubić, V., Petrović, M.D. (2020). Uticaj sezone na koncentraciju proteina toplotnog šoka hsp70 u krvi krava. *Veterianrski pregled*, 1, 112-120.

Petrović, M.Ž., Cincović, M., Starič, J., Djoković, R., Belić, B., Radinović, M., Majkić M., Ilić, Z.Ž. (2022). The correlation between extracellular heat shock protein 70 and lipid metabolism in a ruminant model. *Metabolites*, 12(1), 19.

Polsky, L., von Keyserlingk, M.A. (2017). Invited review: Effects of heat stress on dairy cattle welfare. *Journal of Dairy Science*, 100(11), 8645-8657.

Poulin, C., Kane, M.B. (2021). Infrastructure resilience curves: Performance measures and summary metrics. *Reliability Engineering & System Safety*, 216, 107926.

Raymond, C., Matthews, T., Horton, R.M. (2020). The emergence of heat and humidity too severe for human tolerance. *Sci Adv.*, 8. 6(19):eaaw1838. doi: 10.1126/sciadv.aaw1838).

Rivera, R.M., Kelley, K.L., Erdos, G.W., Hansen, P.J. (2003). Alteration in ultrastructural morphologyof two-cells bovine embrions in vitro and in vivo following a physiologically relevant heat schock, University of Florida, Center of Biotechnology research, 10.1095/ biolreprod.103.020347.

Roth, Z., Arav, A., Bor, A., Zeron. Y., Braw-Tal, R., Wolfenson, D. (2001). Improvement of quality of oocytes collected in the autumn by enhanced removal of impaired follicles from previously heat-stressed cows. *Reproduction*, 122, 737-744.

Roth, Z., Meidan, R., Shaham-Albalancy, A., Braw-Tal, R., Wolfenson, D. (2001a). Delayed effect of heat stress on steroid production in medium-sized and preovulatory bovine follicles. *Reproduction*, 121, 745–751.

Schneider, P. L., Beede, D. K., Wilcox, C. J. (1988). Nycterohemeral patterns of acid-base status, mineral concentrations and digestive function of lactating cows in natural or chamber heat stress environments. J Anim Sci, 66(1), 112-125.

Sherwood, S.C., Huber, M. (2010). An adaptability limit to climate change due to heat stress. *Proc Natl Acad Sci USA*, 107(21), 9552-9555.

Silanikove, N. (1992). Effects of water scarcity and hot environ-ment on appetite and digestion in ruminants: a review. *Livest. Prod. Sci*., 30, 175–194.

Silanikove, N. (2000). Effects of heat stress on the welfare of extensively managed domestic ruminants. *Livest. Prod. Sci.*, 67, 1–18.

Sinha, R., Kamboj, M. L., Ranjan, A., Devi, I. (2019). Effect of microclimatic variables on physiological and hematological parameters of crossbred cows in summer season. *Indian Journal of Animal Research*, 53(2), 173-177.

Spasojević, J., Majkić, M., Cincović, M., Stanojević, J., Blond, B., Kovačević, D., Radinović, M. (2023). Povezanost temperature površine tela izmerene infracrvenom termografijom i indeksa temperature i vlažnosti vazduha u proceni toplotnog stresa krava. *Letopis naučnih radova*, in press.

Stelwagen, K., van Espen, D.C., Verkerk, G.A., McFadden, H.A., Farr, V.C. (1998). Elevated plasma cortisol reduces permeability of mammary tight junctions in the lactating bovine mammary epithelium. *Journal of Endocrinology*, 159, 173–178.

Vranić, P., Milutinović, S. (2016). From local sustainable development towards climate change adaptation: a case study of Serbia. *International Journal of Sustainable Development World Ecology*, 23, 71-82.

West, J.W. (1999). Nutritional strategies for managing the heat-stressed dairy cow. *Journal of Animal science*, 77, 21-35.

Wheelock, J.B., Rhoads, R.P., VanBaale, M.J., Sanders, S.R., Baumgard, L.H. (2010). Effects of heat stress on energetic metabolism in lactating Holstein cows. *J Dairy Sci*, 93(2), 644-655.

Wolfenson, D., Flamenbaum, I., Berman, A. (1988). Hyperthermia and body energy store effects on estrous behavior, conception rate, and corpus luteum function in dairy cows. J. Dairy Sci., 71, 3497-3504.

Wolfenson, D., Thatcher, W.W., Badinga, L., Savio, J.D., Meidan, R., Lew, B.J., Braw-tal, R., Berman, A. (1995). Effect of heat stress on follicular development during the estrous cycle in lactating dairy cattle. *Biol. Reprod.* 52, 1106-1113.

Yadav, B., Singh, G., Verma, A. K., Dutta, N., Sejian, V. (2013). Impact of heat stress on rumen functions. *Veterinary World*, 6(12), 992.

Yan, G., Liu, K., Hao, Z., Shi, Z., Li, H. (2021). The effects of cow-related factors on rectal temperature, respiration rate, and temperature-humidity index thresholds for lactating cows exposed to heat stress. *Journal of Thermal Biology*, 100, 103041.