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INFLUENCE OF CERTAIN ENVIRONMENTAL FACTORS ON BASIC PHYSIOLOGICAL, HEMATOLOGICAL AND BLOOD CELL PARAMETERS IN FREE-RANGE DAIRY COWS

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Abstract

We studied the influence of the temperature-humidity index (THI) on some basic physiological, hematological and blood cell parameters in free-range dairy cows from three cattle farms with different capacity and breeding techniques for a period of one year. Weak to significant variations in the hematological parameters and indices were found in connection with changes in THI. Despite the reported high THI during the light part of the summer days, the animals retain their immune protection: respiration increased by 10-13%, but the heart rate and body temperature remained within the upper reference range, the erythrocytes increased by 7-8%, the leukocytes by 6%, hemoglobin by 10-15% as well as the eosinophils and the monocytes. The values of the blood cell index (BCI), the lymphocyto-granulocyte index (LGI) and the neutrophil-lymphocyte index (NLI) also increased. Almost all studied physiological parameters, blood cell types and indices were dependent on the season and the type of farm - $P < 0.05$ - $P < 0.001$.

Key words: *basic physiological, hematological and blood cell parameters, dairy cows, temperature-humidity index.*

INTRODUCTION

The maintenance of temperature tolerance is of vital importance for the comfort of the cows (West et al., 2003). However, the high temperatures during the summer, even those during the transitional periods, disturb this temperature comfort as well as the well-being of the highly productive cows. The resulting disorders in the thermoregulation of the body lead to changes in the physiological processes, the behaviour and also to milk secretion disruptions (Kekana et al., 2018; Kim et al., 2018; Sejian et al., 2018; Mylostyvyi & Chernenko, 2019; Ouellet et al., 2021).

The heat exchange between the animal organism and the environment is a complex process aimed at maintaining the temperature homeostasis. The processes related to it depend on both a range of biological and many physical factors. Different physical and biological coefficients for their evaluation have been developed and introduced in an effort to minimize the complexity of the respective processes (Jendritzky et al., 2002). Thom (1959) index adapted for cattle is most often used for the assessment of the physical factors of the environment. Based on this index assessment of the environment, the effect i.e. the biological response of the animal is sought so as a conclusion to be made for the comfort, health, productivity and well-being (Hahn et al., 2009).

Physiological and biochemical indicators are most commonly used for the ascertainment of the degree and the nature of the biological response. The blood parameters are one of the most sensitive and fastest-reacting ones when it comes to changes in the cells and the organs in the event of a disease or a stress situation. These changes occur a lot earlier than the manifestation of the first clinical signs. Therefore, according to Mazzullo et al. (2014), Wood & Quiroz- Rocha (2010) their analysis is a suitable indicator for the organism response assessment.

Evtimov & Konstantinov (1968) also consider that the fastest response regarding the effect of the external factors on the animals may be obtained by taking the interior indicators. The publications of other authors (Mazzullo et al., 2014, Wood & Quiroz-Rocha, 2010) also fully support the above mentioned. According to Chulichkova (2017), some blood cell indexes can be used far less frequently for now.

Taking all these into account, we set ourselves the goal to monitor the reactions of dairy cows during winter, summer and transitional periods by using some interior and blood cell parameters.

MATERIALS AND METHODS

The studies were carried out in the course of one year in three cattle breeding farms with a different capacity. The breeding technology in two of them was free in individual boxes, and in the third one- in groups on deep litter bedding.

The cows in the first farms were reared in open buildings with metal construction and a thermo-panel roof. Each animal was provided space of 9.4 m². The area of the individual boxes was 1.25/2.20 m. The floor was cement and in the boxes, it was covered with a solid rubber mat. The chest rails were made of planks. The open spaces providing natural light in each building had a total area of 170 m². The mechanical ventilation was regulated automatically with all devices turning on when the temperature was above 25ºC. The manure cleaning was performed via delta scraper device starting at an interval of 6 hours. The feeding was unlimited with a total mixed ration and a permanent access to water. The milking was performed twice a day in a 'herring bone' milking hall 2 x 12, equipped with herd management software product.

The animals in the second farm were reared in reinforced concrete buildings with concrete walls and roof panels. The side windows and the ridge vents were covered with polyethylene sheets during the winter period. Each cow was provided with space of 11.5 m². The area of the individual boxes was 1.10/2.10 m. The floor of the building was cement and in the boxes, it was covered with a soft rubber mat. The feeding was in the morning and in the evening through a mechanical mixer. The manure cleaning was performed via delta scraper device every three hours. Nipple drinkers were used for watering. The milking was performed twice a day in a 'herring bone' hall 2 x 8.

The cows in the third farm were reared on deep litter bedding in a semi-open building with brick walls without inner or outer screed. Each animal was provided space of 8.06 m². The open areas of the building ensured natural ventilation similar to the tunnel type. The additional mechanical ventilation was turned on gradually at temperatures over 18 and 25ºC. The feeding was unlimited with a total mixed ration and a permanent access to water. The manure cleaning was performed twice a year with a periodic addition of hay. The milking was done twice a day in 'DeLaval' hall 2 x 5.

All microclimatic parameters in the buildings as well as the physiological indicators of the animals were measured at 14.00 h in the course of three days every month. The temperature and the relative humidity in the controlled buildings were measured with an aspiration psychrometer by Assmann. Meanwhile, a weekly thermo-hygrograph reported the diurnal fluctuations of these factors. The temperature-humidity index (THI) was calculated following Kelly and Bond (1971):

$$THI = T - (0.55 - 0.0055 \times RH) \times (T - 58),$$

where:

T is the temperature, ^{0}F

RH - relative humidity, %

Six animals at the same age and physiological condition were selected from each building. Their blood was sampled with Vacutainer K2E 5-4 mg, REF- 368856. The hemoglobin levels, the number of the red and white blood cells as well as the different classes of the white blood count were reported with an automatic hematology analyzer Dymind D7 CRP. The data regarding the different leukocyte types were used so as to calculate the leukocyte indexes (Chulichkova, 2017).

The body temperature was measured with a Kerbl digital thermometer, model 2130, and the respiratory and pulse frequency - with a chronometer in accordance with the approved propaedeutic methods.

The results were statistically processed via SPSS-21. We used a linear model of the following type:

$$Y_{ijk} = \mu + S_i + F_j + e_{ijk},$$

where:

Yijk- observation vector, μ- total mean, S and F are fixed effects of respectively i-th season, j-th- farm; eijk- residuals (dispersion).

RESULTS AND DISCUSSIONS

The appropriate temperature and humidity ensuring convenience, comfort, good health condition and productivity of the dairy cows in accordance with Regulation 44 are at values of respectively 10 and 15°C and 65-75%. Igono et al. (1992) consider temperatures from 24 to 27ºC to be upper threshold ones, while Ozhan et al. (2001) reckon that heat stress might be expected when the temperature exceeds 18-20°C. However, the breakpoint might vary depending on the degree of acclimatization, productivity, physiological condition, the air flow and the relative humidity (Mader et al., 2006).

The temperature-humidity index is used for studying the animals tolerance towards the high air temperatures and it also reflects the overall comfort of the dairy cows (Mahdy et al., 2014). 72 is considered to be the limit value of the index; it corresponds to temperature of 25°C and humidity of 50% (Igono et al., 1992; Ravagnolo et al., 2000).

Referring to the data above, the temperature and relative humidity means regarding the buildings studied by us and displayed in Table 1 show that there were longer or shorter periods with unwanted temperature-humidity regime, especially during the summer and the transitional periods. The temperature-humidity index representing the combined effect of the temperature and the humidity of the air followed the same trend. This index reached 85 during the summer period. Generally, it was within its reference values (70-71) during the transitional periods, while in the winter it fell below 50. Rao et al. (2014) comment that even when the temperature and the relative humidity are within the reference values (27°C and 80%), the THI is higher than that considered to be the limit (72). In this sense, we are inclined to agree with the conclusion reached by Vitali et al. (2009) that the upper minimum value of the coefficient should be 77 and the upper maximum - 87.

Table 1. Means of the microclimatic factors in the controlled buildings

	Transitional period			Summer			Winter		
Parameters	Farm 1	Farm 2	Farm 3	Farm 1	Farm 2	Farm 3	Farm 1	Farm 2	Farm 3
Temperature, °C	21.8	22	22.5	27.8	28.2	27.6	5.8	7.1	6.9
Relative humidity, %	68	73	70	79.0	64.8	75	73	85	76
Temperature-humidity index /THI/	69.8	71	71.4	78.3	82.4	85	43.9	46.3	48.6

According to Raushenbah & Erohin (1975) the barn environment and the rearing technology affect the interior parameters of the animals. The data of our studies show that at THI values between 72 and 78, the body temperature was within the maximum reference values (Table 2), while the respiration accelerated by 10-13%. Sabuncuoglu (2004) states that it is considered one of the most sensitive physiological indicators which follow the change of the temperature and the other physical factors of the environment. Thanks to the sensitive respiratory mechanism, the organism homeostasis is retained. The respiratory movements accelerate and so does the blood circulation in the blood vessels. In addition, the pulse is also quickened but remains within the its reference values. More blood is redirected towards the peripheral parts of the body and the lungs, which leads to quick release of the excess heat.

The hemopoiesis accelerated during the summer period, and as a result there was an increase in the erythrocytes number of the animals of the three farms by 7-8%, while in the transitional and the winter periods, their number experienced no significant change. The same was observed with reference to the hemoglobin (10-15%) and the leukocyte number (more than 6%).

Table 2. Physiological and hematological parameters

Parameters	Physiological limits	Farm 1	Farm 2	Farm 3
Body temperature, °C	37.5-38.5	38.42 ± 0.03	38.33 ± 0.04	38.45 ± 0.06
Breathing, n/min	10-30	32.7 ± 0.02	33.8 ± 0.6	33 ± 0.2
Pulse, n/min	32-80	77 ± 2.1	79.3 ± 0.8	76.7 ± 1.3
Erythrocytes x 10^{12}/L	5.85±0.9	7.4±0.2	6.8±0.4	7.3±0.6
Leukocytes x 10^{9}/L	6.15±1.6	9.4±2.1	11.1±2.1	10.6±2.7
Hemoglobin, g/L	89.6±12.8	121±2.1	112±1.9	125±2.1

Unlike the season, the <<farm>> factor influenced the hematological status of the cows to a much lesser extent. Omran et al. (2011) also report increased erythrocytes and hemoglobin in animals subject to heat stress. Habeeb (1987), however, ascertain decrease of the red blood cells considering that this is a result of a thyromolymphatic involution. Apart from a decrease in the erythrocytes, Al-Haidary (2004) reports increase in their volume. According to the author, this is an adaptation mechanism for ensuring the water, necessary for the organism evaporative cooling. Srikandakumar & Johnson (2004) even assume that the erythrocytes decrease may be caused by the free radicals attack against them as well as the insufficient nutrient intake due to the heat stress. The erythrocytes increase probably stems from the accelerated metabolic processes in the organism. Other authors presume that the increase of the blood elements and the hemoglobin is a result of the developing dehydration (Mirzadeh et al., 2010).

The leukocytes, which according to Nenashev & Bikteev (2008) vary widely, provide abundant information about the blood. The leukocyte increase ascertained by us was relatively high (more than 53%) when compared to that indicated by Abdel-Samee (1987) - 21-26%. Similar to our trend was reported by Lallawmkimi (2009) with reference to pregnant and suckler buffalo cows. The increase observed during the summer period, however, was not related to a change in the leukocyte groups. The fluctuations registered by us in most of the leukocyte groups were within the reference values (Table 3). This makes us consider that despite the high THI during the summer period, the animals immunological protection was retained. This is also proved by the increasing percentage of the leukocytes, neutrophils and eosinophils taking part in the blood cell parameter formation (BCC).

The eosinophils have various functions but resembling those of the other leukocytes. They are a source of many cytokines and growth factors related to immunomodulation functions of the organism. The slight increase (around 14% on average) of the eosinophils during the hot period ascertained by us is also confirmed by Ciaramella et al. (2005) in buffaloes aged over ten years. According to Mayengbam (2008), the changes in the eosinophil and basophil count caused by thermal exposition (40 and 45°C) are also negligible.

The highest values of the blood cell coefficient (BCC) were reported during the summer period (from 0.98 to 1.08), followed by the winter one (0.83-0.93). It remained the lowest during the transitional period (0.68-0.84). Regardless of the slight fluctuations, the coefficient demonstrated a certain dependence on the season and the type of farm ($p<0.001$). Most probably, this was a result of not only the high and the low temperatures of the barn environment which stimulated the cell immunity, but also of the antigen influence of the growing fetus (Chulichkova, 2017).

Table 3. Means of the blood cell indexes examined in the different farms during the different seasons

№	Parameters	Season 1			Season 2			Season 3			SD
		F1	F2	F3	F1	F2	F3	F1	F2	F3	
1	BCC*, conditional units	0.81	0.68	0.84	1.08	0.89	0.98	0.92	0.83	0.93	0.11
2	RNR*, conditional units	1.1	0.38	1.7	0.58	0.50	0.53	0.49	0.49	0.50	0.41
3	LGI*, conditional units	10.5	13.2	9.7	8.1	10.3	9.4	9.5	10.8	9.6	1.3
4	I neutr/lymph, conditional units	0.94	0.73	1	1.2	0.94	1	1	0.89	1	0.12
5	I lymph/eosin, conditional units	67.1	31.3	88.4	23.3	26.8	25.9	24.3	28.4	25.7	22.2
6	I neutr/eosin, conditional units	63.1	22.8	90	27.9	25.2	26.6	24.7	25.4	25.7	22.4
7	Leukocytes, 10^9/l	7.95	7.2	8.2	11.4	10.9	11.7	9.5	8.2	8.6	1.9
8	Erythrocytes, 10^{12}/l	5.93	6.03	6.07	7.38	7.45	6.93	6.05	5.69	5.53	0.76
9	Hemoglobin, g/l	109	116	108	125	122	121	108	107	109	7.32

10	Neutrophils, %	44.2	38.7	45	50.2	45.3	47.8	44.5	43.2	46.2	5.4
11	Eosinophils, %	0.67	1.67	0.50	1.83	1.83	1.83	1.83	1.67	1.83	0.9
12	Lymphocytes, %	47	53	44	42	48	47	44	48	46	5
13	Monocytes, %	8.2	6.5	10	6	4.5	3.7	6.3	6	5.5	2.1

*Note: *THI - temperature and humidity index; BCC - blood cell count; RNR - reactive neutrophil response; LGI - lymphocyte-granulocyte index; NLI – neutrophil – lymphocyte index; LEI – lymphocyte-eosinophil index; NEI- neutrophil- eosinophil index*

The antimicrobial neutrophil factors might provisionally be subdivided into two groups: components of the mature neutrophil whose quantity depend not on the degree of cell stimulation but on substances which are synthesized in the granulopoiesis process (lysozyme, lactoferrin et al), and substances which are formed or sharply activated upon neutrophil stimulation i.e. their quantity depends on the extent of the cell reaction. Our results showed that the coefficient characterizing the reactive neutrophil response (RNR) had the highest values during the transitional period when the temperature-humidity regime in the barn environment was within relatively acceptable values. Only the cows from building 2 made an exception by indicating lower coefficient values throughout the whole year. Despite that, the season and farm factors affected its values ($p<0.001$).

The animals immunological status maintenance is a result of not only the neutrophil activity but also that of the lymphocytes. In our case, the fact that the lymphocyte count was retained within the physiological norms during the different seasons means that normal phagocyte processes were maintained. This was also confirmed by the two-phase orientation of the changes in the lymphocyte-granulocyte index values (LGI) and the correlation between neutrophils and lymphocytes (NLI). Both indexes once again show a high-grade dependence on the season and the type of farm- $P<0.001$ (Table 4).

Table 4. Analysis of variance with reference to the influence of the season and the farm on the hematological parameters and the blood cell indexes

Hematological parameters and leukocyte Indexes	Model	
	Season F and significance	Farm F and significance
BCC, conditional units	267.013***	135.487***
RNR, conditional units	25.860***	13.495***
LGI	53.918***	78.777***
NLI	46.309***	72.065***
LEI	54.382***	9.725***
NEI	35.310***	13.260***
Leukocytes, 10^9/l	43.926***	2.865*
Neutrophils, %	5.309**	4.000*
Eosinophils, %	6.963**	0.896
Lymphocytes, %	1.504	7.819**
Monocytes, %	23.749***	2.610*
Erythrocytes, 10^{12}/l	82.052***	2.725*
Hemoglobin, g/l	89.135***	1.804

Note: ***$P<0.001$; **$P<0.01$; *$P<0.05$

The lymphocyte/eosinophil (LEI) and the neutrophil/eosinophil (NEI) indexes exhibited similar dependence on the season and the farm. In addition, the numerical expression of these indexes was almost equal both in the hot and in the cold winter period. The same indexes were 2-3 times higher during the spring and the autumn but only with reference to the animals bred in the open buildings. The more frequent and sharper fluctuations of the environmental factors in these buildings maintained the adaptive tension by stimulating the antigen/ antibody reactions and stabilizing the homeostatic indicators typical for their physical and chemical thermoregulation.

The monocytosis demonstrated during the different seasons might be attributed to the strong organism resistance to the THI fluctuations. Migrating in the tissues, the monocytes stimulate or suppress the lymphocyte proliferation and differentiation. The seasonal activation observed by us might be a result of both immunological and non-immunological irritation. Abdelatif & Alameen (2012) consider that the higher monocyte percentage found in the cows reared in open spaces during the transitional periods (6.5-10) is connected to the increased cortisol secretion. Using the leukocyte indexes to analyze the different variants of intercellular ratios in the cows blood, Safonov (2013) ascertains that during the foeto-placental complex formation, the NLI decreases by 23.4%, NEI diminishes twice, LEI- by 35.4%, and the LMI increases 2.7 times. According to the author, there is an activation of the immunity effector cell unit and the microphage protection system.

CONCLUSIONS

The results of our studies displayed weak or more strongly exhibited variations of the hematological parameters and indexes in dairy cows in connection with the THI changes. Despite the high THI during the daytime in the summer, the animals retained their immunological protection by increasing their erythrocytes by 7-8%, leukocytes by 6%, hemoglobin by 10-15%, eosinophils and monocytes as well as the values of the blood cell index (BBI), the lymphocyte-granulocyte index (LGI), the neutrophil-lymphocyte index (NLI). Almost all blood cell groups and indexes tested were dependent on the season and the type of farm - $P<0.05$- $P<0.001$.

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