DOI: 10.22620/agrisci.2021.28.003

INFLUENCE OF THE TEMPERATURE-HUMIDITY REGIME IN DAIRY CATTLE BUILDINGS ON SOME HEMATOLOGICAL PARAMETERS AND LEUKOCYTE INDEXES

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Abstract

The present study examines the influence of the temperature-humidity index (THI) on some hematological parameters and leukocyte indexes in dairy cows during transitional (spring and autumn), summer and winter periods. It was ascertained that in the summer the hematopoiesis intensifies and as a result, the number of the erythrocytes of the animals from all three farms builds up by 7 - 8%, while during the transitional and winter periods their number remains almost unchanged. Similar increase is also observed with reference to the haemoglobin (10 - 15%) and the number of leukocytes (more than 6%). Unlike the season, the «farm» factor has a considerably weaker influence.

The leukocytes increase during the summer is not linked to any change in their groups. In most of the leukocyte groups the fluctuations are within the physiological norms. This gives grounds for assuming that despite the high THI during the summer, the immunological reactivity of the animals remains stable. Monocytes increase (6,5 - 10) is observed during the transitional period in the animals which are bred in open houses.

The blood cell coefficient (BCC) also marks its highest values during the summer (from 0,98 to 1,08), followed by the winter (0,83 - 0,93) and the transitional period (0,68 - 0,84). The lymphocyte- granulocyte index (LGI) as well as the neutrophils and lymphocytes ratio are to a large extent dependent on the season and the type of farm - $P<0,001$.

The lymphocyte granulocyte index (LGI) and the ratio between neutrophils and lymphocytes (NLI), lymphocyte/eosinophils (LEI), and neutrophils/ eosinophils (NEI) depend both on the season and the type of farm $P<0,001$. During the transitional period these indexes are 2-3 times higher only with reference to the animals bred in the open buildings.

The lymphocyte /eosinophils and neutrophils/ eosinophils indexes are almost equal both during the hot summer and cold winter periods.

Keywords: dairy cattle, heat stress, blood cell parameters and leukocyte indexes

INTRODUCTION

The lactation period of cows is parallel to the new pregnancy. This requires the animals to be placed under the most comfortable conditions possible. During the entire period the dairy cattle organism experiences changes related to the hormonal balance, metabolism and immunity protection (Nekrasov, 2007). Metabolism, especially protein metabolism, is intensive in order to both ensure maximum milk production levels and form a healthy fetus (Vasilenko, 2008). This condition of the animals is not only related to changes in the blood cell profile but also in their immunobiological reactivity which, according to Chulichkova (2017), are still relatively poorly examined.

The morphological content of the blood is highly sensitive and is subject to the fastest change upon interaction with different external or internal factors such as age, season, year, breed, productivity, physiological condition et al. (Kocharyan, 2008, Koryakina, 2009, Kobec, 2003, Derho, 2014). Therefore, the blood cell elements examination is a good base for ascertainment of occurrence of physiological changes in animals at the earliest stage possible (Jain, 1993, Mazzullo et al., 2014).

Data regarding the influence of the temperature and the relative humidity of the farm environment on the haematological status of dairy cattle can be found in the literature of (Abdelatif and Alameen, 2012; Mazzullo et al., 2014) but such regarding the influence of these ecological factors on the leukocytic indexes and the overall resistance of the animals are scarce (Chulichkova, 2017). This motivated us to study the influence of the temperature and the relative humidity through the complex temperature-humidity index (THI) on some haematological parameters and leukocytic indexes of dairy cows.

MATERIALS AND METHODS

The studies were carried out in the course of one year in three different- capacity cattle farms located in Plovdiv region. The results were grouped in three seasons- transitional (spring and autumn), summer and winter. The cow breeding technology in two of the farms is free in separate boxes, and in the third one- group living on a permanent litter bedding. The buildings examined were nominated F 1, F 2 and F 3 in order to be protected their confidentiality.

Building F 1 shelters 130 dairy cows, divided into two groups of 65 animals each. It is an open metal construction with a thermopanel roof. The side walls are made of concrete with width of 0,25 m and height of 1,5 m. The end walls are also made of concrete and are 3,0 m high. The feeding lane zone has no doors and is entirely open. The total area of the building is 1 248 m², and each animal has 9,4 m² individual area ensured.

The dairy cows reared in building F 2 are 200, divided into 4 groups. It has a total area of 2 310 m², and the individual area per animal ensured is 11,5 m². The building is a reinforced concrete construction with concrete walls and roof panels.

Building F 3 accommodates 67 dairy cows bred free in a group living on deep litter bedding and has a total area of 598,5 m². The area provided for movement and rest is 540 m². Each cow is ensured 8,06 m². The building is semi-open and the roofs are made of double bricks without inner or outside coating.

Each month in the course of three to five days, we measured the temperature and relative humidity in the controlled buildings at 8 a.m., 2 and 9 p.m using Assmann psychrometer. At the same time, a weekly thermo-hygrograph recorded the daily fluctuations of these factors. The calculation of the temperature- humidity index (THI) was performed under Kelly and Bond (1971):

$$THI = T - (0,55 - 0,0055 \times RH) \times (T - 58)$$

where

T is the measured temperature, ^{0}F

RH- relative humidity, %

Six animals at the same age and physiological condition were selected from each of the farms. Their blood samples were taken with Vacutainer K2E 5 - 4 mg, REF – 368856. The hemoglobin content, the number of the red and white blood cells as well as the separate classes of the white blood count, were reported via automated hematology analyzer Dymind D7 CRP. The data about the different leukocyte types were used to calculate the leukocytic indexes (Chulichkova, 2017).

The results were statistically processed via SPSS – 21 and the following linear model was used: Yijk=μ+Si+Fj+eijk, where Yijk is an observation vector, μ is a total average constant, S and F are fixed effects of respectively i-season and j-farm; eijk- residuals (dispersion).

RESULTS AND DISCUSSION

The average values of the temperature-humidity index and the parameters examined in the different farms during the different seasons are displayed in Table 1. It can be seen that THI is the highest during the summer period when it reaches 85. During the winter it varies between 44 and 48,6. The values of this coefficient during the transitional period are 70 - 71. Some authors assume that these values are optimal and believe that the animals are in a state of welfare (Dimov, 2017). However, when the index exceeds 78, the maintenance of temperature homeostasis will be impeded (Miteva, 2012). Rao et al. (2014) presume that even if the temperature and the relative humidity are within the norms (27ºC and 80%), the THI is equal to 78. Therefore, we are likely to support the conclusion made by Vitali et al. (2009**)** with reference to the upper minimal value of the coefficient- 77 and upper maximum- 87. What is more, upon THI calculation, the air flow does not take any part. In further support, it can be also added that the grouping of heat stress performed by Chase (2006) based on the so called heat load index (HLI): when the index is 72 there is a zero influence, when it is 72 - 79 it is mild, when it is 80 - 89- moderate, and above 90- high.

The changes in the environment during the seasons can affect the haematological parameters of the farm animals (Feldman et al., 2000). It can be noticed that the hemopoiesis intensifies in the summer. As a result, in that period the number of erythrocytes increases by 7 - 8% in the animals from the three farms, while during the transitional and the winter period, their number remains relatively unchanged. The same trend is observed with reference to the haemoglobin (10 - 15%) and the number of leukocytes (more than 6%). Unlike the season, the «farm» factor has a considerably weaker influence.

Taking into account that the erythrocytes are oxygen carriers, their increase, according to Lutsenko (2012), is prompted by the increased metabolic processes in the organism as a result of the influence of the barn environment. Other authors assume that this increase of the blood elements and the haemoglobin is a result of a progressing dehydration (Mirzadeh et al., 2010). Omran et al. (2011) presume that the dehydration itself is the reason for additional erythrocytes release from the spleen.

The leucopoiesis organs constantly synchronise their work under the influence of various external and internal factors (Nezhdanov, 2003). Therefore, it may be considered that one of blood parameters carrying the richest information is that of the leukocytes, which in the case of cows vary within wide ranges (Nenashev, 2008). The protective function of the organism is based namely on them (Amagirova, 2010, Hristev, 2007). The leukocyte increase during the summer, however, does not lead to any changes in their groups. In most of the leukocyte groups, the fluctuations are within the physiological norms (Table 2). This provides grounds for considering that despite the high THI levels in the summer, the immunological reactivity of the animals remains stable.

In the recent years, the leukocyte indexes find their application upon monitoring the immunological status of the animals (Tkachenko, 2014; Mustafina, 1999;

Tihonchuk, 1992). They provide the opportunity to evaluate the work of the immune system effector mechanisms which determine the processes related to the formation of the non-specific adaptation reactions.

The results of the present study reveal that the blood cell count (BCC) exhibits its highest values in the summer (from 0,98 to 1,08) followed by the winter (0,83 - 0,93) period. The transitional period has the lowest blood cell parameter (0,68 - 0,84).

Table 1. Average values of the parameters 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	THI	69,8	71	71,4	78,3	82,4	85	43,9	46,3	48,6	15,2
2	BCC, conditional units	0,81	0,68	0,84	1,08	0,89	0,98	0,92	0,83	0,93	0,11
3	RNR, conditional units	1,1	0,38	1,7	0,58	0,50	0,53	0,49	0,49	0,50	0,41
4	LGI, conditional units	10,5	13,2	9,7	8,1	10,3	9,4	9,5	10,8	9,6	1,3
5	I neutr/lymph, conditional units	0,94	0,73	1	1,2	0,94	1	1	0,89	1	0,12
6	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
7	I neutr/eosin, conditional units	63,1	22,8	90	27,9	25,2	26,6	24,7	25,4	25,7	22,4
8	Leukocytes, 10^9/l	7,95	7,2	8,2	11,4	10,9	11,7	9,5	8,2	8,6	1,9
9	Neutrophils, %	44,2	38,7	45	50,2	45,3	47,8	44,5	43,2	46,2	5,4
10	Eosinophils, %	0,67	1,67	0,50	1,83	1,83	1,83	1,83	1,67	1,83	0,9
11	Lymphocytes, %	47	53	44	42	48	47	44	48	46	5
12	Monocytes, %	8,2	6,5	10	6	4,5	3,7	6,3	6	5,5	2,1
13	Erythrocytes, 10^{12}/l	5,93	6,03	6,07	7,38	7,45	6,93	6,05	5,69	5,53	0,76
14	Hemoglobin, g/l	109	116	108	125	122	121	108	107	109	7,32

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

Regardless of the minor fluctuations, this parameter displays a certain dependence not only on the season but also on the type of farm (p<0,001). The increase of the BCC is a result of the increase of the number of leukocytes, the percentage of eosinophils and of the neutrophils. In all likelihood, this is due not only to the high and low temperatures of the barn environment but also to the antigenic influence of the growing fetus stimulating phagocytic protection (Chulichkova, 2017).

Table 2. Results of the parameters examined in the three farms during the three seasons.

	N	Minimum	Maximum	Mean		Std. Deviation	Variance
	Statistic	Statistic	Statistic	Statistic	Std. Error	Statistic	Statistic
THI	54	43,9	85,0	66,30	2,062	15,153	229,619
BCC	54	0,68	1,08	0,88	0,015	0,108	0,012
RNR	54	0,4	1,7	0,69	0,056	0,408	0,167
LGI	54	8,1	13,2	10,12	0,181	1,327	1,761
NLI	54	0,73	1,20	0,97	0,016	0,118	0,014
LEI	54	23,3	88,4	37,91	3,020	22,193	492,551
NEI	54	22,8	90,0	36,82	3,049	22,408	502,111
Leukocytes	54	5,6	13,7	9,32	0,263	1,931	3,728
Neutrophils	54	35	58	45,00	0,737	5,418	29,358
Eosinophils	54	0	3	1,52	0,120	0,885	0,783
Lymphocytes	54	36	58	46,65	0,687	5,048	25,478
Monocytes	54	2	12	6,30	0,292	2,142	4,590
Erythrocytes	54	5,12	8,24	6,34	0,103	0,760	0,578
Hemoglobin	54	100	128	113,91	0,997	7,329	53,708

The standard deviation is low with the lowest being observed in the BCC, RNN, NLI indexes and the eosinophils leukocytes. More significant fluctuation was observed in LEI, NEI and the hemoglobin. The blood provides a relative stability in the internal environment and as such it changes its composition upon high environmental temperatures, although this alteration is slight. In one of the works of Noura El-Shahat Attia (2016) it is proved that the heat stress leads to significant changes in the physiological, some hematological and biochemical parameters.

The antimicrobial factors of the neutrophil can be provisionally divided into two groups: substances of the adult, mature neutrophil, whose amount depends not on the degree of stimulation of the cell but on the components synthesised during the process of granulopoiesis /lysocim, lactoferon and others/ and substances which are formed or are sharply activated upon neutrophil stimulation i.e. their amount depends on the intensity of the cell reaction.

In the particular case, the coefficient characterising the reactive neutrophil response (RNR) is the highest during the transitional period when the temperature-humidity regime of the barn environment is also within more acceptable levels. The only exception of the above mentioned are the cows from building F2.

The maintenance of the immunological status of the animals is a result not only of the activity of the neutrophils but also of that of the lymphocytes. This is also proved by the two-phase orientation of changes in the values of the lymphocyte- granulocyte index (LGI) and the ratio of the neutrophils and lymphocytes (neutr/lymph- NLI). To a large extent both indexes are dependent on the season and the type of farm- P<0,001 (Table 3).

Table 3 Seasonal and farm influence on some hematological parameters and leukocyte 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

***P<0.001; **P<0.01; *P<0.05

The indexes characterizing the ratio lymphocytes /eosinophils (LEI) and neutrophils/ eosinophils (NEI) also display dependence on the season and the type of farm. What is more, the numeric values of these indexes are almost equal both during the hot summer and the cold winter periods. The indexes are 2 - 3 times higher during the transitional period but only with reference to the animals reared in the open buildings. The data show that due to the more sudden and more common fluctuation of the environmental factors in these buildings, the adaptation pressure of the animals is higher and it remains such until the stabilization of the homeostatic parameters typical of their physical and chemical thermoregulation.

The monocytes derive from the stem cells of the bone marrow. They have the capacity to migrate in the tissues as well as to stimulate or suppress the proliferation and the differentiation of the lymphocytes. Their activation is definitely seasonal and may be a result of immune or non-immune stimuli.

The higher monocyte percentage of the animals reared in the open farms (6,5- 10) during the transitional period may be attributed to the increased secretion of cortisol (Abdelatif and Alameen, 2012).

CONCLUSIONS

During the summer period the hemopoiesis intensifies and as a result there is an increase in the number of erythrocytes by 7 – 8%, the hemoglobin values (10-15%) and the number of erythrocytes (more than 6%) with reference to the animals of all three farms. The influence of the 'farm' factor on them is weak.

During the summer is observed an increase in the levels of the haemoglobin (10-15%) and the number of the leukocytes (more than 6%). They are also in a positive and high correlation dependence on THI: (r= 0,545; r= 0,480 and r = 0,695 respectively).

Despite the high THI during the summer, the immunological reactivity of the animals is retained (the leukocyte increase during the summer is not related to any changes in their groups).

The season influences the monocytes increase. During the transitional period, this increase is from 6,5 to 10% with reference to the animals reared in open buildings.

The blood cell coefficient (BCC) also exhibits its highest values during the summer (from 0,98 to 1,08), followed by the winter (0,83- 0,93) and the transitional period (0,68- 0,84)

The lymphocyte- granulocyte index (LGI) and the ratio neutrophils/ lymphocytes (NLI, lymphcyte/eosinophils (LEI) and neutrophils/eosinophils (NEI) are to a large extent dependent on the season and the type of farm- $P<0,001$. Furthermore, they also display high but mutually inverse correlation with THI (r= -0,939 and r= 0,940 respectively).

The lymphocyte /eosinophil and neutrophil/ eosinophil indexes are almost equal both during the hot summer and cold winter periods.

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