

Implications of seasonal leukocyte blood formula fluctuations in stallions for breeding management

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Abstract. The influence of seasons of the year on the leukocyte blood formula parameters in stallions was studied. The study involved 14 stallions of different breeds; the average age of animals was 9.8 ± 2.0 years. It was found that the composition of leukocytic formula in stallions can change in different seasons of the year. We observed an increase in the number of segmented neutrophils in the studied stallions in spring and summer compared to the fall-winter period. In spring and summer periods we observed a decrease in the number of lymphocytes in the blood compared to the fall and winter periods. We found an increase in the number of eosinophils in the blood in summer and fall. The number of segmented neutrophils and lymphocytes in the blood of stallions by 13% and 12%, respectively, depended on the season of the year ($p < 0.05$).

1 Introduction

Animal health is one of the most important factors to ensure effective work in horse breeding aimed at increasing the productivity and performance of horses. Regular clinical examinations and laboratory research are performed to monitor animal health. The most accessible for obtaining and informative biomaterial is blood [1].

Blood consists of a liquid part - plasma and cells suspended in it, the formational elements: erythrocytes, leukocytes and platelets. Each type of blood cells performs a large number of functions necessary for the vital activity of the body.

Organs responsible for hematopoiesis, are highly sensitive to various physiological changes and pathological effects on the body. The composition of blood in the norm is fairly constant, but in response to internal or external factors can change. Therefore, hematologic and biochemical blood analysis reflects various influences on the body and provides important information about the animal's health status, metabolic changes in the body and often helps to identify health disorders already at the preclinical stage [2].

In some diseases, changes in the body may not be detected clinically, but blood tests can reveal hidden processes and possible complications [3, 4]. Blood research helps to distinguish between similar infectious and non-infectious diseases, as well as to assess the

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general condition of the body and the functioning of specific organs [5]. In addition, results analysis makes it possible to monitor the effectiveness of treatment and make necessary adjustments. With the help of quantitative and qualitative blood parameters, combined with clinical data, it is possible to perform differential diagnosis of such conditions as anemias of various types, leukemia, and diseases caused by blood parasites and other causes [6].

One of the important methods of clinical laboratory diagnostics that make it possible to assess the health status of animals is the general blood count (GBC). The general blood count includes determination of the number of erythrocytes, hemoglobin, leukocytes, platelets, the color index, hematocrit, erythrocyte sedimentation rate (ESR) and leukocyte formula (the number of leukocyte forms expressed as a percentage) are also determined.

Leukocytes or white blood cells are cells of the immune system that perform a protective function in the body. They are divided into granulocytes and agranulocytes. In turn, granulocytes include neutrophils, basophils, eosinophils, while agranulocytes include lymphocytes and monocytes. Deviations in the content of the number of leukocytes in the blood acts as an important indicator of the presence of a pathological process. Also, no less important is the change in the ratio of leukocyte species in the leukocyte formula.

Unlike erythrocytes, leukocytes are very labile cell populations, and changes in their content can be associated with numerous both physiological and damaging factors. In addition, leukocytes can respond significantly to changes in the external and internal environment under physiological conditions, being an important criterion for proper feeding and animal care [7].

Leukocyte formula is a numerical designation of the number of certain types of leukocytes in the blood, which can reflect both genetic and individual physiological features of the animal, as well as the presence of pathological processes in their body. Leukocytes are known to play an important role in the immune system of animals and help in defense against infections and other pathological conditions. Leukocytes originate from stem cells in the bone marrow and have a short life span, which requires their constant renewal. The process of their formation is accelerated in response to various tissue injuries, which is part of the normal inflammatory process. Although each type of white blood cell has specific objectives, they can interact with each other through cytokine-mediated "messages." In contrast to the homogeneous population of red blood cells, white blood cells are divided into five different types, each with their own characteristics and functions. Under normal conditions, their number may vary due to various factors [8].

To interpret the results of blood research, including the leukocyte formula, it is necessary to take into account the sex, age [9, 10] and breed of the animal [11]. In addition, blood values may also be influenced by the season of the year [12]. Weather changes can affect the immune system of animals, which in turn causes changes in the leukocyte blood formula [13]. Seasonal fluctuations in the number of different types of leukocytes may indicate rearrangements in the immune system of animals in response to the season and environment [14].

Regular blood analysis, conducted consecutively and at a certain interval during 12 months, makes it possible to study the influence of the seasonal factor on the leukocyte formula indices, while single research is used mainly to compare the obtained values with physiological norms [15]. Therefore, studying the influence of the seasonal factor on the indicators of biological fluids of the organism seems to us to be a rather topical research direction.

The objective of this research was studying the changes of leukocyte formula parameters in blood in stallions in different seasons of the year.

2 Methods and materials

The research was conducted during the period from 2020 to 2021 on the basis of the All-Russian Research Institute for Horse Breeding (ARRIH, Ryazan Region). The experiments involved 14 stallions of different breeds; the average age of the animals was 9.8 ± 2.0 years. The conditions of keeping and feeding of animals at the time of research corresponded to the established norms and requirements.

Blood collection. Blood was collected once a month (in the middle of each month) during the calendar year. A sample of blood from each stallion was taken from the jugular vein before morning feeding into Vacuette Premium Series vacuum plastic tubes (4 ml) with anticoagulant (K3EDTA) (Greiner BioOne GmbH, Austria). Before sample collection, the intended puncture site was disinfected with 70% alcohol.

Blood research. Leukocyte formula - the percentage of different types of leukocytes - was counted in stained blood smears. Blood smears were stained by Pappenheim staining. The preparations were examined using an Olympus BX41 phase-contrast microscope (Olympus Corporation, Japan). At least 150 cells were examined in each sample.

Statistical analysis. For statistical processing of the data obtained, the average value for each of the studied parameters for a season of the year was taken. To process the obtained materials, Microsoft Office Excel 2016 ("Microsoft", USA) and Statistica 13.3 (Stat Soft., Russia) application program packages were used. The results are presented in the form of arithmetic mean (M) and its standard error (m). Duncan's posterior rank criterion was used to assess different averages between paired groups. To reflect the proportion of variation in the effective feature, we calculated the determination coefficient R^2 and R^2 adjusted. The significance level (p) was used to assess the reliability of the obtained relationships. Differences were considered statistically significant at $p \leq 0.05$.

3 Results and discussion

In healthy horses, the number of leukocytes in the peripheral blood ranges from 7.0 to 12.0 x 10⁹/l. Among these cells, basophils constitute 0-1%, eosinophils 2-6%, segmented neutrophils 45-62%, rod-shaped neutrophils 3-6%, lymphocytes 24-44%, and monocytes 2-4% [16].

As a result of the conducted research, data obtained on the quantitative changes of different types of leukocytes in stallions showed that the white blood cells were within the normal values for leukocyte formula in different seasons of the year (Table 3). It was also found (Table 1) that the percentage of segmented neutrophils and lymphocytes in the blood in summer differed significantly from similar values in fall and winter, and the percentage of eosinophils in spring from summer and fall.

Table 1. Approximate probabilities for a posteriori criteria for the dynamics of leukocyte formula indices in the blood of stallions depending on the season of the year.

Indicator	Season	Season			
		Spring	Summer	Autumn	Winter
Bacillary neutrophils	Spring		0.396009	0.791650	0.777546
	Summer	0.396009		0.510371	0.537304
	Autumn	0.791650	0.510371		1.000000
	Winter	0.777546	0.537304	1.000000	
Segmented neutrophils	Spring		0.464973	0.062374	0.062806
	Summer	0.464973		0.014566	0.013771
	Autumn	0.062374	0.014566		0.922636
	Winter	0.062806	0.013771	0.922636	
Lymphocytes	Spring		0.288534	0.133235	0.102948
	Summer	0.288534		0.016219	0.011055
	Autumn	0.133235	0.016219		0.820388
	Winter	0.102948	0.011055	0.820388	
Monocytes	Spring		0.725268	0.672421	0.960029
	Summer	0.725268		0.920042	0.707049
	Autumn	0.672421	0.920042		0.651307
	Winter	0.960029	0.707049	0.651307	
Eosinophils	Spring		0.042928	0.031051	0.086269
	Summer	0.042928		0.829977	0.667700
	Autumn	0.031051	0.829977		0.546641
	Winter	0.086269	0.667700	0.546641	

Thus, based on the data in Table 2, it was found that the number of segmented neutrophils and lymphocytes by 13% and 12%, respectively, depended on the season of the year ($p < 0.05$).

Table 2. Determination coefficients and significance levels of leukocyte formula indices in the blood of stallions depending on the season of the year.

Indicator	R ²	R ² correc	p
Bacillary neutrophils	0.018293	-0.038344	0.808711
Segmented neutrophils	0.179020	0.131655	0.015788
Lymphocytes	0.170314	0.122448	0.020345
Monocytes	0.007279	-0.049994	0.943630
Eosinophils	0.120266	0.069512	0.081166

Note: R² is the determination coefficient, which shows the proportion of variation that is explained by the conjugacy of variation between the signs. Like the original determination coefficient, the adjusted coefficient (R²) makes it possible to assess the accordance of the regression model with the original data, as well as to compare models with a different number of independent variables. The level of significance (p) reflects the probability of error in the conclusions of the statistical calculation.

An increase in the percentage of segmented neutrophils in the blood was found% in spring - $52.04 \pm 1.65\%$, and in summer - $53.55 \pm 1.43\%$ compared to the fall-winter period. We also observed a decrease in lymphocytes in spring and summer periods - $46.29 \pm 1.64\%$ and $44.07 \pm 1.38\%$, respectively, compared to the number of lymphocytes in fall and winter (Table 3). Similar results were obtained in the research of Dmoch M. et al. (2008) and Ono T. et al. (2021) [17, 18]. Such patterns may be related to the increase in physical activity and heat stress in horses during the summer period [19]. According to Fedosova O.A. (2010) the number of leukocytes in the blood of horses was higher in spring and summer compared to the fall-winter period, which may indicate an increase in the level of metabolic and protective processes during the active functional state of the sexual system [20].

Table 3. Leukocytic formula indices in the blood of stallions in different seasons of the year.

Indicator, units.	Season			
	Spring	Summer	Autumn	Winter
Neutrophils bacillary, %	0.09±0.05	0.02±0.02	0.08±0.05	0.07±0,07
Segmented neutrophils, %	52.04±1.65	53.55±1.43	48.14±1.52	47.94±1.14
Lymphocytes, %	46.29±1.64	44.07±1.38	49.44±1.56	49.90±1.22
Monocytes, %	0.26±0.13	0.31±0.10	0.32±0.08	0.25±0.08
Eosinophils, %	1.16±0.19	1.89±0.28	1.96±0.22	1.74±0.24

Bakai A.B. and Mugniev E.P. (2006) in their research found that the content of neutrophils and eosinophils in cattle increases in the spring period, while the number of lymphocytes decreases during the same period [21].

According to Oshkina L.L. and Trifonov G.A. (2013) the number of neutrophils in the blood of bulls-producers in different seasons of the year changes insignificantly and unreliably. The number of neutrophils increases in spring up to the level of 27.4±0.5%, also there are insignificant fluctuations of this indicator in other seasons of the year within the limits of 25.6±0.5% - 26.0±0.5%. The lowest number of lymphocytes in the blood of bulls-producers is observed in the spring period of the year (63.0±0.7%), in other periods there are slight fluctuations in the range from 64.0±0.7% to 65.0±0.7% [22]. The data obtained by these researchers in the conditions of the Middle Volga region agree with our results obtained in the average strip of Russia.

We observed an increase in the number of eosinophils in summer and fall - 1.89±0.28% and 1.96±0.22%, respectively. The increase in the number of eosinophils during these periods may be associated with allergic reactions to insect bites [18].

According to the results of the conducted research and studying the scientific literature, it can be said that changes in leukocytic formula in horses as well as in other animal species occur depending on the season of the year. Seasonal changes in leukocytic formula can be considered based on both changes in ambient temperature and a number of other factors:

- in spring, with plant growth and the onset of flowering, antigenic load increases, which may affect the number of lymphocytes and granulocytes in the blood;
- in summer, high temperature, humidity, flowering of plants and increase in the number of insects can cause both a load on the body's thermoregulation and antigenic reactions, which, in turn, can affect the number of lymphocytes and granulocytes (neutrophils and eosinophils);
- in the fall, there is a decrease in immune reactions caused by reduced antigenic load and wilting vegetation, which may result in a slight decrease in the total number of granulocytes and lymphocytes;
- in winter time, when antigenic load is minimal, leukocytic formula indices are more stable in most animals, however, exceptions are possible.

Studying the influence of exogenous factors on the animal organism, in this case the seasonal factor, made it possible to obtain new data obtained about the physiology of horses, which can be used both in planning research and in organizing the conditions of animal housing, as well as in controlling their health status.

4 Conclusions

The data obtained show that the composition of leukocytic formula can change under the influence of season. We found an increase in the percentage of segmented neutrophils in the

blood in spring - $52.04 \pm 1.65\%$ and in summer - $53.55 \pm 1.43\%$ compared to the fall-winter period. We observed a decrease in lymphocytes in spring and summer periods - $46.29 \pm 1.64\%$ and $44.07 \pm 1.38\%$, respectively, compared to the number of lymphocytes in fall and winter. We found an increase in the number of eosinophils in summer and fall, $1.89 \pm 0.28\%$ and $1.96 \pm 0.22\%$, respectively. Also, according to the research results we found that the number of segmented neutrophils and lymphocytes by 13% and 12%, respectively, depended on the season ($p < 0.05$).

Such changes in leukocytic formula indicators can be explained by the adaptation of the organism to different weather conditions and exposure to various pathogenic factors. Thus, the research results confirm the theory about the influence of seasonal factor on animal organism, including on leukocytic composition of stallion blood.

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