Evaluation of the phagocytizing blood cells functional activity of cattle (Bous Taurus)

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Abstract. Reactions of free radicals’ formation are integral parts of many metabolic processes in the body, but in a number of states under the influence of external and internal factors there occurs intensification of these reactions accompanied by excessive generation of phagocytizing blood cells activity (PBCA), therefore the study of the antioxidant activity of biological fluids is of clinical (diagnostic, predictive) value. The component analysis of the blood chemiluminescent kinetics of Bous Taurus of different age and sex groups was carried out. The functional activity of venous unfractionated blood cells taken from the jugular vein was evaluated by the Tono-Oke method using the “Chemiluminometer CL-3604” hardware-software complex. The analysis of the chemiluminescent kinetics of PBCA generation and the state of the organism was evaluated by the method of Magrisso M.Y. A component analysis of the blood chemiluminescent kinetics of animals of different sex, age and physiological state revealed a number of specific characteristics and pointed out the relationship between some parameters that can complete the picture of the antioxidant potential possibilities.

1. Introduction
Planning is one of the main elements of the cattle breeding process management. However, a far-sighted forecast is impossible without an objective assessment of the state of animals’ health, which, in its turn, is difficult without the timely identification of problem areas and the initial stages of pathological processes. As reproduction is a significant biological component of breeding programs and it is closely related to the state of animals’ health, it is necessary to develop and apply rapid methods for assessing the physiological state of cattle. The determination of total antioxidant activity (AOA) can serve as an indicator of the state of the body as a result of the action of a functional system that determines the course of metabolic processes reflecting homeostasis and adaptation to changing environmental conditions [1-4]. In this case, the shift in the equilibrium oxidants - antioxidants is unfavorable in both directions.

Evaluation of the functional phagocytizing blood cells activity (PBCA) which determines the nonspecific resistance of the organism as a primary response to the invasion of antigenic agents and
plays an important role in the cooperative interactions of specialized immune cells of the body during the immune response is one of the indicators reflecting the state of the immune status of animals, including Bous Taurus. The data on the absence of influence of sex and age factors on the variability of the blood plasma AOA and the presence of the season influence were obtained [5-7]. The intensity of chemiluminescence correlates with oxygen consumption by cells and the degree of phagocytosis completion, so it makes sense to evaluate the integral component reflecting the potential possibility of antioxidant potential.

There is a need for rapid methods for determining the total AOA, which would allow conducting research not in separate fractions but in biological fluids for different components simultaneously. At the same time, there are few direct methods for detecting radicals in cells and tissues, and among them the chemiluminescence (CL) method is of great importance, first of all - CL in the presence of chemical activators such as luminol and lucigenin. The intensity of chemiluminescence correlates with the oxygen consumption by the cells and the degree of completion of phagocytosis. It makes sense to evaluate the integral component that reflects the potential possibility of the antioxidant potential, taking into account the cumulative effect of rarely occurring, or minor antioxidants (AOs). The molecular level of monitoring the functional activity (FA) of immunocompetent cells (ICC) recorded by the kinetics of PBCA generation using the CL method reveals subtle changes in the mechanisms of the immunogenesis system functioning, which do not always appear at the subcellular and cellular levels, but at the same time play a key role in the formation of pathogenetic mechanisms. From the point of view of practice, it is important to know: what information can be extracted from data analysis of biological fluids? In this regard, research was conducted with the aim of identifying prognostically significant criteria when analyzing blood chemiluminescence data of cattle. [8, 9]

The purpose of the research is to characterize the peculiarities of the cattle blood cells chemiluminescence. The object of the research was the blood of clinically healthy bulls-spermodonors of the breeding enterprise “Krasnoyarskagroplem” and the cows of the commercial farm“Agroyarsk”.

2. Object and methods
Peculiarities of the functional activity of venous blood cells were analyzed using the hardware-software complex "Chemiluminometer CL-3604" - the PC method of Tono-Oke modified by Zemskov with co-authors [10] and Makarskaya with co-authors [11]. Component analysis was carried out by Magrisso M.Y. et al [12].

The object of the research was the blood of cows of AIH CJSC “Agroyarsk”, Sukhobuzimsky district of Krasnoyarsk Krai, Russia; the total number of animals examined was 52 heads. The following groups were singled out according to the physiological state: 1 gr. - cows are not inseminated, 7 days after calving; 2 gr. - cows are not inseminated, 30-45 days after calving; 3g. – cows with 30 days of pregnancy; 4 gr. – cows with 60 days of pregnancy.

When analyzing the condition of the bulls the age characteristics of the bulls were taken into account. For the study, animals were divided according to their age: up to 17 months (group 1), from 18 to 24 months (group 2), from 25 to 38 months (group 3), from 47 to 50 months (group 4), from 58 to 61 months (group 5), from 69 to 72 months (group 6) and from 70 to 100 months (group 7).

To solve the problems posed, each chemiluminescent kinetic curve was divided into three statistical distributions. The first component represents the processes associated with phagocytosis and occurring near the plasma membrane; it reflects the kinetics of extracellular PBCA generation. The second component characterizes intracellular processes associated with phagocytosis and represents the intracellular generation of PBCA. The third component describes the kinetics of the generation of intracellular PBCA not associated with phagocytosis directly.

The algorithm of the component analysis of PBCA products integral characteristics. The first stage includes the determination of the model components of the chemiluminescent response, the identification of three statistical distributions characterizing the contribution of the respective components into the overall picture of the chemiluminescence and adequately reflecting the representative chemiluminescence kinetics, and recording the chemilumogram in minutes. The second
stage is the calculation of parameters and determination of threshold levels for each component. The third stage is the direct characterization of the states and their interpretation.

3. Results
In different age periods bulls have significant degree of variability in the efficiency of their physiological processes, which reflects both the general trend of increasing efficiency with age and dependence on physiological loads, in this case an increase in the productive use of spermodonors. The rate of PBCA formation during phagocytosis in the process of postnatal ontogenesis does not change significantly (from 0.16 to 0.55) and is probably genetically determined. For comparison: for humans this indicator is from 0.5 to 3 [5]. The minimum contribution of the first component under the influence of the processes associated with phagocytosis and extracellular generation of PBCA is shown in figure 1.

![Figure 1](image1.png)

**Figure 1.** Characteristics of the chemiluminescence kinetics and its components, the blood of bulls of different age groups.

In general, in terms of age variability there is a significant superiority of the third component over the other two separately and in the sum of two components associated with phagocytosis. It is reasonable to assume that the species peculiarity of the Bous Taurus chemiluminogram (double peak) is related to the ratio of the components in favor of the third one, which transmits a high level of PBCA not directly associated with phagocytosis (figure 2).

The ratio between the 1st and the 2nd components ranges from 19 to 25%, while the influence share of phagocyte-related CL tends to decrease in older age.

![Figure 2](image2.png)

**Figure 2.** The ratio of the components in the integral blood chemiluminescence of bulls of different age groups, months.
Cows have lower chemiluminescence components characteristics; however, there is a pattern similar to that of bulls, that is, significant variability of the third component in different groups of animals (figure 3).

![Figure 3. Characteristics of the chemiluminescence kinetics and its component, the blood of cows of different physiological states, not pregnant: 1gr. – newly-calved, 2g - 1-1.5 months after calving; pregnant: 3gr. – one month, 4 gr. - two months.](image)

Cows in the first month of pregnancy have a sharp decrease in the CL response power. The share of extracellular influence in total phagocyte-related chemiluminescence is within 20% and is not significantly different in cows after calving and at the beginning of pregnancy (figure 4). Analysis of the parameters of blood of cows after calving and in the early stages of pregnancy revealed an increase in the amplitude of the first maximum and the area under the chemiluminescence curve in the group of cows in the second month of pregnancy.

![Figure 4. The ratio of the components in the integral chemiluminescence of the blood of cows of different physiological states.](image)

4. Conclusion

Thus, the component analysis of the chemiluminescent blood kinetics of animals of different sex, age and physiological state revealed that the increase in the production of all types of PBCA is determined by the growth of the maximum intensity and volume of the third component of the generation of free oxygen radicals - intracellular and extracellular ones, unrelated to the phagocytosis process.

Antioxidant processes associated with phagocytosis are more effective; extracellular and intracellular antioxidants not directly associated with phagocytosis are less effective, unbalanced, and therefore the intensity of PBCA (luminescence area) significantly increases.
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