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Platelet Activity of Ireshire Heifers Being on Supplementary Breeding

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Authors' contributions

This work was carried out in collaboration between all authors. Author JLO designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. Authors INM and LLF managed the analyses of the study. Author LLF managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

The course of all the ontogenesis in cattle is closely connected with the dynamics of blood system indices which are largely integrated in the animals' bodies. Interest to peculiarities of platelet activity in high-productive cows increases because of its great signification for the optimum of microcirculation, anabolism and maximal productivity. The aim of the research: to study peculiarities of platelet activity in healthy Ireshire heifers being on supplementary breeding. We examined 36 Ireshire heifers at the age of 12, 15 and 18 months which were on supplementary breeding. We applied hematological and statistical methods of investigation. The most active platelets' aggregation was noted in response to adenosine diphosphate being maximal to the end of observation. Collagenic and ristomicinic aggregation had less evidence and similar directivity.

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Summarizing index value of platelets' aggregation with three used inductors was equal to the end of observation to $25.6 \pm 0.05\%$, $10.0 \pm 0.26\%$ and $10.3 \pm 0.39\%$, respectively. It indirectly pointed at high integrity of vessels' endothelium in the animals and little concentration of von Willebrand's Factor in their plasma. Disaggregative platelets' activity in respect of all the applied inductors had an upward trend in the observed heifers counterbalancing the rise of aggregation. Detected low platelets' activity in Ireshire heifers should be considered as the necessary condition for optimal provision of anabolic processes in their growing tissues and of the basis for their future high productivity. Received results can be used in future researches of Ireshire heifers as normative values.

Keywords: Platelets; aggregation; heifers; supplementary breeding; Ireshire breed.

1. INTRODUCTION

Sequential realization of all the stages of cattle ontogenesis is inseparably connected with the dynamics of the systems, including blood [1,2], which regulate and integrate their bodies. Hemostasis [3,4] is physiologically very important biological subsystem of blood which provides, on the one hand, preservation of its liquid state, and, on the other hand, prevention and reduction of bleedings.

Being a complicated system, hemostasis includes some various components. Platelets [5,6] are the most significant of them and can influence its other mechanisms [7]. The efficiency of tissue blood supply, prevention of thromboses, ischemia and infarcts of organs, reduction of hemorrhages, protection degree from dissemination of bacteria and toxins out of lesion focuses through the whole body [8,9] mostly depend on platelets' functional activity.

Estimation of hemostasis indices of productive animals has great practical signification for practical biology. It is caused by its detected impact on somatic characteristics [10] and on the activity of functioning of many body's systems [11]. Studying of platelet activity allows working out its age-specific norms [12] what will help to detect in proper time the developing hemostasiopathy at any states [13]. These researches can have especially great significance for the biology of high-productive animals' breeds as their practical application should provide preservation of their productive potential and lengthening of animals' economic usage [14].

Taking into account high productivity of Ireshire cows and large signification of platelet activity for its provision the need in conducting of researches on physiology of the platelet section of their hemostasis rises. In this respect we put the following aim in our research: to study

peculiarities of platelet activity in healthy Ireshire heifers being on supplementary breeding.

2. MATERIALS AND METHODS

The research was conducted in strict accordance with ethical principles established by the European Convent on protection of the vertebrate used for experimental and other scientific purposes (adopted in Strasbourg in March, 18th, 1986, and confirmed in Strasbourg in June, 15th, 2006) and approved by the local Ethics Committee of Federal State Budgetary Educational Institution of Higher Education "Vologda State Dairy Farming Academy by N.V. Vereshchagin" (Record №12 dated December, 3rd, 2015), the local Ethics Committee of All-Russian SII of Physiology, Biochemistry and Animals' nutrition (Record №11, dated December, 4th, 2015) and the local Ethics Committee of Russian State Social University (Record №16, dated December, 7th, 2015).

The study was conducted on 36 Ireshire heifers being in optimal conditions of keeping on supplementary breeding at the farm "Plemzavod Majskiy" in Vologda region (Russia). The animals were examined thrice: at the age of 12 months, at the age of 15 months and at the age of 18 months. Only healthy animals (which were planned for insemination) were taken under observation.

Blood samples were collected from jugular vein of all the heifers in the morning for studying platelet parameters. Sampling was made into a plastic tube containing 3.8% citrate of sodium dilution in the ratio of blood volumes and citrate of sodium –9:1.

The number of platelets in animals' blood was determined by electron- automatic method on hematological analyzer BC-3000 PLUS (the firm "Shenzhen Mindray Bio-Medical Electronics Co., Ltd.", China).

Platelets' aggregative activity was determined by quantitative method with application of photo-electro-colorimeter KFK-2 (Russia) with such aggregation inductors as ADP, collagen and ristomicin in standard concentrations. Platelets' aggregation was estimated according to the values of summarizing index for platelets' aggregation (SIPA), speed of aggregation (SA) and index of platelets' disaggregation (IPD).

The value of SIPA was found with the help of the formula:

$$SIPA = \frac{E1 - E2}{E1 - E} \times 100\%$$

where:

E - optical density of rich in platelets plasma in units of optical density;

E1 - optical density of platelet depleted plasma before aggregation in units of optical density;

E2 - optical density of platelet depleted plasma after aggregation in units of optical density.

The value of platelets' aggregation speed was found according to the formula:

$$SA = \frac{E1 - E2}{T}$$

where:

E1 - optical density of platelet depleted plasma before aggregation in units of optical density;

E2 - optical density of platelet depleted plasma after aggregation in units of optical density;

T - period of time, during which maximal fall of optical density took place, in min.

The index value of platelets' disaggregation was calculated according to the formula

$$IPD = \frac{E3 - E2}{E3} \times 100\%$$

where:

E2 - optical density of platelet depleted plasma after aggregation in units of optical density;

E3 - maximal optical density of platelet depleted plasma, measured in 10 minutes after the addition of an aggregation inductor.

The results were processed by Student's criterion (t). Statistical processing of received information was made with the help of a programme package "Statistics for Windows v.

6.0", "Microsoft Excel". Differences in data were considered reliable in case of $p < 0.05$.

3. RESULTS AND DISCUSSION

Accounting in the research common platelet indices (quantity of platelets, average volume of platelets and thrombocrit) were within the limits of the conventional norm in the examined animals and didn't change in the course of observation (Table 1).

The strongest aggregative response of platelets was noted to ADP. At the same time, SIPA with ADP in heifers in the course of supplementary breeding increased reaching to its end $25.6 \pm 0.05\%$. In response to collagen SIPA in animals in the course of supplementary breeding also gradually increased till $10.0 \pm 0.26\%$. It pointed at the rise of platelets' sensitivity to aggregation inductors in Ireshire heifers at the intensification of their secretory process out of platelets [15]. The activity of platelets' aggregation under the impact of ristomicin also rose in the observed heifers – SIPA increased in the course of observation by 24.1%.

The speed of aggregates' formation in Ireshire heifers in response to ADP in the course of supplementary breeding decreased doubly. Similar dynamics was experienced by SA under the impact of collagen and ristomicin being equal in heifers by the end of observation to 0.09 ± 0.007 min and 0.09 ± 0.004 min, respectively.

Estimation of the index value of platelets' disaggregation which shows the stability of forming aggregants, allowed finding out that the most stable aggregates were those which were formed in response to ristomicin – the value of IPD (index of platelets' disaggregation) with it in the course of supplementary breeding rose by 31.8% reaching, however, minimal values ($2.9 \pm 0.11\%$). Aggregates which were formed under the impact of ADP and collagen, were less stable in the course of supplementary breeding: IPD in respect of both inductors also rose reaching with collagen $3.2 \pm 0.31\%$, with ADP – $14.2 \pm 0.42\%$.

Collected by the present moment rather extensive knowledge about the role of hemostasis in functional activity of the internals allows considering this system to be especially significant in maintenance of physiological optimum of a body [16,17]. Platelets occupy

Table 1. Platelet indices in Ireshire heifers being on supplementary breeding

Indicators	Age of heifers, n=36, M±m		
	12 months	15 months	18 months
Quantity of platelets, thousand/mcl	346.8±12.62	357.2±9.70	362.1±6.25
Average platelet count, fl	7.2±0.44	7.2±0.26	7.3±0.19
Thrombocyte, %	0.27±0.07	0.27±0.05	0.27±0.04
Inductor of aggregation ADP			
SIPA, %	22.5±0.34	23.1±0.86	25.6±0.05 p<0.05
SA, min	0.02±0.007	0.03±0.006 p<0.05	0.04±0.007 p<0.01
IPD, %	12.2±0.36	13.3±0.46	14.2±0.42 p<0.05
Inductor of aggregation collagen			
SIPA, %	8.1±0.35	9.1±0.40	10.0±0.26 p<0.05
SA, min	0.07±0.004	0.08±0.003	0.09±0.007 p<0.05
IPD, %	2.6±0.22	3.0±0.24 p<0.05	3.2±0.31 p<0.01
Inductor of aggregation - ristomicin			
SIPA, %	8.3±0.45	9.5±0.50	10.3±0.39 p<0.05
SA, min	0.07±0.003	0.08±0.006	0.09±0.004 p<0.05
IPD, %	2.2±0.08	2.5±0.12	2.9±0.11 p<0.05

Explanation: p – reliability of indices' dynamics in comparison with the beginning.

rather important place in hemostasis as they mostly determine its level of activity in different sections of vascular course [18].

Last years' researches significantly widened our notions about the factors influencing platelets' aggregation, and also preservation of blood in liquid state. These processes are well studied at many states in people and animals [19]. At the same time, a lot of aspects of hemostasis platelet component in cattle at different ages and in different environmental conditions remain to be studied rather poorly. Their breed peculiarities are left unclear, particularly in Ireshire breed, including the course of the stage of a heifer's preparation to the realization of its productivity – the term of supplementary breeding.

It is known that physiologically necessary anabolic processes take place in heifers' bodies in the course of the whole supplementary breeding. These processes rise the capacity of functioning of all the organs and systems of their bodies [20]. At the same time, all the tissues are rather sensitive at this period to the impact of unfavorable factors of the environment and need

maximal inflow of blood having good liquid properties [2,21].

The conducted researches on Ireshire heifers being on supplementary breeding, detected that platelets' quantity and their average volume in them don't surpass the limits of conventional normative values [22]. At that, platelets' aggregative activity in them rose in the course of supplementary breeding. Heifers' platelets reacted most actively on ADP. While animals' aging SIPA with this inductor increased. In response to collagen and ristomicin the values of SIPA reached in them less and comparable between each other values. It indirectly pointed at their little availability level of vascular wall's collagen for platelets and low content of von Willebrand's Factor in blood. As it's known, this factor can interact simultaneously with ristomicin and glycoproteins GP Ib and GP IIb/IIIa of platelets' membranes providing interaction between aggregating platelets [23]. Found in the observed heifers rise of aggregation speed in response to all the applied inductors points at number increase of appropriate receptors on platelet membranes in them.

Disaggregative capabilities of heifers' platelets in response to all the agonists didn't reach high values in the course of supplementary breeding. Given effect can be also explained by receptor structural changes of platelets' membranes and little weakening of intraplatelet mechanisms of activation (synthesis of thromboxane, phosphatidic acid and factor of platelets' activation) [24,25].

Summarizing the received data in the examined animals we can come to the conclusion that strengthening of receptor mechanisms of adhesive-aggregative processes in platelets takes place in Ireshire heifers in the course of supplementary breeding. It is balanced by the rise of their disaggregative capability. Detected peculiarities of platelet activity are physiologically rather significant for the growth and development of Ireshire heifers in the course of supplementary breeding as they mostly determine the level of microcirculation in their tissues.

4. CONCLUSION

Signification of platelet activity in provision of optimal conditions for microcirculation and metabolism determining the productive qualities of cows is acknowledged at present. It caused the rise of interest towards researches of various aspects of functioning of hemostasis platelet section in cattle of milk breeds. In the course of the conducted research we detected little strengthening of platelets' aggregation in healthy Ireshire heifers which were kept in optimal conditions of the environment. It was physiologically balanced by little strengthening of platelets' disaggregative properties in the observed animals. Received in the research values of platelet aggregation can be used in future researches as normative ones.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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