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Platelets' Aggregative Properties of Ireshire Calves in the Phase of Dairy-vegetable Nutrition

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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 aim of research is to determine the dynamics of platelets' ability to aggregation in healthy Ireshire calves during the phase of dairy-vegetable nutrition. We examined 67 Ireshire calves of dairy-vegetable nutrition. We applied hematological and statistical methods of investigation in our research. At the age of 45 days the animals had short-time strengthening of aggregation in response to all the applied inductors. This strengthening of platelets' aggregation in calves was removed by the 3rd month of their life. They had the most active platelets' aggregation in response to adenosine diphosphate. Platelets' aggregation with collagen and ristomicin was less evident in them. Ireshire calves had strengthening of disaggregating platelets' capabilities in response to all the applied inductors of aggregation by the 45th day of life. But its evidence returned to the initial level by the end of observation. In the course of our research we determined the dynamics of indices of platelet hemostasis in Ireshire calves in the phase of dairy-

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vegetable nutrition. Short-time strengthening of platelets' activity in calves of this breed at the beginning of observation has in its basis the processes of adaptation to changing of nutrition character.

Keywords: Aggregation; platelets; calves; the phase of dairy-vegetable nutrition; Ireshire breed.

1. INTRODUCTION

Growth and development of living beings are inseparably connected with dynamics of the systems which regulate and integrate them. Blood [1] is considered to refer to these systems. Hemostasis is its very important biological subsystem from the point of view of physiology. It provides, from one hand, preservation of liquid state of blood, and, from the other hand, prevents and arrests bleeding [2]. Hemostasis is presented by a number of different components. Platelets [3] are among the most significant components. Their activity can influence the processes of microcirculation in tissues [4]. Intensity of anabolic processes in tissues and state of mechanisms of local and common resistance [5,6] mostly depend on platelets' functional state.

At present their aggregation [7] is most often registered for monitoring of their activity and determining their dynamics. Its estimation has great practical signification for biology in the context of age-specific changes in productive animals' bodies. It is connected with the dynamics of somatic characteristics [8] and different mechanisms of a body's functioning on the whole [9]. Given studies allow working out the age-specific norms of platelets' aggregative properties for different kinds of productive animals. It is necessary for early detection of the hemostasiopathy beginning in them in different pathological states [10].

These studies have especially great signification for high-productive breeds of cattle as they can serve the basis for preservation of their economic properties.

At present researchers just begin to study platelet activity of cattle's separate breeds. In previous works platelet properties were often estimated in groups of calves composed of representatives of different breeds [11]. The authors managed to find just one work conducted on newborn Holstein calves [12].

Because of high productivity of Ireshire cows and importance of preservation of this breed in the course of the whole ontogenesis, we find it

urgent to track changes of platelet aggregation in healthy Ireshire calves during the phase of dairy-vegetable nutrition.

The aim of research: to determine the dynamics of platelets' ability to aggregation in healthy Ireshire calves during the phase of dairy-vegetable nutrition.

2. MATERIALS AND METHODS

2.1 Materials

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).

A pilot project was developed in the course of preparation for the work to assess the dynamics of platelet activity in purebred calves in the phase of dairy-vegetable nutrition. The interest to this stage of calves' life was caused by the rearrangements in the system of their digestion occurring in the course of it. These rearrangements affect many indices of their bodies. The activity of platelets affects the state of microcirculation and, therefore, it was important to evaluate it at this transitional stage of purebred calves.

The study used 67 healthy Ireshire calves of dairy-vegetable nutrition which were kept in a calf-house of the breeding farm "Mayskiy" in Vologda region (Russia). The animals were examined 5 times: on the 31st day, on the 45th day, on the 60th day, on the 75th day and on the 90th day of life.

2.2 Methods

Blood samples were collected from jugular vein of all the calves 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 program package "Statistics for Windows v. 6.0", "MicrosoftExcel". Differences in data were considered reliable in case of $p < 0.05$.

3. RESULTS AND DISCUSSION

The quantity of platelets in blood of examined animals was within the norm and didn't change in the course of observation (Table 1). At the same time, Ireshire calves had reliable changes in platelets' aggregative activity in the course of the phase of dairy-vegetable nutrition (Table 1).

In the course of our research Ireshire calves had peak increase of indices of platelets' aggregation by the 45th day of life. The largest platelets' response was noted on ADP. At the same time, SIPA with ADP during the phase of dairy-vegetable nutrition rose till 27.15±2.86% but on the 90th day of life returned to the level which was near to the initial one. SIPA of animals also had peak rise in response to collagen by the 45th day of life, then gradually lowered till the initial level. The activity of platelets' aggregation under the impact of ristomicin in Ireshire calves at the beginning of the phase of dairy-vegetable nutrition also had short- term increase. At the age of 45 days their SIPA reached 13.01±0.62%, being equal to the end of observation to 7.71±0.26%.

The speed of aggregates' formation in Ireshire calves in response to ADP had short-term increase by the 45th day of life [46.1%], but on the 3rd month of life it reached the values near to the initial ones. SA had similar dynamics in response to collagen and ristomicin, being by the end of observation 0.0060±0.0008 min and 0.0065±0.004 min, respectively.

Table 1. Platelet indices of Irish calves of dairy-vegetable nutrition

Indicators	Age of calves, n=67, M±m				
	31 days	45 days	60 days	75 days	90 days
Quantity of platelets, thousand/mcl	302.1±12.26	305.4±9.34	310.6±7.11	308.9±9.62	315.0±7.07
Inductor of aggregation ADP					
SIPA, %	17.40±1.49	27.15±2.86**	22.80±2.31*	19.60±1.98*	17.60±1.35
SA, min	0.026±0.012	0.038±0.016**	0.033±0.009*	0.028±0.008*	0.026±0.005
IPD, %	10.08±0.63	16.40±1.08**	13.82±0.89*	12.17±0.72	10.15±0.68
Inductor of aggregation collagen					
SIPA, %	7.02±0.57	12.05±0.96**	10.04±0.80*	8.90±0.68	7.05±0.49
SA, min	0.0062±0.005	0.0089±0.014**	0.0077±0.009*	0.0069±0.008	0.0060±0.008
IPD, %	2.52±0.17	4.68±0.31**	4.02±0.26*	3.21±0.22*	2.49±0.19*
Inductor of aggregation - ristomicin					
SIPA, %	7.68±0.28	13.01±0.62**	11.07±0.51*	8.60±0.36*	7.71±0.26
SA, min	0.0066±0.005	0.0085±0.014**	0.0078±0.009*	0.0071±0.007	0.0065±0.004
IPD, %	2.15±0.07	5.26±0.13**	4.61±0.10*	3.02±0.08*	2.16±0.08

Note: Reliability of dynamics of accountable indices with the level on the 31st day of life: *-p<0.05, **-p<0.01

The index value of platelets' disaggregation, which showed the stability of aggregates' appearance in observed calves, also had short-term increase by the 45th day of life. But the most stable aggregates were those ones which were formed in response to ristomicin. The value of IPD with it during the phase of dairy-vegetable nutrition was the least one. The aggregates, which were formed under the impact of ADP and collagen, were less stable during the phase of dairy-vegetable nutrition. The values of IPD in response to all the applied inductors reached the values, which were near to the initial ones, by the end of observation.

Present information collected about the role of hemostasis in provision of internals' functional activity allows considering this system very significant in maintenance of physiological optimum of the whole body [13]. Platelets play an important role in it [10]. Last years' observations significantly widened the ideas about factors influencing the level of their aggregation. These processes are now studied at many states of people and animals [2,14]. At the same time, a number of aspects of platelet component's hemostasis in cattle of different ages and in many environmental conditions are studied rather poorly. Their breed peculiarities are not yet fully studied. Their peculiarities in Irish breed were not determined earlier, including the course of the most adaptive- significant period – the phase of dairy-vegetable nutrition. Given transition deserves special attention as very important, physiologically necessary processes, accompanying the beginning of vegetable food

consumption [11], take place in calves' bodies during this phase. At this period the animals are easily influenced by unfavorable environmental factors and need maximal perfusion of internals and good micro-rheological blood features [15].

Conducted researches on Irish calves of dairy-vegetable nutrition found out that the quantity of platelets in their blood didn't exceed the borders of conventional normative values. At the same time, platelets' aggregative activity in calves of this breed at the beginning of the phase of dairy-vegetable nutrition had short-term increase. Their platelets reacted most actively on the addition of ADP into plasma. Platelets' aggregation also rose in response to collagen and ristomicin and reached a bit less values. It pointed at short-duration of strengthening of receptors' expression on platelets of these calves during this period and at brief strengthening of their platelets' ability to secretion. Received results also indirectly pointed at brief rise of platelets' sensitivity to collagen and brief increase of von Willebrand Factor's content in blood of these calves. It was provided by brief expression strengthening of glycoproteins Ib and IIb/IIIa on platelets' membranes [2].

The evidence of disaggregative platelets' abilities in Irish calves during the phase of dairy-vegetable nutrition in response to all the applied agonists rose at the beginning of the phase and lowered by its end. Found regularity can be explained by short-term strengthening of receptors' expression to disaggregates on platelets' membranes [2].

Estimating the received data about the examined animals, we can come to the conclusion that Irish calves at the beginning of vegetable food consumption have brief increase of platelets' adhesive-aggregative activity which is removed in the course of adaptation to new way of nutrition.

Available in literature fragmentary data about age-specific calves' dynamics of separate hemostasis components (including platelets [16,17]) haven't yet been practically studied in the aspect of animals' breed differences. It is not possible at present to compare the received and available information. Finding out the differences in platelet activity between cattle breeds in the course of ontogenesis – is the theme for future researches.

4. CONCLUSION

In the course of our research we determined the dynamics of indices of platelet hemostasis in Irish calves passing from dairy nutrition to vegetable food consumption. Brief strengthening of platelets' activity at the beginning of calves' observation has the processes of adaptation to new character of nutrition in its basis.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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