Hemostasis and Rheological Blood Features Dynamics of Black-many Coloured Lactating Cows at the Inclusion into their Ration of Antioxidant Liposomal Preparation “Lipovitam-beta”

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ABSTRACT

One of modern vitamin sources for lactating cows is Lipovitam-beta. It was made on liposomes’ basis and can provide maximum vitamins’ assimilation by the whole organism’s cells. Aim of investigation: to estimate Lipovitam-beta impact on the indices of hemostasis system and blood rheology of high-productive cows during the first 5 months of lactation. We examined 207 healthy black-many coloured cows having begun the process of lactation. They were of the 3-4th calving and were kept in conditions of fastened support having traditional ration. The animals were casually divided into experimental and control groups. in the experimental group (104 cows) we examined the impact of Lipovitam-beta feeding on the basis of 1 capsule (0,17gr) on 30 kg of body mass once in 5 days during the first 5 months of lactation on accountable indices. The control group was composed of 103 lactating cows. We estimated the basic indices of hemostasis, blood viscosity, erythrocytes’ aggregation and deformability on the 5th day and in 5 months of lactation. Lipovitam-beta application led in case of cows from experimental group to reliable decrease of spontaneous and stimulated platelets’ aggregation and weakening of plasma hemostasis activity. On the background of Lipovitam-beta feeding the cows were found to have decrease of blood viscosity and erythrocytes’ aggregation index what was accompanied by increase of erythrocytes’ deformability index. The cows from the control group during the first 5 months of lactation were noted to have rise of platelets’ aggregation and increase of hemocoagulation. It was accompanied by the tendency to increasing of blood viscosity and erythrocytes’ aggregation with inclination to the decrease of their deformability. High-productive cows by the middle of lactation are noted to have developing tendency to hemostasis activation and worsening of blood rheological features what weakens microcirculation processes and may be one of the factors gradually blocking lactation. Lipovitam-beta application to lactating cows led to activity decrease of plasma hemostasis and platelets, increase of rheological features what can positively influence microcirculation and the level of dairy producing capacity.

Keywords: Cows, Lactation, Lipovitam-beta, Hemocoagulation, Platelets, Blood rheology.

INTRODUCTION

At present cattle breeding is one of very significant branches of modern agriculture providing population with dairy products of full value and dietary meat1,2. Nowadays in the whole world the task of its intensification is established. It should be realized at the expense of high-productive livestock growth and application of progressive feeding technologies and keeping of lactating cows3. Science gradually comes to understanding that great significance for the provision of vitality
and productive capacity of cattle belongs to the state of one of its integrating systems - blood\(^4,5\). Its hemostatic and rheological features mostly define the degree of tissues’ perfusion and, consequently, anabolism level in an animal’s organism and its productive characteristics at any age\(^5,6\). Because of great physiological significance and vulnerability of hemostasis and blood rheology they are started to be investigated more and more actively\(^7\).

One of valid ways causing intensification of anabolic processes in an organism and, thereby, increase of phenotypic manifestation of genetically conditional features\(^8,9\) is application of different biologically significant impacts\(^10,11\) and biologically active substances\(^12\). In their number we include rise of physical activity\(^13\) and also application of vitamins. The need in some vitamins in case of high-productive cows is very high and with the help of ration can’t sometimes be covered entirely\(^5\). One of modern vitamin sources for lactating cows is Lipovitam-beta which, being made on the basis of liposomes, can provide maximum vitamins’ assimilation by an organism’s cells\(^14\).

It is known that blood - is the most labile indicator of an organism’s state quickly reacting on different ingredients’ inflow\(^15,16\). The more metabolism in an organism increases under their impact, the more evident will be changes in blood\(^17,18\). Besides, blood changing its composition, hemostatic and rheological features, can itself influence the functional state of the whole organism \(^19,20\). In this connection we considered to be approved to fulfil estimation of Lipovitam-beta impact in case of high-productive lactating cows on physiologically significant indices of hemostasis and blood rheology.

Aim of investigation: to estimate Lipovitam-beta impact on the indices of hemostasis system and blood rheology of high-productive cows during the first 5 months of lactation.

**MATERIALS AND METHODS**

In our investigation we took 207 healthy black-many coloured cows of pure breed of 3-4th calving being kept at the farms of Samarsk region having given during the previous lactation 7,5-8 thousand of litres of milk each. The cows were taken under investigation on the 5th day of lactation. All the cows were kept in conditions of fastened support and were milked thrice. The ration of taken into investigation lactating cows was composed of corn silage, wheat straw, schrot of sunflower, fodder syrup, mixture of concentrates. The animals were casually divided into experimental and control groups. The experimental group was composed of 104 cows who additionally received Lipovitam-beta (“Biodom”, Russia) on the basis of one capsule (0,17gr) on 30 kg of animal’s body mass by mixing it with concentrates. The preparation was given once in 5 days during the first 5 months of lactation. The control group was composed of 103 lactating cows of similar age being kept in similar conditions and receiving the same ration. The examination of animals in both groups was fulfilled twice: on the 5th day after calving and in 5 months of lactation.

All the cows were examined with the object to the content of fibrinogen in blood by Klause’ modified method\(^21\). Estimation of plasminogen level was defined by kinetic method with the help of the device FP-901 (“LabSystems”, Finland) with chromogen substrates (“Dade Behring”, Germany). The concentration of soluble fibrinomeric complexes (SFMC) was defined by visual method with the help of reagents by the firm “Technology-standard”, Russia\(^21\). Activated partial thromboplastin time (APTT) was examined with the help of coagulometre “HumaClot” (“HUMAN GmbH”, Germany) with a set of reagents HemoStat aPTT-EL. The definition of international normalized relation (INR) was made by Quick’s method\(^21\). Platelets’ aggregative ability was examined with the help of two-channel laser analyzer of platelets’ aggregation (“Biola”, Russia) by turbo-dymetric method. As inductor we applied 0,5mkM ADP\(^22\).

Registration of blood viscosity was fulfilled by rotary viscosimeter AKP-2 (“Melt”, Russia) at shift speeds 200 sec\(^{-1}\) and 20 sec\(^{-1}\) with the consequent calculation of erythrocytes’ aggregation index and deformability index\(^22\). Statistical processing of the received results was made with the help of Student’s t-criterion.
RESULTS

During the first 5 accountable months of lactation the cows, having received Lipovitam-beta, gave on average 4420.0±17.4 kg of milk each (3.8±0.03% fat content), while the cows from the control group gave on average 4140.0±27.7 kg of milk each (3.9±0.04% fat content).

Estimation of hemostasis state on the 5th day of lactation in cows of both groups didn’t find reliable differences in values of defined indices of coagulant and anticoagulative systems (table).

Platelets’ functional activity before the start of investigation also had no differences in both groups of animals.

At the beginning of investigation we also didn’t find any reliable differences between both groups of animals as far as indices of blood rheological features are concerned. Blood viscosity at shift speed 200 sec⁻¹ in the group which later began to receive Lipovitam-beta was equal to 4.27±0.19 centipoise, in the control group - 4.29±0.22 centipoise. At shift speed 20 sec⁻¹ the values of blood viscosity were also comparable composing 6.30±0.35 and 6.28±0.38 centipoise, correspondingly. Initially indices’ values of erythrocytes’ deformability and erythrocytes’ aggregation of animals in both groups statistically didn’t differ from each other.

To the end of investigation period the cows receiving Lipovitam-beta were noted to have reliable inhibition of APTT, tendency to increasing of INR and plasminogen indices, decrease of fibrinogen and SFMC concentrations. In the control group of cows in 5 months of lactation we noticed increase of APTT, tendency to decreasing of INR and plasminogen activity at increase in blood of fibrinogen and SFMC.

In 5 months cows from experimental group were noted to have decrease of platelets’ aggregation - spontaneous on 11.5% - stimulated on 11.8%. In the control group of lactating cows spontaneous and ADP-inducible platelets’ aggregation reliably rose, having exceeded initial values on 14.7% and 24.1%, correspondingly. At the same time differences in platelets’ aggregation in both groups to the end of investigation composed for spontaneous - 26.9% (p<0.01), for stimulated - 39.4% (p<0.01).

At repeated investigation of accountable indices of blood rheological features in 5 months of lactation we noticed multidirectional tendency of

| Table 1: Indices’ dynamics of hemostasis and blood rheology of lactating cows |
|-----------------------------|-----------------------------|-----------------------------|-----------------------------|-----------------------------|
| **Indices** | **Lipovitam-beta, n=104** | **Control, n=103** | **Lipovitam-beta, n=104** | **Control, n=103** |
| **on the 5th day of lactation** | **in 5 months of lactation** | **on the 5th day of lactation** | **in 5 months of lactation** |
| indicators of hemostasis | | | | |
| INR | 1.14±0.10 | 1.21±0.08 | 1.13±0.11 | 1.10±0.16 |
| APTT, sec | 29.6±1.1 | 36.9±0.6* | 29.8±1.0 | 26.2±0.8* |
| Fibrinogen, g/l | 3.1±0.24 | 2.6±0.19* | 3.0±0.30 | 3.6±0.32* |
| SFMC, mg/decilitre | 3.0±0.32 | 2.5±0.28* | 3.1±0.36 | 3.7±0.24* |
| Plasminogen, % | 89.2±1.8 | 95.2±1.5 | 90.8±1.9 | 85.8±1.3 |
| Spontaneous platelets’aggregation, Units | 1.16±0.13 | 1.04±0.06* | 1.15±0.12 | 1.32±0.14* |
| Platelets’aggregation 0.5 mM ADP, Units | 2.27±0.15 | 2.03±0.19* | 2.28±0.13 | 2.83±0.22** |
| indices of blood rheology | | | | |
| Index of erythrocytes’aggregation | 1.27±0.06 | 1.22±0.08 | 1.27±0.05 | 1.33±0.10 |
| index of erythrocytes’deformability | 1.10±0.03 | 1.13±0.05 | 1.11±0.04 | 1.09±0.12 |
| Blood viscosity at 200 sec⁻¹, centipoise | 4.27±0.19 | 4.06±0.26 | 4.29±0.22 | 4.42±0.28 |
| Blood viscosity at 20 sec⁻¹, centipoise | 6.30±0.35 | 5.94±0.39 | 6.28±0.38 | 6.52±0.45 |

Notations: reliability of indices’ dynamics * - p<0.05, ** - p<0.01.
blood viscosity common for animals of both groups. The cows from experimental group were noted to have tendency to decrease of blood viscosity at shift speeds 20 sec\(^{-1}\) and 200 sec\(^{-1}\) on 5.2% and 6.1%, correspondingly. Given indices of control animals showed tendency to growth on 3.0% and 3.8%, correspondingly. Experimental cows were also noted to have tendency to value decrease of erythrocytes’ aggregation index on 4.0%, and control animals were found to have tendency to its increase on 4.7%. At the same time index of erythrocytes’ deformability of lactating cows having received Lipovitam-beta had tendency to increase and in case of control animals had tendency to decrease.

**DISCUSSIONS**

Being clear genetically coded all the features of a living organism\(^{23,24}\) can change the degree of their phenotypic survival depending on the environment\(^{25}\). In this connection high urgency of profound investigation continuation of living organisms’ physiology different aspects\(^{26}\) and a human being is still kept, including negative environmental conditions\(^{27,28}\) with obligatory registration of their social consequences\(^{29}\). In this connection additional investigations devoted to calves’ and cows’ physiology can provide solid basis for further perfection of their keeping and feeding technologies\(^{1,3}\). As the result of summation of received during these investigations knowledges and their consequent practical application we can reach intensification of cattle breeding\(^{21}\).

In previous investigations they showed with the help of different biological objects that hemostasis\(^{30,31}\) and hemorheology\(^{32,33}\) rather keenly respond to environmental impacts including unfavourable factors\(^{34}\) and development of different dysfunctions\(^{35}\) and evident pathology\(^{15,16}\) in an organism. It is also known that inhibition of lipid peroxidation and suppression of lack of vitamins can influence a living organism rather variously and favourably\(^{38}\). It was noted that on this background activity decrease of many components of hemostasis system\(^{39}\) and improvement of blood rheological parameters on the whole and erythrocytes in particular\(^{40,41}\) are developing. With these very changes of hematological indices we connect improvement of micrcirculation processes and intensification of metabolic processes on the background of different variants’ impacts on an organism having in its basis antioxidant impact\(^{42}\).

As the result of fulfilled investigation on the background of Lipovitam-beta application the cows were noted to have weakening of hemostasis activity and improvement of blood rheology. At the same time control results showed reverse regularity having provided to the end of investigation reliability of different accountable indices in both groups of animals.

Received results allow us to consider that additional inflow of balanced vitamin complex into an organism of a lactating cow weakens the process of hemocoagulation along both ways of its realization\(^{37}\). It is evidently connected with activity decrease of most coagulation factors participating in it. Most probably that some decrease of thromboplastin generation and weakening of XII factor’s contact activation were also developing in these animals’ blood. Lipovitam-beta addition into animals’ feeding led also to decrease of fibrinogen and SFMC in blood what pointed at inhibition of its polymerization which was actively restrained by physiologically activated system of fibrinolysis. Lactating control cows were noted to have opposite phenomena promoting increase of all the hemocoagulation stages and decrease of fibrinolysis activity.

Relying on literature data we can consider that Lipovitam-beta application stimulates organism’s antioxidant protection\(^{12,14}\), what, as it is known, decreases platelets’ ability to both spontaneous and stimulated aggregation\(^{43,44}\). We have some basis to think that addition of the given preparation into animals’ feeding rises their level of cyclic adenosine monophosphate in platelets and decreases thromboxan A\(_2\) formation. Given situation blocks the formation of thrombocyte aggregates in the lumen of the vascular bed\(^{45,46}\). Noted in control cows aptitude to the growth of platelets’ aggregative ability may be connected with the decrease of cyclic adenosine monophosphate in them\(^{47}\) and increase of thromboxan A\(_2\) synthesis in them\(^{18,19}\), naturally leading to increase of platelets’ dynamic aggregates’ number in these animals’ blood\(^{50,51,52}\).

Found in control cows increase of erythrocytes’ aggregation can mostly be provided by coming changes of their membranes’ charge because
of degradation on their surface of some glycoproteins under the influence of always strengthening in conditions of low physical activity lipid peroxidation. Intensification of oxygen active forms’ generation in given conditions provides oxidative alteration of erythrocyte membranes’ structures in these animals at simultaneous damage of plasma globular proteins having the ability to be connected in the kind of “bridges” between erythrocytes and realize their aggregation. Given situation promotes the growth of disaggregation threshold of control cows’ red platelets.

It can be supposed that found in experimental cows weakening of erythrocytes’ aggregation is mostly provided by strengthening of antioxidant plasma activity as the result of Lipovitam-beta application. On this background we noticed decrease of \( \alpha_1 \)-receptors activity, weakening of Ca\(^{2+}\)-calmodulin system and cascade of phosphatidyl inositol intracellular reactions. Taking place at this time decrease of \( \alpha_2 \)-adrenoreceptors’ activity leads to strengthening of adenylate cyclase during physiological impacts from receptors on Gi-proteins what causes rise of cyclic adenosine monophosphate quantity in erythrocytes, blocks Ca\(^{2+}\) inflow into them, providing not high erythrocytes’ aggregation.

We can consider that erythrocytes of animals having received Lipovitam-beta are more able to deformation what is an important factor of maintenance in them of necessary microcirculatory bed’s perfusion. It is possible that in these animals’ erythrocytes there is rise of ATP content what positively influences the interaction of spectrin, actin and other integral proteins of the erythrocyte membrane playing an important role in its features’ maintenance. Besides, rise of erythrocytes’ deformability in experimental cows is mostly explained by lowering of unbounded ions Ca\(^{2+}\) concentration what minimizes its interaction with membrane’s proteins and makes it more deformed. At the same time in erythrocytes of control animals there is evidently lowering of ATP content what negatively changes the character of interaction of spectrin, actin and erythrocyte membrane’s integral proteins. Erythrocytes’ deformability lowering in control cows is evidently mostly caused by the rise of unbounded ions Ca\(^{2+}\) level in them which interacting with membrane’s proteins make it firmer and less deformed.

In modern literature the opinion is gradually forming about close connection of somatic status and animals’ producing capacity with their hematological indices. At the same time in this investigation we didn’t trace the whole lactation and, that’s why it’s too early to make conclusions about the impact of Lipovitam-beta feeding on lactation level trying to explain the results from the position of hemostasis activity dynamics and state of blood rheology. At the same time, found tendency to higher level of dairy productive capacity at more physiologically profitable state of accountable indices makes us think that such dependence can be found in future investigations.

**CONCLUSION**

High-productive black-many coloured cows to the middle of lactation get the tendency to hemostasis activation and worsening of blood rheological features what negatively influences microcirculation and can become one of the factors gradually inhibiting lactation. Lipovitam-beta application to lactating cows led to decrease of plasma hemostasis and platelets’ functional activity, weakening of erythrocytes’ aggregative ability, strengthening of their deformability, improvement of blood fluidity at different shift speeds what can positively influence microcirculation and level of dairy producing capacity.

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