AGGREGATION OF REGULAR BLOOD ELEMENTS IN MILK FED CALVES

ABSTRACT

Aim. The aim was to examine aggregation activity of regular blood elements of milk fed calves.

The study design. The study used 39 calves of black and white breed which were taken into the research on the 11th day of life. They were examined on the 11th, 15th, 20th, 25th and 30th days of life.

Place and duration of the study. The study was conducted on “Kolos” farm of Fatezh district in Kursk region, Russia, in spring, 2014.

Methodology. We used biochemical, hematological and statistical methods of investigation. We estimated the intensity of lipids' peroxidation in plasma, aggregation of erythrocytes, platelets and neutrophils.

Results. Milk fed calves were noted to have an upward trend of erythrocytes’ spontaneous aggregation. It could be judged by a light upward trend of erythrocytes’ summary quantity in an aggregate, quantity rise of aggregates themselves and number lowering of disaggregated erythrocytes. All the milk fed calves were noted to have a trend to strengthening of platelets' aggregation. So, on the 11th day of life their period of platelets' aggregation development under collagen impact was equal to 30.7±0.12s. It decreased to some extent during the research. Similar state of platelets' aggregation of healthy animals was noted for adenosine diphosphate (to the end of the phase 38.1±0.15s) and ristomicin (to the end of the phase 46.2±0.17s). In later period developed platelets' aggregation for thrombin and adrenaline also had a trend to light acceleration during the research and to its end was equal to 51.3±0.18s and 98.0±0.34s, respectively. Milk fed calves were also noted to have a little trend to strengthening of neutrophils' aggregation. So, their neutrophils' aggregation during the research rose with lectin on 4.6%, with concanavalin A - on 6.4%, with phytogemagglutinin - on 3.2%.

Conclusion. During the phase of milk feeding the calves were noted to have shown stable values of lipids' peroxidation in plasma. The calves of the age between 11 to 30 days of life were found to have little strengthening of regular blood elements' aggregation.

Key words: phase of milk feeding, calves, aggregation, erythrocytes, platelets, white blood cells.

1. INTRODUCTION

Blood consists of regular elements and plasma. It continuously circulates along vessels in a living body [1]. It provides gas metabolism and delivery of nutrients and biologically active substances to tissues [2,3]. It also provides removal of metabolic waste products out of them [4,5]. The efficiency of hemocirculation, especially in microcirculation system, mostly depends on regular blood elements' aggregation [6,7]. Its evidence is under constant control from the side of a vascular wall [8,9]. It was noted that surplus aggregation of erythrocytes, platelets and leucocytes could inhibit metabolic processes in a body [10,11]. In this connection, we are sure that estimation of the degree of regular blood elements' aggregation in calves at the beginning of their ontogenesis - in the phase of milk feeding - is very urgent [12]. Given researches are important for both fundamental science and practice as abnormalities in the processes of aggregation and disaggregation in blood play essential role in pathogenesis of many diseases [13,14]. Both physiology of animals and veterinary science need precisely adjusted normative indices of basic regular blood elements' aggregation [15]. These norms are necessary for estimation of dynamics of cattle state, including milk fed calves, in case of application of various impacts on their bodies [16].

The following aim was put in our research - to examine aggregation activity of regular blood elements in milk fed calves.

2. MATERIALS AND METHODS

The research was conducted in strict accordance with ethical principles established by the European Convent on protection of the vertebrata 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 Kursk Institute of Social Education, a branch of Russian State Social University (record №12, dated December, 3rd, 2015) and the local Ethics Committee of All-Russian Scientific Research Institute of Physiology, Biochemistry and Animals' Feeding (record №11, dated December, 4th, 2015).

The study used 39 calves of black and white breed, taken into the research on the 11th day of life. All the calves were received in autumn. The animals were kept in Kursk region (Central Russia) in calf-sheds of the farm “Kolos” without special heating. They drank whole milk in the amount of 6-7 liters a day from the teaspoon drinking bowls, which amounted to approximately 12-14% of their body weight. They were examined five times during the phase of milk feeding - on the 11th, 15th, 20th, 25th and 30th days of life.

The activity of the processes of lipids' peroxidation (LPO) in plasma was estimated according to the content of thiobarbituric acid (TBA)-active products with the help of a set "Agat-Med" and acyl hydroperoxides (AHP). Antioxidant potential of liquid part of blood was determined according to its antioxidant activity (AOA) [17].

The evidence of erythrocytes' aggregation was determined with the help of a light microscope in Gorjaev's box. We registered the quantity of erythrocytes' aggregates, the number of aggregated and disaggregated erythrocytes [18].

Platelets' aggregation (AP) was estimated with the help of visual micromethod of AP estimation [19] with the usage of adenosine diphosphate (ADP) (0.5×10^{-4} M), collagen (dilution 1:2 of basic suspension), thrombin (0.125 un/ml), ristomicin (0.8 mg/ml) and adrenaline (5.0×10^{-6} M) in rich in platelets plasma with standardized platelets' quantity 200×10^{9} tr. Activity of neutrophils' aggregation was estimated with the help of a photoelectrocolorimeter. We used lectin of wheat foetus in a dose of 32 mkg/ml, concanavalin A - 32 mkg/ml and phytogemagglutinin - 32 mkg/ml as inductors.

Statistical processing of received data was made with the help of a program package “Statistics for Windows v. 6.0”, “Microsoft Excel”. A single-factor analysis of variance was used with application of the F-reliability criterion of Fisher. Differences in data were considered reliable in case of $p<0.05$.

3. RESULTS AND DISCUSSION

Examined calves were noted to have little LPO activity of plasma with a slight trend to strengthening during the period of the research. The content of AHP in it rose from 1.44±0.17 D_{233}/1ml to 1.47±0.25 D_{233}/1ml, TBA-active products - from 3.59±0.15 umol/l to 3.64±0.28 umol/l. It was accompanied by a trend to some weakening of plasma AOA from 33.5±0.38% on the 11th day of life to 33.0±0.34% on the 30th day of life (table 1).

During the phase of milk feeding the calves were noted to have unexpressed upward trend of spontaneous erythrocytes' aggregation. It could be judged by a slight upward trend of summary erythrocytes' quantity in an aggregate (on 1.9%), quantity rise of aggregates themselves (on 2.4%) and number lowering of disaggregated erythrocytes (on 2.2%) (table 1).

All the milk fed calves were noted to have a trend to strengthening of platelets' aggregation. So, on the 11th day of life their period of AP development under the impact of collagen was equal to 30.7±0.12s. It decreased to some extent during the research. Similar AP state of healthy animals was noted for ADP (to the end of the phase - 38.1±0.15s) and ristomicin (to the end of the phase - 46.2±0.17s). In later period developed thrombin and adrenaline AP also had a trend to light acceleration during the research and to its end were equal to 51.3±0.18s and 98.0±0.34s, respectively (table 1).

During the phase of milk feeding the calves were also noted to have a little trend to strengthening of neutrophils' aggregation. So, during the research their neutrophils' aggregation rose with lectin on 4.6%, with concanavalinA - on 6.4%, with phytogemagglutinin - on 3.2% (table 1).

The consumption of milk and beef by the population of the planet increases. It dictates the necessity of constant development of this agricultural branch. It can be achieved in the result of continuation of active scientific researches in the field of cattle physiology [15,20]. In this connection, special significance is given to researches of calves' blood physiology at the beginning of ontogenesis [21,22]. Much attention is paid to studying of calves which prepare to switch to the consumption of vegetable feeding. In our work it was found that calves at the age between 11 and 30 days of life had stable plasma AOA. It was accompanied by a stable level of LPO products in plasma. Found facts were supported by the results of earlier researches [23]. It is known that intensity of freely-radical processes in plasma influences significantly the morpho-functional state of erythrocytes, platelets and leucocytes [24,25]. It can explain the slight ability of milk fed calves to aggregation of basic regular blood elements.

In our work special attention was paid to aggregation of uniform elements of blood. Intra vascular formation of units and success of microcirculation in many respects depended on its level. In this regard, processes of metabolism and intensity of animals' growth depended on the activity of uniform blood elements' aggregation.

It is obvious that a large number of electronegative proteins on erythrocytes' surface [26,27] largely provides low activity of erythrocyte aggregation in calves during the phase of milk feeding. High control over generation of oxygen active forms in calves provides minimization of oxidative damages of membrane erythrocyte proteins and globular plasma proteins which participate in aggregation [28,29]. In this connection, we can come to the conclusion that the phase of milk feeding of calves is characterized by optimum of metabolic and receptor processes in erythrocytes. Received estimation results of erythrocytes' aggregation are confirmed by the single work. It contains information about the trend to its strengthening in calves of the given age [30]. We should compare received results with literature data with great caution. In previous researches the groups were mixed, as far as breed was concerned, but calves of Simmental breed prevailed. Besides, they were received in autumn. It also makes comparison of results difficult.

Noted in milk fed calves trend to strengthening of platelets' aggregative activity was connected with activity increase of their receptors and postreceptor mechanisms of aggregation [31]. Concentration of von Willebrand Factor - cofactor of platelets' adhesion - gradually rose in calves' blood at the age of 11-30 days. It was accompanied by little number increase of receptors to it - (GPIb) on platelets' surface. It was pointed by a downward trend of AP period in calves in response to ristomicin. Found AP dynamics in response to strong and weak agonists of aggregation could be explained by physiologically approved activity changes of platelet phospholipase A_2 and C. They provided functioning of thromboxane and phosphoinositol ways of platelets' activation [32,33]. In literature there is rather poor information about platelets' activity in milk fed calves [34]. Famous sources confirm that milk fed calves have a trend to strengthening of platelets' aggregation. But comparison of these results with received ones should be done with great caution. It's connected with the fact that experimental calves in previous researches were kept in Central Russia in calf-sheds with special heating, and they received substitutes of whole milk and fodder concentrated products.

It is known that activity of neutrophils' aggregation in mammals is provided by locuses' quantity in their glycoprotein receptors' composition. These receptors can connect lectins [35]. It is firmly established that phytogemagglutinin can mostly interact with parts of bD-galactose of glycoproteins, lectin of wheat foetus - with N-acetyl-D-glycosamin и N-acetyl-neuraminic (sialic) acid, and concanavalin A – with N-glycans containing mannose [11]. That's why, the state of lectin stimulated neutrophils' aggregation of calves is determined by the expression level of receptors' adhesion. These receptors have such parts in their composition. Taking it into consideration, we can come to the conclusion that found growth trend of neutrophils' aggregation at calves' age of 11-30 days was, evidently, connected with the rise of sensitivity and density of leucocytes' glycoprotein receptors. It happened simultaneously with changing of their composition. Gradual strengthening of lectin - and concanavalin A - induced neutrophils' aggregation in experimental calves was provided by expression increase of adhesion receptors on their surface and by some growth of areas containing N-acetyl-D-glucosamine, N-acetyl-neuraminic acid and mannose. Strengthening increase of aggregation, induced by phytogemagglutinin in calves between the 11th and the 30th days of life, was provided by an upward trend of areas of glycoproteins, containing bD-galactose [11], in their neutrophils' receptors. Neutrophils' aggregation was not studied earlier on productive animals and, moreover, on calves. With the help of available literature sources, containing information about researches aimed at human beings, it becomes clear that the role of receptor mechanisms in its realization is great, and that it can be quickly damaged in case of unfavorable environmental and metabolic conditions [11,32].

Noted strengthening of aggregative activity of erythrocytes, platelets and neutrophils in milk fed calves was mostly caused by processes of growth and strengthening of environmental impacts against their background [36]. Sufficient activity of adaptive mechanisms keeps the balance of aggregation and disaggregation in calves' blood in these conditions on the level which is necessary for optimum of internals' blood supply [37].

4. CONCLUSION

The phase of milk feeding is an important stage in the development of hematological indicators in cattle. During the phase of milk feeding, the calves showed stability of lipids' peroxidation in plasma. It was found that calves at the age of 11-30 days had a weak upward trend in aggregation of the basic blood elements. This situation is, in many respects, the basis for the optimal bloodstream through small vessels in milk fed calves and the processes of their growth.

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Table 1. The activity of the processes of lipids' peroxidation in plasma and aggregation of blood elements in milk fed calves

Registrated parameters	Age of calves (n=39, M±m)				
	11 days	**15days**	**20 days**	**25 days**	**30 days**
acyl hydroperoxides, D_{233}/1ml	1.44±0.17	1.46±0.12 F= 0.357 (p≤0.425)	1.47±0.20 F= 1.102 (p≤0.282)	1.47±0.15 F= 1.124 (p≤0.271)	1.49±0.25 F= 1.348 (p≤0.249)
TBA-active products, umol/l	3.59±0.15	3.63±0.22 F= 0.218 (p≤0.615)	3.60±0.26 F= 0.416 (p≤0.431)	3.62±0.19 F= 1.320 (p≤0.232)	3.64±0.28 F= 2.264 (p≤0.096)
AOA, %	33.5±0.38	33.3±0.36 F= 1.220 (p≤0.252)	33.1±0.34 F= 1.758 (p≤0.189)	32.9±0.29 F= 1.974 (p≤0.192)	32.4±0.32 F= 2.126 (p≤0.174)
sum of all the erythrocytes in an aggregate	40.1±0.19	40.2±0.24 F= 0.123 (p≤0.726)	40.4±0.29 F= 1.117 (p≤0.294)	40.6±0.25 F= 1.112 (p≤0.295)	40.9±0.32 F= 1.344 (p≤0.250)
quantity of aggregates	8.2±0.12	8.2±0.10 F= 0.017 (p≤0.896)	8.3±0.16 F= 0.019 (p≤0.890)	8.4±0.19 F= 1.286 (p≤0.260)	8.4±0.11 F= 2.912 (p≤0.092)
quantity of free erythrocytes	245.7±2.19	244.2±2.25 F= 3.122 (p≤0.0.081)	241.8±2.01 F= 2.284 (p≤0.135)	242.0±1.90 F= 1.529 (p≤0.220)	240.4±2.46 F= 1.032 (p≤0.313)
AP with ADP, s	39.2±0.16	39.0±0.12 F= 0.645 (p≤0.424)	38.7±0.13 F= 1.779 (p≤0.186)	38.4±0.10 F= 3.110 (p≤0.081)	38.1±0.15 F= 3.189 (p≤0.078)
AP with collagen, s	30.7±0.12	30.5±0.10 F= 0.025 (p≤0.876)	30.3±0.09 F= 0.295 (p≤0.588)	30.1±0.11 F= 0.724 (p≤0.397)	29.7±0.14 F= 1.704 (p≤0.196)
AP with thrombin, s	52.7±0.15	52.6±0.10 F= 0.238 (p≤0.627)	52.2±0.16 F= 1.207 (p≤0.275)	51.7±0.10 F= 2.505 (p≤0.117)	51.3±0.18 F= 3.039 (p≤0.085)
AP with ristomicin, s	47.5±0.12	47.2±0.16 F= 0.771 (p≤0.383)	46.9±0.22 F=0.877 (p≤0.352)	46.6±0.26 F= 2.505 (p≤0.117)	46.2±0.17 F= 3.057 (p≤0.084)
AP with epinephrine, s	97.8±0.42	97.4±0.36 F= 0.504 (p≤0.479)	97.1±0.32 F= 0.798 (p≤0.374)	98.5±0.45 F= 1.008 (p≤0.318)	98.0±0.34 F= 1.167 (p≤0.283)
Aggregation of neutrophils with lectin, %	14.5±0.16	14.5±0.17 F= 0.716 (p≤0.399)	14.7±0.15 F= 1.010 (p≤0.318)	14.9±0.26 F= 1.467 (p≤0.229)	15.2±0.22 F= 1.781 (p≤0.186)
Aggregation of neutrophils with concanavalin A, %	14.5±0.10	14.6±0.12 F= 0.529 (p≤0.469)	14.9±0.16 F=1.037 (p≤0.312)	15.1±0.11 F= 1.349 (p≤0.249)	15.5±0.13 F= 1.982 (p≤0.163)
Aggregation of neutrophils with phytogemagglutinin, %	27.1±0.19	27.2±0.23 F= 0.693 (p≤0.408)	27.4±0.14 F=0.877 (p≤0.352)	27.8±0.26 F= 1.104 (p≤0.297)	28.0±0.21 F=2.683 (p≤0.106)

Note:
F – the value of Fisher test when the indicators are compared with their values at the age of 11 days throughout the entire observation,
p – possibility of unmistakable prognosis.