AGGREGATION OF REGULAR BLOOD ELEMENTS IN MILK FED CALVES

ABSTRACT

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

Study design. The study used 39 calves of black and white breed which were taken into the research on the 11th day of life. Examination was made 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, spring of 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. During the phase of milk feeding the 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, decreasing to some extent during the research. Analogical 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 there developed platelets' aggregation for thrombin and adrenaline, also having a trend to light acceleration during the research and being equal to its end to 51.3±0.18s and 98.0±0.34s, respectively. During the phase of milk feeding

the 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 a little trend to strengthening 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, consisting of regular elements and plasma, 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], and also 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 find the estimation of the degree of regular blood elements' aggregation in calves at the beginning of their ontogenesis - in the phase of milk feeding - to be 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 the estimation of dynamics of cattle state, including calves of milk feeding in case of application of various impacts on their bodies [16].

The following aim was put in our research - to find out 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. Examination was made 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 small 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, decreasing to some extent during the research. Analogical 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 there developed thrombin and adrenaline AP, having also a trend to light acceleration during the research and being equal to its end 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 small 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, and 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]. So, great attention is devoted to researches of calves' preparing for transition to vegetable feeds' consumption. In our work the calves were noted to have gradual strengthening of plasma AOA at the age between the 11th and 30th days of life which was accompanied by gradual weakening of LPO activity. Found facts were supported by the results of earlier researches [23]. It

139 is known that intensity of freely-radical processes in plasma influences
140 significantly the morpho-functional state of erythrocytes, platelets and
141 leucocytes [24,25]. It can explain the slight ability of milk fed calves to
142 aggregation of basic regular blood elements.
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144 In our work special attention is paid to aggregation of uniform elements of
145 blood. Intra vascular formation of units and success of microcirculation in many
146 respects depend on its level. In this regard, processes of metabolism and
147 intensity of animals' growth depend on the activity of uniform blood elements'
148 aggregation.
149
150 It is evident that the great quantity of electronegative proteins on the surface of
151 erythrocytes [26,27] mostly lies in the basis of small activity of erythrocytes'
152 aggregation in milk fed calves. High control over generation of oxygen active
153 forms in calves provides minimization of oxidative damages of membrane
154 erythrocyte proteins and globular plasma proteins which participate in
155 aggregation [28,29]. In this connection, we can consider that the phase of milk
156 feeding of calves is characterized by optimum of metabolic and receptor
157 processes in erythrocytes. Received estimation results of erythrocytes'
158 aggregation are confirmed by the single work, containing information about the
159 trend to its strengthening in calves of the given age [30]. We should compare
160 received results with literature data with great caution as in the previous

researches the groups were mixed, as far as breed is concerned, but calves of Simmental breed prevailed. Besides, they were received in autumn, and it also makes comparison of results difficult.

Noted in milk fed calves trend to strengthening of platelets' aggregative activity is connected with activity increase of their receptors and postreceptor mechanisms of aggregation [31]. Concentration of von Willebrand Factor - cofactor of platelets' adhesion - gradually rises in calves' blood at the age of 11-30 days. It is accompanied by weak 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 can be explained by physiologically approved activity changes of platelet phospholipase A_2 and C. They provide 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]. In spite of the fact that famous sources confirm that milk fed calves have a trend to strengthening of platelets' aggregation, comparison of these results with received ones should be done with great caution. It is 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 is 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 consider 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 [25].

4. CONCLUSION

During the phase of milk feeding the calves were noted to have a slight trend to strengthening of lipids' peroxidation in plasma. The calves at the age of 11-30 days were found to have little strengthening of regular blood elements' aggregation.

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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.