

1 AGGREGATION OF BASIC REGULAR BLOOD ELEMENTS IN**2 CALVES OF MILK FEEDING****3 ABSTRACT**

4 Aim - to find out aggregation activity of regular blood elements of calves during
5 the phase of milk feeding. There was formed a group of calves of black-many
6 coloured breed which were examined five times at the age between the 11th and
7 the 30th days of life with the usage of biochemical, hematological and statistical
8 methods of investigation. The farm "Kolos" of Fatezh district in Kursk region,
9 Russia, spring of 2014. The work was conducted with the help of 39 calves of
10 black-many coloured breed, taken into investigation on the 11th day of life.
11 Examination was made on the 11th, 15th, 20th, 25th and 30th days of calves'
12 life. We estimated the intensity of lipids' peroxidation in plasma, aggregation of
13 erythrocytes, platelets and neutrophils. During the phase of milk feeding the
14 calves were noted to have a tendency to the increase of spontaneous aggregation
15 of erythrocytes. It could be judged by a light tendency to the increase of
16 summary quantity of erythrocytes in an aggregate, quantity rise of aggregates
17 themselves and number lowering of disaggregated erythrocytes. All the calves
18 during milk feeding were noted to have a tendency to strengthening of platelets'
19 aggregation. So, on the 11th day of life their period of AP development under
20 collagen impact was equal to 30.7 ± 0.12 s, decreasing to some extent during
21 investigation. Analogical AP state of healthy animals was noted for ADP (to the

22 end of the phase $38.1 \pm 0.15s$) and rhystomicin (to the end of the phase
23 $46.2 \pm 0.17s$). In later period there developed thrombin and adrenalin AP, also
24 having a tendency to light acceleration during investigation and being equal to
25 its end to $51.3 \pm 0.18s$ and $98.0 \pm 0.34s$, correspondingly. During the phase of milk
26 feeding the calves were also noted to have a little tendency to strengthening of
27 neutrophils' aggregation. So, their neutrophils' aggregation during investigation
28 rose with lectin on 4.6%, with concanavalinA - on 6.4%, with
29 phytohemagglutinin - on 3.2%. During the phase of milk feeding the calves
30 were noted to have a little tendency to strengthening of lipids' peroxidation in
31 plasma. The calves of the age of 11-30 days of life were found to have little
32 strengthening of regular blood elements' aggregation.

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

35 **1. INTRODUCTION**

36 In a living body blood, consisting of regular elements and plasma, continuously
37 circulates along vessels [1]. It provides gas exchange and delivery of nutrients
38 and biologically active substances to tissues [2,3], and also removal of
39 metabolic products out of them [4,5]. The efficiency of hemocirculation,
40 especially in microcirculation system, mostly depends on regular blood
41 elements' aggregation [6,7]. Its evidence is under constant control from a
42 vascular wall [8,9]. It was noted that surplus aggregation of erythrocytes,
43 platelets and leucocytes can break metabolic processes in a body [10,11]. In this

44 connection we find to be very urgent the estimation of the degree of regular
45 blood elements' aggregation in calves at the beginning of their ontogenesis - in
46 the phase of milk feeding [12]. Given investigations are important for both
47 fundamental science and practice as abnormalities in the processes of
48 aggregation and disaggregation in blood play essential role in pathogenesis of
49 many diseases [13,14]. Both physiology of animals and veterinary science need
50 precisely adjusted normative indices of basic regular blood elements'
51 aggregation [15]. These norms are necessary for the estimation of dynamics of
52 cattle state, including calves of milk feeding in case of application of various
53 impacts on their bodies [16].

54

55 The following aim was put in our investigation - to find out aggregation activity
56 of regular blood elements in calves during the phase of milk feeding.

57 **2. MATERIALS AND METHODS**

58 The work was conducted with the help of 39 calves of black-many coloured
59 breed, taken into investigation on the 11th day of life. Examination was made
60 five times during the phase of milk feeding - on the 11th, 15th, 20th, 25th and
61 30th days of life.

62

63 The activity of the processes of lipids' peroxidation (LPO) in plasma was
64 estimated by the content of thiobarbituric acid (TBA)-active products with the

65 help of a set "Agat-Med" and acylhydroperoxides (AHP). Antioxidant potential
66 of liquid part of blood was defined by its antioxidant activity (AOA) [17].

67

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

71

72 Platelets' aggregation (AP) was estimated with the help of visual micromethod
73 of AP estimation [19] with the usage of ADP (0.5×10^{-4} M), collagen (dilution
74 1:2 of basic suspension), thrombin (0.125 un/ml), rhystomicin (0.8 mg/ml) and
75 adrenalin (5.0×10^{-6} M) in rich in platelets plasma with standardized platelets'
76 quantity 200×10^9 tr. Activity of neutrophils' aggregation was estimated with the
77 help of a photoelectrocolorimeter. As inductors we used lectin of wheat foetus
78 in a dose of 32 mkg/ml, concanavalinA - 32 mkg/ml and phytohemagglutinin -
79 32 mkg/ml. Statistical processing of received results was made by Student's t-
80 criterion.

81

82 **3. RESULTS AND DISCUSSION**

83 Examined calves were noted to have small LPO activity of plasma with alight
84 tendency to strengthening during the period of investigation - the content of
85 AHP in it rose from 1.44 ± 0.17 D₂₃₃/1ml to 1.47 ± 0.25 D₂₃₃/1ml, TBA-active
86 products - from 3.59 ± 0.15 umol/l to 3.64 ± 0.28 umol/l. It was accompanied by a

87 tendency to some weakening of plasma AOA from $33.5\pm 0.38\%$ on the 11th day
88 of life to $33.0\pm 0.34\%$ on the 30th day of calves' life.

89

90 During the phase of milk feeding the calves were noted to have unexpressed
91 tendency to the rise of spontaneous erythrocytes' aggregation. It could be judged
92 by a light tendency to the increase of summary erythrocytes' quantity in an
93 aggregate (on 1.9%), quantity rise of aggregates themselves (on 2.4%) and
94 number lowering of disaggregated erythrocytes (on 2.2%) (table).

95

96 All the calves during milk feeding were noted to have a tendency to
97 strengthening of platelets' aggregation. So, on the 11th day of life their period of
98 AP development under the impact of collagen was equal to $30.7\pm 0.12s$,
99 decreasing to some extent during investigation. Analogical AP state of healthy
100 animals was noted for ADP (to the end of the phase - $38.1\pm 0.15s$) and
101 rhytomicin (to the end of the phase - $46.2\pm 0.17s$). In later period there
102 developed thrombin and adrenalin AP, having also a tendency to light
103 acceleration during investigation and being equal to its end to $51.3\pm 0.18s$ and
104 $98.0\pm 0.34s$, correspondingly (table).

105 During the phase of milk feeding the calves were also noted to have a small
106 tendency to strengthening of neutrophils' aggregation. So, during investigation
107 their neutrophils' aggregation rose with lectin on 4.6%, with concanavalinA - on
108 6.4%, with phytohemagglutinin - on 3.2% (table).

109 The consumption of milk and beef by the population of the planet increases, and
110 it dictates the necessity of constant development of this agricultural branch. It
111 can be achieved in the result of continuation of active scientific investigations of
112 cattle physiology [15]. In this connection special significance is given to
113 investigations of calves' blood physiology at the beginning of ontogenesis [20].
114 So, great attention is devoted to investigations of calves preparing for the
115 beginning of vegetable feeds' consumption. In our work the calves were noted
116 to have gradual strengthening of plasma AOA at the age between the 11th and
117 30th days of life which was accompanied by gradual weakening of LPO
118 activity. Found facts were supported by the results of earlier investigations [21].
119 It is known that intensity of freely-radical processes in plasma influences
120 significantly the morphofunctional state of erythrocytes, platelets and
121 leucocytes [22,23]. It can explain found in calves at milk feeding small ability
122 to aggregation of basic regular blood elements.

123

124 It is evident that in the basis of small activity of erythrocytes' aggregation in
125 calves at milk feeding mostly lies the great quantity of electronegative proteins
126 on the surface of erythrocytes [24]. High control over generation of oxygen
127 active forms in calves provides minimization of oxidative damages of
128 membrane erythrocyte proteins and globular plasma proteins, participating in
129 aggregation [25]. In this connection we can consider that the phase of milk

130 feeding of calves is characterized by optimum of metabolic and receptor
131 processes in erythrocytes.

132

133 Noted in calves during the phase of milk feeding tendency to strengthening of
134 platelets' aggregative activity is connected with activity increase of their
135 receptors and postreceptor mechanisms of aggregation. Evidently, in calves'
136 blood at the age of 11-30 days gradually rises concentration of Willybrand's
137 factor - cofactor of platelets' adhesion. It is accompanied by weak number
138 increase of receptors to it - (GPIb) on platelets' surface. It was pointed in calves
139 by a tendency to decrease of AP period in response to rhytomicin. Found AP
140 dynamics in response to strong and weak agonists of aggregation can be
141 explained by physiologically approved activity changes of platelet
142 phospholipase A₂ and C. They provide functioning of thromboxan and
143 phosphoinositol ways of platelets' activation [26,27].

144

145 A tendency to growth of neutrophils' aggregation at calves' age of 11-30 days
146 was, evidently, connected with the rise of sensitivity and density of leucocytes'
147 glycoprotein receptors. It happens simultaneously with changing of their
148 composition. Gradual strengthening of lectin - and concanavalin A - induced
149 neutrophils' aggregation in experimental calves was provided by expression
150 increase of adhesion receptors on their surface and by some growth of areas
151 containing N-acetyl-D-glucosamine, N-acetyl-neuraminic acid and mannose.

152 Strengthening increase of aggregation induced by phytohemagglutinin in calves
153 between the 11th and the 30th days of life was provided by a tendency to the
154 rise of areas of glycoproteins, containing bD-galactose [11], in their neutrophils'
155 receptors.

156

157 Noted strengthening of aggregative activity of erythrocytes, platelets and
158 neutrophils in calves during the phase of milk feeding was mostly caused by
159 processes of growth and strengthening of environmental impacts on their
160 background [21]. Sufficient activity of adaptive mechanisms in these conditions
161 keeps the balance of aggregation and disaggregation in calves' blood on the
162 level necessary for optimum of blood supply of internal organs [25].

163

164 **4. CONCLUSION**

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

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258
259**Table. Aggregation of blood elements in calves of dairy nutrition**

Registered parameters	Age of calves (n=39, M±m)				
	11 days	15days	20 days	25 days	30 days
sum of all the erythrocytes in an aggregate	40.1±0.19	40.2±0.24	40.4±0.29	40.6±0.25	40.9±0.32
quantity of aggregates	8.2±0.12	8.2±0.10	8.3±0.16	8.4±0.19	8.4±0.11
quantity of free erythrocytes	245.7±2.19	244.2±2.25	241.8±2.01	242.0±1.90	240.4±2.46
AP with ADP, s	39.2±0.16	39.0±0.12	38.7±0.13	38.4±0.10	38.1±0.15
AP with collagen, s	30.7±0.12	30.5±0.10	30.3±0.09	30.1±0.11	29.7±0.14
AP with thrombin, s	52.7±0.15	52.6±0.10	52.2±0.16	51.7±0.10	51.3±0.18
AP with ristomycin, s	47.5±0.12	47.2±0.16	46.9±0.22	46.6±0.26	46.2±0.17
AP with epinephrine, s	97.8±0.42	97.4±0.36	97.1±0.32	98.5±0.45	98.0±0.34
Aggregation neutrophils with lectin, %	14.5±0.16	14.5±0.17	14.7±0.15	14.9±0.26	15.2±0.22
Aggregation neutrophils with concanavalin A, %	14.5±0.10	14.6±0.12	14.9±0.16	15.1±0.11	15.5±0.13
Aggregation neutrophils with phytohemagglutinin, %	27.1±0.19	27.2±0.23	27.4±0.14	27.8±0.26	28.0±0.21

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