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Aggregation of Basic Regular Blood Elements in Calves during the Milk-feeding Phase

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Authors' contributions

This work was carried out in cooperation between both authors. Author TIG has developed the study, carried out the statistical analysis of the material and literature searches. Author SYZ wrote the minutes and the first draft of the manuscript. Both authors together carried out a set of material and conducted the analysis of the study. Both authors prepared the final version of the manuscript, read it and approved it.

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ABSTRACT

Aim: This study aimed to examine the aggregation activity of basic regular blood elements of calves during the milk-feeding phase.
Study Design: The study was initiated in 39 black and white breed calves, which were examined at the ages of 11, 15, 20, 25 and 30 daysat Kolos farm in the Fatezh district of the Kursk region, Russia, in spring, 2014.
Methods: We used biochemical, haematological and statistical methods of investigation. We estimated the intensity of lipid peroxidation (LPO) in the plasma, as well as the aggregation of

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erythrocytes, platelets and neutrophils.

Results: It was found that the calves had an upwards trend of spontaneous erythrocyte aggregation during the milk-feeding phase. This was identified by a slight upwards trend in the total quantity of erythrocytes in an aggregate, an increase in the quantity of the aggregates themselves and a decreased number of disaggregated erythrocytes. All the calves had a trend towards an increase of platelet aggregation during the milk-feeding phase; at the age of 11 days, their period of platelet aggregation development under collagen impact was equal to 30.7±0.12s. This decreased to some extent during the study. A similar healthy animal platelet aggregation was observed 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 the later period, platelet aggregation that was developed with thrombin or adrenaline also showed trends towards slight acceleration during the study period, and was equal to 51.3±0.18s and 98.0±0.34s, respectively, at the end of the study. The calves also had a slight trend towards increasing neutrophil aggregation during the milk-feeding phase; it increased by 4.6% with lectin, by 6.4%, with concanavalin A and by 3.2% with phytogemagglutinin.

Conclusion: During the milk-feeding phase, the calves showed low LPO activity in plasma, with a slight upwards trend. These calves, aged between 11 and 30 days, had little increase in the aggregation of regular blood elements.

Keywords: Milk-feeding phase; calves; aggregation; erythrocytes; platelets; white blood cells.

1. INTRODUCTION

Blood consists of regular elements and plasma, and it continuously circulates via the vessels in a living body [1]. It provides gas metabolism and the delivery of nutrients and biologically active substances to tissues [2,3], while removing metabolic waste products [4,5]. The efficiency of haemocirculation, particularly in the microcirculation system, primarily depends on the aggregation of regular blood elements [6,7], and evidence has shown that this is under constant control from the side of a vascular wall [8,9]. It has been observed that surplus aggregation of erythrocytes, platelets and leucocytes can inhibit metabolic processes [10,11]. In this respect, we are certain that estimation of the level of the aggregation of regular blood elements in calves at the beginning of their ontogenesis - in the milk-feeding phase - is critical [12]. Therefore, studies are important for both fundamental science and practice, as abnormalities in the processes of aggregation and disaggregation in the blood play an essential role in the pathogenesis of many diseases [13,14]. Both animal physiology and veterinary science require precisely adjusted normative indices of aggregation of basic regular blood elements [15]. These norms are necessary for estimation of cattle state dynamics, including milk-fed calves, in case of application of various impacts on their bodies [16].

Our aim was to examine the aggregation activity of regular blood elements in calves during the milk-feeding phase.

2. MATERIALS AND METHODS

The study was conducted in strict accordance with the ethical principles established by the European Convention for the Protection of Vertebrate Animals used for experimental and other scientific purposes (adopted in Strasbourg on 18 March, 1986, and confirmed in Strasbourg on 15 June, 2006) and approved by the local Ethics Committee of Kursk Institute of Social Education, a branch of the Russian State Social University (record no. 12, dated 03 December, 2015) and the local Ethics Committee of the All-Russian Scientific Research Institute of Physiology, Biochemistry and Animals' Feeding (record no. 11, 04 December, 2015).

The study was initiated in 39, black and white breed calves aged 11 days, and all the calves were received in autumn. The animals were kept in calf-sheds with no special heating, at Kolos farm in the Kursk region of central Russia. They drank 6–7 litres of whole milk per day from teaspoon drinking bowls, and this amounted to approximately 12–14% of their body weight. They were examined five times during the milk-feeding phase – at ages 11, 15, 20, 25 and 30 days.

The activity of the lipid peroxidation (LPO) processes in the plasma was estimated according to the content of thiobarbituric acid (TBA)-active products, using an Agat-Med set and acyl hydroperoxides (AHP). The antioxidant potential of the liquid part of blood was determined according to its antioxidant activity (AOA) [17].

Evidence of erythrocyte aggregation was determined using a light microscope in Gorjaev's box. We registered the quantity of erythrocyte aggregates, as well as the number of aggregated and disaggregated erythrocytes [18].

Platelet aggregation (AP) was estimated via the visual micromethod of AP estimation [19], with the use 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 platelet-rich plasma with a standardised platelet quantity of 200×10^{9} tr. Neutrophil aggregation activity was estimated via a photoelectrocolorimeter. Lectin of wheat foetus (32 mkg/ml), concanavalinA (32 mkg/ml) and phytogemagglutinin (32 mkg/ml) were used as inductors.

Statistical processing of the data obtained was carried out using Statistics for Windows software, version 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 statistically significant at a value of $p<0.05$.

3. RESULTS AND DISCUSSION

It was observed that the calves had little plasma LPO activity, with a slight trend towards an increase during the study period; the AHP content increased from 1.44±0.17 D_{233}/1 ml to 1.47±0.25 D_{233}/1 ml, and the TBA-active products increased from 3.59±0.15 umol/l to 3.64±0.28 umol/l. This was accompanied by a trend towards a reduction in plasma AOA from 33.5±0.38% at the age of 11 days to 33.0±0.34% at the age of 30 days (Table 1).

It was found that the calves showed an upwards trend in spontaneous erythrocyte aggregation during the milk-feeding phase. This was identified by a slight upwards trend in total erythrocyte quantity in an aggregate (1.9%), an increase in the quantity of aggregates themselves (2.4%) and a reduction in the number of disaggregated erythrocytes (2.2%), as shown in Table 1.

It was observed that all of the milk-fed calves had a trend towards an increase of platelet aggregation; at the age of 11 days, their period of AP development under the impact of collagen was equal to 30.7±0.12s. It decreased to some extent during the study. A similar AP state of healthy animals was observed with regard to ADP (at the end of the phase - 38.1±0.15s) and ristomicin (at the end of the phase - 46.2±0.17s). In a later period, AP that had been developed with thrombin or adrenaline also showed a trend toward a slight acceleration during the study, and was equal to 51.3±0.18s and 98.0±0.34s, respectively, at the end of the study (Table 1).

The calves also showed a slight trend towards an increase of neutrophil aggregation during the milk-feeding phase. During the study, neutrophil aggregation increased by 4.6% with lectin, by 6.4% with concanavalin A and by 3.2% with phytogemagglutinin (Table 1).

Global consumption of milk and beef is increasing, and dictates the necessity of constant development of this branch of agriculture. This can be achieved as a result of continuation of active scientific research in the field of cattle physiology [15,20]. In this respect, particular significance is given to studies of calves' blood physiology at the beginning of ontogenesis [21,22]. A great deal of attention is paid to the study of calves that are in preparation for a switch to the consumption of vegetable feeding. In the present study, we found that calves aged between 11 and 30 days had stable plasma AOA, which was accompanied by a stable level of LPO products in the plasma. These findings are in accordance with the results of previous studies [23]. It is known that the intensity of freely-radical processes in the plasma significantly influences the morpho-functional state of erythrocytes, platelets and leucocytes [24,25]. It can explain the slight ability of milk fed calves in aggregation of basic regular blood elements.

In the present study, we paid particular attention to the aggregation of basic regular blood elements; the intra vascular formation of units and the success of microcirculation depends on this in many respects. In this regard, metabolism processes and the intensity of animal growth depends on the activity of the aggregation of regular blood elements.

It is obvious that a large number of electronegative proteins that exist on the surface of erythrocytes [26,27] largely provide low erythrocyte aggregation activity in calves during the milk-feeding phase. A high level of control over the generation of forms of active oxygen in calves minimises oxidative damage to membrane erythrocyte proteins and globular plasma

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

Registered parameters	Age of calves (n=39, M±m)				
	11 days	**15 days**	**20 days**	**25 days**	**30 days**
acyl hydroperoxides, D_{233}/1 ml	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.

proteins, which are involved in aggregation [28,29]. In this respect, we can conclude that the milk-feeding phase of calves is characterised by optimal metabolic and receptor processes in erythrocytes. The obtained erythrocyte aggregation estimation results have been confirmed in a single study, which contains information regarding the trend towards its increase in calves of the given age [30]. We should compare our results with those of previous studies with great caution, as the latter used mixed breeds, although calves of the Simmental breed prevailed. In addition, the calves were received in autumn, which also makes a comparison of results difficult.

It was observed that the trend towards an increase in platelet aggregative activity during the milk-feeding phase was associated with an increase in receptor activity and post-receptor mechanisms of aggregation [31]. The concentration of von Willebrand Factor – a cofactor of platelet adhesion – gradually increased in the calves' blood from the age of 11–30 days, and it was accompanied by a small increase in the number of its receptors (GPIb) on the platelet surface. This was identified by a downwards trend in the AP period in calves in response to ristomicin. We found that the response of AP dynamics 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]. The information regarding platelet activity in milk-fed calves in previous studies is rather poor [34], but well-known sources have confirmed that there is a trend towards increasing platelet aggregation in calves during the milk-feeding phase. However, great caution should be exercised in making a comparison of these results with those of the present study. This is related to the fact that the experimental calves used in previous studies were kept in specially heated calf-sheds in Central Russia, and they received whole milk substitutes and fodder-concentrated products.

It is known that neutrophil aggregation activity in mammals is provided by the quantity of their loci in the composition of their glycoprotein receptors, which can connect lectins [35]. It is firmly established that phytogemagglutinin primarily interact with parts of bD-galactose of glycoproteins, lectin of wheat foetus - with N-acetyl-D-glycosaminи N-acetyl-neuraminic (sialic) acid, and concanavalinA – with N-glycans-containing mannose [11]. This is why the level of lectin-stimulated neutrophil aggregation in calves is determined by the adhesion receptor expression level. These receptors have such parts in their composition. Therefore, we can conclude that the observed growth trend in neutrophil aggregation in calves aged 11–30 days was evidently associated with the increase in the sensitivity and density of leucocyte glycoprotein receptors, which occurred simultaneously with the changes in their composition. The gradual increase in lectin- and concanavalin A-induced neutrophil aggregation in the calves in the present study was the result of an increase in the expression of adhesion receptors on their surface, and by some growth of areas containing N-acetyl-D-glucosamine, N-acetyl-neuraminic acid and mannose. The increase of aggregation, induced by phytogemagglutinin in calves aged between 11 and 30 days, was due to an upwards trend in areas of glycoproteins, containing bD-galactose [11], in their neutrophil receptors. Neutrophil aggregation has not previously been studied in productive animals or, moreover, in calves. Publications containing information regarding studies conducted in human beings makes clear the fact that receptor mechanisms play a large role in the realisation of neutrophil aggregation, and that the latter can be quickly damaged in the case of unfavourable environmental and metabolic conditions [11,32].

The observed increase in the aggregative activity of erythrocytes, platelets and neutrophils in calves during the milk-feeding phase was primarily caused by processes of growth and background environmental impact [36]. In these conditions, sufficient activity of adaptive mechanisms maintains the balance of aggregation and disaggregation in calves' blood at a level that is necessary for optimal internal blood supply [37].

4. CONCLUSION

The milk-feeding phase is an important stage in the development of haematological indicators in cattle. In the present study, the calves showed stability of LPO in plasma during this phase. It was found that calves aged 11–30 days had a weak upwards trend in aggregation of the basic blood elements. This situation is, in many respects, the basis for the optimal bloodstream along small vessels in milk-fed calves and for the processes of their growth.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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