



## **Aggregative Activity of Basic Regular Blood Elements and Vascular Disaggregating Control over It in Calves of Milk-vegetable Nutrition**

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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**

Estimation of ontogenetic peculiarities of aggregation of regular blood elements and vascular control over it, including calves in the middle of early ontogenesis - in the phase of milk-vegetable nutrition, is of great scientific and practical interest. Clarification of their physiological balance is really significant for receiving the possibility of regulation of hemostasis activity and metabolism intensity in tissues. To estimate aggregative activity of regular blood elements and disaggregating vessels' control over them in calves during the phase of milk vegetable nutrition. We formed a group of calves of black-many-colored breed which were examined 5 times at the age between the 31st and the 90th days of life with the application of biochemical, hematological and statistical methods of

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investigation. Farm "Kolos" of Fatezh district, Kursk region, Russia, spring 2014. The work was conducted with the help of 41 calves of black many-colored breed taken into investigation on the 31st day of life. Examination was conducted on the 31st, 45th, 60th, 75th and 90th days of calves' lives. We estimated the intensity of lipids' peroxidation in plasma, aggregation of erythrocytes, platelets and neutrophils in plasma received without application of a cuff on a vessel and with its usage. The calves of 45 days of life were noted to have short-period increase of summary erythrocytes' quantity in aggregate (on 15.9%) and aggregates' quantity (on 13.4%) at decrease of free erythrocytes (on 9.7%). Given indices returned in calves to the initial level by the 90th day of life. In plasma after temporary venous occlusion in calves by the 45th day of life summary erythrocytes' quantity in aggregates (on 21.7%) and aggregates' number (on 16.3%) had a short-term increase at decrease of free erythrocytes on 13.9%. By the 45th day of life their time of platelets' aggregation development with collagen shortened to  $22.5 \pm 0.12$ s returning by the end of investigation to the initial level. In plasma after temporary venous occlusion we found peak acceleration of platelets' aggregation by the 45th day of life. The calves of 45 days of life were noted to have strengthening of neutrophils' aggregation with lectin on 10.1%, concanavalin A - on 9.4%, phytohemagglutinin - on 19.1% eliminated by the 90th day of life. Found in calves of 45 days of life short-period strengthening of formular blood elements' aggregation was combined with brief weakening of their deaggregating vascular activity.

*Keywords: Calves; the phase of milk-vegetable nutrition; vascular wall; aggregation; regular blood elements.*

## 1. INTRODUCTION

Age-specific dynamics of physiological indices of cattle youngsters is mostly connected with ontogenetic changes of their blood parameters [1]. Continuously moving along vessels blood, composing of plasma and its regular elements, fulfils some functions including provision of gas exchange and transportation to tissues of nutrients, hormones, vitamins and so on, and also -washing of final products of metabolism out of them [2,3]. Hemocirculation optimum including vessels of microcirculation system is mostly connected with evidence of regular blood elements' aggregation [4,5] essentially defined by the level of deaggregating control from a vascular wall side. It is established that high aggregation evidence of erythrocytes, platelets and leucocytes can negatively influence metabolism and damage many physiological processes [6,7]. Estimation of ontogenetic peculiarities of regular blood elements' aggregation and vascular control over it [8], including calves in the middle of early ontogenesis - in the phase of milk-vegetable nutrition [9], is of great scientific and practical interest. Clarification of their physiological balance is really significant for receiving of possibility of hemostasis activity regulation and metabolism intensity regulation in tissues [10]. Besides, practical biology needs precisely adjusted age-specific indices' norms of basic regular blood elements' aggregation and the level of vessels' deaggregating control over them for estimation of their abnormalities' depth in

calves with different pathology and application efficiency of different correction variants in them [11]. The aim of this research is to estimate aggregative activity of regular blood elements and vessels' deaggregating control over them in calves during the phase of milk-vegetable nutrition.

## 2. MATERIALS AND METHODS

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 March 18, 1986, and confirmed in Strasbourg June 15, 2006) and approved by the local ethic committee of Kursk Institute of Social Education, branch of Russian State Social University (Record №12 dated December 3, 2015) and the local ethic committee of All-Russian SII of Physiology, Biochemistry and Animals' feeding (Record №11, dated December 4, 2015).

The research was conducted on 41 calves of black-many-coloured breed taken into investigation on the 31st day of life. All the calves were kept at the farm "Kolos", Fatezh district, Kursk region. The work was conducted in spring period of 2014. Calves' examinations was made 5 times during the phase of milk-vegetable nutrition - on the 31st, 45th, 60th, 75th and 90th days of life. Processes' intensity of lipids' peroxidation (LPO) in plasma 81 was estimated according to the content of thiobarbituric acid

(TBA)-active products by a set "Agat-Med" and acylhydroperoxides (AHP). We also defined antioxidant activity (AOA) of plasma [12].

Activity of vascular control over regular blood elements' aggregation was registered according to its weakening in the probe with temporary venous occlusion [13].

Erythrocytes' aggregation was registered before and after temporary ischemia of a vessel wall in Gorjaev's box by calculating of erythrocytes' aggregates' quantity, number of aggregated and non-aggregated erythrocytes [13]. In all the animals by dividing the sum of all the erythrocytes in aggregates on the value of this sum on the background of temporary venous occlusion we calculated the index of vessels' control over the sum of erythrocytes in an aggregate (ICVSEA), as the result of division of aggregates' quantity without temporary venous occlusion on their quantity on the background of temporary venous occlusion we defined the index of vessels' control over erythrocyte aggregates' quantity (ICVQEA), while dividing the quantity of free erythrocytes on the background of temporary venous occlusion on the quantity of free erythrocytes without it we calculated the index value of vessels' control over the quantity of free erythrocytes (ICVQFE).

Aggregation of platelets (AP) was estimated by visual micromethod [14] before and after venous occlusion with the application of ADF ( $0.5 \times 10^{-4}$  M), collagen (solution 1:2 of the basic suspension), thrombin (0,125 un/ml), rhystomicin (0.8 mg/ml) ("Renam", Russia) and adrenalin ( $5.0 \times 10^{-6}$  M, Gedeon Richter, Hungary) in rich by platelets plasma with standardized platelets' quantity  $200 \times 10^9$  tr. Index of antiaggregative activity of a vascular wall (IAAVW) we calculated by dividing of AP development time after venous occlusion on the time without it.

Neutrophils' aggregation was estimated on the photoelectrocolorimeter in plasma received after application of a cuff on a vessel and without it. As inductors we applied lectin of wheat corcule in the dose of 32 mkg/ml, concanavalin A - 32 mkg/ml and phytohemagglutinin – 32 mkg/ml. In all the calves we calculated the index of neutrophils' aggregation by vascular wall inhibition (IIVWAN) by dividing the value of neutrophils' aggregation in plasma, received without application of a cuff on a vessel, on its value in plasma, taken in conditions of temporary venous occlusion. Statistical processing of

received results was made by t-Student's criterion.

### 3. RESULTS AND DISCUSSION

Calves during the phase of milk-vegetable nutrition were noted to have initially not high activity of LPO plasma with its peak strengthening by the 45th day of life - contents of AHP in it increased from  $1.50 \pm 0.19$  D<sub>233</sub>/1 ml to  $1.82 \pm 0.14$  D<sub>233</sub>/1 ml, TBA-active products - from  $3.62 \pm 0.12$  mkmol/l to  $3.98 \pm 0.18$  mkmol/l with their consequent return to the initial level by the 90th day of life. The dynamics of LPO level was accompanied by an episode of AOA plasma weakening from  $33.2 \pm 0.36\%$  on the 31st day to  $28.8 \pm 0.29\%$  on the 45th day and rise of this index to  $33.7 \pm 0.42\%$  by the 90th day of life.

Calves of 45 days of life were noted to have short-period increase of spontaneous erythrocytes' aggregation what could be judged according to the rise of summary erythrocytes' quantity in aggregate (on 15.9%), quantity increase of aggregates themselves (on 13.4%) and number lowering of freely lying red corpuscles' (on 9.7%). Given indices returned to the level, near the initial one, by the 90th day of life.

Conducting the probe with temporary venous occlusion we noticed that in examined calves by the 45th day of life summary erythrocytes' quantity in aggregates and these aggregates number had short-period increase on 21.7% and 16.3%, correspondingly, at not lasting number decrease of free erythrocytes on 13,9%. It provided short-period lowering of ICVSEA, ICVQEA and ICVQFE with their return by the end of investigation to the initial level (Table 1).

Calves at the beginning of milk-vegetable nutrition phase were noted to have not lasting acceleration of platelets' aggregation. By the 45th day of life their time of AP development under the impact of collagen had short-period reduction to  $22.5 \pm 0.12$ s returning by the end of milk-vegetable nutrition phase to the level, characteristic to its beginning. Analogical AP dynamics in experimental animals was also noted with ADF and rhystomicin. In later periods the calves had developing AP with thrombin and adrenalin, and also short-period of acceleration at the age of 45 days, on 27.9% and on 18,7% (up to  $40.5 \pm 0.14$ s and  $82.1 \pm 0.29$ s, correspondingly) with consequent return of their level by the end of milk-vegetable nutrition phase

to values corresponding to the age of 31 days (Table 1).

Conducting the probe with temporary venous occlusion we found analogical peak of AP acceleration at the age of 45 days. In case of experimental calves it pointed at short-period weakening at this age of a vessel wall's control over thrombocyte aggregation what was supported by found during milk vegetable nutrition phase dynamics of IAAVW values (Table 1).

Calves at the age of 45 days were noted to have a short-period of strengthening of neutrophils' aggregation with lectin on 10.1%, with concanavalin A on 9.4%, with phytohemagglutinin on 19.1% with its consequent weakening by the end of investigation.

In the probe with temporary venous occlusion neutrophils' aggregation also experienced by the 45th day of life short-period strengthening in relation to all the applied inductors. It caused in calves in this period the episode of IIVWAN lowering for lectin on 10.3%, for concanavalin A on 14.3%, for phytohemagglutinin on 6.9% with its gradual return to the initial values by the end of investigation (Table 1).

Gradual demand growth of the planet's population in cattle breeding products dictates the necessity of continuous development of this agricultural branch what is possible in the result of continuation of active scientific investigations in cattle physiology [15]. In this connection rather important significance is given to investigations in blood physiology of calves in the period of nutrition change and active growth [16]. In this connection large attention is devoted to investigations of calves begun to consume vegetable food [1]. In our conducted work the calves were noted to have brief episode of AOA plasma weakening at the age of 45 days which was accompanied by not lasting strengthening of LPO activity. Found facts were supported by the results of earlier investigations [17].

It is known that intensity of freely radical processes in plasma has essential impact on morphofunctional state of erythrocytes, platelets, leucocytes and vascular endothelium [18-20]. It can explain found in experimental calves at the beginning of vegetable food consumption brief strengthening of ability to aggregation and not lasting weakening of vessels' deaggregating activity.

It's evident that not lasting strengthening of erythrocytes' aggregation at the beginning of vegetable food inflow into calves' bodies can be mostly explained by quantity lowering of electronegative proteins on erythrocytes' surface [21].

Quick restoration after the 45th day of life of effective control over generation of oxygen active forms provides minimization of oxidative damage of membrane erythrocyte proteins and globular plasma proteins participating in aggregation [22]. In this connection there is some ground to consider that milk vegetable nutrition phase of calves is characterized by complicated metabolic and receptor changes in erythrocytes. At the same time, registered in calves at the age of 45 days brief rise of erythrocytes' aggregation at quantity lowering of free erythrocytes [23] doesn't influence perfusion sufficiency of their internals.

Weakening with consequent evidence restoration of vessels' control over erythrocytes' aggregation in calves at the beginning of milk-vegetable nutrition phase has, evidently, in its basis brief lowering of prostacyclin and NO production in vascular endothelium.

By the 45th day of life the calves were noted to have brief strengthening of platelets' aggregative activity. Found in calves quick consequent lowering of platelets' activity is mostly connected with lowering of their receptors' activity and weakening of aggregation postreceptor mechanisms. Besides, in calves' blood at the elder age than 45 days we found gradual concentration lowering of Willybrand's factor - cofactor of platelets' adhesion at lowering of receptors' number to it - (GPIb) on blood platelets' surface. It was pointed by lengthening of calves' AP time in response to rhytomicin. Found AP dynamics in response to strong and weak aggregation agonists can be explained by physiologically approved activity variations of thrombocyte phospholipase A<sub>2</sub> and C, providing functioning of thromboxan and phosphoinositol ways of platelets' activation [24,25]. Found brief AP acceleration in calves' plasma after temporary venous occlusion also points at brief weakening in them at the age of 45 days of deaggregating vascular impacts. Later the level of vascular control over AP strengthened what pointed at activation of prostacyclin and NO production in endothelium [26], reaching in calves by the 90th day of life the level relevant to the beginning of milk-vegetable nutrition phase.

**Table 1. Aggregation of regular blood elements and vascular control over it in calves of milk-vegetable nutrition (n=41, M±m)**

Indicators under consideration	Ontogenesis				
	31	45	60	75	90
The sum of all the erythrocytes in an aggregate	40.8±0.16	48.5±0.20**	45.2±0.23*	42.4±0.19*	45.4±0.17*
ICVSEA	1.29±0.004	1.20±0.007**	1.25±0.003**	1.28±0.005*	1.32±0.004**
The quantity of erythrocyte aggregates	8.4±0.08	9.7±0.06**	9.0±0.09**	8.5±0.12*	8.5±0.07
ICVQEA	1.17±0.004	1.13±0.003**	1.15±0.005*	1.16±0.002	1.18±0.003*
The quantity of free erythrocytes	243.7±1.86	222.1±2.09**	231.1±2.36*	238.5±1.96	239.5±2.12
ICVQFE	1.23±0.009	1.18±0.007**	1.19±0.010	1.21±0.012*	1.24±0.008*
Aggregation of platelets with ADF, s	38.2±0.14	29.1±0.10**	33.0±0.08*	35.6±0.14*	36.2±0.18
IAAVW with ADF	1.62±0.005	1.32±0.009**	1.39±0.012**	1.48±0.005**	1.65±0.007**
Aggregation of platelets with collagen, s	30.2±0.16	22.5±0.12**	26.7±0.14**	29.5±0.19*	29.0±0.15
IAAVW with collagen	1.60±0.004	1.41±0.006**	1.47±0.005*	1.52±0.003*	1.61±0.006**
Aggregation of platelets with thrombin, s	51.8±0.17	40.5±0.14**	45.8±0.20**	49.4±0.16**	48.2±0.13
IAAVW with thrombin	1.54±0.006	1.42±0.004**	1.46±0.009*	1.49±0.004	1.54±0.002*
Aggregation of platelets with rhytomicin, s	46.3±0.14	36.8±0.14**	40.8±0.17*	43.2±0.20	44.8±0.15
IAAVW with rhytomicin	1.54±0.005	1.24±0.012**	1.32±0.008**	1.46±0.007**	1.56±0.006**
Aggregation of platelets with adrenalin, s	97.5±0.36	82.1±0.29**	87.0±0.34*	94.6±0.42*	96.1±0.29
IAAVW with adrenalin	1.65±0.003	1.44±0.006**	1.52±0.007**	1.59±0.005**	1.67±0.003*
Aggregation of neutrophils with lectin, %	15.1±0.12	16.8±0.14**	16.0±0.18*	15.4±0.15*	15.6±0.19
IIVWAN with lectin	1.28±0.004	1.16±0.003**	1.25±0.005**	1.28±0.006*	1.29±0.004
Aggregation of neutrophils with concanavalin A, %	15.4±0.12	17.0±0.10**	16.5±0.08*	15.8±0.07*	16.1±0.12
IIVWAN with concanavalin A	1.28±0.005	1.12±0.004**	1.19±0.006**	1.25±0.007*	1.29±0.004
Aggregation of neutrophils with phytohemagglutinin, %	27.9±0.14	34.5±0.20**	31.6±0.18*	28.4±0.13*	29.6±0.17
IIVWAN with phytohemagglutinin	1.23±0.004	1.15±0.002**	1.19±0.005**	1.22±0.006*	1.24±0.003

Conventional signs of reliability of age-specific dynamics: \* -  $p < 0.05$ ; \*\* -  $p < 0.01$

Brief rise of neutrophils' aggregation at the age of nearly 45 days changed in calves by its weakening, evidently, because of sensitivity and density rise of leucocytes' glycoprotein receptors at changes of their composition. Gradual weakening of lectin- and concanavalin A-induced neutrophils' aggregation in calves elder than 45 days was provided by expression lowering of adhesion receptors on their surface and decrease in their composition of parts containing N-acetyl-D-glucosamine, N-acetyl-neuraminic acid and mannose. Coming to calves elder than 45 days of life weakening of aggregation induced by phytohemagglutinin was provided by the tendency to decrease in neutrophils' receptors of glycoprotein's parts containing bD-galactose. Brief weakening of vascular disaggregation control over neutrophils was quickly eliminated after the 45th day of life, evidently, on behalf of

strengthening of prostacyclin and NO production in animals' vessels [27].

#### 4. CONCLUSION

Significant rise of aggregative activity of erythrocytes, platelets and neutrophils and lowering of vessels' abilities to disaggregation in calves at the beginning of milk vegetable nutrition phase are mostly caused by stressful situation in their body connected with the beginning of vegetable food consumption. Quick insertion of adaptive mechanisms in these conditions keeps the balance of aggregation and disaggregation in calves' blood on the level which is necessary for optimum of internals' perfusion and metabolism in tissues. Consequent quick lowering of aggregative features of regular blood elements and strengthening of vascular control over them

are the basis for the adaptive processes successfully passing in calves' body in the second part of early ontogenesis.

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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