Annals of R.S.C.B., ISSN: 1583-6258, Vol. 25, Issue 5, 2021, Pages. 5492 - 5500

Received 15 May 2021; Accepted 20 May 2021.

The Physiological Characteristics of Platelets in Calves Dairy Nutrition Holstein Breed

Ilya Nikolaevich Medvedev[1*], NadezhdaViktorovnaVorobyeva[2]
[1]Faculty of Physical Education/Russian State Social University/
Moscow, Russia
[2]Department of Physical Education/ South-West state University/ Kursk, Russia

***Corresponding author:** Medvedev Ilya Nikolaevich, Scopus ID 7004513523,
http://orcid.org/0000-0002-9263-2720
Tel +79102732263
E-mail address: ilmedv1@yandex.ru

ABSTRACT

The level of platelet activity in the blood affects the course of metabolic processes, which is of great biological importance, especially at the beginning of ontogenesis. There is reason to believe that in animals of different breeds there are differences in the level of platelet activity. The work was performed on 48 purebred calves in Holstein breed, which were obtained from completely healthy cows, after normal pregnancy. All young animals were examined and examined on the 11th, 15th, 20th, 25th, 30th day of his life. During the study, biochemical, hematological and statistical research methods were applied. In animals, a decrease in platelet aggregation was noted during the phase of dairy nutrition. In the blood of calves of the Holstein breed, discocytes increased by 2.9%. Moreover, the total number of active platelets in them decreased by 20.0%, and the levels of platelet aggregates circulating in the blood of small and large sizes decreased during the observation period by 20.0% and 40.0%, respectively. Apparently, this was largely due to a weakening of the synthesis of thromboxane calves in platelets, a decrease in the amount of adenosine phosphate in them and a weakening of its secretion. During the second phase of early ontogenesis, the amount of actin and myosin in the platelets of animals also decreased, which further weakened platelet hemostasis. In addition, their generation of actin and myosin was weakened under the conditions of induced platelet aggregation. It is clear that Holstein calves in the phase of milk nutrition are characterized by a high functional perfection of the hemostatic properties of platelets. This provides them with physiologically very favorable conditions for microcirculation in their tissues. This is ensured in them by a certain weakening of the activity of mechanisms providing platelet processes of adhesion, aggregation, and secretion. It is clear that a decrease in the intravascular activity of platelets in calves of the Holstein breed during the milk feeding phase forms the optimum of perfusion and metabolism in all their tissues, which is extremely important for the rapid growth, development and creation of the basis for the future high productivity of this breed of cattle.

Keywords: Calves; Phase lactispascens; Holstein breed; Platelets; Aggregation; Secretion.

Introduction

The general functional state of the body very much depends on the activity of hemocirculation processes in its capillaries [1]. Of great importance in this process belongs to the activity of platelets [2], which differs in different species of mammals [3]. It was established that the functional parameters of platelets are able to experience dynamics against the background of growth and development [4], in the case of the appearance of many dysfunctions [5], against the background of the development of various pathologies [6] and during the healing effects [7]. Until now, many aspects of platelet function remain poorly studied in cattle.

Until now, platelet activity in calves and cows of optimal functional status has not been adequately studied [8]. This makes it impossible to compose a holistic view of it and forms the need for research in this direction. At the same time, it can be considered that the activity of capillary blood flow and the intensity of the

Annals of R.S.C.B., ISSN: 1583-6258, Vol. 25, Issue 5, 2021, Pages. 5492 - 5500

Received 15 May 2021; Accepted 20 May 2021.

functioning of the morphological structures of their body are very significantly associated with the level of functional properties of platelets [9]. Due to the presence of genetic and physiological characteristics of individual cattle breeds, it is of great scientific interest to determine the level of platelet activity in young animals with a high milk yield of Holstein breed, including during the phase of dairy nutrition.

The goal is to find out changes in platelet activity in Holstein calves of optimal functional status during the milk feeding phase.

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).

The study was conducted on 48 purebred calves in Holstein breed, which were obtained from healthy cows after normal flowing style. All calves were examined and examined for the phase of dairy nutrition 5 times: 11, 15, 20, 25 and 30 days of ontogenesis.

All animals underwent an indirect assessment of the activity of thromboxane synthesis in platelets and indirectly determined the level of enzymatic properties of cyclooxygenase and thromboxane synthetase in them. This was carried out using 3 portable samples on a photoelectrocolorimeter. The calf blood plates showed the levels of adenosine triphosphate (ATP) and adenosine diphosphate (ADP), the degree of their secretion under the influence of collagen platelets, as well as the amount of actin and myosin in intact platelets and in platelets subjected to ADP activation [10].

The degree of platelet aggregation (AP) in the observed calves was evaluated using a visual micromethod with a number of agonists: ADP (0.5 x 10^{-4} M), collagen (dilution 1: 2 of the main suspension), thrombin (0.125 units/ml), adrenaline ($5.0x10^{-6}$ M) and ristomycin (0.8 mg/ml) in plasma, which was standardized by the number of platelets to a level of $200x10^{9}$ platelets in 1 liter [11]. Intravascular platelet activity was recorded by applying a phase-contrast nozzle to a light microscope [12]. Statistical processing of received information was made with the help of a programme packet "Statistics for Windows v. 6.0", "MicrosoftExcel". Differences in data were considered reliable in case $p<0.05$.

Results

In calves of Holstein breed taken under observation during the milk feeding phase, weakening of the initially small platelet activity was found. In calves included in the observation group, on the 11th day of life, AP developed under the action of collagen for 37.9±0.17 s, subsequently slowing down to 30 days of life up to 42.2±0.14 s. Similar changes in antibodies by the end of the observation were noted with the other inducers: in response to ADP and ristomycin up to 51.4±0.13 s and 59.9±0.26 s, respectively, antibodies with thrombin up to 64.0±0.18 with antibodies with adrenaline up to 112.6±0.17s (table 1).

Table 1. Platelets in Holstein calves during the dairy phase

Takenintoaccountindicators	Holstein calves, n=42, M±m				
	11 day	15 day	20 day	25 day	30 day
Restoring platelet aggregation during a collagen-aspirin test, %	73.8±0.16	73.0±0.14	71.2±0.09	69.5±0.15	67.0±0.12 p<0.05
Restoring platelet aggregation during a collagen-imidazole test, %	35.1±0.10	34.5±0.09	33.2±0.08	32.6±0.05	30.5±0.07 p<0.05

Annals of R.S.C.B., ISSN: 1583-6258, Vol. 25, Issue 5, 2021, Pages. 5492 - 5500

Received 15 May 2021; Accepted 20 May 2021.

Platelet Aggregation in a Simple Transfer Sample, %	25.6±0.05	25.0±0.07	24.2±0.10	23.5±0.09	21.4±0.12 p<0.01
The ATP content in platelets before secretion, μmol/10^9 platelets	5.26±0.016	5.19±0.014	5.02±0.012	4.90±0.009	4.82±0.007 p<0.05
The ADP content in platelets before secretion, μmol/10^9 platelets	3.14±0.004	3.10±0.008	3.02±0.006	2.94±0.005	2.80±0.003 p<0.05
Level secretion ATP,%	24.6±0.10	24.0±0.07	23.2 ±0.11	22.3±0.14	21.0±0.10 p<0.01
Levelsecretion ADP,%	31.9±0.12	31.3±0.13	30.2±0.08	29.6±0.16	28.4±0.08 p<0.05
The amount of actin in inactive platelets,% of the total protein in platelets	20.3±0.14	19.9±0.15	19.1±0.09	18.4±0.06 p<0.05	17.5±0.08 p<0.05
The amount of actin in platelets with ADP-aggregation,% of total protein in platelets	31.9±0.12	31.4±0.09	30.6±0.07	29.8±0.09	28.7±0.13 p<0.05
The amount of myosin in inactive platelets,% of the total protein in platelets	9.3±0.16	8.9±0.14	8.6±0.12	8.1±0.10 p<0.05	7.8±0.14 p<0.01
The amount of myosin in platelets during ADP-aggregation,% of the total protein in platelets	21.7±0.10	21.2±0.13	20.5±0.07	19.7±0.05 p<0.05	19.1±0.06 p<0.05
APwith ADP, s	47.2±0.09	47.8±0.16	48.5±0.12	49.7±0.14	51.4±0.13
APwithcollagen, s	37.9±0.17	38.7±0.10	39.9±0.09	41.0±0.07	42.2±0.14 p<0.05
APwiththrombin, s	59.6±0.12	60.2±0.16	60.09±0.14	62.3±0.22	64.0±0.18
APwithristomycin, s	55.5±0.19	56.4±0.14	57.2±0.21	58.7±0.23	59.9±0.26
APwithadrenaline, s	107.2±0.19	107.9±0.25	109.1±0.23	110.3±0.19	112.6±0.17
The number of small platelet aggregates, per 100 free platelets	2.4±0.03	2.3±0.06	2.3±0.02	2.2±0.04 p<0.05	2.0±0.05 p<0.05
The number of medium and large platelet aggregates, per 100 free platelets	0.07±0,018	0.06±0.014	0.06±0.020 p<0.05	0.05±0.016 p<0.01	0.05±0.019 p<0.01

Note: p – is the reliability of the dynamics of the recorded indicators in relation to 11 days of age.

The content of discocytes in the blood of the calves included in the study during the phase of dairy nutrition tended to increase. This was accompanied by a decrease in the sum of active platelet species by 20.0% (picture 1). The amounts of small, as well as medium and large platelet aggregates freely moving in the blood also experienced a decrease during the observation.

Annals of R.S.C.B., ISSN: 1583-6258, Vol. 25, Issue 5, 2021, Pages. 5492 - 5500

Received 15 May 2021; Accepted 20 May 2021.

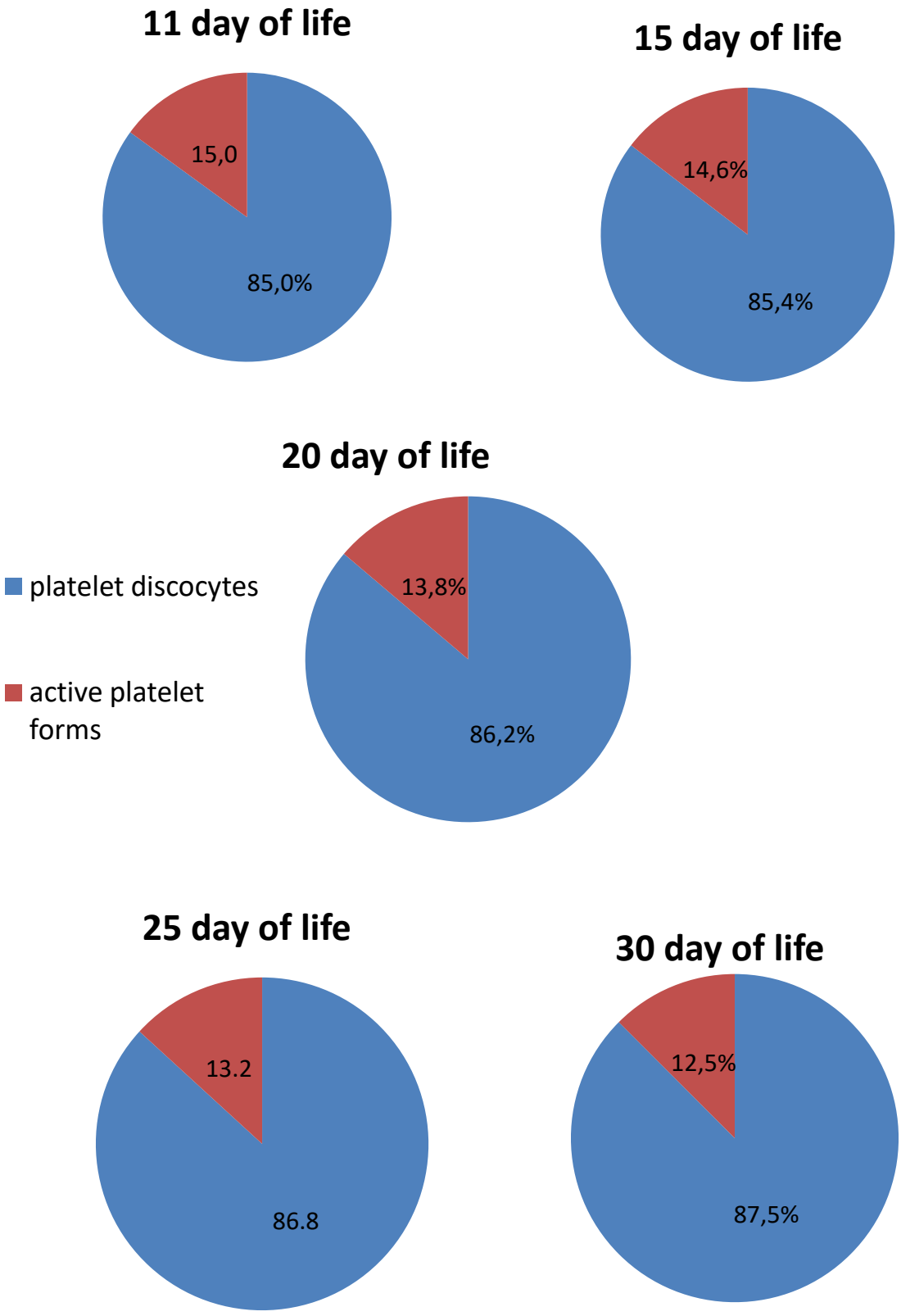

Figure 1. Intravascular platelet activity in Holstein calves during the dairy plant phase.

Annals of R.S.C.B., ISSN: 1583-6258, Vol. 25, Issue 5, 2021, Pages. 5492 - 5500

Received 15 May 2021; Accepted 20 May 2021.

One might think that the decrease in the synthesis of thromboxane in platelets found in this work is the basis for lengthening the time of development of antibodies in calves in the phase of milk feeding of the Holstein breed. This was indicated by a decrease in AP in a simple transfer request (for 30 days of ontogenesis 21.4±0.12%). This was based on the observed calves in lowering the level of activity in their platelets of both enzymes that realize its synthesis - cyclooxygenase and thromboxane synthetase. The activity of the first enzyme was judged by a decrease in the degree of restoration of antibodies during a collagen-aspirin test, which was 67.0±0.12% at the end of the study. This was accompanied by a decrease in the degree of restoration of antibodies in a collagen-imidazole test, which allowed us to indirectly determine the decrease in the level of thromboxanesynthetase activity in platelets taken from calves (up to 30 days to 30.5±0.07%) (table 1).

The initially small amount of ATP and ADP calves in platelets during the second phase of their early ontogenesis somewhat decreased, reaching 4.82±0.007 and 2.80±0.003 μmol/10^9 platelets by the age of 30 days. The severity of their secretion from dense platelet granules during the observation decreased by 17.1% and 12.3%, reaching 21.0±0.10 and 28.4±0.08% by the end of the observation.

The number of actin and myosin calves in intact platelets on day 11 was 20.3±0.14 and 9.3±0.16%, the total protein in platelet, at the end of the observation, reached 17.5±0.08 and 7.8±0.14% of the total protein in the platelet. This was accompanied in calves of the Holstein breed by a gradual weakening of their additional self-assembly during the observation.

Discussion

Among modern researchers, there remains a high interest in various aspects of hematological changes in productive animals [13]. It becomes clear that the results of these works will contribute to a further understanding of the regulatory mechanisms in mammals [14,15]. Despite the great biological significance of platelet activity in young cattle of highly productive breeds, it still remains very poorly studied. In the performed study, for the first time it was possible to identify the level of platelet functional capabilities in calves of the Holstein breed during the phase of milk feeding.This breed is considered as very promising in terms of productivity [16.17], which dictates the need for its systematic cultivation [18].

Considering the results of the assessment in calves of the time, the development of antibodies in response to collagen and ristomycin can be said about the initially small adhesive ability of their platelets, which was somewhat weakened during the milk feeding phase. Apparently, at least two realizing mechanisms were the basis of this [19]. The presence of the first mechanism could be judged by the weakening of platelet aggregation under the action of collagen. This indicated a decrease in calf platelets located on membranes at this age, initially a small number of receptors capable of contacting collagen (glycoproteins Ia-VI) [20]. The second mechanism for the implementation of the weakening of the adhesive ability of platelets was associated in Holstein calves with inhibition of their AT response to ristomycin [21]. This was due to a decrease in the concentration of Willibrand factor in their blood during the phase of milk nutrition and as a result of this decrease in the participation of platelet receptors in it (GPI в) [22].

It was found that for Holstein calves, during the phase of milk feeding, a weakening of platelet aggregation is characteristic, which can enhance the microcirculation process in organs and tissues. With an initially unexpressed sensitivity of platelets to various aggregation inducers in the observed calves, its additional weakening was noted. This is caused by the realization of effects after binding of aggregation inducers to their receptors on platelets [23]. After exposure to strong inducers, a decrease in the activity of phospholipase C and inhibition of the processes of the phosphoinositol pathway, as well as a decrease in the phosphorylation of proteins involved in the contractile platelet system, were noted. The onset of a slight decrease in the synthesis of inositol triphosphate in platelets caused a decrease in Ca^{2+} intake from its depot, which can be considered an important reason for the weakening

Annals of R.S.C.B., ISSN: 1583-6258, Vol. 25, Issue 5, 2021, Pages. 5492 - 5500

Received 15 May 2021; Accepted 20 May 2021.

of their assembly activity and a decrease in the severity of the reduction of the actomyosin complex [24].

ADP and adrenaline, considered weak inducers in the process of platelet aggregation, ensured the development of inactive, somewhat weakening platelet aggregation in Holstein calves during the milk feeding phase. Obviously, this was based on a decrease in the number of receptors for them on the surface of platelets, a physiologically acceptable decrease in the expression of fibrinogen receptors (GPIIb-IIIa), and a decrease in the enzymatic activity of phospholipase A_2, which is very important for the implementation of platelet aggregation in response to weak inducers [25]. The latter mechanism contributed to a decrease in the receipt of platelet membrane molecules from phospholipids of arachidonic acid molecules, which reduced the level of thromboxane A_2 formation [3]. Found in Holstein calves, an unexpressed decrease in the functional properties of platelet cyclooxygenase and thromboxane synthetase during the milk feeding phase. This also led to an additional weakening of the generation of thromboxane A_2 in the platelets of these animals. These changes in the study were able to prove by the dynamics of the results of carried out transfer tests, which revealed a low level of cyclooxygenase and thromboxane synthetase activity in the platelets of the observed calves and a decrease in the amount of thromboxane synthesized in the platelets [26]. Another very significant mechanism of AP attenuation in Holstein calves during the milk feeding phase can be considered as a slight decrease in their actinogenesis and myosin formation and the intensity of ATP and ADP secretion from platelets under the action of the aggregation inducer.

The decrease in the number of active platelet forms in calves found in the study showed a decrease in their degree of insensitivity to aggregation agonists. The small intravascular activity of platelets was also evidence of the poor availability of collagen, which is part of the vascular wall as a result of an increase in the degree of preservation of endothelial cells. Apparently, this was due to a decrease in the number of activated platelets and their aggregates of different sizes circulating in the blood of calves at this age. In addition, a decrease in the intravascular activity of platelets indicated a decrease in the blood of the observed calves in the number of aggregation inducers (ADP, adrenaline, thrombin) dissolved in their plasma, as confirmed by data from previous work [27,28].

The weakening of platelet aggregation ability found in animals is the physiological basis for a decrease in the amount of their active forms and different sizes of their aggregates in their blood [29]. These changes should be considered very significant mechanisms of weakening platelet hemostasis activity in calves of the Holstein breed, and, therefore, excluding the possibility of even partial blockade by capillary aggregates in growing tissues. This created physiological conditions for the manifestation of very high milk yield in these animals in the future [30]. The revealed decrease in the intravascular activity of platelets in Holstein calves during the milk feeding phase not only indicates a high degree of adhesive and aggregation properties of platelets in the bloodstream, but also gives reason to suspect that they have a high degree of platelet disaggregation ability, apparently due to the high sensitivity of their receptors todisaggregants of vascular origin [31].

Conclusion

The calves of the Holstein breed during the dairy phase are characterized by a high degree of functional perfection of platelets. The level of their activity provides physiologically very favorable conditions for hemocirculation in the capillaries. The basis of this in these animals is a small activity of the platelet adhesion, aggregation and secretion processor. The weakening of intravascular platelet activity in Holstein calves during the milk feeding phase creates conditions for optimal capillary perfusion and active metabolism in all tissues and internal organs. This is especially important for their active growth, development and the creation of the foundations of their high productivity in the future.

Conflict of interest

No conflict of interest is declared.

Received 15 May 2021; Accepted 20 May 2021.

Sources of financing
The study was conducted at the expense of the authors.

Ethics Committee Resolution
The study was approved by the local ethics committee of the Russian State Social University on September 15, 2018 (protocol №11).

Acknowledgement
The authors are grateful to all those who participated in the study and their direct leaders for the organizational assistance in conducting the study.

REFERENCES

[1] Momot AP (2006) Pathology of hemostasis. St. Petersburg, 210.
[2] Glagoleva TI, Zavalishina S Yu (2017) Aggregative Activity of Basic Regular Blood Elements and Vascular Disaggregating Control over It in Calves of Milk-vegetable Nutrition. Annual Research & Review in Biology. 12(6), 1-7. Articleno.ARRB.33767 DOI: 10.9734/ARRB/2017/33767
[3] Shitikova AS (2008)Thrombocytopathy is congenital and acquired. St. Petersburg, 384.
[4] Tsvetkova NA, Kulakova SV (2020) Life orientations and aspirations of the penal system's employees, depending on their level of self-efficacy. Russian journal of criminology. 14(1), 128-138.
[5] Skoryatina I A, Zavalishina SYu (2017) Impact of Experimental Development of Arterial Hypertension and Dyslipidemia on Intravascular Activity of Rats' Platelets. Annual Research & Review in Biology. 14(5), 1-9. DOI: 10.9734/ARRB/2017/33758
[6] Mal GS, Zavalishina SYu (2019) Functional Platelet Activity During Ontogeny in Rats. Indian Journal of Public Health Research & Development. 10(8), 1915-1919.DOI:10.5958/0976-5506.2019.02131.4
[7] Tkacheva ES (2018)Physiological Features of Platelets in Milk and Vegetable Nutrition Piglets. Biomedical & Pharmacology Journal.11(3),1437-1442. http://dx.doi.org/10.13005/bpj/1508 (2018)
[8] Mal GS, Makhova AV, Makurina ON, Tkacheva ES (2021)Physiological changes in the body of young cattle when the feed additive "Bacitox" is introduced into their diet. IOP Conf. Series: Earth and Environmental Science. IV International Scientific Conference: AGRITECH-IV-2020: Agribusiness, Environmental Engineering and Biotechnologies. 677(4), 042067. https://iopscience.iop.org/article/10.1088/1755-1315/677/4/042067
[9] Zavalishina S Yu (2017) Physiological Features of Hemostasis in Newborn Calves Receiving Ferroglukin, Fosprenil and Hamavit, for Iron Deficiency. Annual Research & Review in Biology, 14(2), 1-8. DOI: 10.9734/ARRB/2017/33617
[10] Ermolaeva TA, Golovina OG, Morozova TV (1992)The program of clinical and laboratory examination of patients with thrombocytopathy. St. Petersburg, 25.
[11] ShitikovaAS (1999) Visual micromethod for platelet aggregation studies. Hemostasis. Physiological mechanisms, principles of diagnosis of the main forms of hemorrhagic diseases. St. Petersburg, 49-52.
[12] Shitikova AS, Tarkovskaya LR, Kargin VD (1997) A method for determining intravascular platelet activation and its significance in clinical practice. Clinical and laboratory diagnostics.2, 23-35.

Received 15 May 2021; Accepted 20 May 2021.

[13] Hulsman HannaLL, Hieber JK, Haipeng Yu, CelestinoEF, Dahlen CR, WagnerSA, Riley DG (2019)Blood collection has negligible impact on scoring temperament in Angus-based weaned calves. Livestock Science. 230,103835. DOI:10.1016/j.livsci.2019.103835

[14] Noya A, Casasús I, Rodríguez-Sánchez JA, Ferrer J, Sanz A (2020) A negative energy balance during the peri-implantational period reduces dam IGF-1 but does not alter progesterone or pregnancy-specific protein B (PSPB) or fertility in suckled cows.Domestic Animal Endocrinology. 72,106418. http://dx.doi.org/10.1016/j.domaniend.2019.106418

[15] Bin-Jumah M, Abd El-Hack ME, Abdelnour SA, Hendy YA, Ghanem HA, Alsafy SA, Khafaga AF, Noreldin AE, Shaheen H, Samak D, Momenah MA, Allam AA, AlKahtane AA, Alkahtani S, Abdel-Daim MM, Aleya L (2020)Potential use of chromium to combat thermal stress in animals: A review. Science of the Total Environment. 707,135996. http://dx.doi.org/10.1016/j.scitotenv.2019.135996

[16] Aragona KM, Rice EM, EngstromM, Erickson PS (2020)Supplementation of nicotinic acid to prepartum Holstein cows increases colostral immunoglobulin G, excretion of urinary purine derivatives, and feed efficiency in calves.Journal of Dairy Science.103(3), 2287-2302.DOI: 10.3168/jds.2019-17058

[17] Fahey MJ, Fischer AJ, Steele MA, Greenwood SL (2020) Characterization of the colostrum and transition milk proteomes from primiparous and multiparous Holstein dairy cows.Journal of Dairy Science, 103(2), 1993-2005. DOI:10.3168/jds.2019-17094

[18] ZavalishinaSYu (2017)PhysiologicalDynamicsofSpontaneousErythrocytes' AggregationofRatsatLastOntogenesis.AnnualResearch&ReviewinBiology. 13(1), 1-7. DOI: 10.9734/ARRB/2017/33616

[19] Glagoleva T I, Zavalishina S Yu (2017)Aggregation of Basic Regular Blood Elements in Calves during the Milk-feeding Phase. Annual Research & Review in Biology. 17(1), 1-7.doi: 10.9734/ARRB/2017/34380

[20] Zavalishina S Y (2017) Restoration of Physiological Activity of Platelets in New-Born Calves With Iron Deficiency. BiomedPharmacolJ. 10(2), 711-716. DOI: http://dx.doi.org/10.13005/bpj/1160

[21] Tkacheva ES, Zavalishina SYu (2019) Functional Features of Platelet Secretion in Piglets During Early Ontogenesis. Biomedical & Pharmacology Journal. 12(1), 485-489.http://dx.doi.org/10.13005/bpj/1665

[22] Mal GS, Zavalishina SYu, Makurina ON, Zaitsev VV, Glagoleva TI (2019) Functional Features of Vascular Endothelium with Developing Arterial Hypertension. Prensa Med Argent. 105(1),1000331.

[23] Makurina ON, Mal GS, Zavalishina SYu, Medvedev IN (2019)Functional Features of Platelets in Rats Fed a Standard Diet with Low Antioxidant Content During Ontogenesis. Indian Journal of Public Health Research & Development.10(10), 999-1003.

[24] TóthováC, Nagy O, KováčG, NagyováV (2016)Changes in the concentrations of serum proteins in calves during the first month of life.Journal of Applied Animal Research, 44(1), 338-346.https://doi.org/10.1080/09712119.2015.1031791

[25] Zavalishina SY (2020) Physiological characteristics of cattle of different ages. IOP Conference Series: Earth and Environmental Science. Biological Technologies in Agriculture: from Molecules to Ecosystems. 548(4) : 042066. https://iopscience.iop.org/article/10.1088/1755-1315/548/4/042066

[26] Glagoleva TI, Zavalishina SYu (2017) Physiological Peculiarities of Vessels' Disaggregating Control over New-Born Calves' Erythrocytes. Annual Research & Review in Biology. 19(1), 1-9. DOI:10.9734/ARRB/2017/37232

[27] NagyO, TóthováC, KováčG (2014)Age-related changes in the concentrations of serum proteins in calves.Journal of Applied Animal Research.42(4), 451-458.https://doi.org/10.1080/09712119.2013.875918

[28] Kamal R, Dutt T, Patel M, Dey A, Chandran PC, Bharti PK, Barari SK (2016)Behavioural,

Received 15 May 2021; Accepted 20 May 2021.

biochemical and hormonal responses of heat-stressed crossbred calves to different shade materials. Journal of Applied Animal Research.44(1), 347-354.https://doi.org/10.1080/09712119.2015.1074076

[29] Karpov VYu, Zavalishina SYu, Dorontsev AV, Voronova NN, Shulgin AM, Sharagin VI, Koz'yakov RV (2020)Influence of Regular Feasible Physical Activity on the Platelet's Functional Activity of the Second Mature Age People. Systematic Reviews in Pharmacy. 11(8), 439-445.

[30] Kachenkova ES, Zavalishina SY, Makurina ON, Kulkova IV, Tkacheva ES (2020) Physiological Reaction of the Cardiovascular System of Men 50-59 Years to Feasible Regular Physical Activity. Biomedical & Pharmacology Journal. 13(4), 1719-1727. DOI : https://dx.doi.org/10.13005/bpj/2046

[31] Zavalishina SYu (2021)Physiological reaction of blood counts in lactating cows to the use of catosal. IOP Conf. Series: Earth and Environmental Science. IV International Scientific Conference: AGRITECH-IV-2020: Agribusiness, Environmental Engineering and Biotechnologies. 677, 042068.