

## Physiological Activity of Platelet Aggregation in Calves of Vegetable Feeding

JU.L. OSHURKOVA<sup>1</sup> and T.I. GLAGOLEVA<sup>2\*</sup>

<sup>1</sup>Federal State Budgetary Educational Institution for Higher Professional training "Vologda State Dairy Farming Academy named after N.V. Vereshchagin", Vologda, Russia.

<sup>2</sup>All-Russian Research Institute of Physiology, Biochemistry and Nutrition of Animals, Institute of village, Borovsk, Russia.

\*Corresponding author E-mail: ti.glagoleva@yandex.ru

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### ABSTRACT

At present it is acknowledged that platelets' activity is very significant in provision of blood microrheology. In this connection, monitoring of their indices in different fields of medicine and biology is used more and more actively. The aim of our research is to clear out peculiarities of platelet activity in healthy Irish calves during the phase of vegetable feeding of early ontogenesis. The study used 42 Irish calves of vegetable feeding at stock-farm "Plemzavod Majskiy" in Vologda region (Russia). The animals were examined 4 times: at the age of 3 months, at the age of 6 months, at the age of 9 months and at the age of 12 months. We applied hematological and statistical methods of investigation. While aging the animals were noted to have strengthening of platelets' aggregation in response to all the applied aggregation inductors. The most active aggregation of their platelets was noticed in response to adenosine diphosphate. Collagen and ristocetin aggregation had less evidence in them and less degree of rising. It pointed indirectly at low availability of collagen and little concentration of von Willebrand Factor in it. Deaggregating platelets' abilities in response to all the applied inductors in examined calves had a tendency to growth. Found low platelets' aggregation in Irish calves at the age between 3 and 12 months of life provided optimal conditions for blood supply of their internals during their growth and development.

**Keywords:** Platelets, Calves, Vegetable feeding, Irish (breed).

### INTRODUCTION

Creation of genetic programme of growth and development is taking place nowadays under the impact of environment<sup>1,2</sup>. It is inseparably connected with the dynamics of systems which regulate and integrate living beings<sup>3</sup>. Blood is among them<sup>4,5</sup>. Hemostasis is its physiologically very important biological subsystem<sup>6,7</sup>. It provides, from one side, preservation of its liquid state and, from the other side, prevention and arrest of bleeding. Hemostasis system is formed by numerous components. Platelets<sup>8</sup> are the most significant of

them. They are functionally closely connected with hemocoagulation<sup>9</sup>. It becomes clear that efficiency of tissue blood supply<sup>10</sup> and prevention of different pathological states in the whole body<sup>11,12</sup> mostly depend on the level of their activity.

At present the great role of platelets in provision of blood rheology is acknowledged<sup>13,14</sup>. That's why, their monitoring is starting to be widely used in different fields of medicine<sup>15,16</sup> and biology<sup>17</sup>. Estimation of blood indices and, especially, hemostasis elements has great practical significance for biology. Hemostasis elements are

closely connected with somatic characteristics<sup>18</sup> and functioning of the whole body<sup>19,20</sup>. Their estimation will allow working out aging norms of relevant indices<sup>21,22</sup> and finding the beginning of hemostasiopathy at separate states<sup>23,24</sup>. These researches have especially great significance for high productive breeds of cattle. Such researches are already started on cattle of black-many-colored breed<sup>25,26</sup>.

Taking into consideration high productivity of Ireshire cows and the fact that platelet activity is very important for optimality of animals' status and their productivity, it was decided to estimate platelets' activity.

The aim of research: to clear out peculiarities of platelet activity in healthy Ireshire calves of vegetable feeding.

## MATERIALS AND METHODS

The research was conducted in strict accordance with ethical principles established by the European Convention on protection of the vertebrata used for experimental and other scientific purposes (adopted in Strasbourg in March 18, 1986, and confirmed in Strasbourg in June 15, 2006) and approved by the local ethic committee of Federal State Budgetary Educational Institution of Higher Education "Vologda State Dairy Farming Academy by N.V. Vereshchagin" (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 study used 42 healthy Ireshire calves being in the phase of vegetable feeding. The animals were kept on the farm "Plemzavod Majskiy" in Vologda region (Russia). The calves were examined 4 times: at the age of 3 months, at the age of 6 months, at the age of 9 months and at the age of 12 months.

Blood out of jugular vein was taken from all the calves in the morning for studying platelet parameters. This sampling was made into plastic tube containing 3.8% dilution of sodium citrate, in ratio of blood volume and volume of sodium citrate – 9:1.

The number of platelets, their average volume and thrombocrit (the index which characterizes the factor of platelet mass in blood volume) were determined by electronic-automatic method with the help of hematological analyzer BC-3000 PLUS (the firm "Shenzhen Mindray Bio-Medical Electronics Co.,Ltd.", China).

Aggregative platelets' activity was found by quantitative method with application of photoelectron-colorimeter KFK-2 (Zagorsk optic-mechanical plant, Russia). We used ADP, collagen and ristomicin in standard concentrations<sup>27</sup> as inductors of aggregation. Platelets' aggregation was estimated according to the summary index of platelets' aggregation (SIPA), speed of aggregation (SA) and index of platelets' disaggregation (IPD) which were calculated by traditional ways.

The results were processed by Student's criterion (t). Statistical processing of received information was made with the help of a programme package "Statistics for Windows v. 6.0", "MicrosoftExcel". Differences in data were considered reliable in case of  $p < 0.05$ .

## RESULTS

Common platelet indices of examined animals (quantity of platelets, average volume of platelets and thrombocrit) were within norms in the course of investigation (table 1).

In the result of the study of aggregative platelets' activity in Ireshire calves of vegetable feeding there were found reliable changes (table 2).

In the course of conducted research we cleared out the dynamics of relevant indices of platelets' aggregation in Ireshire calves during the phase of vegetable feeding at early ontogenesis. The largest response of platelets was noticed to ADP. At the same time, SIPA with ADP in the course of investigation had a tendency to growth reaching  $22.40 \pm 2.16\%$  to its end. SIPA in animals between 3 and 12 months of life also gradually rose till  $8.14 \pm 0.81\%$  in response to collagen. In case of Ireshire calves it pointed at the growth of platelets' sensitivity to aggregation inductors at intensification of secretory process out of platelets during platelets'

activation. Activity of platelets' aggregation under the impact of ristomicin in Ireshire calves during the phase of vegetable feeding also had a tendency to growth – SIPA at its beginning was equal to  $7.79 \pm 0.23\%$ , reaching to its end  $8.23 \pm 0.27\%$ .

The speed of aggregates' formation in Ireshire calves in response to ADP gradually rose during the phase of vegetable feeding from  $0.026 \pm 0.009$  min till  $0.031 \pm 0.007$  min to its end. SA had analogical dynamics under the impact of collagen and ristomicin. To the end of research it was equal to  $0.0070 \pm 0.0007$  min and  $0.0075 \pm 0.006$  min, respectively.

Estimation of index value of platelets' disaggregation which showed stability of appearing aggregates, allowed finding out that the most stable

aggregates were those ones which were formed in response to ristomicin. The value of IPD with it had an inclination for growth in the course of vegetable feeding and reached minimum values ( $2.21 \pm 0.09\%$ ). Aggregates formed under the impact of ADP and collagen, were less stable in the course of vegetable feeding: IPD in relation to bothy inductors gradually rose reaching with collagen  $2.70 \pm 0.24\%$ , and with ADP –  $12.22 \pm 0.76\%$ .

### DISCUSSION

Reached level of knowledge about the role of hemostasis in functional activity of internals allows considering this system to be especially significant in supporting of physiological optimum of the whole body<sup>28,29</sup>. It is assumed that hemostasis activity is nonuniform in different parts of vascular bed. Definite

**Table 1: Common platelet characteristics of Ireshire calves in the course of vegetable feeding phase**

indicators	Age of calves, n=42, M±m			
	3 months	6 months	9 months	12 months
Quantity of platelets, thousand/mcl	311,0±10,19	320,3±6,88	324,2±4,20	340,7±3,17*
Average volume of platelets, femtolitr	7,2±0,36	7,2±0,19	7,3±0,24	7,3±0,33
Thrombocrit, %	0,27±0,09	0,27±0,11	0,27±0,16	0,27±0,11

Note: reliability of indices' dynamics in comparison with the age of 3 months: \* -  $p < 0.05$ .

**Table 2: Aggregative platelets' activity in Ireshire calves in the course of vegetable feeding phase**

indicators	Age of calves, n=42, M±m			
	3 months	6 months	9 months	12 months
Inductor of aggregation ADP				
SIPA, %	17,20±1,26	20,07±1,38*	21,54±2,24*	22,40±2,16*
SA, min	0,026±0,009	0,028±0,008	0,030±0,005*	0,031±0,007*
IPD, %	10,22±0,81	10,36±0,65	11,02±0,89	12,22±0,76*
Inductor of aggregation collagen				
SIPA, %	7,00±0,62	7,39±0,57	8,01±0,04	8,14±0,81**
SA, min	0,0061±0,003	0,0065±0,004	0,0070±0,005*	0,0070±0,007*
IPD, %	2,50±0,19	2,53±0,20	2,67±0,25	2,70±0,24*
Inductor of aggregation ristomicin				
SIPA, %	7,79±0,23	8,03±0,40	8,10±0,36*	8,23±0,27**
SA, min	0,0067±0,004	0,0069±0,005	0,0072±0,006	0,0075±0,006*
IPD, %	2,15±0,09	2,18±0,10	2,20±0,14	2,21±0,09

haemostatic level is established in functionally active at the given moment internals. It differs from the common bloodstream what is connected with mosaicism of hemostasis system in different parts of vascular bed<sup>30</sup>.

Researches of last years significantly widened our ideas about factors which influence platelets' aggregation and preservation of blood in liquid state. These processes are well studied at many states of human beings and animals<sup>31,32</sup>. It becomes clear that we deal with dynamics of platelets' activity while aging<sup>33</sup>. At the same time, many aspects of hemostasis platelet component in cattle at different age and in different environmental conditions are still studied rather poorly. We know nothing about peculiarities of any separate breed, in particular – Ireshire breed, including the course of vegetable feeding phase which is very important in the sense of future productive period.

It is acknowledged that anabolic, physiologically necessary processes actively take place in a mammal's body during early ontogenesis. It causes definite shifts in functioning of all the internals and systems<sup>25</sup>. At this very period all the tissues are mostly exposed to the impact of unfavorable environmental factors. They need maximum blood inflow to them and its good liquid features<sup>34</sup>. Conducted researches on Ireshire calves of vegetable feeding found out that platelets' quantity and their average volume didn't surpass standard normative values. At the same time, platelets' aggregative activity gradually rose in them during the phase of vegetable feeding. Platelets reacted most actively on the impact of ADP. SIPA with this inductor increased while aging. But SIPA reached less and comparable between each other values in response to collagen and ristomicin. It pointed indirectly at low level of collagen availability during the phase of vegetable feeding at low content of von Willebrand Factor in their blood<sup>35</sup>. Given factor, as it is known, can interact simultaneously with ristomicin and glycoproteins GP Ib and GP IIb/IIIa of platelets' membranes. It provides interaction between

aggregating platelets<sup>36</sup>. Speed of aggregation in experimental animals during the phase of vegetable feeding in response to all the applied inductors had an inclination for increase. Judging by literature data<sup>37</sup>, it pointed at the rise of receptors' number to them on platelet membranes.

Evidence of platelets' deaggregating abilities in response to all the agonists rose to the same degree during the whole phase of vegetable feeding. Given phenomenon can also be explained by receptor rearrangements of platelets' membranes and by the dynamics of initial hemostasis' mechanisms<sup>38</sup>.

Estimating received data about the examined animals, we can come to the conclusion that Ireshire calves had increase of adhesively-aggregative platelets' activity during the phase of vegetable feeding. It was the most evident to its end. Taking into consideration that growth and development of calves take place simultaneously for a long time, it becomes clear that both these processes influence adhesively-aggregative activity of platelets<sup>39</sup>.

## CONCLUSION

In the course of the given research we found out indices' dynamics of platelet hemostasis in Ireshire calves in the phase of vegetable feeding. Low platelets' activity supplied animals of this breed during their growth and development with optimal conditions for blood supply of growing and maturing internals.

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## REFERENCES

1. Amelina, I.V. and Medvedev, I.N. Transcriptional activity of chromosome nucleolar organizing regions in population of Kursk region. *Bulletin of Experimental Biology and Medicine*, 147(6) : 730-32 (2009)
2. Amelina, I.V. and Medvedev, I.N. Evaluation

- of the dependence of mutagenesis intensity on activity of nucleolus organizer regions of chromosomes in aboriginal population of Kursk region. *Bulletin of Experimental Biology and Medicine*, **145**(1) : 68-71 (2008)
3. Medvedev, I.N. and Amelina, I.V. An association between human morphological phenotypical characteristics and the activity of chromosomal nucleolar organizer regions in the interphase cell nucleus in the population of indigenous people of Kursk region. *Morfologii*, **142**(4): 87-91 (2012)
  4. Medvedev, I.N. and Gromnatskii, N.I. Correction of thrombocyte hemostasis and biological age reduction in metabolic syndrome. *Klinicheskaiia meditsina*, **83**(8): 54-7 (2005)
  5. Medvedev, I.N. and Gromnatskii, N.I. Effect of amlodipine on intravascular thrombocyte activity in patients with arterial hypertension and metabolic syndrome. *Klinicheskaiia meditsina*, **83**(2): 37-9 (2005)
  6. Medvedev, I.N. Microrheology of erythrocytes in arterial hypertension and dyslipidemia with a complex hypolipidemic treatment. *Russian Journal of Cardiology*, **4**(144): 13-7 (2017)
  7. Medvedev, I.N. and Gromnatskii, N.I. The influence of nebivolol on thrombocyte aggregation in patients with arterial hypertension with metabolic syndrome. *Klinicheskaiia meditsina*, **83**(3): 31-3 (2005)
  8. Gromnatskii, N.I. and Medvedev, I.N. Non-pharmacological correction of impaired platelet hemostasis in hypertensive patients with metabolic syndrome. *Klinicheskaiia meditsina*, **81**(4) : 31-4 (2003)
  9. Simonenko, V.B., Medvedev, I.N. and Tolmachev, V.V. Effect of irbesartan of the function of hemocoagulative component of hemostasis in patients with arterial hypertension during metabolic syndrome. *Klinicheskaiia meditsina*, **88**(6): 27-30 (2010)
  10. Zavalishina, S.Yu., Kutafina, N.V., Vatnikov, Yu.A., Makurina, O.N. and Kulikov E.V. Platelet-Activity Dependence on the Age of Rats with Experimental Dyslipidemia. *Biol Med (Aligarh)*, **8**: 326. doi: 10.4172/0974-8369.1000326 (2016)
  11. Zavalishina, S.Yu. 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 (2017)
  12. Medvedev, I.N. and Gromnatskii, N.I. The influence of hypocaloric diet on thrombocyte rheology in patients with metabolic syndrome. *Klinicheskaiia meditsina*, **84**(3): 49-52 (2006)
  13. Zavalishina, S.Yu. Restoration of Physiological Activity of Platelets in New-Born Calves With Iron Deficiency. *Biomed Pharmacol J*, **10**(2) <http://biomedpharmajournal.org/?p=14568> (2017)
  14. Zavalishina, S.Yu. Physiological Dynamics of Spontaneous Erythrocytes' Aggregation of Rats at Last Ontogenesis. *Annual Research & Review in Biology*, **13**(1) : 1-7 doi: 10.9734/ARRB/2017/33616 (2017)
  15. Medvedev, I.N. and Kumova, T.A. Reduced platelet aggregation in losartan-treated patients with arterial hypertension and metabolic syndrome. *Russian Journal of Cardiology*, **1**: 40-2 (2008)
  16. Medvedev, I.N. and Skoriatina, I.A. Effect of lovastatin on adhesive and aggregation function of platelets in patients with arterial hypertension and dyslipidemia. *Klinicheskaiia meditsina*, **88**(2): 38-40 (2010)
  17. Zavalishina, S.Yu., Vatnikov, Yu.A., Makurina, O.N., Kulikov, E.V., Sotnikova, E.D., Parshina, V.I., Rystsova, E.O., Kochneva, M.V. and Sturov, N.V. Diagnostical Appreciation of Physiological Reaction of Intravascular Thrombocytes Activity of Two-Years-Old Mice to Regular Physical Loads. *Biomedical & Pharmacology Journal*, **10**(1) : 129-36. <http://dx.doi.org/10.13005/bpj/1090> (2017)
  18. Medvedev, I.N., Lapshina, E.V. and Zavalishina, S.Yu. Activity of platelet hemostasis in children with spinal deformities. *Bulletin of experimental biology and medicine*, **149**(5): 645-46 (2010)
  19. Medvedev, I.N. and Zavalishina, S.Yu. Platelet Activity in Patients With Third Degree Arterial Hypertension and Metabolic Syndrome. *Kardiologiia*, **56**(1): 48 (2016)
  20. Sizov, .. and Zavalishina, S.J. Russian Criminal Legislation in Prevention of Sexually Transmitted Diseases in the Territory of the Russian Federation. *Biology and Medicine*

- (*Aligarh*), **7**(5): BM-142-15, 5 pages (2015)
21. Kutafina, N.V. and Medvedev, I.N. Platelet aggregation in clinically healthy persons of the second coming of age living in the Kursk region. *Advances in gerontology*, **28**(2): 321-25 (2015)
  22. Simonenko, V.B., Medvedev, I.N. and Tolmachev, V.V. Comparative evaluation of the influence of sulfhydryl and phosphate ACE inhibitors on thrombocyte aggregation in patients suffering from arterial hypertension with metabolic syndrome. *Klinicheskaja meditsina*, **85**(4): 24-7 (2007)
  23. Medvedev, I.N. and Skoryatina, I.A. Fluvastatin effects on blood cell aggregation in patients with arterial hypertension and dyslipidemia. *Cardiovascular Therapy and Prevention*, **12**(2): 18-24 (2013).
  24. Simonenko, V.B., Medvedev, I.N., Mezentseva, N.I. and Tolmachev, V.V. The antiaggregation activity of the vascular wall in patients suffering from arterial hypertension with metabolic syndrome. *Klinicheskaja meditsina*, **85**(7): 28-30 (2007)
  25. Glagoleva, T.I. and Zavalishina, S.Yu. 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 doi: 10.9734/ARRB/2017/33767 (2017)
  26. Medvedev, I.N. Vascular-platelet interaction in pregnant cows. *Bulg. J. Agric. Sci.*, **23**(2): 310-14 (2017)
  27. Medvedev, I.N., Savchenko, A.P., Zavalishina, S.Yu., Krasnova, E.G., Kumova, T.A., Gamolina, O.V., Skoryatina, I.A. and Fadeeva, T.S. Methodology of blood rheology assessment in various clinical situations. *Russian Journal of Cardiology*, **5**: 42-5 (2009)
  28. Medvedev, I.N., Maksimov, V.I., Parakhnevich, A.V., Zavalishina, S.Yu. and Kutafina, N.V. Rapid assessment of aggregation abilities and surface properties of platelets and red blood cells. *International Journal of Pharma and Bio Sciences*, **7**(2): (B) 793-97 (2016)
  29. Simonenko, V.B., Medvedev, I.N. and Kumova, T.A. Pathogenetic aspects of hypertension in case of metabolic syndrome. *Voenno-meditsinskii zhurnal*, **331**(9): 41-4 (2010)
  30. Simonenko, V.B., Medvedev, I.N. and Tolmachev, V.V. Pathogenetic aspects of arterial hypertension in metabolic syndrome. *Klinicheskaja meditsina*, **89**(1): 49-51 (2011)
  31. Medvedev, I.N. and Skoriatina, I.A. Dynamics of microrheologic properties of erythrocytes in patients with arterial hypertension and dyslipidemia treated with atorvastatin. *Klinicheskaja meditsina*, **90**(6): 42-5 (2012)
  32. Simonenko, V.B., Medvedev, I.N. and Gamolina, O.V. Primary hemostasis activity in patients with arterial hypertension and impaired glucose tolerance treated with trandolapril. *Klinicheskaja meditsina*, **89**(2): 29-31 (2011).
  33. Kutafina, N.V. and Medvedev, I.N. Platelet Aggregation in Clinically Healthy Persons of the Second Coming-of-Age Living in the Kursk Oblast. *Advances in Gerontology*, **5**(4): 267-70. (2015).
  34. Simonenko, V.B., Medvedev, I.N. and Tolmachev, V.V. Dynamics of primary hemostasis activity in patients with arterial hypertension and metabolic syndrome treated with candesartan. *Klinicheskaja meditsina*, **89**(3): 35-8 (2011).
  35. Medvedev, I.N. and Kumova, T.A. Reduced platelet aggregation in losartan-treated patients with arterial hypertension and metabolic syndrome. *Russian Journal of Cardiology*, **5**: 53-5 (2008).
  36. Simonenko, V.B., Medvedev, I.N. and Kumova, T.A. Losartan for correction of thrombocyte activity in patients suffering from arterial hypertension with metabolic syndrome. *Klinicheskaja meditsina*, **86**(1): 38-41 (2008).
  37. Medvedev, I.N. and Bryukhovetsky, A.G. The use of verospiron and the degree of platelet aggregation in arterial hypertension with abdominal obesity. *Klinicheskaja meditsina*, **92**(3): 50-3 (2014).
  38. Medvedev, I.N. and Danilenko, O.A. Effectiveness of vascular wall activity correction in patients with arterial hypertension, metabolic syndrome, and oculo-vascular occlusion. *Russian Journal of Cardiology*, **83**(3): 64-7 (2010).
  39. Medvedev, I.N. Dynamics of violations of intravascular platelet activity in rats during the formation of metabolic syndrome using fructose models. *Problems of nutrition*, **85**(1): 42-6 (2016).