

**INFLUENCE OF MEMBRANE-REPAIRING MEDICATIONS ON THE
EXPRESSION OF PROTEINS OF PLASMOLEMMMA OF ENTEROCYTES
DURING THE FORMATION OF COLOSTRAL IMMUNITY**

S. I. GOLOPURA, candidate of veterinary sciences, associate professor

M. I. TSVILIKHOVSKY, doctor of biological sciences, professor,

B. V. POPADIUK, postgraduate student

National University of Life and Environmental Sciences of Ukraine

E-mail: golopura@ukr.net

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Abstract. *The indices of the content of proteins with molecular mass of 10 – 15 and 15 – 24 kDa in the plasmolemma of enterocytes of the small intestine of newborn calves in the dynamics from the birth till the age of 1 day were investigated. The study was performed on three groups of calves (the control group and two experimental ones) of Ukrainian black-and-white dairy breed by drawing the samples of enterocytes of jejunum and determining protein content of membrane lysate fraction by electrophoresis. The application of native liposomes based on soybean lecithin and “Membranostabil” medication for newborn calves with colostrum increases the expression of plasmolemic proteins of enterocytes with a molecular mass of 10 – 15 kDa. The dynamics of comparative analysis between fractions of protein expression with molecular mass of 10 – 15 and 15 – 24 kDa in the plasmolemma of the enterocytes of the jejunum of control and experimental groups of calves is shown. The result of application of native liposomes with phospholipid bilayer based on soybean lecithin and “Membranostabil” medication allows to assume the positive effect of these means on the level of colostral immunoglobulins in the serum of newborn calves.*

Key words: *newborn calves, small intestine, enterocyte, plasmolemma, proteins, phospholipids, native liposomes, colostral immunity*

Nowadays it is known that there are proteins with identical molecular masses but different functions in the apical membrane of plasmolemma of enterocytes. For example, Cuprum-binding protein with a molecular mass of 15 kDa and one of the subunits of glucose-transporting protein (10 kDa) [9]. However, according to other studies [4], proteins of apical membrane of

small intestine enterocytes of newborn calves with molecular mass of 14.5 and 11 kDa are not detected in adult animals. This may indicate that the proteins of enterocyte membranes of newborn calves with molecular mass of 10 to 15 kDa are receptors involved in the transfer of colostral immunoglobulins from the intestinal lumen to the lymphatic system and bloodstream. This

Голопура С. І., Цвіліховський М. І., Попадюк Б. В. is supported by the fact that the currently known FcR- γ receptor, which carries IgG₁ and IgG₃ in mature animals has a molecular mass of 15 kDa [10]. According to the authors [6], it is known that immunoglobulin G (IgG) comprises a significant portion of total Ig of cow colostrum. However, the IgG₁ isoform comprises approximately 80% of total IgG [7], whereas the ratio of IgG, IgM and IgA in dairy cows is 85 – 90%, 7% and 5%, respectively [8]. Therefore, determining the expression of proteins with a molecular mass of 10 – 15 kDa, which may correspond to the FcR- γ receptor protein, and the correction of their content in plasmolemma of enterocytes of calves by membrane-repairing medications may be one of the key values in the formation of colostrum immunity in these animals.

The aim of the study was to investigate the content of proteins with molecular mass of 10 – 15 and 15 – 24 kDa in the plasmolemma of jejunum enterocytes of newborn calves under the application of macrocapsules from phospholipid bilayer based on soybean lecithin and “Membranostabil” medication with colostrum.

Materials and methods of the study. Research was conducted in scientific research center “Velykosnitinske n.a. O. V. Muzychenko” NULES of Ukraine on the new-born calves of Ukrainian

black-and-white breed during the period from birth till the age of 1 day.

Calves were separated into three groups: one control and two experimental ones, each with 6 animals. Calves of all groups were fed colostrum in the amount of 2 l after birth, and then 1.5 l every 6 hours during the first day of life of the animals. The calves of each control group received the colostrum only. The calves of the first experimental group received native liposomes from phospholipid bilayer based on soybean lecithin in the dose of 5 ml 20 minutes prior to colostrum; the calves of the second experimental group received a medication “Membranostabil” developed by this research team on the basis of soybean lecithin in the dose of 5 ml. The medication “Membranostabil” constitutes macrocapsules of phospholipid bilayer filled with water-soluble forms of vitamins A – 1.2 mg and E – 15 mg (patent for utility model No. 92841 dated September 10, 2014, Bul. # 17 [1]).

The prototypes of the jejunum enterocytes were drawn from the newborn calves before the first colostrum feeding, then 6 and 24 hours after birth of the animals. The experimental studies on newborn calves adhered to all bioethical requirements in relation to animals that comply with the Law of Ukraine "On the Protection of Animals against Cruelty" from 28.03.2017, the "European Convention

Голопура С. І., Цвіліховський М. І., Попадюк Б. В. "for the Protection of Vertebrate Animals" from 13.11.1987, and the Council Regulation (EC) No 1099/2009 from the 24th of September 2009 on the protection of animals during slaughter.

Determination of protein content in the plasmolemma of jejunum enterocytes of newborn calves was performed by an electrophoretic division in 7.5 % polyacrylamidegel from the sodiumdodecylsulphate by modified method with addition of tricine [2].

The percentages of individual protein fractions were determined by densitometry using TotalLab software.

The statistical processing of the results was performed using a Microsoft Excel 2003 computer program.

Results of the research.

The significant number of proteins with molecular mass of 10 – 15 kDa was

isolated during the separation of proteins of enterocyte membranes by electrophoresis. About $9.45 \pm 0.35\%$ of proteins with molecular mass of 10 – 15 kDa was detected among the total amount of proteins in the membrane during investigation of the composition of plasmolemic proteins of enterocytes in newborn calves that got no colostrum (up to 1 hour after birth).

An increase by 80,7 % ($p \leq 0.001$) was found in the content of proteins with molecular mass of 10 – 15 kDa in the plasmolemma of enterocytes of calves of control group 6 hours after the birth, compared with the calves before feeding of colostrum (Fig. 1). The protein content of this fraction to the total content of all proteins in the membrane comprised 21.86% (Fig. 2).

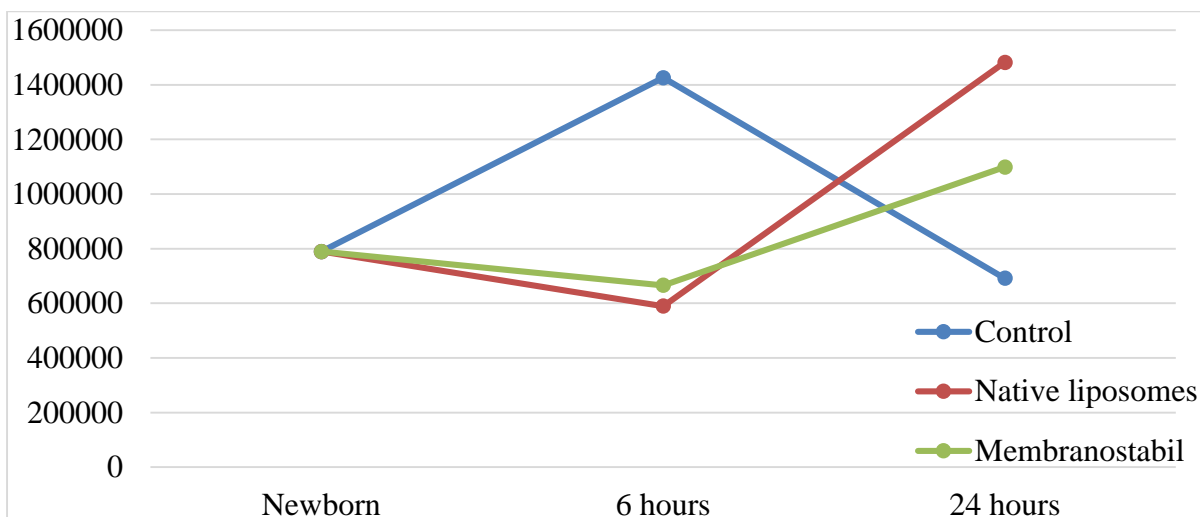


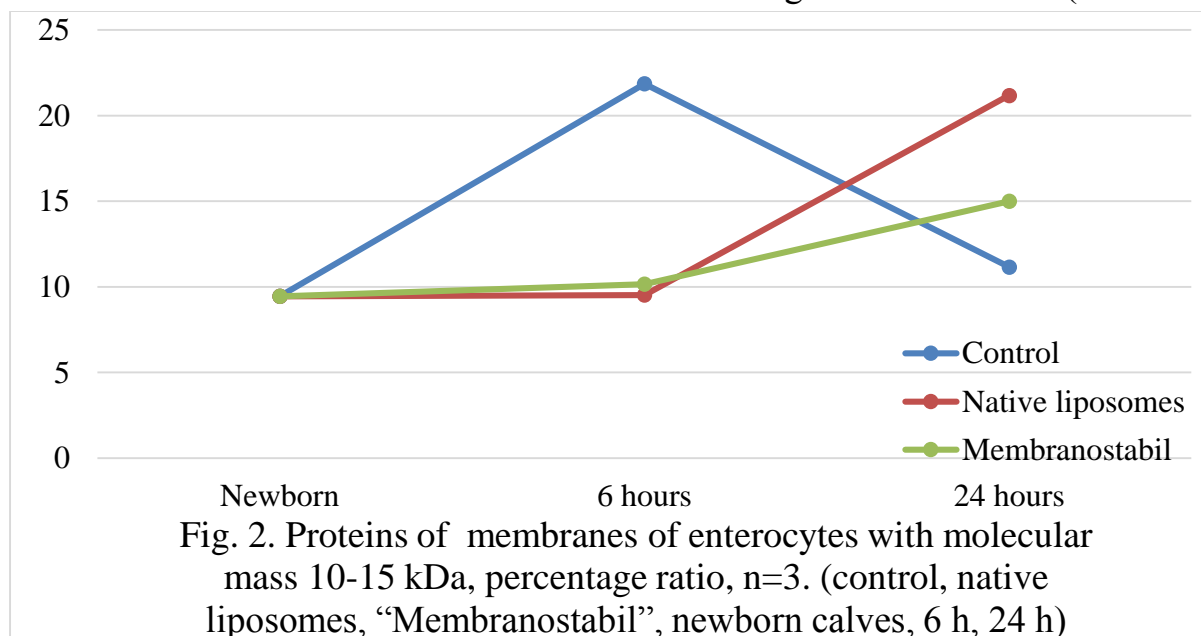
Fig. 1. Proteins of membranes of enterocytes with molecular mass 10-15 kDa, volume units, n=3. (control, native liposomes, “Membranostabil”, newborn calves, 6 h, 24 h)

Instead, in the plasmolemma of enterocytes of calves of the first and second experimental groups during this

period there was observed a decrease in the protein content of this fraction by 25.3 and 15.6%, respectively, compared

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to the calves before feeding of colostrum. However, compared to the total protein content in the enterocyte membrane, the percentage of proteins with molecular mass of 10 – 15 kDa

tended to increase by $9.52\% \pm 0.56$ and $10.16\% \pm 0.35$ in calves of the first and second experimental groups, respectively, compared to calves before feeding of the colostrum ($9.45\% \pm 0.35$).



A significant decrease in the fraction of proteins with molecular mass of 10 – 15 kDa by 12.4% ($p \leq 0.05$) was observed in the plasmolemma of enterocytes of calves in the control group 24 hours after birth, compared to calves before feeding of colostrum (Fig. 1). On the other hand, this fraction increased by 11.16% in the total volume of proteins of plasmolemma of enterocytes, compared to calves before feeding of colostrum ($9.45\% \pm 0.35$) (Fig. 2). Therefore there can be assumed a relative increase in the content of proteins with molecular mass of 10 – 15 kDa compared to proteins of other fractions due to the overall decrease of proteins in the composition of plasmolemma of enterocytes.

An absolute increase of the content of proteins with molecular mass of 10 – 15 kDa by 87.8% ($p \leq 0.01$) and 39.2 % ($p \leq 0.05$) was found in the plasmolemma of enterocytes of calves from the first and second experimental group, respectively, 24 hours after birth, compared to calves before feeding of colostrum, and compared to the animals of the control group 24 hours after birth 2.14 times ($p \leq 0.001$) and 1.59 times ($p \leq 0.01$), respectively. It can be concluded, 24 hours after birth of calves, the increase in the content of proteins with molecular mass of 10 – 15 kDa in the membranes of enterocytes of calves of the first and second experimental groups up to $21,18\% \pm 1,49$ and $15,0\% \pm 1,35$, respectively, compared to the calves

Голопура С. І., Цвіліховський М. І., Попадюк Б. В. before feeding colostrum ($9.45\% \pm 0.35$) and calves in the control group at the age of 24 hours ($11.16\% \pm 0.19$) may indicate an increase of the part of FcR- γ receptor proteins, which transfer IgG₁ and IgG₃. This is confirmed by the results of previous studies done by this research group [3, 5], where a significant increase of total proteins and immunoglobulins transported from the intestinal lumen into the bloodstream by the FcR- γ protein of this fraction was found in serum of blood 24 hours after birth of calves.

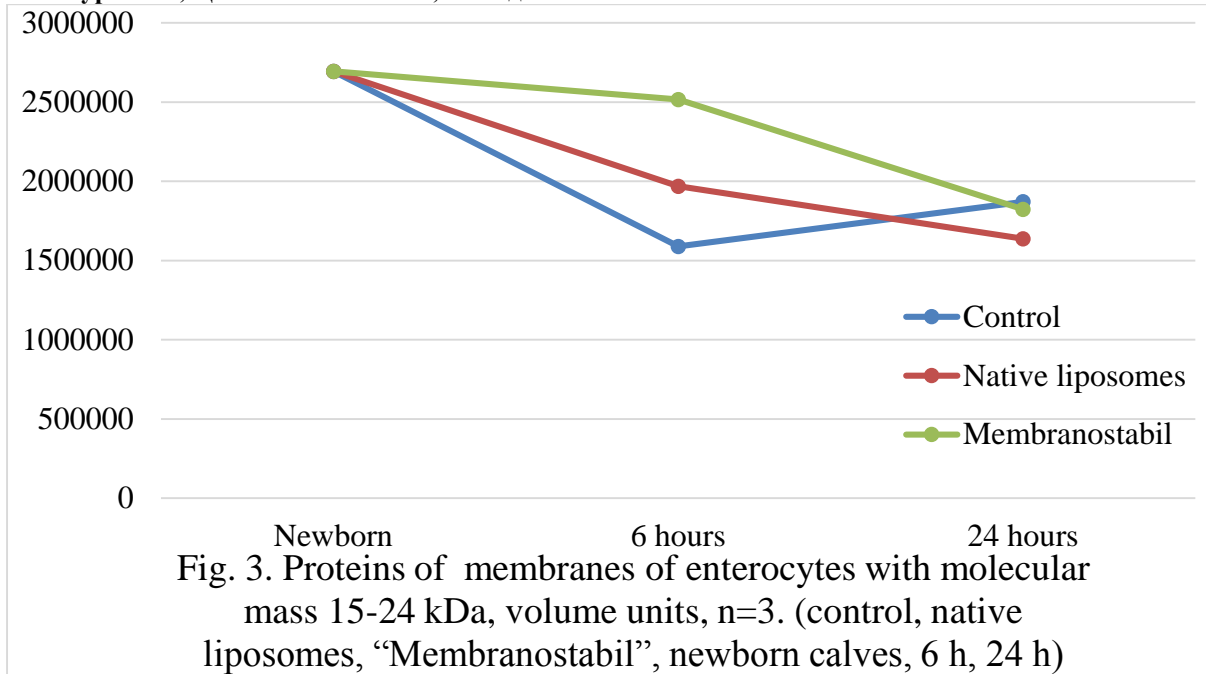
The allocation of plasmolemic proteins of enterocytes of newborn calf in polyacrylamide gel has shown that proteins with molecular mass of 15 – 24 kDa have a quantitative advantage on a given macromolecule of the cell membrane. The part of these proteins comprises 32.27%.

The fraction of proteins with a molecular mass of 15 – 24 kDa also includes calmodulin (16.7 kDa). It is involved in the regulation of ion transport in the intestine, binds and activates more than 40 targets. Calmodulin of the plasma membrane of the intestine performs a leading role in

the regulation of transport of Na⁺, Cl⁻, and Ca²⁺ in the terminal net area. For some pathologies (diarrhea), the activity of calmodulin increases, Ca²⁺ flow increases, and Na⁺ and Cl⁻ flow decreases [4].

Within 6 hours after birth, the content of proteins of the fraction with a molecular mass of 15-24 kDa in the enterocyte plasmolemma of the control group significantly decreased by 41.0%, ($p \leq 0.001$), and in the calves of the first and second experimental groups it decreased only by 26.9 % and 6.59%, respectively, compared with calves before feeding of colostrum (Fig. 3). Also, during this period there was a significant decrease of this protein fraction from $32.27\% \pm 0.49$ to $24.37\% \pm 0.31$ ($p \leq 0.001$) in the total macromolecule of plasmolemma of enterocytes of calves from the control group (Fig. 4). This index remained almost unchanged in the calves of the first experimental group 6 hours after birth, and in calves of the second experimental group it significantly increased ($p \leq 0.001$) from $32.27\% \pm 0.49$ to $38.42\% \pm 0.6$ compared to calves before feeding of colostrum.

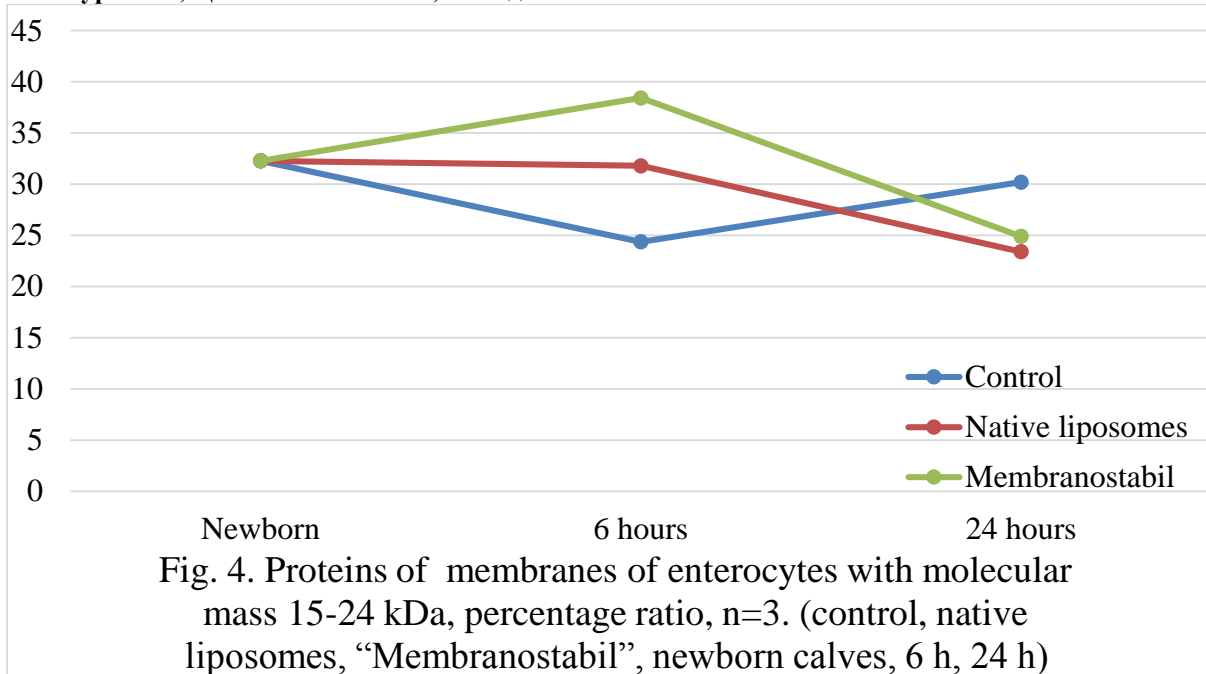
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The content of fraction of proteins with a molecular mass of 15 – 24 kDa in calves 24 hours after the birth was lower than in calves before feeding of colostrum: for calves from the control group by 30.5%, for calves from the first experimental group by 39.2%, and for calves from the second experimental group by 32.3% (Fig. 3). In the total macrodomain of plasmolemma of enterocytes of calves of the control group, the percentage of these proteins 24 hours after birth was: in the calves of the control group $30.2\% \pm 0.35$, in the

calves of the first experimental group $23.4\% \pm 0.32$, and in the calves of the second experimental groups $24.9\% \pm 0.33$ (Fig. 4). Thus, there was a steady decrease in the protein content of this fraction, both in volume units and in relation to other fractions. During this period, there has been observed a significantly lower ($p \leq 0.001$) protein content of this fraction in the plasmolemma of jejunum enterocytes of the first and second experimental groups (1.23 and 1.18 times respectively) compared to the control group of calves.

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It can be assumed, that the reason is the increase in the pool of protein fractions that content receptor proteins binding colostral immunoglobulins and promoting their transport to the bloodstream of calves.

Conclusions

1. Significant changes occur in the expression of proteins in the plasmolemma of the enterocytes of the jejunum of newborn calves during the formation of colostral immunity during the first day of life.

2. By molecular mass, proteins from the fraction of 10 – 15 kDa correspond to the protein receptor FcR- γ , which provides transmembrane transfer of IgG₁ and IgG₃ from the lumen of the intestine into the bloodstream of the calf.

3. The use of native liposomes from phospholipid bilayer based on soybean lecithin and “Membranostabil”

medication in newborn calves significantly stimulates till the end of the first day, compared with control 2.14 times ($p \leq 0.001$) and 1.59 times ($p \leq 0.001$), respectively, the synthesis and expression of proteins with molecular mass of 10 – 15 kDa on plasmolemma of enterocytes of the empty intestine of newborn calves.

4. Increased expression of plasmolemic proteins of enterocytes with molecular mass of 10 – 15 kDa under the influence of native liposomes from phospholipid bilayer and the “Membranostabil” medication promotes the increase of colostrum immunoglobulins in serum of newborn calves, apparently predominantly due to IgG₁.

Prospects for further research.

Further investigation of the protein composition of the small intestine plasmolemma in newborn calves will

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practically in the field of dairy farming in order to prevent the emergence of early calf pathology.

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ВПЛИВ МЕМБРАНРЕПАРУЮЧИХ ЗАСОБІВ НА ЕКСПРЕСІЮ БІЛКІВ ПЛАЗМОЛЕМИ ЕНТЕРОЦИТІВ ТЕЛЯТ ПІД ЧАС ФОРМУВАННЯ КОЛОСТРАЛЬНОГО ІМУНІТЕТУ

С. І. Голопура, М. І. Цвіліховський, Б. В. Попадюк

Анотація. Досліджені показники вмісту білків з молекулярними масами 10-15 та 15-24 кДа у плазмолемі ентероцитів тонкої кишки новонароджених телят у динаміці — від народження до 1-добового віку. Дослідження проведені на трьох групах телят (контроль на та дві дослідні групи) української чорно-рябої молочної породи з відбором у них зразків ентероцитів порожньої кишки та визначенням вмісту білків мембранної фракції лізатів за допомогою електрофорезу. Застосування новонародженим телятам із молозивом нативних ліпосом на основі соєвого лецитину та препарату «Мембраностабіл»

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підвищує експресію білків плазмолемі ентероцитів з молекулярною масою 10-15 кДа. Показана динаміка з порівняльним аналізом між окремими фракціями експресії білків з молекулярними масами 10-15 та 15-24 кДа у плазмолемі ентероцитів порожньої кишки телят контрольної і дослідних груп. Застосування нативних ліпосом з фосфоліпідного бішару на основі соєвого лецитину та препарату «Мембраностабіл» дозволяє зробити припущення щодо позитивного впливу застосованих нами засобів на рівень колостральних імуноглобулінів у сироватці крові новонароджених телят.

Ключові слова: новонароджені телята, тонкий кишечник, ентероцит, плазмолема, білки, фосфоліпіди, нативні ліпосоми, колостральний імунітет