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INFLUENCE OF THE MEDICATIONS CONTAINING PHOSPHOLIPIDS ON THE SERUM IMMUNOGLOBULIN G LEVEL IN CALVES DURING FORMATION OF COLOSTRAL IMMUNITY

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Abstract. *The results of the application of the medication "Membranostabil" and native phospholipid bilayer liposomes based on soybean lecithin developed by this research group for correction of serum immunoglobulin G (IgG) content in newborn calves during colostral immunity formation are presented. The indices of IgG content in blood serum of newborn calves have been investigated in dynamics - from the birth till the age of 11 days. Studies were performed on newborn calves of three groups (control, and two experimental ones) of the Ukrainian black-and-white dairy breed. The level of IgG was investigated by electrophoresis in polyacrylamide gel Quantitative estimation of protein fractions was performed by scanning the electrophoregram, with their subsequent graphical reconstruction and calculation by relative units or area using a computer program. It is established that "Membranostabil" medication and native liposomes from phospholipid bilayer based on soybean lecithin activate transport of immunoglobulins in small intestine and promote a significant increase of content of serum IgG compared to calves of the control group. The content of IgG in the serum of newborns calves of both experimental groups at 6 hours of age significantly increased and remained higher throughout the duration of the experiment, except for the calves of the first experimental group at the age of 7 days, compared with calves in the control group. The dynamics with comparative analysis of serum IgG content between calves of individual groups is shown. The increase of content of serum IgG in newborn calves is one of factors preventing early immunodeficiency, sepsis, development of digestive disorders, and other diseases of young animals.*

Keywords: *colostral immunity, colostrum, immunoglobulin G (IgG), newborn calves, Membranostabil*

Introduction and analysis of recent researches and publications

The newborn's immune system has no humoral response. Therefore, the transfer of maternal antibodies to it is an important mechanism of protection against infectious diseases (Borghesi et al., 2014; Palmeira et al., 2014). A number of factors affect ability of newborn calf to assimilate antibodies. And that has an effect on the level of the passive immunity that the calf receives from the mother's colostrum. The calves should consume at least 150 g of IgG with colostrum after birth as soon as possible (Jim Quigley, 2007).

The quality of colostrum is determined by the concentration of immunoglobulins in it, preferably IgG. IgG constitute 85 % of colostrum immunoglobulins. The immunity formed due to colostrum consumption is the most common form of protection for calves and other ruminants, because immunoglobulins in these species are not transferred from mother to fetus during prenatal period (Borghesi et al., 2014). Transfer of immunity with colostrum is unsuccessful in case if there is less than 10 mg IgG/ml in the serum of calves at the age of 1-2 days (Jim Quigley, 2007; Silper et al., 2012). It depends largely on the method of determination of serum Ig. Thus, according to Davis and Dracli (Jim Quigley, 2007; Davis & Dracley, 1998), the average concentration of immunoglobulins in the serum of calves within three days of life was 7 g / dl with spectrophotometry measurement and 10.1 g/dl with application of the refractometric method.

Biological activity of IgG is specified by its ability to carry specific antibodies (Dzhanabekova et al., 2012). It is also present in tissue spaces and involved in the control of infection throughout the body, associated with a variety of pathogens: viruses, bacteria, fungi etc (Mallery et al., 2010). Binding of IgG to pathogens causes their immobilization and agglutination as well as opsonization, which allows to recognize, absorb, and destroy them by phagocytes. Binding to neutrophils, macrophages, mononuclear phagocytes, and other specialized cells of the immune system after such interaction carries out phagocytosis of foreign agents or destruction of infected cells due to antibody-dependent cytotoxicity (Ofitserov, 2005). IgG molecules are also capable of binding and neutralization of toxins. Antibodies can also activate a cascade of enzymes called complement which can perform the following duties: a) generate a membrane attack complex which lyses the infectious organism; b) produce a series of mediators, such as anaphylatoxins, which produce an inflammatory response capable of killing the pathogen; c) attach an enzyme derivative called C3b to the pathogen, which then allows the pathogen to be recognised by phagocytic cells in much the same manner as if coated with antibody (Butler, 1998). This type of antibody plays an important role in the cellular cytotoxicity dependent on antibodies (Atkinson et al., 2006). As is known, the IgG from blood serum of farm animals has two subclasses – IgG_1 and IgG_2 (Mallery et al., 2010). All domesticated ruminants have an IgG_1 which is highly cross-reactive among species. IgG_1 is the major Ig in the colostrum of cows, ewes and nannies and the high concentration of IgG_1 in this secretion (60 mg to 100 mg / ml) is the consequence of a selective transport mechanism involving IgG_1-specific transport receptors in the mammary gland (Butler, 1998; Mallery et al., 2010). IgG_1 is selectively transported by the udder from the

circulation to the lacteal secretions by a mechanism yet to be elucidated. Hence, IgG_1 is the principal immunoglobulin for passive immunization of the calf. IgG_2 is generally ascribed as the most important opsonin for both neutrophil and macrophage phagocytosis (Butler, 1998).

Pires-Junior studies (Pires Junior, 2009) have shown that calves do not have any immunoglobulins in their body before receiving colostrum and that their level increases in the blood of these animals shortly after its consumption, reaching its maximum level in 12-48 hours. Own IgG begin to be synthesized in the body of calves much later, but they are produced for a very long time (responsible for long-term immunity).

Consumption of enough IgG is important for good health of a newborn calf. Lots of calves do not get the enough amount of colostrum in the first day of life. As a result, morbidity and mortality of calves on dairy farms remains unacceptably high. The data of researchers (Wang et al., 2015) confirms this, claiming that the content of IgG in the serum of calves with diarrhea is significantly lower than in healthy calves, and there is a positive relationship between serum IgG concentration and incidence of diarrhea in calves.

The aim of this study was to determine the concentration of serum immunoglobulin G (Ig G) of newborn calves using native phospholipid bilayer liposomes and "Membranostabil" medication with colostrum.

Materials and methods of research

Research was conducted in scientific research center "Velykosnitinske n.a. O.V. Muzychenko" NULES of Ukraine on the cows of Ukrainian black-and-white breed 3 days prior to and 7 days after parturition and on their calves during the period from birth till the age of 11 day. Calves were separated into three groups: one control and two experimental ones, each with 5 animals. Calves of all groups were fed by 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 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 (Tsvilikhovsky et al., 2014).

Blood for research was drawn from the jugular vein into vacuum tubes with EDTA from cows 3 days prior to parturition, after milking the first colostrum, on the 3rd and 7th days after parturition, and from newborn calves prior to the first feeding of colostrum, and 6 hours after birth of the animal, as well as on the 1st, 3rd, 7th, and 11th days of their lives. The experimental studies on cows and 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, and the "European Convention for the Protection of Vertebrate Animals" from 13.11.1987.

Investigation of protein fractions in serum of cows and newborn calves were

performed by electrophoretic separation in 7.5 % polyacrylamide gel with sodium dodecyl sulfate modified with tricin (Schägger & Jagow, 1987).

Protein zones were identified using a reagent for amino groups Kumasi G-250 (Serva), and the molecular mass of the proteins was determined according to the markers of Bioscience (Amersham), Sweden.

Quantification of protein zones was performed by method of electrophoregram scanning, with their subsequent graphical reconstruction and calculating by relative units or area by computer program. The total amount was taken as 100 %.

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

Results of the research and their discussion

For a more detailed study of the mechanism of colostrum immunity formation in newborn calves, namely transmission of mother's colostral antibodies with colostrum, it has been needed to determine the serum immunoglobulin content in cows.

Serum immunoglobulin G content in cow blood was the lowest 3 days prior to expected parturition and constituted 10.96 g / L (Fig. 1).

After parturition of cows and milking of the first colostrum, the content of serum IgG reliably ($P \leq 0.001$) increased 1.37 times compared with one 3 days prior to the parturition, and amounted 15.05 g / L.

On the 3rd and 7th days after parturition there were slight fluctuations of content of serum IgG of cows from 16,33 ± 0,24 ($P \leq 0,001$) to 13,85 ± 0,37 g / L ($P \leq 0.01$) respectively, but compared to this index 3 days prior to parturition these indices remained significantly higher 1.49 and 1.26 times, respectively.

It can be assumed, that significantly lower serum IgG content in cows before parturition indicates the elimination of them from the bloodstream by the mammary gland in colostrum for further formation of colostral immunity in newborn

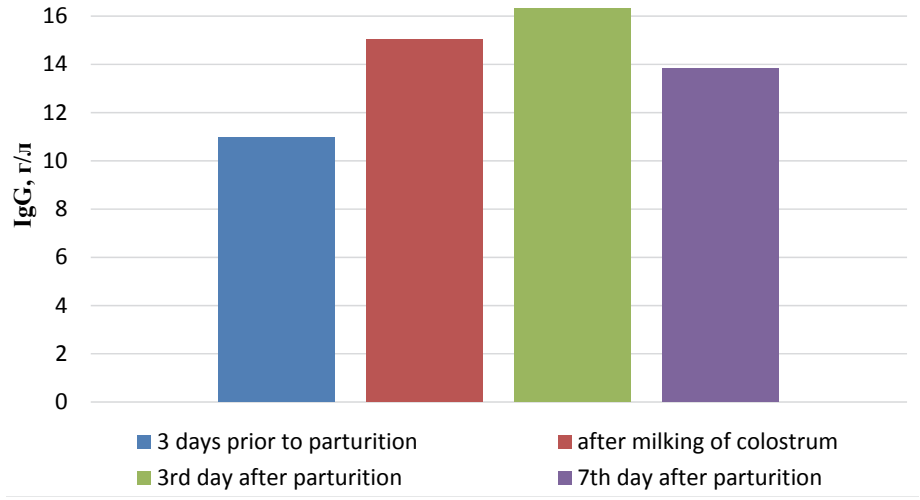

Fig.1 Level of serum IgG of cows 3 days prior to parturition, after colostrum milking, 3, and 7 days after parturition.

calf. This is consistent with the data of other authors (Brandon et al., 1971), who indicate that the transition of IgG from the mother to the mammary gland begins in several weeks prior to parturition and ends just before the parturition.

Before feeding of newborn calves by colostrum, the serum IgG content in the blood was only 2.51 ± 0.06 g / L, which is consistent with the data of others researchers (Ježek et al., 2012). The reason for its low content in the serum of calves just after birth is little immunoglobulin synthesis by one's own immune system and peculiarity of the structure of the desmochoric type of placenta in ruminant animals that does not transfer IgG to the fetus.

Feeding of the first portion of colostrum to calves after birth contribute reliable ($P \leq 0.001$) increase of serum IgG content 6 hours later in animals of all groups compared to the pre-colostrum feeding period (Fig. 2).

Thus, in blood serum of calves of the control group, this index increased 1.94 times, and in calves of the first and second experimental groups 2.16 and 2.73 times, respectively. The use of the medication "Membranostabil", at the age of 6 hours contributed to the more intense transfer of IgG in native condition from the lumen of the intestine into the bloodstream (calves of the second experimental group). This is indicated by reliably ($P \leq 0.01$) 1.41 times higher content of immunoglobulins in the blood of these animals compared with that of the control group. This is also consistent with other studies (Golopura et al., 2019; Golopura et al., 2019) concerning receptor proteins that have the ability to transport IgG in the native state through the small intestine enterocyte plasmolema in the blood of newborn calf. It can be assumed, that more intensive transfer of immunoglobulins in calves of second experimental group is conditioned by the influence of vitamin A, which constitutes the medication "Membranostabil". This is also consistent with other studies (Kurtyak & Yanovich, 2004) indicating that vitamin A contribute to the RNA and

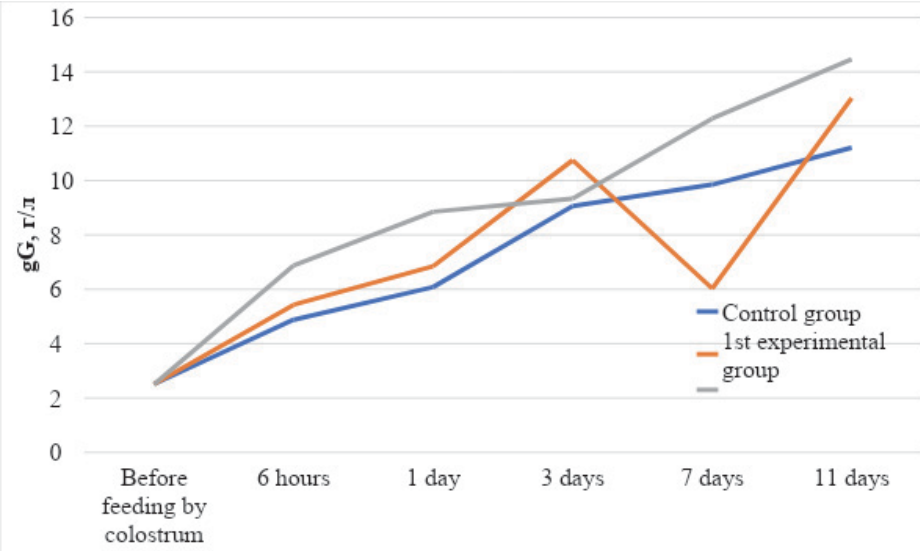

Fig. 2. Level of serum IgG of calves after birth (before colostrum feeding) and at the age of 6 hours, 1, 3, 7, and 11 days after birth

sulfated glycosaminoglycans synthesis, which, in turn play an important role in cell membrane permeability (Silper et al., 2012; Davis & Dracley, 1998). According to data of other studies almost all retinol in cell membranes is tightly bound to proteins and only part of it is extracted during lipids extraction. Besides retinol in the cell membranes the retinic acid was found, which is tightly bound to its components. According to this data it was assumed that the structure and transport function of cell membrane depends on the content of retinol in it. Nonetheless its influence on the molecular mechanisms wasn't sufficiently investigated. There is only presumption, that the content of vitamin A in cell membranes influences the micro viscosity of lipid bilayer.

24 hours after birth, serum IgG content of calves from all groups continued to grow: in control group 2.42 times, and in first and second experimental groups 2.73 and 3.53 times, respectively, compared with the calves before colostrum feeding, and 1.25, 1.26 and 1.29 times in calves of the control, first and second experimental groups, respectively, by comparison with calves at the age of 6 hours. However, reliably ($P \leq 0.01$) 1.46 times higher serum IgG content in calves of the second experimental group compared with calves of the control group was found.

According to other authors (Nonnecke et al., 2012), 36 hours after birth in calves the Ig penetration through the epithelium of the small intestine completely stops. Therefore, determination of IgG content in calf serum at the age of 3 days should show how animals were able to maximally absorb this immunoglobulin from the mother's colostrum.

Thus, 72 hours after birth, the serum IgG content in calves from control group was 9.06 ± 0.27 g / L, and in calves of the first and second experimental groups it was 10.75 ± 0.37 and 9.34 ± 0.3 g / L, respectively. In particular, the serum IgG content in the blood of calves of the first experimental group during this period was reliably ($P \leq 0.05$) higher compared to that in the control group. It can be assumed, that native liposomes applied to calves per os due to the phospholipids promote the stable structure and viscosity of the enterocyte plasmolemma. This, in turn, activates immunoreceptor proteins of plasmolemma of enterocytes up to colostrum immunoglobulins and promotes sufficient IgG formation in the serum of newborn calves and prevents them from sepsis and digestive disorders.

Nowadays, there are many different studies regarding the beginning of synthesis of own IgG in calves. According to various authors (Singh et al., 2011), the endogenous production of IgG in calves with a high initial IgG concentration begins at the age of 4 weeks, whereas in calves with hypogammaglobulinemia endogenous IgG production starts at the age of 1 week. Using IgG_1 labeled I^{125} it was found that in calves at the age of 36 hours the synthesis of own IgG_1 begins in the amount of 1 g per day and lasts till the age of 3 weeks.

In this study, on the 7th day after birth in calves the content of serum IgG in animals of the control group remained almost at the same level as on the 3rd day of life. In calves of the first experimental group this index reliably decreased to 6.03 ± 0.45 g / L ($P \leq 0.001$), which may be an evidence of intense use of colossal IgG in the absence of own synthesis at this age. Instead, in the serum of the calves of the second experimental group at the same period the IgG content significantly increased to 2.94 g / L, ($P \leq 0.01$) and amounted 12.28 g / L. It may be assumed that at the 7th day of life the synthesis of

own IgG in the body of calves of the second experimental group was stimulated by vitamins A and E, which are the constituents of the medication "Membranostabil". This is also consistent with other studies, indicating that with addition of vitamin E to the ration of mice the content of IgG in their blood increases. And the addition of vitamin E to the ration of pregnant cows during winter period increases the concentration of immunoglobulins in the blood serum (Kurtyak & Yanovich, 2004). There is also data regarding a positive influence of short period vitamin A application on the activity of immune system in animals. In particular, under application if vitamin A to white mice, white rats, and rabbits during three days with their subsequent immunization by influenza virus PR-8, the increasing of mass of thymus and spleen, potentiation of immune reaction, and rising of antibodies, antibody-forming cells, and serum agglutinins amount was detected (Kurtyak & Yanovich, 2004).

The serum IgG content in calves from control, first, and second experimental groups on the 11th day of age increased 1.14, 2.16 ($P \leq 0.001$), and 1.18 ($P \leq 0.01$) times, respectively, compared with this index at the age of 7 days. Also, during this period a reliably ($P \leq 0.01$) 1.29 times higher serum IgG level in calves of the second experimental group compared to ones of the control group was found. Based on obtained results, it can be claimed that native liposomes and "Membranostabil" medication administered internally (per os) significantly enhance the synthesis of own IgG. This is also consistent with studies with different species of laboratory and farm animals (Kurtyak & Yanovich, 2004), indicating on stimulating effect of vitamin E on immune function of animals with its addition to ration or parenteral application.

Conclusions

Lower serum IgG level of cows 3 days before parturition comparing to post parturition period indicates the elimination of immunoglobulins from the mother's bloodstream to the colostrum for later formation of colostral immunity in a newborn calf.

The per os application of medication from native liposomes based on soybean lecithin for calves promote reliably ($P \leq 0.05$) higher serum IgG levels of newborn calves, what is indicated by their level in the blood of animals on the 3rd day of life.

The application of the medication "Membranostabil" to the newborn calves reliably ($P \leq 0.01$) 1.29 times increases the synthesis of own IgG in their organism.

Research findings indicate a positive effect of native liposomes and the medication "Membranostabil" on the formation of colostral immunity and synthesis of own IgG in the early postnatal period. This makes it possible to strengthen the effect of colostrum immunity and avoid the development of early immunodeficiency, sepsis, digestive disorders, and other diseases in calves.

Prospects for further research

In the future, the researches on the effect of native liposomes and the medication "Membranostabil" on the level of immune proteins of other classes in the blood of newborn calves will be continued.

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Анотація. *Наведені результати застосування розробленого авторами препарату «Мембраностабіл» і нативних ліпосом з фосфоліпідного бішару на основі соєвого лецитину для корекції показників вмісту імуноглобуліну G (IgG) у сироватці крові новонароджених телят у період формування колострального імунітету. Досліджені показники вмісту IgG у сироватці крові новонароджених телят у динаміці — від народження до 11-добового віку.*

Дослідження проводили на новонароджених телятах трьох груп (контрольна, та дві дослідні) української чорно-рябої молочної породи. Рівень IgG досліджували методом гель-електрофорезу в поліакриламідному гелі. Кількісну оцінку білкових фракцій проводили скануванням електрофореграми, з наступною реконструкцією їх графічно і обчисленням за відносними одиницями або площею з використанням комп'ютерної програми. Встановлено, що препарат «Мембраностабіл» і нативні ліпосоми з фосфоліпідного бішару на основі соєвого лецитину активують транспорт імуноглобулінів у тонкому кишечнику і сприяють достовірному зростанню вмісту IgG порівняно з телятами контрольної групи. Вміст IgG у сироватці крові новонароджених телят обох дослідних груп у віці 6 годин достовірно зріс і залишався вищим впродовж всього періоду досліду, за винятком телят першої дослідної групи у віці 7 діб, порівняно з телятами контрольної групи. Показана динаміка з порівняльним аналізом вмісту IgG в сироватці крові між показниками телят окремих груп. Зростання вмісту IgG в крові новонароджених телят є одним із факторів, що забезпечує профілактику раннього імунодефіциту, сепсису, розвитку розладів травлення та виникнення інших хвороб молодняку.

Ключові слова: *колостральний імунітет, молозиво, імуноглобулін G, новонароджені телята, Мембараностабіл*

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