Part 1. Veterinary medicine

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MODULATION OF INNATE IMMUNITY OF CALVES IN THE EARLY NEONATAL PERIOD WITH PROBIOTIC NANOMETAL GLOBULIN DRUG

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Summary. The article highlights the results of studying the effect of a new probiotic nanometal globulin drug (PNMGD) on biomarkers of innate immunity of newborn calves. The experiment was performed on two groups of calves. Animals of the experimental group from the 2nd day of life were fed the drug for 5 days at a dose of 0.25 g/kg of body weight with milk, calves of the control group received milk without its addition. Before the experiment, and on the 10th, 20th, 35th day of the experiment, blood was taken from calves for clinical and biochemical studies. The obtained results show that the use of PNMGD causes an increase in the natural resistance of calves. This is indicated by an increase in the expression of such markers of innate immunity as globulins, circulating immune complexes and nitrogen metabolites by an average of 17–25%, as well as inhibition of seromucoid synthesis by 16.9%. Signs of anti-stress effect of the drug on the calves in the early postnatal period have been found. The positive effect of PNMGD on the state of innate immunity of calves can be regarded as one of the factors increasing the average daily weight gain by 32.2% in the first 36 days of life

Keywords: immune resistance, clinical and biochemical parameters, anti-stress effect

Introduction. Health protection of young farm animals and poultry at an early age is one of the most topical problems of animal husbandry in Ukraine. The economic losses from diseases and deaths of productive livestock, especially young animals, are quite significant. This is due to their low level of resistance caused by insufficient and unbalanced feeding, unsatisfactory maintenance of cows during pregnancy, which leads to impaired embryonic development, reduced content of immune globulins, immune-competent cells, vitamins, macro-and micronutrients in colostrum and milk (Van de Perre, 2003). The polyetiological nature of diseases of newborn animals necessitates the development of effective complex drugs that would have diverse stimulating and immune modulatory properties (Lytvyn V. P. et al., 2002; Chornyi et al., 2016; Cuttance and Laven, 2019). Today, there is a trend towards the use of drugs of endogenous origin, which can activate the immune system by enhancing the proliferation and function of immune competent cells, as well as stimulating innate and adaptive immunity (Lavelle and McLachlan, 2018; Noh et al., 2019).

In this regard, the development of effective complex drugs, including the use of nanoparticles of macro- and micronutrients, the study of the biological effect of these drugs on the state of natural resistance to markers of humoral factors of the immune response remains a problem (Smith, Simon and Baker, 2013; Lee et al., 2010).

Previous studies have found a positive effect of developed probiotic nanometal globulin drug (PNMGD) on the state of nonspecific resistance of chickens (Kovalenko et al., 2017). The study of its effect on the body of young cattle is also of considerable scientific and practical interest.

The aim of the study was to determine the direction and levels of the effect of PNMGD on the functional state of innate immunity and health status, indicator of which is the weight gain in calves up to 36 days of age.

Materials and methods. PNMGD, which was used in the experiment, contains serum globulins, aqueous solutions of nano-iron (Fe) aquachelates and metal salts ($CoSO_4$, $CuSO_4$, $MnCl_2$, and $ZnSO_4$), as well as a mixture of cultures of lactobacteria (*Lactobacillus plantarum* No. 7) and bifidobacteria (*Bifidobacterium adolescentis* No. 17).

The research was conducted in the conditions of cattle breeding farm on newborn calves. Thus two groups of five animals in each were formed. Calves of the 1st group from the 2nd day of life for 5 days were fed the drug at a dose of 0.25 g/kg body weight with milk (optimal dose has been selected in previous experiments on laboratory animals), calves of the 2nd (control) group were fed milk without the drug.

Each animal was weighed before and at the end of the experiment. Before the administration of the drug and on the 10th, 20th, and 35th day of the experiment, the blood

from calves was examined by clinical and biochemical methods.

The level of leukocytes was determined in the blood, the level of total protein, protein fractions (Kondrakhin et al., 1985), circulating immune complexes of average molecular weight and seromucoids (Men'shikov, 1987), nitric oxide were determined in blood plasma (Lee et al., 2009), lysozyme activity (Labinskaya, 1978) and the state of the oxidant-antioxidant system by the level of diene conjugates, malonic dialdehyde and catalase activity (Stegniy et al., 2007).

Experiments on animals were carried out in accordance with the rules of the 'European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes' (CE, 1986) and Council Directive 86/609/EEC (CEC, 1986).

The obtained results were processed by methods of variation statistics using Microsoft Excel for Windows 2007. Student's *t*-test was used to compare mean values (Van Emden, 2019).

Results and discussions. Analysis of the results obtained when feeding PNMGD to calves, shows that the drug has a positive effect on the weight gain of animals, the dynamics of which is shown in Fig. 1. According to calculations, the average daily weight gain of calves in the control group was 0.62 ± 0.08 kg, and in calves of the experimental group — 0.94 ± 0.07 kg, which is higher by 32.2% ($p \leq 0.05$). Exceeding the average body weight of calves in the experimental group on the 10th, 20th, and 35th day of the experiment reached 14.2%, 17.8% and 27.8%, respectively. The level of safety of newborn calves in both groups was 100%.

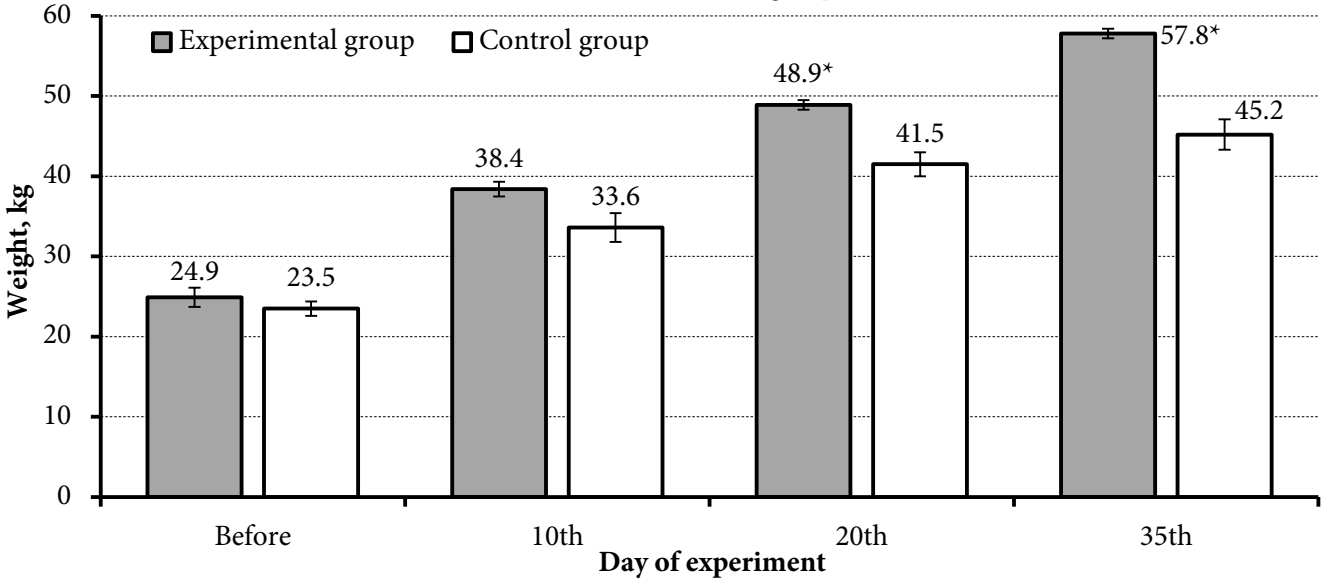

Figure 1. Dynamics of live weight of calves with the use of PNMGD (* — the difference is statistically significant relative to the indicators of the control group at $p \leq 0.05$)

During the experiment, a dynamic increase in the level of leukocytes in the blood of calves of both groups was observed (Table 1), which, according to Koryakina and Borisov (2015), is the evidence of the body's response to stressors in the early postnatal period. However, if in animals of the control group this index increased by 54.5%, in calves of the experimental group — only by 16.3%. The established differences between the indicators can be explained by the increased efficiency of adaptive reactions of the body of newborn calves under the action of the drug.

Analysis of the dynamics of the plasma protein profile presented in Table 1 shows an increase in the level of total protein in the plasma of calves of the experimental group by 17.6% on the 10th day of the experiment and by 13.8% on the 20th day. The concentration of globulins in the 2nd and 3rd experiments increased by 17.9% and 24.6%, respectively, and on the 35th day, this index remained increased by 14.4% relative to the level of the control.

It was also found that the use of PNMGD causes an increase in concentration of a number of mediators of innate immunity in the plasma. Thus, the level of circulating immune complexes on the 10th and 20th days of the experiment increased by 17.1% ($p \leq 0.05$) and 9.5%, respectively, compared with the control. A similar direction of changes was established with respect to the level of nitric oxide metabolites — their concentration in calves of the experimental group exceeded the control values by 23.9% and 14.2% ($p \leq 0.05$), respectively. Considering the biological role of the circulating immune complexes of average molecular weight and NO metabolites, it can be stated that the developed drug promotes the induction of mediators of cellular immunity and increase the body's natural resistance (Bogdan, Röllinghoff and Diefenbach, 2000; Weigert et al., 2018).

The level of seromucoids, which are considered inhibitors of the humoral part of specific immunity, in the blood plasma of calves of the experimental group on the

10th day was lower by 16.9% ($p \le 0.05$) relative to the values of the control group, in the following terms a gradual approximation of values to the control level was observed.

At the same time, it was found that PNMGD does not significantly affect the activity of lysozyme in the blood plasma of experimental calves, which, in addition to direct antimicrobial action, is one of the modulators of the body's immune response to infection (Ragland and Criss, 2017).

The most pronounced effect on the lipoperoxidation process was recorded on the 10th day of the experiment, when the level of diene conjugates was 28.1 ± 0.1 mmol/l and malonic dialdehyde — 5.1 ΔD, which is lower by 15.9% and 18.6% ($p \le 0.05$) relative to the control values, respectively. In subsequent studies, significant differences in lipid peroxidation activity were not observed. At the same time, an increase in catalase activity was found, up to the 10th day of the experiment, this indicator was reliably increased by 24.2% in animals of the experimental group relative to the control group (Fig. 2).

Considering the biological role of the lipid peroxidation system and antioxidant protection, the established signs of its slight activation also indicate an increase in innate immunity under the action of PNGMD (Weigert et al., 2018; Maldonado Galdeano et al, 2019).

Table 1 — Dynamics of markers of innate immunity of calves when using PNMGD (M ± m; n = 5)

Indexes	Before experiment		10th day of experiment		20th day of experiment		35th day of experiment	
	Experimental group	Control group	Experimental group	Control group	Experimental group	Control group	Experimental group	Control group
The number of leukocytes, $\times 10^9$/l	7.6 ± 0.2	6.6 ± 0.4	6.8 ± 0.5	7.5 ± 0.7	7.6 ± 0.4*	8.9 ± 0.7	9.1 ± 0.5	10.2 ± 0.8
Total protein, g/l	54.6 ± 2.6	53.8 ± 1.5	64.7 ± 1.7*	55.0 ± 0.9	69.3 ± 2.7	60.9 ± 2.4	63.6 ± 1.1	62.5 ± 1.7
Globulin, g/l	26.8 ± 0.8	24.6 ± 1.2	29.9 ± 1.5	25.4 ± 0.9	32.3 ± 1.6*	25.9 ± 1.5	31.6 ± 1.2*	27.6 ± 0.5
Circulating immune complexes, mg/ml	0.110 ± 0.003	0.110 ± 0.005	0.128 ± 0.006*	0.112 ± 0.006	0.110 ± 0.010	0.100 ± 0.005	0.110 ± 0.002	0.110 ± 0.005
Seromucoids, mg/ml	0.13 ± 0.002	0.12 ± 0.005	0.13 ± 0.005	0.16 ± 0.005	0.15 ± 0.002	0.14 ± 0.002	0.13 ± 0.006	0.12 ± 0.005*
Nitric oxide, μMol/l	378.5 ± 3.5	367.8 ± 4.2	342.1 ± 9.8*	260.4 ± 5.6	239.8 ± 5.5*	205.7 ± 2.1	206.3 ± 2.1	196.8 ± 1.9

Note. * — the difference is statistically significant relative to the indicators of the control group at $p \le 0.05$.

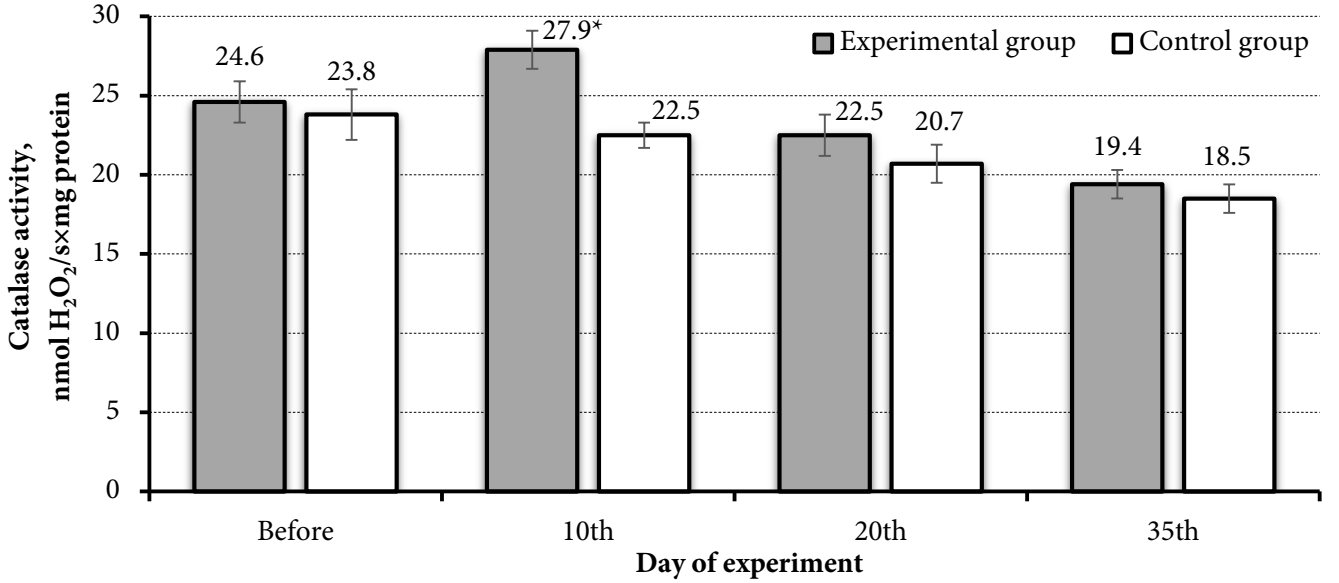

Figure 2. Dynamics of catalase activity in the blood plasma of calves when feeding PNMGD (M ± m; n = 5; * — the difference is statistically significant relative to the indicators of the control group at $p \le 0.05$)

Conclusions. 1. The use of PNMGD helps to increase the level of natural resistance of newborn calves, as evidenced by the increase in expression, on average by 17–25%, its markers such as globulins, circulating immune complexes and nitrogen metabolites, as well as inhibition of seromucoid synthesis by 16.9%.

2. Signs of anti-stress effect of the test drug on the body of calves in the early postnatal period have been established, which is manifested in a decrease in the manifestation of leukocyte adaptive-compensatory response, as well as a decrease in lipoperoxidation, one of the factors of which may be a compensatory increase in catalase activity, which on the 10th day of the experiment was 24.2%.

3. The revealed positive effect of PNMGD on the state of innate immunity of calves can be regarded as one of the factors increasing the average daily weight gain of calves by 32.2% in the first 36 days of life.

References

Bogdan, C., Röllinghoff, M. and Diefenbach, A. (2000) 'The role of nitric oxide in innate immunity', *Immunological Reviews*, 173(1), pp. 17–26. doi: 10.1034/j.1600-065X.2000.917307.x.

CE (The Council of Europe). (1986) *European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes*. (European Treaty Series, No. 123). Strasbourg: The Council of Europe. Available at: https://conventions.coe.int/treaty/en/treaties/html/123.htm.

CEC (The Council of the European Communities). (1986) 'Council Directive 86/609/EEC of 24 November 1986 on the approximation of laws, regulations and administrative provisions of the Member States regarding the protection of animals used for experimental and other scientific purposes', *The Official Journal of the European Communities*, L 358, pp. 1–28. Available at: http://data.europa.eu/eli/dir/1986/609/oj.

Chornyi, M. V., Shchepetilnikov, Yu. O., Bondar, A. O. and Panasenko, Ye. O. (2016) 'Influence of abiotic factors on the cows health and productivity and on calves resistance' [Vplyv abiotychnykh faktoriv na produktyvnist ta zdorovia koriv i rezystentnist teliat], *Ukrainian Black Sea Region Agrarian Science [Visnyk ahrarnoi nauky Prychornomoria]*, 2(2), pp. 161–170. Available at: http://nbuv.gov.ua/UJRN/vanp_2016_2(2)__23. [in Ukrainian].

Cuttance, E. and Laven, R. (2019) 'Estimation of perinatal mortality in dairy calves: A review', *The Veterinary Journal*, 252, p. 105356. doi: 10.1016/j.tvjl.2019.105356.

Kondrakhin, I. P., Kurilov, N. V., Malakhov, A. G., Arkhipov, A. V., Belov, A. D., Belyakov, I. M., Blinov, N. I., Korobov, A. V., Frolova, L. A. and Sevast'yanova, N. A. (1985) *Clinical Laboratory Diagnostics in Veterinary Medicine [Klinicheskaya laboratornaya diagnostika v veterinarii]*. Moscow: Agropromizdat. [in Russian].

Koryakina, L. P. and Borisov, N. I. (2015) 'Indices of autarcesis and blood physiological and biochemical status of newborn calves' [Pokazateli estestvennoy rezistentnosti i fiziologo-biokhimicheskiy status krovi u novorozhdennykh telyat], *Vestnik of the North-Eastern Federal University Named After M. K. Ammosov [Vestnik Severo-Vostochnogo federal'nogo universiteta imeni M. K. Ammosova]*, 5, pp. 23–30. Available at: https://www.elibrary.ru/item.asp?id=24905579. [in Russian].

Kovalenko, L. V., Boiko, V. S., Rudenko, O. P., Krotovska, Yu. M. and Doletskyi, S. P. (2017) 'Affect of complex probiotic nanometal globulin preparation on level of nonspecific resistance indicators of chickens' [Vplyv kompleksnoho probiotychno nanometalohlobulinovoho preparatu na riven pokaznykiv nespetsyfichnoi rezystentnosti kurchat], *Veterinary Medicine [Veterynarna medytsyna]*, 103, pp. 335–339. Available at: http://www.jvm.kharkov.ua/sbornik/103/6_81.pdf. [in Ukrainian].

Labinskaya, A. S. (1978) *Microbiology with Microbiological Research Technique [Mikrobiologiya s tekhnikoy mikrobiologicheskikh issledovaniy]*. 4th ed. Moscow: Meditsina. [in Russian].

Lavelle, E. C. and McLachlan, J. B. (2018) 'Editorial overview: Immunomodulation: Striking the right balance: using immunomodulators to target infectious diseases, cancer, and autoimmunity', *Current Opinion in Pharmacology*, 41, pp. vii–ix. doi: 10.1016/j.coph.2018.07.013.

Lee, S. H., Lillehoj, H. S., Jang, S. Ik., Kim, D. K., Ionescu, C. and Bravo, D. (2010) 'Effect of dietary *Curcuma*, *Capsicum*, and *Lentinus*, on enhancing local immunity against *Eimeria acervulina* infection', *The Journal of Poultry Science*, 47(1), pp. 89–95. doi: 10.2141/jpsa.009025.

Lytvyn, V. P., Oliinyk, L. V., Korniienko, L. Ye., Yarchuk, B. M. and Dombrovskyi, O. B. (2002) *Factor Diseases of Farm Animals [Faktorni khvoroby silskohospodarskykh tvaryn]*. Kyiv: Ahrarna nauka. ISBN 9665400738. [in Ukrainian].

Maldonado Galdeano, C., Cazorla, S. I., Lemme Dumit, J. M., Vélez, E. and Perdigón, G. (2019) 'Beneficial effects of probiotic consumption on the immune system', *Annals of Nutrition and Metabolism*, 74(2), pp. 115–124. doi: 10.1159/000496426.

Men'shikov, V. V. (ed.) (1987) *Laboratory Research Methods in Clinic [Laboratornye metody issledovaniya v klinike]*. Moscow: Meditsina. [in Russian].

Noh, E.-M., Kim, J.-M., Lee, H. Y., Song, H.-K., Joung, S. O., Yang, H. J., Kim, M. J., Kim, K. S. and Lee, Y.-R. (2019) 'Immuno-enhancement effects of *Platycodon grandiflorum* extracts in splenocytes and a cyclophosphamide-induced immunosuppressed rat model', *BMC Complementary and Alternative Medicine*, 19(1), p. 322. doi: 10.1186/s12906-019-2724-0.

Ragland, S. A. and Criss, A. K. (2017) 'From bacterial killing to immune modulation: Recent insights into the functions of lysozyme', *PLoS Pathogens*, 13(9), p. e1006512. doi: 10.1371/journal.ppat.1006512.

Smith, D. M., Simon, J. K. and Baker Jr, J. R. (2013) 'Applications of nanotechnology for immunology', *Nature Reviews Immunology*, 13(8), pp. 592–605. doi: 10.1038/nri3488.

Stegniy, B. T., Kovalenko, L. V., Ushkalov, V. O., Doletskyi, S. P., Romanko, M. Ye., Boiko, V. S., Matiusha, L. V. and Krotovska, Yu. M. (2007) *Methods for Estimating the Intensity of Lipid Peroxidation and its Regulation in Biological Objects: Methodological Recommendations [Metody otsinky intensyvnosti perekysnoho okysnennia lipidiv ta yoho rehuliatsii u biolohichnykh ob'iektakh: metodychni rekomendatsii]*. Kharkiv: NSC 'Institute of Experimental and Clinical Veterinary Medicine'. [in Ukrainian].

Van de Perre, P. (2003) 'Transfer of antibody via mother's milk', *Vaccine*, 21(24), pp. 3374–3376. doi: 10.1016/S0264-410X(03)00336-0.

Van Emden, H. F. (2019) *Statistics for Terrified Biologists*. 2nd ed. Hoboken, NJ: John Wiley & Sons. ISBN 9781119563679.

Weigert, A., Von Knethen, A., Fuhrmann, D., Dehne, N. and Brüne, B. (2018) 'Redox-signals and macrophage biology', *Molecular Aspects of Medicine*, 63, pp. 70–87. doi: 10.1016/j.mam.2018.01.003.