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SYSTEM OF PREVENTION OF METABOLIC SYNDROME IN HORSES USING A COMPLEX OF DISINFECTANTS AND PROBIOTICS

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Abstract. *The article discusses the results of research on the development of a system for the integrated use of a probiotic and two disinfectants in horse husbandry. The scheme, regimens and terms of application of spore probiotic in the feed and disinfectants in the presence of horses for 50 days for the prevention of metabolic syndrome are proposed. The doses and concentrations of the studied drugs are also summarized. The effect of the studied drugs on the hematological parameters of horses was analyzed.*

The results of the studies showed that feeding spore probiotics with feed and the use of disinfectants in their presence did not have a negative effect on the horse's body.

Production studies have determined the modes of disinfection of horse farm premises in the presence of horses, using iodine-based disinfectants " Iodesol" and "Geocide" based on benzalkonium chloride and deltamethrin, and established a bactericidal effect when disinfecting walls, floors and feeders of the premises when used in aerosol form. For prophylaxis, after thorough mechanical cleaning of the surfaces of the premises of the experimental group, once every 2 weeks, disinfection was carried out with a 0.2 % solution of "Iodesol" at the rate of 0.3 l of solution per 1 m^2until complete drying at an exposure time of 30 minutes. Also, once a week, the premises were disinfected using a cold fog generator, using the aerosol method with a 0.5 % working solution of the Geocide preparation for a one-hour exposure at a consumption of 0.05 l/m^3. Disinfection was carried out with ventilation turned on. Sanitary and hygienic studies were conducted according to current methods.

The bactericidal effect when disinfecting the surface of the room in the presence of horses during their maintenance after disinfection with disinfectants " Iodesol" and "Geocide" was almost 100%, which confirms their effectiveness.

Under the influence of the studied drugs, a positive effect on the blood parameters of horses was revealed. On day 35 of the study, the hemoglobin level in the experimental group exceeded the control values by 8.2 % ($p<0.05$), and on day 50 by 11.3 % ($p<0.05$). The number of red blood cells in the experimental group also increased during the same period: on day 35 it was 17.5% higher ($p<0.01$), and on day 50 by 12.2% ($p<0.01$) compared to the control.

At the same time, no significant changes in the number of leukocytes and the ratio of their individual species in the blood of horses of the experimental group compared to the control group were found. These data indicate that aerosol treatment of premises in the presence of horses is not toxic and has no negative effect on the physiological state, which is confirmed by studies of blood morphological parameters.

Key words: horses, probiotics, disinfectant, prevention, nonspecific resistance, hematological parameters, metabolic syndrome.

Introduction. Antibiotic resistance is an important problem of animal health worldwide due to the accelerated recovery of pathogens and their resistance to drugs, which complicates therapeutic measures.

A large number of different feed additives and preparations containing proteins, amino acids, vitamins, macro- and microelements, antibiotics and other biologically active substances are used to feed horses and balance diets in terms of nutrients, increase digestibility, metabolism, and prevent stressful conditions of animals (Mandal, 2021; Stefańska, 2021).

Methods of enriching diets with various additives are being developed to improve the digestibility of nutrients, especially the introduction of a mixture of biologically active substances into feed (Kovalenko, 2010; Schostel, 2014; Chechet, 2022).

The problem of probiotics' using for horses covers both scientific and practical aspects: efficacy, safety, composition and selection of strains, quality control, regulatory standards and potential risks (Jaimee, 2016; Markowiak, 2017).

Although probiotics for horses are widely advertised and used in practice, a convincing evidence base for their clinical efficacy remains insufficient. A review of the scientific and publication landscape shows that a number of studies demonstrate only partial or modest positive effects, such as improved fecal biochemical composition or reduced fecal enteropathogenic bacterial DNA without significant impact on clinical status or significant health outcomes (Ishizaka, 2014; Humphrey, 2013). In addition, some studies have a limited sample size and short follow-up period, which makes it difficult to extrapolate the results to the general horse population. Thus, the question of the validity of probiotic application remains open, and currently no specific strain or combination has been recognized as sufficiently effective or universally recommended for horses (Schoster, 2014; Ishizaka, 2014).

The microbiota of the equine gastrointestinal tract is highly individual and sensitive. The application of standard human or general veterinary probiotic species strains (e.g., *Lactobacillus spp., Bifidobacterium spp., Enterococcus spp*.) does not guarantee synergy with the microflora profile of a particular horse and may not provide the expected effect (Schoster, 2014). In addition, the mechanisms of action of probiotics are strain-specific, and mechanistic studies for horses have been performed only partially (Schoster, 2014; Plaza- Díaz, 2019; Wieërs, 2020). Compared to other animals, even successful colonization in the gastrointestinal tract of horses remains questionable, as most probiotic microorganisms do not become a stable part of their microbiome, but rather have a transient effect (Wieërs, 2020).

Studies of the mechanisms, both in veterinary and medical contexts, show that probiotics have a marked immunomodulatory effect. They affect the immune function of the mucosa by modulating cytokine profiles, stimulating secretory IgA production, and enhancing phagocyte activity, which can support host defense against pathogens (Azad, 2018; Plaza-Díaz, 2019; Chechet, 2022). In horses, these effects are thought to contribute to resistance to gastrointestinal infections or inflammation; however, most results are extrapolated from laboratory models, and clinical relevance for horses remains to be determined (Schoster, 2014).

Most probiotic strains used in veterinary medicine are considered safe, with minimal reports of adverse reactions in healthy animals (Schoster, 2014; Liong, 2008). However, caution should be exercised in immunocompromised horses, as there are rare but reported cases of infections associated with probiotic use, even in healthy horses (Ishibashi, 2001; Liong, 2008; Ibrahim, 2021). In addition, the equine gut acts as a reservoir for antibiotic resistance genes, and horizontal gene transfer can occur between probiotics and commensal or pathogenic bacteria, raising concerns about the potential contribution of probiotics to the spread of antibiotic resistance (Daniali, 2020).

Another issue is the quality and regulatory oversight of probiotics effects for horses. Many over-the-counter products do not have strict standardization in terms of strain content and viability, which makes their efficacy and safety unpredictable (Henriksson, A, 2005).

Our previous studies have established the effectiveness of the complex use of biocidal preparations, especially in the presence of horses, in order to improve the quality of the microbial background.

Probiotics and biocidal preparations are a prospect of replacing antibiotics used as a feed additive. However, there is no research on their integrated use in the technological process of horse keeping. Especially in the general technological scheme, which includes: preliminary disinfection of premises, introduction of probiotics into the diet, disinfection in the presence of horses (Guarner, 2008; Tang, 2017). This new method is interesting and therefore needs to be investigated and implemented in production in order to improve the prevention of horse keeping.

The aim of the study was to investigate in production conditions in the presence of horses the biocidal preparations "Iodesol" of the private enterprise "Kronos Agro" and "Geocide" of LLC SPE "Ecological Capital" for aerosol disinfection and a spore probiotic preparation in the feed on the hematological profile, the viability of the horse's body.

Materials and methods. The study was conducted in the conditions of the horse breeding farm of PE Rymchuk, Bashtanka district, Mykolaiv region, as well as in the conditions of the Institute of Veterinary Medicine (Kyiv) and the National Scientific Center "Institute for Experimental and Clinical Veterinary Medicine" (Kharkiv). The horses were kept in animal facilities with free access to feed and water, and the technological parameters of their rearing (temperature and light conditions) were observed in accordance with the standards. For the research, 2 groups of horses were formed (control and experimental), 7 animals per group. For the experiments, the animals were selected according to the principle of analogous pairs, taking into account age, live weight, sex and physiological condition, the average age of the animals was 5.4 ± 2.3 years, body weight was 505 ± 45.3 kg.

The premises were kept clean at all times. The walls and floors of the premises were well cleaned and washed before the horses were introduced. Feeders, drinkers, lighting fixtures, and thermometers were disinfected. Sawdust bedding was used on the floor.

The experiment was carried out as follows: during the maintenance of horses for prophylaxis, once every 2 weeks after thorough mechanical and sanitary cleaning of the surfaces of the objects, the room was disinfected in the presence of horses with "Iodesol". A 0.2 % aqueous solution of the product was prepared at the rate of 0.3 l of solution per 1 m^2until it dried completely at an exposure time of 30 min. Once a week in the experimental group, the premises were disinfected using a cold fog generator, using the aerosol method with a 0.5 % working solution of "Geocide" for a one-hour exposure at a consumption of 0.05 l/m^3. Disinfection was carried out with ventilation turned on. The products are intended for preventive, current and final disinfection in the presence of animals. These products have high bactericidal, virucidal, and fungicidal properties (Kovalenko, 2020). Bactericidal control of the premises was carried out before and after disinfection: washing off the walls, floor, and feeders. Sanitary and hygienic studies were performed according to current methods (Kotsyumbas, 2019).

Horses in the control group were fed a standard diet according to the existing standards recommended for horses.

A spore probiotic was used in the experiment: the experimental animals were administered orally at a dose of 20 billion CFU/ml. CFU/ml by mixing with a small amount of feed in the morning for 45 days. The spore probiotic includes 5 strains: *Bacillus subtilis* UNCSM - 020, *Bacillus licheniformis* UNCSM - 033, *Bacillus amyloliquefaciens* ALB 65, *Bacillus pumilus* UNCSM - 026, *Bacillus subtilis variant mesentericus* UNCSM - 031 (Koltchyk, 2025).

Bacteriological studies of sanitary flushes were performed in accordance with DSTU EN 1656:2009. To conduct the rinses, swabs were made on wire rods embedded in a cotton gauze cork, which closed the tube, with distilled water previously poured into it in a volume of 2.0 cm^2, and sterilized in an autoclave at a pressure of 0.5 atmospheres for 30 minutes (Harkavenko, 2020). Samples of flushes from the surfaces of technological equipment and inventory were collected from an area of 100 cm^2using a metal frame-stencil measuring 10 × 10 cm, which limited the required area. Before each application to the surface of the object

under study, the stencil frame was flamed on an alcohol flame. When the area was limited to 100 cm^2 of the test surface was wiped with a swab on a rod dipped in distilled water and reinserted into the tube (DSTU EN 1040:2004).

In whole blood, the following were determined: hemoglobin concentration - by the hemiglobin cyanide method; total number of leukocytes and erythrocytes - by counting under a microscope in a chamber with a Goryaev grid; Biochemical studies were performed according to generally accepted methods as described in the reference book (Kotsiumbas, 2006; Vlizlo, 2012).

Statistical analysis of the data was performed using Minitab 19 and Minitab Inc. Based on the results of statistical processing, the tables show the following indicators: Mean ± SD, A significant difference between the study groups was established based on the calculation of the Mann-Whitney test ($P < 0.05$).

Results. In order to improve the immune system of horses, improve the quality of cleanliness of premises against microorganisms, a complex of biocidal preparations "Iodesol" and "Geocide" for aerosol disinfection and a spore probiotic preparation with a complete rejection of antibiotics in the experimental group were used.

In the general scheme of keeping horses in the control group, their usual natural state, feed consumption, and the formation of unstable immunity were observed.

In the experimental group, when using a spore probiotic preparation in the feed and disinfecting the premises in the presence of horses according to the scheme with the drugs "Jodezol" and "Geocide", which showed very high preventive efficacy. A better clinical condition was observed in comparison with the control group in terms of hematological profile.

The analysis of the results showed that the concentration of hemoglobin in the blood of horses of the control group gradually increased with age (Table 1). At the same time, changes in the number of red blood cells in the blood of animals of this group during the study period were less pronounced.

Table 1

Hematological profile of the studied horses (Mean ± SD, n=7)

Indicator	Group	7 days	21 days	35 days	50 days
Hemoglobin, g/l	Control (C)	115,4 ± 2,8	117,2 ± 2,5	118,1 ± 2,3	119,0 ± 2,6
	Experimental (E)	116,7 ± 2,6	121,9 ± 2,7	127,8 ± 3,1 *	132,4 ± 2,9 *
Erythrocytes, T/l	Control (C)	6,85 ± 0,19	6,72 ± 0,22	6,91 ± 0,17	7,03 ± 0,21
	Experimental (E)	6,91 ± 0,24	7,34 ± 0,26	8,12 ± 0,30 **	7,89 ± 0,28 **

Note: In this and the following tables: * - statistically significant differences between the studied indicators in horses of the experimental group compared to the control group: * - $p<0.05$; ** - $p<0.01$

The use of the complex of drugs in the presence of horses of the experimental group led to an increase in the concentration of hemoglobin and an increase in the number of red blood cells in the blood. Thus, on day 35 of the study, the hemoglobin level in the experimental group exceeded the control values by 8.2 % ($p<0.05$), and on day 50 by 11.3 % ($p<0.05$). The number of red blood cells in the experimental group also increased during the same period: on day 35 it was 17.5% higher ($p<0.01$), and on day 50 by 12.2% ($p<0.01$) compared to the control.

The data obtained indicate a positive effect of the studied complexes of drugs on the processes of hematopoiesis and improvement of oxygen supply to the tissues of the horse body.

The general dynamics of changes in leukocytes in the blood is one of the important indicators for the diagnostic determination of the condition of animals, but for a complete analysis of morphological changes in the blood of animals, a differential count of the ratio of individual types of blood leukocytes is performed. According to the results of the research (Table 2), it was found that the total number of leukocytes in the blood of horses of the control

group increased during the experiment, which corresponds to physiological norms. At the same time, during the study period, no significant changes were found in the number of eosinophils, lymphocytes and blood monocytes in the control group.

Table 2

Blood leukogram of horses, % (Mean ± SD; n=7)

Indicator	Group	7 days	21 days	35 days	50 days
Leukocytes, G/l	Control (C)	8,9 ± 0,42	9,5 ± 0,38	10,2 ± 0,45	11,3 ± 0,47
	Experimental (E)	9,2 ± 0,50	10,4 ± 0,52	11,6 ± 0,48 *	12,8 ± 0,51 *
Basophils, %.	Control (C)	0,4 ± 0,05	0,4 ± 0,05	0,3 ± 0,04	0,3 ± 0,05
	Experimental (E)	0,3 ± 0,04	0,3 ± 0,04	0,3 ± 0,04	0,3 ± 0,04
Eosinophils, %.	Control (C)	1,7 ± 0,14	1,9 ± 0,16	2,0 ± 0,17	2,0 ± 0,16
	Experimental (E)	1,8 ± 0,15	2,5 ± 0,19 *	2,3 ± 0,17	2,1 ± 0,15
Neutrophils, %.	Control (C)	44,6 ± 1,7	44,0 ± 1,6	42,5 ± 1,5	41,4 ± 1,7
	Experimental (E)	45,9 ± 1,9	44,5 ± 1,6	41,2 ± 1,7	42,1 ± 1,6
Lymphocytes, %.	Control (C)	52,3 ± 1,5	52,9 ± 1,6	53,4 ± 1,5	53,3 ± 1,5
	Experimental (E)	52,1 ± 1,6	50,8 ± 1,5	50,7 ± 1,6	49,6 ± 1,6 *
Monocytes, %.	Control (C)	0,9 ± 0,05	0,8 ± 0,04	0,8 ± 0,05	0,8 ± 0,04
	Experimental (E)	0,9 ± 0,05	0,9 ± 0,05	0,9 ± 0,04	0,9 ± 0,04 *

During the experiment, the total number of leukocytes in the blood of horses of the experimental group increased compared to the control group. It should be noted that at the same time, the ratio of different types of leukocytes in horses of the experimental group on days 35 and 50 showed a decrease in the relative number of lymphocytes and an increase in monocytes. The percentages of neutrophils, basophils and eosinophils fluctuated but remained stable within physiological norms. The results indicate a positive effect of the complex of disinfectants and probiotic on the immune system of the horse body, since monocytes and lymphocytes are the main immunocompetent cells to protect the body and maintain immune homeostasis.

Thus, the results of the research indicate the effectiveness of the complex use of drugs for horses to improve their living conditions. The drugs used contributed to better absorption of feed nutrients, which had a positive effect on their immune status and viability.

When disinfecting the premises in the presence of horses with “Iodesol” and “Geocide” on the surfaces, the total bacterial contamination decreased by 99.9% (Table 3).

As a result of production experiments to study the effectiveness of the use of a complex of disinfectants for preventive and forced disinfection in the room (wall, floor, feeders) in the presence of horses, it was confirmed that their effectiveness is manifested in 0.2% "Iodesol" and 0.5% "Geocide" concentrations, at a solution consumption of 200 ml/m^2.

Thus, according to the results of the research, it was found that the spore probiotic preparation has a positive effect on the immunity of the horse's body, and the bactericidal preparations "Iodesol" and "Geocide" effectively affect the stabilization of the microbial background of the room in their presence.

Table 3

Efficiency of premises disinfection in the presence of horses with bactericidal preparations "Iodesol" and "Geocide" (n=5)

Disinfection objects and microbial test cultures microbial test cultures	Bacterial contamination of the surface, 10^3CFU/cm^2		
	Before disinfection	After disinfection	% disinfection
Surface of the room			
Wall	0,8±1,12	<10*	100
Floor	1,5±1,01	25	99,9
Feeding troughs	1,1±1,11	0	100

Note: *- p≤0.05 - against the initial values

Discussion. The use of disinfectants based on iodine, benzalkonium chloride and others makes it possible to effectively disinfect premises.

It is known that the stabilization of normal microflora in the intestine of animals affects their condition, avoidance of immunodeficiencies, stimulation of body resistance (Volosianko, 2006; Klaenhammer, 2012), maintaining a constant balance of the quantitative composition of the microbial ecosystem and the conditions of its stable existence. This is made possible by the use of environmentally safe probiotic preparations and bactericidal preparations "Iodesol" and "Geocide" throughout the entire period of keeping horses under this scheme.

Our research has revealed the complex effect of the drugs on the hematological profile and indicators of immune defense of the horse's body. The results of morphological and biochemical studies showed that in the control group, changes in the number of red blood cells and hemoglobin concentration in the blood increased, indicating physiological changes in their body during the maintenance process (Zhang, 2012; Zhang, 2003).

Since the quantitative and qualitative composition of peripheral blood is maintained at a certain level and reflects the physiological or pathological state of the organism, the degree of reactivity and resistance of animals to exogenous factors (Flehontova, 2004), respectively, the results obtained indicate the absence of disturbances in the functioning of homeostatic systems in the body of horses under the influence of the studied drugs. At the same time, the stated trends indicate an increase in protective functions in the body of horses under the influence of the studied complex of drugs. It is known that monocytes are the precursors of immunocompetent cells, they are involved in phagocytosis, and lymphocytes are key cells for the immune function of the body. These results are partially consistent with the data available in the literature on the stimulating effect of probiotic preparations on erythropoiesis and hemoglobin synthesizing function of the body, on the processes of cell proliferation and differentiation (Klaenhammer, 2012).

In general, the studies have shown that the use of these drugs in horses had a positive effect on hematological parameters, which contributed to the strengthening of immunity and the growth of their immune status. From this point of view, an integrated approach to the use of the studied drugs can be considered as a new promising direction of increasing the immune potential by reducing the influence of indoor microorganisms and improving the microbiota of the gastrointestinal tract of horses.

Conclusions. The results of the research have established the positive effect of a complex of disinfectants and probiotics on hematological parameters of horse blood to ensure the protection of the body and maintain immune homeostasis.

In studies using environmentally safe disinfectants "Yodezol" for aerosol disinfection at 0.2% and "Geocide" at 0.5% concentrations and spore probiotic, a scheme for increasing economic efficiency and profitability of keeping was substantiated.

The developed scheme of disinfection with the use of a probiotic preparation for horses during the period of life in industrial conditions provided optimal protection of the body from various pathogenic factors by 100% without the use of antibiotics and increased immunity.

Perspectives for further research. The next stage of work is to determine the quality and safety of horse breeding products after the use of disinfectants and probiotic preparations.

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СИСТЕМА ПРОФІЛАКТИКИ МЕТАБОЛІЧНОГО СИНДРОМУ КОНЕЙ ПРИ ВИКОРИСТАННІ КОМПЛЕКСУ ДЕЗІНФІКУЮЧИХ ЗАСОБІВ ТА ПРОБІОТИКІВ.

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Резюме. *У статті обговорюються результати дослідження розробки системи комплексного застосування пробіотика та двох дезінфікуючих засобів при утриманні коней. Пропонується схема, режими та терміни застосування спорового пробіотика в складі корму і дезінфікуючих засобів у присутності коней упродовж 50 діб для профілактики метаболічного синдрому. Також узагальнено дози та концентрації досліджуваних препаратів. Проаналізовано вплив досліджуваних препаратів на гематологічні параметри коней.*

Результати проведених досліджень показали, що згодовування разом з кормом спорового пробіотика та застосування дезінфікуючих засобів у їх присутності не мало негативного впливу на організм коней.

Виробничими дослідженнями визначено режими дезінфекції приміщень конеферми у присутності коней, під час використання дезінфікуючих засобів «Йодезоль» на основі йоду та «Геоцид» на основі бензалконія хлориду та дельтаметрину і встановлено бактерицидний ефект при дезінфекції стін, підлоги та годівниць приміщення за їх аерозольного застосування. З метою профілактики після ретельної механічної

очистки поверхонь приміщень дослідної групи один раз на 2 тижня проводили знезараження 0,2 %-й розчином препарату «Йодезоль» з розрахунку 0,3 л розчину на 1 м^2 до повного висихання за експозиції 30 хв. А також один раз на тиждень здійснювали дезінфекцію приміщень за допомогою генератора холодного туману, аерозольним методом 0,5 % робочим розчином препарату «Геоцид» за одногодинної експозиції при витраті засобу 0,05 л/м^3. Дезінфекцію здійснювали при включеній вентиляції. Санітарно-гігієнічні дослідження проводили згідно чинних методик.

Бактерицидний ефект при дезінфекції поверхні приміщення в присутності коней під час їх утримання після проведення дезінфекції дезінфікуючими препаратами «Йодезоль», «Геоцид» становило майже 100 %, що підтверджує їх ефективність.

За дії досліджуваних препаратів виявлено позитивний вплив на показники крові коней. На 35 добу дослідження рівень гемоглобіну в дослідній групі перевищував контрольні значення на 8,2 % ($p<0,05$), а на 50 добу на 11,3 % ($p<0,05$). Кількість еритроцитів у дослідній групі в цей же період також зростала: на 35 добу вона була вищою на 17,5 % ($p<0,01$), а на 50 добу на 12,2 % ($p<0,01$), порівняно з контролем.

При цьому вірогідних змін у кількості лейкоцитів і співвідношення окремих їх видів у крові коней дослідної групи стосовно контрольної не виявлено. Ці дані свідчать, що аерозольна обробка приміщень у присутності коней не токсична та не має негативного впливу на фізіологічний стан, що підтверджено дослідженнями морфологічних показників крові.

Ключові слова: коні, пробіотики, дезінфектант, профілактика, неспецифічна резистентність, гематологічні показники, метаболічний синдром.

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