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Effect of phytobiotic - Germivit on the functional state of cattle

Efecto del fitobiótico - Germivit sobre el estado funcional del ganado

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ABSTRACT

Keywords:
Cattle
Feed supplement
Germivit
Immunity
Metabolism

Plant-based feed additives, also known as phytobiotics, show great promise to compensate deficiencies of important biologically active substances in the diet. Therefore, this study aimed to evaluate the effect of Germivit on the feed supplement of cattle of different ages. In the first experiment, four groups of 9-month-old Simmental calves (10 animals in each group) were fed with Germivit at 0.0, 0.5, 0.7, and 0.9 g kg^{-1} live body weight, respectively. In the second experiment, three groups of pregnant cows (10 animals in each group) received Germivit at 0.0, 0.25, and 0.50 g kg^{-1} live body weight, respectively. The morphological composition of the collected blood was studied using a PCE-90vet automatic hematology analyzer. The blood biochemical composition was studied using a Stat Fax 1904 biochemical analyzer, and the immunological status of the animals was evaluated using generally accepted methods in veterinary medicine: immunocompetent cells by spontaneous rosette formation; immunoglobulins by the radial immunodiffusion method; phagocytosis by using *S. aureus* culture; serum lysozyme activity by the photoelectrocolorimetric method; and bactericidal activity of serum by determining the degree of growth inhibition of the mixture of daily culture of *E. coli* in a nutritive culture broth. Germivit contributed to the improvement of the biochemical and immunological parameters in the calves. In the cows, an increase in the morphological parameters of blood was observed, their immune status improved, and their calves were born with high rates of natural resistance and health. Germivit had a positive effect on the functional state of cattle.

RESUMEN

Palabras clave:
Ganado
Suplemento alimenticio
Germivit
Inmunidad
Metabolismo

Los aditivos de origen vegetal para alimento animal, también conocidos como fitobióticos, son muy prometedores para compensar una deficiencia de sustancias biológicamente activas importantes en la dieta. Este estudio tuvo como objetivo evaluar el efecto del Germivit como suplemento alimenticio del ganado de diferentes edades. En el primer experimento, cuatro grupos de terneros (10 animales en cada grupo) Simmental de 9 meses de edad fueron alimentados con Germivit a una dosis de 0,0; 0,5; 0,7 y 0,9 kg^{-1} de peso corporal vivo, respectivamente. En el segundo experimento, tres grupos de vacas preñadas (10 animales en cada grupo) recibieron Germivit a una dosis de 0,0; 0,25 y 0,50 g kg^{-1} de peso corporal vivo, respectivamente. La composición morfológica de la sangre recogida se estudió utilizando un analizador hematológico automático PCE-90vet. La composición bioquímica de la sangre se estudió utilizando un analizador bioquímico Stat Fax 1904, y el estado inmune de los animales se evaluó utilizando métodos generalmente aceptados en veterinaria: células inmunocompetentes por formación espontánea de rosetas; inmunoglobulinas por el método de inmunodifusión radial; fagocitosis usando cultivo de *S. aureus*; actividad de la lisozima sérica por el método fotoelectrocolorimétrico; y actividad bactericida del suero determinando el grado de inhibición del crecimiento de la mezcla de cultivo diario de *E. coli* en un medio nutritivo líquido. Germivit contribuyó a una mejora de los parámetros bioquímicos e inmunológicos en los terneros. En las vacas se observó un aumento de los parámetros morfológicos de la sangre, mejoró su estado inmunológico y sus terneros nacieron con altos índices de resistencia natural y salud. Germivit tuvo un efecto positivo sobre el estado funcional del ganado.

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At the animal husbandry stage of development, special attention should be paid to the feed in order to obtain the maximum genetic potential of animal productivity. The most antimicrobial agents by their nature can be attributed to xenobiotics in relation to the body of the animal. Depending on the dose, they can exhibit toxic effects, have an immunosuppressive effect, disrupt metabolic processes, promote intestinal dysbiosis, and accumulate and pollute raw materials in animal products. This makes necessary to find preparations and feed additives devoid of these negative properties and introduce them into the practice of feeding productive animals (Trukhachev *et al*., 2015).

The attention of researchers and practitioners has recently turned to plant-based feed additives, also known as phytobiotics, which have diverse positive effects on the animal body. Plants contain a complex of biologically active substances that have low toxicity and act comprehensively. They also offer economic benefits, particularly in terms of the availability and low prices of plant materials as well as the relatively uncomplicated technology for the preparation of phytobiotics (Franciosini *et al*., 2016; Mohiti-Asli and Ghanaatparast-Rashti, 2017). Numerous scientific studies have shown the positive effects of phytobiotics on the productivity and quality of livestock while reducing the cost of feed for unit of production. These positive effects are achieved due to the high level of vitamins, minerals, flavonoids, essential oils, phytoncides, polysaccharides, tannins, among others, in many plants (Castillo-López *et al*., 2017; Mohammadi Gheisar and Kim, 2018; Oguey and Wall, 2016; Windisch *et al*., 2008).

The biologically active substances of plants which have immuno-stimulating activity, are able to suppress pathogenic microbiota, have antioxidant effect, and improve the digestibility of feed nutrients (Borda-Molina *et al*., 2018; Stanley *et al*., 2014). The ability of phytobiotics to improve the chemical composition and increase the biological value of meat has also been established (Ramiah *et al*., 2014). Furthermore, herbal preparations, which often contain probiotics, have a positive effect on the microbiota of the gastrointestinal tract of animals (Currò *et al*., 2017; Drouillard, 2018; Samii *et al*., 2016; Valero *et al*., 2014). The inclusion of phytobiotics in the diet of poultry, cows, young cattle, and sheep has shown to increase their productivity (Al-Yasiry *et al*., 2017; Brogna *et al*., 2014; Kumar *et al*., 2014; Samii *et al*., 2016; Tosi *et al*., 2013; Wall and Bravo, 2016), and herbal feed additives can serve as a worthy substitute for synthetic drugs, especially antibiotics (Friedman *et al*., 2002). When choosing feed additives, preference should be given to drugs and biologically active substances that do not pollute the environment, do not accumulate in the animal's body, are capable of being rapidly metabolized, improve the metabolism, and have a positive effect on animal productivity and livestock production quality.

Germivit produced by the company "Pink-Lotus", is a preparation obtained from wheat germ that takes the form of a beige powder. The product contains minerals (potassium, calcium, magnesium, sodium, phosphorus, iron, copper, manganese, zinc), vitamins (B1, B2, B5, B3, B6, B12, E, A, D), amino acids (alanine, arginine, aspartic acid, valine, histidine, glycine, leucine, isoleucine, lysine, methionine, proline, serine, tyrosine, threonine, phenylalanine, cysteine), and polyunsaturated fatty acids (arachidonic, behenic, linoleic, linolenic, myristic, oleic, palmitic, erucic) (Pink-Lotus, 2020). The latter are the precursors of prostaglandins, which take part in the neurohumoral regulation of the tissues and systems of the body. Germivit has antitoxic, antioxidant, and hepatoprotective effects, has immuno-stimulating properties, and has positive effects on the reproductive function of animals. Furthermore, experiments on newborn calves, pigs, and poultry have shown a high therapeutic and prophylactic activity of Germivit in animal diseases (Donnik *et al*., 2010).

The phytobiotic Germivit meets several aspects of a healthy animal diet. However, the effect of this food supplement on the bodies of cows and calves has not been studied. Therefore, this study aimed to examine the effect of the Germivit feed supplement on cattle of different ages.

MATERIALS AND METHODS

This research consisted of Simmental breed cattle, which were bred in farm conditions of the Orenburg region of the Russian Federation. Animal care and experimental studies were performed in accordance with the instructions and recommendations of Russian

Regulations, 1987 (Order No. 755 on 12.08.1977 the USSR Ministry of Health) and "The Guide for Care and Use of Laboratory Animals (National Academy Press Washington, D.C. 1996)". When performing the research, efforts were made to minimize the suffering of the animals and to reduce the number of samples used.

Two experiments were carried out; in the first one, the effect of different doses of Germivit on the bodies of calves was studied. For this purpose, four groups of 9-month-old calves were formed: three experimental groups and one control group – each group contained 10 animals. The animals of the first experimental group were fed from the 9 months of age with Germivit once a day at a dose of 0.5 g kg^{-1} live weight, with the morning feeding along with concentrated food. For the animals of the second experimental group, the dosage was increased up to 0.7 g kg^{-1}, and for the third experimental group, it was 0.9 g kg^{-1}. The animals of the control group did not receive Germivit. The experiment continued until the animals were 18 months of age. Blood samples were taken from the jugular vein of 9-, 15-, and 18-month calves to assess the effect of Germivit on their body.

The second experiment consisted of evaluating the effect of Germivit on the body of cows 4-5 years-old two months before calving, as well as its effect on the newborn calves. For this, two experimental groups and one control group with 10 animals each were formed. The cows of the first experimental group for two months daily received Germivit once a day at a dose of 0.25 g kg^{-1} of live weight, while for the animals of the second experimental group, the dose was increased to 0.50 g kg^{-1}. The Germivit was provided every day in the morning with concentrated feed for two months. The cows from the control group did not receive any Germivit. Blood sampling was performed on the cows at 60, 30, and 10 days before calving and 7 days after giving birth. Blood was also taken from the calves obtained from the cows of the experimental and control groups at the ages of 1 day and 1 month. In addition, any case of gastrointestinal pathology in the calves and any calf mortalities over 30 days were recorded.

The morphological composition of the collected blood was studied using a PCE-90vet automatic hematology analyzer (USA), the blood biochemical composition was studied using a Stat Fax 1904 biochemical analyzer (USA), and the animals' immune status was evaluated using methods generally accepted in veterinary medicine: immunocompetent cells by spontaneous rosette formation; immunoglobulins by the method of radial immunodiffusion; phagocytosis by using a *S. aureus* culture; serum lysozyme activity by the photoelectrocolorimetric method; and bactericidal activity of serum by determining the degree of growth inhibition of the mixture of daily culture of *E. coli* in a liquid nutrient medium (Shakhov, 2015). Statistical processing of the obtained data was carried out using the SPSS Statistics software package. During static data processing, the mean, the standard deviation and the Student's t-test were calculated.

RESULTS AND DISCUSSION

First Experiment

The biochemical composition of the blood in the calves is shown in Table 1.

The inclusion of Germivit in the diet of calves contributed to a significant increase in the amount of total protein in the blood of the animals. At the age of 15 months, the young cattle of the first experimental group had a higher total protein content in the blood serum (1.64% ($P<0.01$)) than the animals from the control group. For the animals of the second experimental group, it was 2.14% ($P<0.01$) higher, and of the third experimental group, it was 2.05% ($P<0.01$) higher. By the end of the experiment, i.e., at 18 months of age, the animals of the test groups had retained an advantage in relation to the amount of total protein in the blood compared to the animals in the control group. For the first experimental group, it was 1.22% ($P<0.05$); for the second one, it was 2.00% ($P<0.001$), and for the third experimental group, it was 2.05% ($P<0.001$).

Glucose is the main source of energy for the cells of the body. When assessing the carbohydrate metabolism in the calves, it was found that feeding Germivit contributed to higher levels of blood glucose at the age of 15 months, with 3.00 mmol L^{-1} in the control group and 3.11 mmol L^{-1} in the second experimental group an increase of 3.67%. By the age of 18 months, the maximum values of glucose were recorded in animals of the third experimental group, which had 4.23% ($P<0.05$)

more blood glucose than the control group. The animals of the first and second experimental groups had 3.26% (*P*<0.05) and 3.91% (*P*<0.05) higher glucose contents, respectively, than the control calves.

Table 1. Biochemical composition of the blood of the calves fed with Germivit daily.

Indicators	Germivit (g kg^{-1} live weight)	Age (months) 9	15	18
Total protein (g L^{-1})	0.0	67.93±0.111	67.75±0.311	67.96±0.101
	0.5	67.96±0.131	68.86±0.172 **	68.79±0.321 *
	0.7	68.02±0.152	69.20±0.163 **	69.32±0.210 ***
	0.9	67.94±0.154	69.14±0.214 **	69.35±0.193 ***
Glucose (mmol L^{-1})	0.0	3.03±0.032	3.00±0.084	3.07±0.022
	0.5	3.01±0.067	3.11±0.011	3.17±0.021 *
	0.7	3.01±0.063	3.11±0.007	3.19±0.011 *
	0.9	3.02±0.055	3.11±0.011	3.20±0.035 *
Total bilirubin (µmol L^{-1})	0.0	3.93±0.122	3.97±0.113	3.96±0.115
	0.5	3.92±0.101	3.93±0.214	3.77±0.024
	0.7	3.91±0.131	3.95±0.180	3.76±0.032
	0.9	3.93±0.096	3.94±0.222	3.75±0.031
Uric acid (µmol L^{-1})	0.0	162.40±1.367	162.90±1.128	164.30±0.251
	0.5	161.40±1.842	162.50±1.325	162.50±0.743
	0.7	161.80±1.771	162.50±1.516	162.80±0.521
	0.9	162.00±1.691	162.70±1.233	162.90±0.556
Cholesterol (mmol L^{-1})	0.0	2.54±0.131	2.63±0.042	2.66±0.042
	0.5	2.56±0.083	2.62±0.032	2.52±0.061 **
	0.7	2.52±0.091	2.64±0.051	2.53±0.021 *
	0.9	2.51±0.082	2.63±0.022	2.54±0.012 *
Triglycerides (mmol L^{-1})	0.0	0.32±0.033	0.35±0.023	0.39±0.009
	0.5	0.31±0.033	0.31±0.007	0.35±0.008 *
	0.7	0.33±0.031	0.30±0.006	0.35±0.011 *
	0.9	0.31±0.031	0.30±0.011 *	0.34±0.011
Total lipids (g L^{-1})	0.0	4.67±0.091	4.47±0.082	4.70±0.122
	0.5	4.63±0.084	4.43±0.091	4.80±0.062
	0.7	4.70±0.101	4.17±0.033 *	4.77±0.194
	0.9	4.73±0.122	4.20±0.101	4.70±0.211

* *P*<0.05; ** *P*<0.01; *** *P*<0.001.

Among the biochemical indicators, the concentration of total lipids is one of the objectives, and it characterizes the metabolism level and the functional state of the body. At the age of 15 months, a decrease in the amount of total lipids in the blood was established in the calves of the experimental groups in comparison with the control group. In the calves of the second and third experimental groups, which were fed Germivit at a dose of 0.7 and 0.9 g kg^{-1}, respectively, the decrease was more noticeable, with differences of 6.04 and 6.71%, respectively. At the same time, the decrease was minimal in the blood of the calves of the first experimental group, at 0.89%. However, by the age of 18 months, there were no significant differences in the number of lipids between the animals of the control and experimental groups. The indicator was in the range of 4.70 to 4.80 g L^{-1}.

The content of cholesterol in the blood is closely related to lipid metabolism. Feeding of the calves by Germivit had a noticeable effect on blood cholesterol only at the age of 18 months. In control group, the blood cholesterol was 5.29% (P<0.05) higher than the calves of the first experimental group and higher than that of the second and third experimental groups by 4.89% (P<0.05) and 4.51% (P<0.05), respectively.

Bilirubin is an important indicator of the functional state of the liver. No significant differences were recorded in the amount of blood bilirubin among the calves of the experimental groups throughout the experiment. At the same time, it is important to note that while at the age of 15 months, the difference between the animals fed Germivit and the control animals was in the range of 0.50% to 1.01%, then at the age of 18 months it was 4.79% to 5.30%.

The product of the purine metabolism is uric acid, and a change in the amount of uric acid in the blood is a diagnostic sign for kidney disease. Feeding the animals Germivit did not affect the amount of uric acid in their blood throughout the experiment. At the age of 15 months and 18 months, the differences between the representatives of different groups were 0.12% to 0.25% and 0.85% to 1.09%, respectively.

Triglycerides are derivatives of glycerol and higher fatty acids. Elevated triglycerides in the blood may indicate liver disease in animals. Under the influence of Germivit, the animals of the first experimental group showed an 11.43% decrease in blood triglycerides at the age of 15 months, and at the age of 18 months, it reached 10.26% (P<0.05) compared to the control animals. The animals of the second experimental group were lower than the control animals in terms of the quantitative content of triglycerides in the indicated period of study at 14.29% and 10.26% (P<0.05), while the animals of the third experimental group showed a decrease of 14.29% (P<0.05) and 12.82% (P<0.05).

In the experiment with calves, the inclusion of Germivit in the diet contributed with a significant increase in the amount of total protein in the serum. Along with protein, an improvement in carbohydrate metabolism was also observed in the animals. A particularly noticeable increase in blood glucose was observed at 18 months of age in the animals of the experimental groups compared to the control animals. When assessing the lipid metabolism, at the age of 15 months, the amount of total lipids in the blood of the calves of the experimental groups had decreased, when compared with control animals, but at the age of 18 months, it was restored to the control values. During this period, Germivit-fed calves showed a significant decrease in blood cholesterol. Based on the content of bilirubin and triglycerides in the blood, it is possible to assess the clinical condition of the liver in the animals. In experiments, feeding Germivit to calves at doses of 0.5, 0.7 and 0.9 g kg^{-1} live weight reduced the amount of bilirubin and triglycerides in the blood, which may indicate a hepatoprotective effect of the feed additive. Germivit did not adversely affect the kidneys; an indicator of kidney disease is a change in the amount of uric acid and between the control and experimental animals, there were no differences in the amount of uric acid in the blood during all periods of the study.

Al-Yasiri *et al.* (2017) found that the uric acid content, the activity of aspartate aminotransferase and alkaline phosphatase in blood plasma decreased in broiler chickens when phytobiotic was included in their diet. Brogna *et al.* (2014) showed no significant metabolic changes in lambs using phytobiotic. Shawle *et al.* (2016) noted a significant decrease in the concentration of glucose, triglycerides and cholesterol in the serum of broilers with a phytobiotic diet. Karásková *et al.* (2015) found a significant reduction in total cholesterol in broiler chickens. Hao *et al.* (2014) the effect of partially purified red yeast rice extract was determined by Xuezhikang (XZK) for lipoproteins. A decrease in total cholesterol was found.

Autoimmune processes, immune defense in infections, and invasive pathologies are the main reactions in which T-lymphocytes are involved. B-lymphocytes that transform into plasma cells that synthesize antibodies are responsible for humoral immunity in the animal body. At the beginning of the experiment, the content of T-lymphocytes in the blood of the animals in all test groups was 28.81% to 29.23%, while the content of B-lymphocytes was 13.61% to 14.22%.

By the age of 15 months, the number of T-lymphocytes in the calves' blood in the control group was 29.2±1.07%, which is 3.01% less than in the animals of the first experimental group, 3.22% less than in animals of the second experimental group, and 4.00% (P<0.01) less than in the animals of the third experimental group. By the time the experiment was completed, the difference in the number of T-lymphocytes for the animals in experimental groups was 4.61% (P<0.001), 4.82% (P<0.01), and 4.21% (P<0.001), respectively.

Similar changes were observed when counting B-lymphocytes. In the animals of the first experimental group, the number of B-lymphocytes exceeded the control values at 15 and 18 months of age by 1.21% and 4.02% (P<0.05), respectively, and in the animals of the second experimental group, the differences with animals of the control group were 1.01% and 4.22% (P<0.001), and for the third experimental group, the values were 1.43% (P<0.01) and 4.41% (P<0.01).

Immunoglobulins have an important function in maintaining the body's homeostasis. The largest role in protecting animals against infectious agents belongs to immunoglobulins G (IgG), which includes antibodies that can neutralize viruses and bacteria (Shakhov, 2015). Another factor of the humoral defense of the body against infection is immunoglobulins M (IgM), which is the first formes in the primary immunological reaction and determines the leading position in the formation of immunity in animals (Shakhov, 2015). IgM antibodies are significantly more effective in hemolysis reactions than IgG antibodies.

In the calves of the experimental groups at the age of 9 months, i.e., before starting of the Germivit feeding, the content of immunoglobulins in the blood was at the same level, amounting to IgG 18.65-19.14 g L^{-1} and IgM 3.45-3.57 g L^{-1} (Table 2). However, at the age of 15 months, the calves of the experimental groups showed a significant increase in serum immunoglobulins compared to the control calves. In the first experimental group during this period, the content of IgG increased by 19.81% (P<0.05) and the IgM increased by 12.40% (P<0.05), in animals of the second experimental group these increased by 13.72% (P<0.05) and 16.11% (P<0.05), respectively, and in the third group by 18.73% (P<0.05) and 10.44% (P<0.05), respectively.

Table 2. The number of immunoglobulins in the blood of the calves.

Indicators	Germivit (g kg^{-1} live weight)	Age (months)		
		9	15	18
IgG (g L^{-1})	0.0	18.72±0.691	18.94±1.211	19.12±0.742
	0.5	18.65±1.133	22.69±0.842*	23.11±0.922*
	0.7	18.96±0.791	21.54±1.134*	22.87±0.691*
	0.9	19.14±0.942	22.48±0.525*	22.50±1.122*
IgM (g L^{-1})	0.0	3.48±0.194	3.54±0.422	3.49±0.255
	0.5	3.51±0.281	3.98±0.296*	4.16±0.154*
	0.7	3.57±0.322	4.11±0.173*	4.21±0.253*
	0.9	3.45±0.123	3.91±0.157*	4.13±0.183*

* P<0.05

By the age of 18 months, the blood of the animals in the experimental groups contained 22.50-23.11 g L^{-1} IgG, which is 17.70-20.81% (P<0.05) more than the control group. In calves of the first experimental group at 18 months of age, the IgM content was higher than in the animals of the control group by 19.22% (P<0.05), the second one 20.65% (P<0.05), and the third one 18.34% (P<0.05).

A study by Kuhn *et al.* (2005) revealed the immunostimulatory effect of Echinacea on sows and their offspring. For

1-day-old piglets, the concentration of IgG and IgA was significantly higher than the controls.

The immune system, along with the nervous and endocrine systems, has an important regulatory function in maintaining the body's homeostasis (Shakhov, 2015).

Under the influence of Germivit in the animals of the experimental groups, the number of T-lymphocytes in the blood significantly increased, indicating an increase in the cellular immunity of the calves. At the same time, the activation of the humoral link of the immune system was also observed due to an increase in the blood of representatives of the experimental groups in the number of B-lymphocytes and, especially, IgG and IgM classes.

Second Experiment

Before the use of the Germivit, the content of red blood cells in the blood of cows of the experimental groups was at the same level with a range of 5.82-5.97×10^{12} L^{-1} (Table 3). Furthermore, 30 and 10 days before the expected calving, no significant differences were found in the number of red blood cells in the cows of the experimental and control groups. The difference was in the range of 0.71-2.11%. However, in the postpartum period, there was a significant increase in the number of red blood cells in the animals of the experimental groups relative to the values for the animals from the control group. Thus, in the cows of the first experimental group, the indicators exceeded the control values by 10.22% (P<0.01) and in the cows of the second experimental group, those exceeded by 10.73% (P<0.01). In the 1-day-old calves obtained from those cows that were additionally fed with Germivit, the number of red blood cells exceeded that from calves born to the cows from the control group by 3.22-4.34%. When studying the morphological composition of blood in the calves at 30 days of age, the difference in the number of red blood cells increased slightly by 9.61% (P<0.05) for the calves of the first experimental group and by 9.01% (P<0.05) for the calves of the second experimental group. The inclusion of Germivit in the diet of pregnant cows did not have a significant effect on the number of leukocytes in the blood of the animals, neither in cows nor in calves. In all the studied periods, the difference between the number of leukocytes in the cows and calves of the experimental groups and the control group was insignificant and unreliable and was in the range of 0.11-2.33% in cows and 0.34-0.62% in the calves obtained from them.

Table 3. Morphological indicators in the blood of the cows and calves.

Indicators	Germivit (g kg^{-1} live weight)	Cows 60 days before calving	Cows 30 days before calving	Cows 10 days before calving	Cows 7 days after calving	One-day calves	30-days calves
Erythrocytes (10^{12} L^{-1})	0	5.86±0.133	5.98±0.164	6.15±0.144	5.89±0.121	6.15±0.131	5.94±0.162
	0.25	5.97±0.091	5.87±0.122	6.23±0.07	6.49±0.140**	6.42±0.09	6.51±0.123*
	0.5	5.82±0.16	6.11±0.081	6.19±0.111	6.52±0.112**	6.35±0.071	6.48±0.075*
White blood cells (10^9 L^{-1})	0	6.82±0.09	6.94±0.06	5.97±0.081	6.13±0.074	6.72±0.03	6.18±0.054
	0.25	6.93±0.151	6.92±0.122	6.11±0.092	6.10±0.05	6.68±0.071	6.20±0.09
	0.5	6.80±0.071	6.89±0.054	5.89±0.133	6.14±0.083	6.75±0.111	6.15±0.122
Platelets (10^9 L^{-1})	0	353.26±4.121	348.62±8.124	360.21±7.924	342.82±8.181	324.61±4.151	329.16±7.111
	0.25	329.11±6.15	350.41±7.823	358.41±4.891	346.18±8.14	329.82±7.122	331.15±8.25
	0.5	342.82±5.784	354.11±9.121	361.29±8.15	349.18±7.67	330.16±8.287	330.18±7.651
Hemoglobin (g L^{-1})	0	98.16±2.842	99.82±3.144	100.25±1.221	102.31±4.621	97.62±4.421	99.82±2.69
	0.25	100.11±3.112	98.87±4.141	108.42±4.821*	109.83±3.620*	108.11±3.121**	111.25±2.321**
	0.5	97.87±4.151	100.12±3.97	106.13±2.123*	111.41±2.644*	106.65±4.150**	110.82±4.251**

* P<0.05; ** P<0.01

A similar pattern was established when calculating the number of platelets in the blood of the cows and calves. Therefore, the difference in the number of platelets between the cows of the experimental and control groups 30 days before delivery was 0.51-1.51%, at 10 days, 0.22-0.54%, after calving 0.91-1, 81%, for 1-day-old calves 1.62-1.70%, and for 1-month-old calves 0.34-0.62% (Table 3).

A completely different picture was observed when determining the amount of hemoglobin. The cows of the both experimental groups at 30 days before calving had minimal differences in the amount of hemoglobin in comparison to the cows of the control group (0.31-1.01%). However, 10 days before giving birth, the amount of hemoglobin in the blood of the cows of the first experimental group was 108.42±4.821 g L^{-1}, which is 8.11% (P<0.05) more than in the control animals. Meanwhile, 7 days after calving, the difference was 7.44% (P<0.05). In the cows of the second experimental group, the studied indicator exceeded the control values at 10 days before birth and 7 days after calving by 5.92% (P<0.05) and 8.92% (P<0.05), respectively. The 1-day-old calves of the experimental groups, along with their mothers, exceeded their control peers by 9.12-10.70% (P<0.01) in terms of hemoglobin by 11.01-11.43% (P<0.01) at the age of one month (Table 3).

In other studies, the use of phytobiotics in birds significantly reduces the level of low-density cholesterol lipoproteins in serum and increases the level of hemoglobin and the number of red blood cells (Ademola *et al.,* 2009). Shawle *et al.* (2016) noted that the use of phytobiotics in diets does not change blood indicators, all hematological indicators were included in the norm. However, in this case, phytobiotics increased hemoglobin and the number of red blood cells. There was a significant decrease in serum glucose, triglycerides and cholesterol in the dietary group that consumed phytobiotics.

The lysozyme activity of the blood serum was 17.69-18.11 µg mL^{-1} and the bactericidal activity of blood serum was 47.59-48.14% 60 days before calving in the animals of the experimental groups (Table 4).

Table 4. Humoral factors of natural resistance of the cows and calves.

Indicators	Germivit (g kg^{-1} live weight)	Cows 60 days before calving	Cows 30 days before calving	Cows 10 days before calving	Cows 7 days after calving	One-day after calving	30-days after calving
Lysozyme activity of blood serum (µg mL^{-1})	0	17.72±0.341	18.14±0.155	17.86±0.121	18.12±0.262	9.82±0.122	13.28±0.171
	0.25	18.11±0.41	18.26±0.212	18.76±0.133*	19.15±0.171*	10.41±0.251*	15.41±0.211**
	0.5	17.69±0.49	18.11±0.17	18.82±0.242*	19.26±0.244*	10.24±0.160*	14.49±0.201**
Bactericidal activity of blood serum (%)	0	47.62±0.522	48.12±0.643	47.98±0.321	47.16±0.244	48.64±0.122	49.98±0.162
	0.25	48.14±0.621	50.49±0.233*	50.64±0.444*	49.89±0.170*	50.13±0.29	52.11±0.133*
	0.5	47.59±0.422	51.72±0.131*	49.18±0.182	48.64±0.133*	51.11±0.191*	51.86±0.111

* P<0.05; ** P<0.01.

In the cows of the first and second experimental groups, 30 days before the birth, no differences were found in the lysozyme activity of the blood serum compared with animals from the control group. In the same period, the bactericidal activity of the blood serum significantly increased in the cows of the experimental groups by 2.37-3.60% (P<0.05). The cows of the first experimental group 10 days before delivery exceeded the control animals in terms of serum lysozyme activity by 5.01% (P<0.05) and regarding bactericidal activity by 2.66% (P<0.05), while the animals of the second experimental group were exceeded by 5.32% (P<0.05) and 1.25%, respectively.

After the birth of the cows of the experimental groups, the state of humoral factors of natural resistance continued to remain at a fairly high level. The lysozyme activity of the blood serum was higher than in the control group by 5.74-6.32% (P<0.05) and bactericidal activity was 1.48-2.73% (P<0.05) higher. The 1-day-old calves of the experimental groups exceeded the lysozyme and bactericidal activity of the blood serum of their control peers by 4.21-6.04% (*P*<0.05) and 1.49-2.47% (*P*<0.05), respectively, and at 30 days of age by 9.10-16.01% (*P*<0.01) and 1.88-2.13% (*P*<0.05), respectively (Table 4).

When evaluating the cellular factors of the natural resistance (Table 5), of the body of cows under the action of Germivit, 30 days before calving, the phagocytic activity and phagocytic index of blood neutrophils in the animals of all experimental groups did not differ significantly. But 10 days before calving and 7 days after calving, the representatives of the first experimental group recorded an increase in the phagocytic activity of blood neutrophils by 3.25% (*P*<0.05) and 2.85% (*P*<0.05), respectively, while the phagocytic index in these study periods increased relative to the control levels by 3.40% (*P*<0.05) and 5.00% (*P*<0.05), respectively. Representatives of the second experimental group exceeded the cows of the control group in terms of the phagocytic activity of blood neutrophils by 1.06% (*P*<0.05) 10 days before calving and by 3.74% (*P*<0.01) 7 days after giving birth, and in terms of the phagocytic index of blood neutrophils by 2.30% and 3.00% (*P*<0.05), respectively. In the one-day calves obtained from cows of the experimental groups, the phagocytic index was at the level of the control values and differed insignificantly. On the contrary, the phagocytic activity of blood neutrophils, was 2.73-4.72% (*P*<0.01) higher than that of their peers from the control. At the age of one month, the calves from the first experimental group had a greater value for the phagocytic activity of neutrophils by 3.63% (*P*<0.01), and for the representatives of the second experimental group it was by 3.01% (*P*<0.001). According to the phagocytic index, the monthly calves of the experimental groups were 12.52-13.04% (*P*<0.01) superior to the monthly calves of the control group (Table 5).

Table 5. Cellular factors of natural resistance of the cows and calves fed daily with Germivit.

Indicators	Germivit (g kg^{-1} live weight)	Cows 60 days before calving	Cows 30 days before calving	Cows 10 days before calving	Cows 7 days after calving	One-day after calving	30-days after calving
Phagocytic activity of neutrophils (%)	0	50.16±1.211	51.82±1.422	49.89±0.711	51.41±1.190	31.46±1.151	41.98±1.271
	0.25	50.45±0.891	52.16±0.741	53.14±0.590*	54.26±0.621*	34.19±0.541**	45.61±0.870**
	0.5	50.79±1.144	52.41±0.890	53.28±0.862*	55.15±0.870**	36.18±0.921**	44.98±0.822**
Phagocytic neutrophil index	0	4.31±0.170	4.39±0.181	4.34±0.190	4.62±0.711	1.29±0.121	3.68±0.090
	0.25	4.37±0.211	4.43±0.160	4.49±0.244*	4.85±0.343*	1.32±0.180	4.16±0.144**
	0.5	4.29±0.422	4.50±0.212	4.44±0.160	4.76±0.222*	1.30±0.150	4.14±0.121**

* - *P*<0.05; ** - *P*<0.01.

The bacteriostatic and bactericidal action of body fluids is provided by the humoral factors of natural resistance. During the experiments, the lysozyme and bactericidal activity of the blood serum was highest in the cows of the experimental groups both before and after calving. Similar changes were recorded in the assessment of the cellular factors of natural resistance. The phagocytic activity of blood neutrophils was also higher in cows from the experimental groups.

The use of different plants in phytobiotics had a significant effect on total cholesterol, total antibody titers and immunoglobulin G (IgG). However, there was no significant effect of the supplements on the production of IgM. Differences in results may be due to numerous factors, for example, the type and part of the plant used, harvest time, methods for preparing phytogenic additives and methods for extracting herbs (Yang *et al.,* 2009).

When evaluating the mineral metabolism in cows, those Germivit-fed did not lead to significant changes in blood levels of calcium, phosphorus, and magnesium 30 days before birth. The blood of the cows of the experimental groups 10 days before calving had 4.81% and 2.82% more magnesium, 2.93% and 1.11% more calcium, and 5.14% (*P*<0.05) and 7.61% (*P*<0.05) more phosphorous than cows without supplement. In the postpartum period, a significant increase in the studied mineral substances in the blood of cows of the experimental groups was found. In terms of the magnesium content, the cows of the first experimental group significantly exceeded the values for the control animals by 7.72% ($P<0.05$), calcium by 9.63% ($P<0.05$), and phosphorus by 8.01% ($P<0.05$). The animals of the second experimental group had 5.84% ($P<0.05$) more magnesium, 10.81% more calcium, and 13.22% ($P<0.05$) more phosphorus in the blood. The calves obtained from the cows of the experimental groups at 1-day-old and 1-month-old did not differ from the calves of the control group of the same ages in terms of the blood content of magnesium, calcium, and phosphorus (Table 6).

Table 6. The mineral composition of the blood of the cows and calves.

Indicators	Groups	Cows 60 days before calving	Cows 30 days before calving	Cows 10 days before calving	Cows 7 days after calving	One-day after calving	30-days after calving
Magnesium (mmol L^{-1})	Control	1.12±0.032	1.10±0.026	1.05±0.013	1.04±0.017	1.06±0.02	1.08±0.019
	First experimental	1.07±0.014	1.09±0.018	1.10±0.044	1.12±0.023*	1.09±0.012	1.12±0.022
	Second experimental	1.08±0.024	1.11±0.02	1.08±0.017	1.10±0.018*	1.05±0.017	1.10±0.015
Calcium (mmol L^{-1})	Control	2.64±0.111	2.69±0.082	2.67±0.137	2.59±0.096	2.42±0.063	2.47±0.088
	First experimental	2.68±0.144	2.71±0.091	2.75±0.171	2.84±0.083*	2.40±0.011	2.45±0.092
	Second experimental	2.63±0.121	2.60±0.08	2.70±0.092	2.87±0.063*	2.38±0.121	2.50±0.101
Phosphorus (mmol L^{-1})	Control	1.58±0.033	1.60±0.044	1.56±0.032	1.59±0.082	1.49±0.033	1.51±0.07
	First experimental	1.60±0.041	1.59±0.07	1.64±0.071*	1.72±0.044*	1.51±0.091	1.48±0.09
	Second experimental	1.57±0.044	1.62±0.052	1.68±0.085*	1.80±0.035*	1.47±0.052	1.50±0.063

* $P<0.05$.

The composition of Germivit includes various vitamins, and it is known that many vitamins have a positive effect on immunity. Under the influence of Germivit, an increase in the mineral content in the cows' blood was observed; in particular, the maximum difference was seen in the content of magnesium, calcium, and phosphorus in the blood after birth, which was associated with the presence of these minerals in the feed supplement. The content of transamination enzymes in the blood of the cows of the experimental groups 60 days before calving was 31.41-33.26 U L^{-1} for ALT (alanine aminotransferase) and 58.54-59.18 U L^{-1} for AST (aspartate aminotransferase). After 30 days from the start of feeding cows with Germivit, the blood levels of these enzymes changed slightly. A significant decrease in the blood of the cows of the experimental groups 10 days before birth was observed in terms of the amount of ALT by 4.81-7.61% ($P<0.05$) relative to the control values. The decrease in the amount of AST was less significant and amounted to 1.82-2.91%. After calving, the amount of ALT and AST was lower in the cows of the first experimental group than in the

Table 7. The content of transamination enzymes in the blood of the cows and calves.

Indicators	Groups	Cows 60 days before calving	Cows 30 days before calving	Cows 10 days before calving	Cows 7 days after calving	One-day after calving	30-days after calving
Alanine aminotransferase (U L^{-1})	Control	31.41±0.266	31.62±0.191	30.58±0.263	31.48±0.232	28.98±0.241	29.97±0.174
	First experimental	33.26±0.181	31.70±0.322	29.11±0.151*	30.32±0.122	28.14±0.422	30.19±0.285
	Second experimental	31.98±0.311	31.59±0.414	28.41±0.290*	30.46±0.545	29.12±0.265	30.41±0.255
Aspartate aminotransferase (U L^{-1})	Control	58.97±0.751	59.18±1.161	58.48±1.144	59.11±1.122	57.48±0.823	57.91±0.92
	First experimental	59.18±0.852	57.11±0.89	57.43±0.433	56.92±0.87	58.03±1.141	58.16±1.133
	Second experimental	58.54±0.491	58.41±0.754	56.82±0.621	57.12±1.15	57.39±0.444	58.21±0.82

* $P<0.05$.

cows of the control group by 3.81%, and for the cows of the second experimental group, it was 3.33% and 3.43% lower, respectively. In the calves of the control and experimental groups, no differences were found in the content of enzymes in the blood (Table 7).

There was a decrease in the blood of animals from the experimental groups in terms of the content of transamination enzymes, especially alanine aminotransferase, which are markers of liver damage. Hence, a decrease in these enzymes indicates a hepatoprotective effect of Germivit. Feeding Germivit to pregnant cows contributed to the birth of more viable offspring, whereby of the 10 calves obtained from the cows of the control group, a gastrointestinal pathology was recorded in four animals. Despite etiotropic and symptomatic treatment, two calves died. In the second experimental group, one calf in mild form became ill. In the group of calves obtained from the cows of the first experimental group, no incidence of calf mortality was observed (Table 8).

Table 8. Clinical indicators of the calves.

Indicators	Groups		
	Control	First experimental	Second experimental
The number of sick calves	4	0	1
The number of dead calves	2	0	0

The state of health of cows has great importance for obtaining healthy and viable young stock. The correlation among the immunological status of young calves, the level of metabolism and natural resistance of mother cows has been scientifically proven by several authors (Kulmakova and Mudarisov, 2019; Kulmakova *et al*., 2019).

Feeding with Germivit to pregnant cows contributed to an improvement in the morphological composition of the blood of their offspring, whereby a significant increase in humoral and cellular resistance factors was observed at the age of 1-day-old and 1-month-old, which in turn contributed to a decrease in the incidence of mortality among the calves.

A positive effect of Germivit on the immuno-biochemical status of animals has been previously established in experiments on calves during the dairy period of growth. In animals, there is an improvement in the clinical condition, which is confirmed by the results of blood monitoring. According to Donnik *et al.* (2010), in the blood of the Germivit-fed cows, the phosphorus-calcium ratio was normalized, the cholesterol content decreased by 13%, the urea content increased, evidencing a normalization of liver function. These results are consistent with this study.

There are currently many herbal preparations that have a positive effect on the functional state and productivity of animals (Patel *et al*., 2016; Shawle *et al*., 2016; Valenzuela-Grijalva *et al*., 2017). The advantage of Germivit compared to its analogs is the low cost and wide availability of its raw materials, the absence of side effects, the safety for animals in cases of overdose, and the pronounced positive effect on the immuno-biochemical status of the animal organism shortly after starting its use. Therefore, Germivit is a good alternative to antibiotics in the context of animal husbandry.

CONCLUSION

The research results confirm the beneficial effects of Germivit in cattle. Therefore, in calves fed with Germivit, an improvement in the metabolism, an increase in immune status, and a normalization of liver function were observed. In cows, an improvement in the morphological composition of blood and the mineral metabolism was observed, while the humoral and cellular factors of natural resistance also increased. Calves born from cows that were fed with Germivit during pregnancy showed a high state of immunity. There was no mortality among the calves of the experimental groups. It should be noted that the morphological, biochemical, and immunological parameters of the cows and calves of the experimental groups did not depend on the dose of Germivit received.

To sum up, the use of Germivit in cattle of different ages normalizes the metabolism, increases the immune status of the animal, and improves the functional state of the liver. The research showed uniformly positive effect of Germivit on experimental animals. On the basis of existing results and economic viability, more appropriate dose was 0.5 g kg^{-1} in the first experiment (in growing calves) and 0,25 g kg^{-1} in the second one (in pregnants cows).

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