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SUSTAINABLE ECOLOGICAL HEALTH OF LIVESTOCK FARMS, THE IMPACT OF A BETULIN-CONTAINING FEED ADDITIVE ON CLINICAL AND HEMATOLOGICAL PARAMETERS IN BREEDING CALVES AND DAIRY COWS

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ABSTRACT

The study was conducted at the dairy complexes of the breeding farm of the Agricultural Enterprise Kolkhoz "Soznatelny," Zubtsovsky District, Tver Region (Russia), in collaboration with the Department of Disease Diagnostics, Therapy, Obstetrics, and Animal Reproduction, and the Medical and Diagnostic Center of the Moscow State Academy of Veterinary Medicine and Biotechnology - MVA named after K.I. Skryabin (Russia). Betulin was administered orally to each animal in the experimental group at a dose of 10 mg/kg of body weight with water, once daily for 14 days. Clinical examinations and hematological analyses were conducted at the experiment's beginning and end to evaluate betulin's effects on the animals and exclude concomitant diseases. An Abacus Junior Vet automatic hematology analyzer (Austria) was used for blood analysis. Parameters assessed included erythrocyte, leukocyte, platelet, lymphocyte, basophil, monocyte, eosinophil, and segmented neutrophil counts, as well as hemoglobin levels, color index, anisocytosis index, and hematocrit values. The results demonstrated that betulin positively influenced the hematological parameters of five- and ten-month-old breeding calves. The drug significantly increased the number of peripheral blood lymphocytes, alleviated neutrophilic leukocytosis, and reliably reduced neutrophil, monocyte, and total leukocyte counts, all within the physiological norm. These findings suggest that betulin stimulates lymphocyte proliferation and enhances phagocytosis, leading to inflammation resolution, a decrease in neutrophil and monocyte levels, and an increase in lymphocyte counts. The observed reduction in erythrocyte and hemoglobin levels by the end of the experiment in all experimental groups was likely due to weather conditions. On the first day of the experiment, intense heat may have caused polycythemia, while cooler temperatures on the final day could have mitigated this effect.

Keywords: betulin, breeding farm, blood analysis, hematological parameters, clinical parameters, oral administration, body weight.

INTRODUCTION

The productivity and reproductive performance of cows are directly influenced by production-related stress. Factors such as an improperly balanced diet, infectious and parasitic diseases, and obstetric or gynecological conditions exacerbate stress and negatively affect health and productivity (Gnezdilova et al., 2023, 2024a). When evaluating feed quality, it is crucial to consider natural contaminants, including mycotoxins, which continue to pose a significant threat to animal health (Gnezdilova et al., 2024b; Kosolapova et al., 2021). The positive application of plant-derived triterpenoids in veterinary practice as immunostimulants for various pathological conditions in animals of different species is of great interest. Particularly promising for the development of new drugs, including veterinary applications, are lupane triterpenoids such as betulonic and betulinic acids (Muradyan et al., 2021; Popova et al., 2017). In veterinary medicine, there is growing interest in addressing infectious diseases through environmentally friendly plant-based preparations. These preparations exhibit bactericidal, bacteriostatic, virucidal, and immunomodulatory effects on diseased organisms (Kemboi et al., 2020; Soldatenko et al., 2020). For practicing veterinarians, it is essential to have access to cost-effective drugs with robust therapeutic activity and polytropic effects. Such drugs can simultaneously target pathogens, influence various stages of pathological processes, and stimulate recovery (Makau et al., 2016). Combining drugs that enhance the body's defenses with those that suppress specific pathological mechanisms offers a comprehensive approach to treatment (Sulzberger et al., 2017). Betulin is a natural pentacyclic triterpenoid of the lupane series. It is present in many plants (e.g., hazel, calendula, licorice) but is primarily obtained industrially through extraction from birch bark—the outer layer of white birch (*Betula alba*) and silver birch (*Betula pendula*) (Valgaeren et al., 2019). Betulin is not found in its free form in nature. Extensive research conducted at more than 40 international and Russian research centers has demonstrated the effectiveness of triterpene compounds as direct regulators of enzymatic systems in the body (Gao et al., 2018; Zhang et al., 2019). Betulin's immunostimulatory activity is evident in its ability to induce the production of endogenous interferon, enhance cellular and humoral immunity, and activate immunocompetent cells. Notably, it increases phagocytic activity-the ability of phagocytes to destroy viruses and bacterial cells-across all indicators (Ahn et al., 2022; Xiong et al., 2015). The aim of this study was to investigate the effect of betulin on the clinical status and hematological parameters of breeding calves and dairy cows.

MATERIALS AND METHODS

The study was conducted at the dairy complexes of the breeding farm of the Agricultural Enterprise Kolkhoz "Soznatelny," located in the Zubtsovsky District of the Tver Region, Russia. The research was carried out in collaboration with the Department of Diagnostics of Diseases, Therapy, Obstetrics, and Reproduction of Animals, and the MDC of the MSA of Veterinary Medicine and Biotechnology, named after K.I. Skryabin, Russia. To investigate the effect of betulin on the clinical and hematological parameters of blood serum, the following groups were formed: Two groups of Sychevskaya calves (experimental and control), each consisting of 10 animals aged 5 months, with a live weight of 150–165 kg; Two groups of Sychevskaya calves (experimental and control), each consisting of 10 animals aged 10 months, with a live weight of 286–316 kg; Two groups of lactating healthy Sychevskaya cows (experimental and control) in their 2nd or 3rd lactation, each consisting of 10 animals, with a live weight of 550–600 kg and a milk yield of 7,000–8,000 liters per year. All planned diagnostic procedures were conducted on the experimental animals. The farm was certified free from leukemia, tuberculosis, and brucellosis. Betulin was administered orally to each animal in the experimental groups at a dose of 10 mg/kg of body weight with water, once daily for 14 days. Throughout the study, the clinical condition of the animals was assessed at the beginning and daily for 14 days. Parameters evaluated included body temperature, pulse rate, respiration rate, behavioral reactions, appetite, defecation, urination, motor activity, and skin condition. For cows, additional assessments included reproductive system health (e.g., nature of discharge, uterine tone) as well as monitoring for side effects and complications. To evaluate the effect of betulin on the animals and to exclude concomitant diseases, clinical examinations and hematological analyses were performed at the start and conclusion of the experiment. A blood test was conducted using the Abacus Junior Vet automatic hematology analyzer (Austria). The parameters assessed included erythrocyte, leukocyte, platelet, lymphocyte, basophil, monocyte, eosinophil, and segmented neutrophil counts, as well as hemoglobin levels, color index, anisocytosis index, and hematocrit values.

Statistical Analysis. Experimental data were processed using biometric methods (Lakin G.F., 1990; Makarova N.V., Trofimets V.Ya., 2002) and the Microsoft Office Excel Data Analysis package. The reliability of differences between groups was evaluated using the following significance levels: $P < 0.05$ (*), $P < 0.01$ (**), $P < 0.001$ (***).

RESULTS AND DISCUSSION

Clinical Indicators. Throughout the experiment, no significant changes were observed in body temperature, pulse rate, or respiratory rate among the animals in the experimental groups. There were no signs of systemic reactions to the administration of the drug. Additionally, no alterations in behavioral responses, appetite, defecation, urination, or milk yield were noted.

Temperature, Pulse, and Respiration Indicators. The results of the clinical assessments, including body temperature, pulse rate, and respiratory rate, conducted before and after betulin administration, are summarized in Table 1.

Table 1. Temperature, pulse, and respiration indicators in calves and dairy cows before and 14 days after oral administration of Betulin.

n/n	Temperature (°C)		Pulse (bpm)		Respiration (breaths/min)	
	Before	After	Before	After	Before	After
	5-Month-Old Calves					
1	38.2	38.4	78	68	26	24
2	37.6	38.3	71	76	28	28
3	38.4	38.9	69	74	27	25
4	38.5	39.0	72	73	24	25
5	37.8	37.8	76	67	25	25
6	38.2	37.6	74	79	20	24
7	39.0	38.5	66	71	28	24
8	39.0	38.3	65	69	24	24
9	38.4	38.7	75	68	22	25
10	38.6	37.8	80	66	20	25
M±m	38.37±0.45	38.33±0.47	72.6±4.9	71.1±4.28	24.4±2.98	24.9±1.2
Norm	38.5-39.5 °C		70–100 bpm		25-45 breaths/min	
	10-Month-Old Calves					
1	38.2	38.4	70	64	24	25
2	37.6	38.3	66	72	22	22
3	38.4	38.9	68	70	26	24
4	38.5	39.0	66	69	18	22
5	37.8	37.8	74	65	25	23
6	38.2	37.6	72	75	26	22
7	39.0	38.5	64	67	22	22
8	39.0	38.3	61	65	28	24
9	38.4	38.7	74	62	23	25
10	38.6	37.8	78	72	20	23
M±m	38.37±0.45	38.33±0.47	69.3±5.25	68.1±4.17	23.4±3.02	23.2±1.23
Norm	38.5–39.5 °C		70–100 bpm		25–45 breaths/min	
	High-Performing Cows					
1	38.0	38.2	66	64	19	20
2	37.4	38.2	64	62	18	18
3	38.2	38.0	58	62	16	18
4	38.2	38.4	64	65	20	18
5	37.6	37.8	64	60	22	19
6	38.2	38.2	65	65	22	20
7	39.0	38.8	58	57	19	25
8	38.5	38.4	55	58	18	20
9	38.2	38.2	64	62	18	18
10	38.3	38.0	71	62	20	19
M±m	38.16±0.52	38.22±0.27	62.9±4.65	61.7±2.71	19.2 ±1.84	19.5±2.12
Norm	37.5–39.5 °C		50–80 bpm		12–25 breaths/min	

Analyzing the obtained data, it can be concluded that the temperature, pulse, and respiration indicators in the experimental animals remained within the physiological norm throughout the experiment and showed no statistically significant differences.

Hematological Indicators

The results of the hematological study for the animals in the experimental group, conducted before and after the administration of the betulin supplement, are presented in Table 2.

Table 2. Results of hematological study of 5-month-old calves before and after oral administration of Betulin at a dose of 10 mg/kg body weight with water, administered individually once per day for 14 days.

n/n	Hemoglobin (g/L)	Erythrocytes (10^{12}/L)	Hematocrit (%)	Leukocytes (10^9/L)	Platelets (10^9/L)	Eosinophils (%)	Eosinophils (10^9/L)	Basophils (%)	Band Neutrophils (%)	Band Neutrophils (10^9/L)	Segmented Neutrophils (%)	Segmented Neutrophils (10^9/L)	Monocytes (%)	Monocytes (10^9/L)	Lymphocytes (%)	Lymphocytes (10^9/L)
	Before / After	Before / After	Before / After	Before / After	Before / After	Before / After	Before / After	Before / After	Before / After	Before / After	Before / After	Before / After	Before / After	Before / After	Before / After	Before / After
Calves aged 5 months, n=10. Experimental Group. Before and 14 days after oral administration of betulin																
1	117/96	7.21/6.2	31.1/27.6	11.5/9.6	316/584	6/0	0.69/0	0/1	0/0-	-	22/25	2.53/2.4	9/8	1.04/0.77	63/66	7.25/6.34
2	116/103	7.38/6.87	32.7/28.6	10.8/10.6	244/315	2/2	0.21/0.21		2/2	0.21/0.21	24/12	2.59/1.27	4/3	0.43/0.31	68/81	7.34/8.58
3	110/106	9.88/6.56	30.2/29.6	15.1/10.3	250/320	2/8	0.3/0.82		10/2	1.51/0.2	48/12	7.24/1.23	9/3	1.35/0.3	31/75	4.68/7.72
4	102/98	6.43/6.16	30.3/29.3	12.1/9.5	348/268	5/4	0.6/0.38		1/2	0.12/0.19	31/17	3.75/1.61	3/1	0.36/0.095	60/76	7.26/7.22
5	106/98	7.97/7.11	29.4/28.4	10.4/10.6	355/260	3/1	0.3/0.1	0/2	2/2	0.2/0.2	29/19	3.01/2.01	3/4	0.3/0.42	63/72	6.55/7.63
6	104/102	7.22/6.91	31.2/29.9	9.2/9.3	498/230	2/4	0.18/0.37		0/0		25/15	2.3/1.39	5/6	0.46/0.55	68/75	3.25/6.97
7	98/94	6.84/6.85	27.8/27.9	11.5/10.2	360/410	5/3	0.57/0.3		0/0		34/21	3.91/2.14	6/2	0.69/0.2	55/74	6.32/7.54
8	110/104	6.88/6.66	27.6/27.5	16.2/9.5	270/205	1/7	0.16/0.66		7/1	1.13/0.09	52/20	8.42/1.9	10/2	1.62/0.19	30/70	4.86/6.65
9	115/98	7.45/6.99	31.1/32.6	9.1/10.1	430/130	6/2	0.72/0.2		3/3	0.36/0.3	28/14	3.38/1.41	3/3	0.36/0.3	58/78	5.26/7.87
10	100/96	7.25/6.2	27.5/27.6	9.2/7.6	620/480	6/1	0.73/0.07		3/0	0.36/	42/17	5.12/1.29	7/2	0.85/0.15	34/80	3.12/6.08
M±m	107.8±6.8 */ 99.5±3.9* P≤0.05	7.4±0.9*/ 6.6±0.3* P≤0.01	29.9±1.7/ 28.9±1.5	11.5±2.4 */ 9.7±0.8* P≤0.01	369±118/ 320±136	3.8±1.98/ 3.2±2.6	0.44±0.2 3/ 0.31±0.2 6		2.8±3.2/ 1.2±1.1	0.4±0.5/ 0.2±0.14	33.5±10* / 17.2±4* P≤0.05	4.2±2.1*/ 1.6±0.4* P≤0.05	5.9±2.7*/ 3.4±2.1* P≤0.01	0.64±0.3*/ 0.32±0.2* P≤0.05	53±15*/ 74.7±4.5* P≤0.05	5.6±1.6*/ 7.26±0.76* P≤0.01
Calves aged 5 months, n=10. Control Group																
1	110/98	7.14/5.64	30.1/30.5	9.6/9.8	360/280	0/1	0/0.09/		0/1	0/0.09	36/35	3.45/3.43	4/5	0.38/0.49	60/58	5.76/5.68
2	108/88	6.75/5.12	30.3/38.4	9.3/9.6	456/426	0/0		0/1	0/2	0/0.19	39/29	3.62/2.78	4/6	0.37/0.57	57/62	5.3/5.95
3	116/102	7.45/5.32	31.2/30.6	7.6/8.6	383/298	2/1	0.15/0.08		3/0	0.22/0	28/35	2.12/3.01	1/2	0.07/0.17	66/62	5.01/5.33
4	96/94	6.15/6.22	27.4/37.3	15.2/15.4	284/188	5/1	0.76/0.15		11/12	1.67/1.84	29/32	4.4/4.92	8/9	1.21/1.38	47/46	7.14/7.08
5	100/96	6.89/6.77	29.4/29.1	8.2/11.3	368/250	8/7	0.65/0.79		0/2	0/0.22	22/37	1.8/4.18	2/4	0.16/0.45	68/50	5.57/5.65
6	100/90	7.25/6.13	27.9/31.3	7.8/9.4	322/402	1/0	0.07/0		0/0		36/43	2.8/4.04	3/2	0.23/0.18	60/55	4.68/5.17
7	112/94	7.25/5.78	31.2/30.2	12.6/11.6	315/289	2/2	0.25/0.23		5/6	0.63/0.69	34/28	4.28/3.24	7/8	0.88/0.92	52/56	6.55/6.49
8	114/98	7.85/6.71	31.2/27.8	7.9/7.8	295/350	2/0	0.15/		2/0	0.15/	27/33	2.16/2.57	2/1	0.15/0.07	67/66	5.29/5.14
9	100/92	6.59/6.75	28.2/27.8	8.9/9.1	262/300	0/1	0/0.09		0/0		35/34	3.11/3.09	4/2	0.35/0.18	61/63	5.42/5.73
10	106/100	7.22/7.23	29.5/28.5	9.8/8.9	219/405	5/4	0.49/0.35		1/0	0.09/0	27/25	2.64/2.22	4/7	0.39/0.62	63/64	6.17/5.69
M±m	106.2±6.8 */ 95.2±4.4* P≤0.05 Differences from the experimental group at the end P ≤ 0.05	7.0±0.4*/ 6.1±0.7* P≤0.05	29.6±1.4/ 31.5±3.7	9.69±2.4/ 10.1±2.1	326.4±68/ 318.8±76	2.5±2.6/ 1.7±2.2	0.25±0.2 8/ 0.18±0.2 4		2.2±3.5/ 2.3±3.8	0.25±0.5/ 0.3±0.5	31.3±5/ 33.1±5* Differences from the experimental group P ≤ 0.05	3.0±0.9/ 3.3±0.8	3.9±2.1/ 4.6±2.8	0.42±0.35/ 0.5±0.4	60.1±6.6/ 58.2±6.4* Differences from the experimental group P ≤ 0.05	5.68±0.74/ 5.79±0.6* Differences from the experimental group P ≤ 0.05

Table 3. Comparative evaluation of hematological indicators in the experimental and control groups of 5-month-old calves before and after Betulin administration.

Indicators	Norm	Experimental Group (n=10)		Control Group (n=10)	
		Before (M±m)	After (M±m)	Before (M±m)	After (M±m)
Hemoglobin (g/L)	90-120	107.8±6.8*	99.5±3.9* P≤0.05	106.2±6.8*	95.2±4.4* P≤0.05 Differences from the exp. group at the end P≤0.05
Erythrocytes (10^{12}/L)	5-7.5	7.4±0.9*	6.6±0.3* P≤0.01	7.0±0.4*	6.1±0.7* P≤0.05
Leukocytes (10^9/L)	4.5–12.0	11.5±2.4*	9.7±0.8* P≤0.01	9.69±2.4	10.1±2.1
Segmented Neutrophils (%)	20–35	33.5±10*	17.2±4* P≤0.05	31.3±5	33.1±5* Differences from the experimental group P≤0.05

Segmented Neutrophils (10^9/L)		4.2±2.1*	1.6±0.4* P≤0.05	3.0±0.9	3.3±0.8
Monocytes (%)	2–7	5.9±2.7*	3.4±2.1* P≤0.01	3.9±2.1	4.6±2.8
Monocytes (10^9/L)		0.64±0.3*	0.32±0.2* P≤0.05	0.42±0.35	0.5±0.4
Lymphocytes (%)	40–75	53±15*	74.7±4.5* P≤0.05	60.1±6.6	58.2±6.4* Differences from the experimental group P≤0.05
Lymphocytes (10^9/L)		5.6±1.6*	7.26±0.76* P≤0.01	5.68±0.74	5.79±0.6* Differences from the experimental group P≤0.05

*Note: * indicates statistically significant differences at P ≤ 0.05 and P ≤ 0.01.*

Analyzing the obtained data, it can be concluded that at the beginning of the experiment, some animals in both the experimental and control groups exhibited an increased leukocyte count. A more detailed examination of the leukograms in both groups revealed neutrophilic leukocytosis, monocytosis, and lymphopenia. By the 14th day of the experiment, the animals in the experimental group (after receiving betulin) showed a decrease in the leukocyte count to within the physiological norm, with a reduction of 1.8 × 10^9/L ($p \leq 0.01$), from 11.5 ± 2.4 to 9.7 ± 0.8 × 10^9/L. This reduction was attributed to decreases in both the absolute (by 2.6 × 10^9/L) and relative (by 16.3%) neutrophil counts, with significant differences ($p \leq 0.05$). Additionally, there was a decrease in the absolute (by 0.32 × 10^9/L, $p \leq 0.05$) and relative (by 2.5%, $p \leq 0.01$) monocyte counts.

Conversely, the absolute and relative lymphocyte counts in the experimental group increased by the end of the experiment. On average, lymphocytes increased by 1.66 × 10^9/L ($p \leq 0.01$) or 21.7% ($p \leq 0.05$), reaching 7.26 ± 0.76 × 10^9/L compared to 5.6 ± 1.6 × 10^9/L at the beginning of the experiment, and 74.7% ± 4.5 compared to 53% ± 15 at the start.

When comparing these indicators between the control and experimental groups at the start of the experiment, no notable differences were observed. However, at the end of the experiment, no significant changes in leukocyte count or leukogram parameters were noted in the control group compared to the beginning of the study. Nevertheless, significant differences between the experimental and control groups were observed for neutrophil and lymphocyte counts.

By the end of the experiment, the percentage of segmented neutrophils in the control group was 15.9% higher than in the experimental group, reaching 33.1% ± 5 compared to 17.2% ± 4 ($p \leq 0.05$). Conversely, lymphocyte counts were 16.5% lower in the control group compared to the experimental group ($p \leq 0.05$), at 58.2% ± 6.4 compared to 74.7% ± 4.5. The absolute lymphocyte counts also showed significant differences, with 5.79 ± 0.6 × 10^9/L in the control group compared to 7.26 ± 0.76 × 10^9/L in the experimental group ($p \leq 0.05$).

An analysis of hemoglobin and erythrocyte counts revealed significant differences at the start and end of the experiment in both the experimental and control groups. At the beginning of the experiment, higher hemoglobin and erythrocyte levels were observed. The hemoglobin levels in the experimental and control groups averaged 107.8 ± 6.8 and 106.2 ± 6.8 g/L, respectively, with no significant differences between the groups. By the end of the experiment, hemoglobin levels in the experimental and control groups averaged 99.5 ± 3.9 and 95.2 ± 4.4 g/L, respectively. A reduction in hemoglobin levels was noted in both groups; however, the experimental group showed higher hemoglobin levels than the control group by 4.2 g/L ($p \leq 0.05$).

Similarly, erythrocyte counts in peripheral blood showed comparable trends. At the beginning of the experiment, erythrocyte counts were higher in both the experimental and control groups. By the end of the experiment, the erythrocyte count had decreased in both groups, though the differences between the experimental and control groups were not statistically significant.

The results of the hematological study for animals in the experimental group before and after the use of the betulin supplement are presented in Table 4.

At the beginning of the experiment, the peripheral blood indices of ten-month-old calves in both experimental groups did not differ significantly. Analyzing the obtained data presented in Table 4, we can conclude that the levels of hemoglobin and erythrocytes prior to the experiment in animals of both groups were not significantly different. By the end of the experiment, a decrease in these indices was observed in both the experimental and control groups of ten-month-old calves ($P \leq 0.05$). A similar trend was observed in five-month-old calves.

Table 4. Results of hematological study of 10-month-old calves before and after oral administration of Betulin at a dose of 10 mg/kg body weight with water, administered individually once per day for 14 days.

n/n	Hemoglobin (g/L)	Erythrocytes (10^{12}/L)	Hematocrit (%)	Leukocytes (10^9/L)	Platelets (10^9/L)	Eosinophils (%)	Eosinophils (10^9/L)	Basophils (%)	Band Neutrophils (%)	Band Neutrophils (10^9/L)	Segmented Neutrophils (%)	Segmented Neutrophils (10^9/L)	Monocytes (%)	Monocytes (10^9/L)	Lymphocytes (%)	Lymphocytes (10^9/L)
	Before / After	Before / After	Before / After	Before / After	Before / After	Before / After	Before / After	Before / After	Before / After	Before / After	Before / After	Before / After	Before / After	Before / After	Before / After	Before / After
Experimental Group (n=10)																
	105/98	8.67/7.88	26.6/26.4	9.2/8.9	647/360	4/3	0.37/0.26	0/0	0/0	0/0	18/20	1.66/1.78	9/3	0.83/0.26	69/74	6.35/6.58
	103/88	7.47/6.77	26.4/28.3	10.4/6.9	353/270	3/1	0.31/0.07	0/1	0/0	0/0	15/23	1.56/1.58	7/3	0.73/0.2	75/72	7.8/4.96
	115/110	8.95/7.46	26.9/25.8	11.2/10.8	425/320	1/1	0.11/0.11	0/0	3/2	0.33/0.22	32/26	3.58/2.81	4/5	0.44/0.54	60/66	6.72/7.13
	107/100	7.99/7.42	26.8/27.1	10.6/10.8	316/275	5/4	0.53/0.43	0/0	0/1	0/0.1	33/25	3.49/2.7	1/2	0.1/0.2	61/70	6.46/7.56
	104/96	7.44/7.12	27.2/27.5	7.8/7.9	456/352	0/1	0/0.07	0/1	1/1	0.07/0.07	26/24	2.02/1.89	6/3	0.46/0.23	67/70	5.22/5.53
	108/100	7.85/7.02	28.1/26.4	6.8/7.3	520/322	2/2	0.13/0.14	0/0	2/1	0.13/0.07	23/22	1.56/1.6	5/1	0.35/0.07	68/74	4.62/5.4
	100/92	6.59/6.32	25.2/25.6	14.2/12.6	214/298	9/2	1.27/0.25	0/0	0/1	0/0	59/28	8.37/3.52	9/4	1.27/0.5	23/65	3.26/8.19
	105/86	8.68/6.48	28.2/27.6	13.1/10.5	236/278	1/1	0.13/0.1	0/0	0/0	0/0	62/32	8.12/3.36	5/1	0.65/0.1	32/66	4.19/6.93
	102/96	7.82/7.02	26.6/26.7	15.7/12.6	368/275	2/4	0.3/0.5	0/1	0/0	0/0	75/32	11.77/4.03	11/4	1.72/0.5	12/59	1.88/7.43
	110/95	8.22/7.65	26.4/26.1	12.3/8.2	442/448	1/3	0.1/0.24	0/0	4/1	0.49//0.08	56/21	6.88/1.72	6/3	0.73/0.24	33/72	4.05/5.9
M±m	105.9±4.3*/ 96.1±6.7 P≤0.05	7.96±0.7*/ 7.11±0.5 P≤0.05	26.8±0.8/ 26.7±0.7	11.1±2.7/ 9.6±2.1	397.7±130/ 319.8±55	2.8±2.6/ 2.2±1.2	0.32±0.36/ 0.21±0.15	0/0.3±0.4	1.0±1.4/ 0.7±0.6	0.1±0.17/ 0.05±0.07	39.9±21*/ 25.3±4.2 P≤0.01	4.9±3.6/ 2.49±0.9	6.3±2.8*/ 2.9±1.2 P≤0.05	0.72±0.4*/ 0.28±0.1 P≤0.01	50±22.6*/ 68.8±4.75* P≤0.01	5.05±1.8*/ 6.56±1.06* P≤0.01
Control Group (n=10)																
	110/100	8.06/7.55	26.7//26.3	13.5/17.7	225/312	5/7	0.67/1.23	0/0	3/9	0.4/1.59	55/67	7.42/11.8	7/8	0.94/1.41	30/9	4.05/1.59
	105/90	8.61/7.26	27.3/27.1	12.6/13.8	362/458	4/9	0.5/1.24	0/0	3/5	0.37/0.69	51/56	6.42/7.72	6/5	0.75/0.69	36/25	4.53/3.45
	108/102	7.75/7.45	26.8/26.4	6.9/7.9	658/452	2/3	0.13/0.23	0/0	1/0	0.06/0	33/35	2.27/2.76	2/2	0.13/0.15	62/60	4.27/4.74
	98/90	7.34/6.12	26.7/26.6	5.8/5.2	278/551	2/2	0.1/0.1	0/0	2/2	0.1/0.1	36/30	2.08/1.56	1/1	0.05/0.05	59/65	3.42/3.38
	103/98	7.66/7.02	26.5/26.3	10.5/11.5	320/341	1/2	0.1/0.23	0/0	2/3	0.2/0.34	27/32	2.83/3.68	1/2	0.1/0.23	69/61	7.24/7.01
	100/88	6.98/6.23	26.6/25.6	13.2/13.6	268/245	5/5	0.66/0.68	0/0	6/5	0.79/0.68	45/39	5.94/5.3	5/5	0.66/0.68	39/46	5.14/6.25
	106/90	8.62/6.56	27.4/27.5	10.9/9.3	416/354	2/1	0.2/0.09	0/0	0/2	0/0.18	25/26	2.72/2.41	4/3	0.43/0.27	69/68	7.52/6.32
	110/102	8.15/7.62	26.2/27.8	8.8/9.6	322/348	3/1	0.26/0.09	0/0	2/1	0.17/0.09	23/27	2.02/2.59	3/4	0.26/0.38	69/67	6.07/6.43
	100/86	7.87/6.55	27.4/27.2	10.2/8.7	284/345	3/3	0.3/0.26	0/0	3/4	0.3/0.34	22/18	2.24/1.56	2/2	0.2/0.17	70/71	7.14/6.17
	106/96	8.66/7.02	27.7/26.2	8.4/8.7	415/314	3/4	0.25/0.34	0/0	0/0	0/0	33/31	2.77/2.69	5/6	0.42/0.52	41/59	3.44/572
M±m	104.6±4.2*/ 94.2±6.1 P≤0.05	8.0±0.53*/ 6.93±0.5 P≤0.05	26.9±0.4/ 26.7±0.8	10.1±2.6/ 10.6±3.6	354.8±123/ 372.0±89	3.0±1.3/ 3.7±2.6	0.31±0.21/ 0.44±0.44	0/0	2.2±1.7/ 3.1±2.7* Differences from the experimental group P≤0.01	0.23±0.24/ 0.4±0.48* Differences from the experimental group P≤0.01	31.3±5/ 36.1±14.7* Differences from the experimental group P≤0.01	3.67±2.07/ 4.2±3.2	3.6±2.1/ 3.8±2.2	0.42±0.35/ 0.5±0.4	54.4±16/ 53.1±20.5* Differences from the experimental group P≤0.01	5.28±1.59/ 5.04±1.74* Differences from the experimental group P≤0.01

When analyzing the leukocyte counts in the animals of the experimental group, a slight decrease was noted by the end of the experiment, remaining within the physiological norm. Before the experiment, the leukocyte count was 11.1 ± 2.7 × 10^9/L, which decreased to 9.6 ± 2.1 × 10^9/L by the end of the experiment. In contrast, in the control group, the leukocyte count remained stable, measuring 10.1 ± 2.6 × 10^9/L at the start and 10.6 ± 3.6 × 10^9/L by the end of the study. The leukogram data for animals in both the experimental and control groups prior to the experiment did not show significant differences (Table 4). However, 14 days after the introduction of betulin, the leukogram indices in the calves of the experimental group exhibited significant changes in the counts of lymphocytes, neutrophils, and monocytes. Specifically, in the experimental group, the absolute lymphocyte count significantly increased from 5.05 ± 1.8 × 10^9/L at the beginning of the experiment to 6.56 ± 1.06 × 10^9/L by the end ($P \leq 0.01$). Even more pronounced changes were observed in the relative lymphocyte values: from 50 ± 22.6% at the start to 68.8 ± 4.75% ($P \leq 0.01$) at the end, representing an 18.8% increase. Conversely, the number of neutrophils and monocytes decreased by the end of the experiment in the same experimental group. The absolute count of segmented neutrophils dropped significantly from 4.9 ± 3.6 × 10^9/L at the beginning to 2.49 ± 0.9 × 10^9/L at the end. Similarly, relative neutrophil values decreased by 14.6%, from 39.9 ± 21% at the start to 25.3 ± 4.2% ($P \leq 0.01$) at the end of the experiment. Additionally, the peripheral blood monocyte count in the experimental group also decreased by the end of the experiment. The absolute monocyte count declined from 0.72 ± 0.4 × 10^9/L at the beginning to 0.28 ± 0.1 × 10^9/L at the end ($P \leq 0.01$), while the relative monocyte percentage decreased from 6.3 ± 2.8% to 2.9 ± 1.2% ($P \leq 0.05$).

Table 5. Comparative evaluation of hematological indicators in the experimental and control groups of 10-month-old calves before and after Betulin administration.

Indicators	Norm	At the Beginning of the Experiment		At the End of the Experiment	
		Experimental Group (n=10) (M±m)	Control Group (n=10) (M±m)	Experimental Group (n=10) (M±m)	Control Group (n=10) (M±m)
Hemoglobin (g/L)	90–120	105.9±4.3*	104.6±4.2*	96.1±6.7 P≤0.05	94.2±6.1 P≤0.05
Erythrocytes (10^{12}/L)	5–7.5	7.96±0.7*	8.0±0.53*	7.11±0.5 P≤0.05	6.93±0.5 P≤0.05

Leukocytes (10⁹/L)	4.5–12.0	11.1±2.7	10.1±2.6	9.6±2.1	10.6±3.6
Segmented Neutrophils (%)	20–35	39.9±21*	31.3±5	25.3±4.2 P≤0.01	36.1±14.7* Differences from the experimental group P≤0.01
Segmented Neutrophils (10⁹/L)		6.3±2.8*	3.0±0.9	2.9±1.2 P≤0.05	2.49±0.9
Monocytes (%)	2–7	0.72±0.4*	3.6±2.1	0.28±0.1 P≤0.01	3.8±2.2
Monocytes (10⁹/L)		0.64±0.3	0.42±0.35	0.32±0.2* P≤0.05	0.5±0.4
Lymphocytes (%)	40–75	50±22.6*	54.4±16	68.8±4.75* P≤0.01	53.1±20.5* Differences from the experimental group P≤0.01
Lymphocytes (10⁹/L)		5.05±1.8*	5.28±1.59	6.56±1.06* P≤0.01	5.04±1.74* Differences from the experimental group P≤0.01

*Note: * Significant differences at P ≤ 0.05 and P ≤ 0.01*

When comparing the results of the experimental group with those of the control group, significant differences in the numbers of neutrophils and lymphocytes were observed at the end of the experiment. In the control group, the relative number of lymphocytes was 15.7% lower, amounting to 53.1 ± 20.5% compared to 68.8 ± 4.75% in the experimental group ($P \leq 0.01$). The absolute lymphocyte count at the end of the experiment was 5.04 ± 1.74 × 10⁹/L in the control group compared to 6.56 ± 1.06 × 10⁹/L in the experimental group ($P \leq 0.01$).
The relative number of neutrophils in the control group at the end of the experiment was 36.1 ± 14.7%, compared to 25.3 ± 4.2% in the experimental group, which is 10.8% higher ($P \leq 0.01$) and correlates with the absolute values. Similar changes were observed in the band neutrophil indices, with significant differences ($P \leq 0.01$).
The results of the hematological study of highly productive cows in the experimental group, before and after the use of the betulin supplement, are presented in Table 6.

Table 6. Results of hematological study of highly productive cows before and after oral administration of Betulin at a dose of 10 mg/kg body weight with water, administered individually once per day for 14 days.

n/n	Hemoglobin (g/L)	Erythrocytes (10¹²/L)	Hematocrit (%)	Leukocytes (10⁹/L)	Platelets (10⁹/L)	Eosinophils (%)	Eosinophils (10⁹/L)	Basophils (%)	Band Neutrophils (%)	Band Neutrophils (10⁹/L)	Segmented Neutrophils (%)	Segmented Neutrophils (10⁹/L)	Monocytes (%)	Monocytes (10⁹/L)	Lymphocytes (%)	Lymphocytes (10⁹/L)
	Before / After	Before / After	Before / After	Before / After	Before / After	Before / After	Before / After	Before / After	Before / After	Before / After	Before / After	Before / After	Before / After	Before / After	Before / After	Before / After
Cows (n=10). Experimental Group. Before and 14 Days After Oral Administration of Betulin																
	108/88	6.9/6.03	28.8/25.9	17.9/8.8	471/453	7/14	1.25/1.23	0/2	1/2	0.18/0.18	52/38	9.31/3.34	9/4	1.61/0.35	31/40	5.55/3.52
	100/101	5.83/6.09	27.2/29.4	11/10.5	460/288	15/13	1.65/1.37	0/1	7/0	0.77/0	51/31	5.61/3.26	3/7	0.33/0.74	24/48	2.64/5.04
	111/102	6.31/6.08	30.7/30.8	10.7/9.5	461/402	11/12	1.17/1.14	0/0	0/0	0/0	49/36	4.65/3.42	8/7	0.76/0.67	32/45	3.04/4.28
	104/107	5.92/6.37	28.2/31.7	13.1/9.3	243/314	18/9	2.36/0.84	0/0	0/0	0/0	33/21	4.32/1.95	4/3	0.52/0.28	45/67	5.89/6.23
	108/110	6.6/7.08	28.8/32	9.7/10.4	343/263	17/19	1.65/1.98	0/0	0/2	0/0.21	47/29	4.56/3.02	8/4	0.75/0.42	28/46	2.72/4.78
	123/117	6.35/6.38	33.5/34.3	16.2/10.6	370/421	18/16	2.91/1.7	0/0	0/0	0/0	51/23	8.26/2.44	5/9	0.81/0.95	26/52	4.21/5.51
	100/103	5.88/6.28	26.9/30.2	7/5.8	643/470	17/8	1.19/0.46	0/0	1/3	0.07/0.17	39/28	2.73/1.62	0/2	0/0.12	43/59	3.01/3.42
	106/91	6.59/5.97	28.7/27.2	13/10.9	463/436	20/15	2.6/1.63	0/0	0/0	0/0	39/31	5.07/3.38	8/1	1.04/0.11	33/53	4.29/5.77
	100/96	5.91/5.88	28.3/29.1	10.4/8.9	365/311	6/4	0.62/1.25	0/0	0/0	0/0	40/23	4.16/2.04	4/6	0.41/0.53	50/57	5.2/5.07
	110/108	7.12/6.71	31/28.5	11/10.6	312/280	5/3	0.55/0.32	0/1	0/1	0/0.1	38/25	4.18/2.65	2/2	0.22/0.21	55/68	6.05/7.2
M±m	107.0±6.9/102.3±8.8	6.34±0.45/6.28±0.37	29.2±1.9/29.9±2.4	12.0±3.1*/9.53±1.5 P≤0.01	413±111/363±79	13.4±5.6/11.3±5.2	1.59±0.8/1.19±0.53	0/0.4±0.7	0.9±2.1/0.8±1.1	0.1±0.2/0.06±0.08	43.9±6.8*/28.5±5.6* P≤0.05	5.28±2.0*/2.7±0.6* P≤0.05	5.1±3.03/4.5±2.6	0.64±0.4/0.43±0.2	36.7±10.7*/53.5±9.3* P≤0.05	4.2±1.3/5.08±1.1

Highly productive cows. n=10. Control group																
	99/90	5.83/5.65	27.1/27.3	9.1/7.3	250/342	18/10	1.64/0.73	0/0	0/0	0/0	33/40	3/2.92	4/9	0.36/0.66	45/41	4.1/2.99
	113/99	6.34/7.63	30.2/28.3	12.2/7.6	512/223	6/6	0.73/0.45	0/0	0/0	0/0	37/38	4.51/2.88	10/11	1.22/0.83	47/45	5.73/3.42
	113/98	6.68/6.33	29.7/28.9	12.8/10	269/197	9/9	1.15/0.9	0/0	0/2	0/0.3	47/55	6.02/5.5	6/2	0.77/0.26	38/32	4.86/3.2
	106/97	6.03/5.87	28.9/29.3	6.3/5.8	426/122	13/11	0.82/0.64	0/0	0/0	0/0	26/40	1.64/2.32	7/6	0.44/0.35	54/43	3.4/2.49
	117/110	6.39/6.41	33.2/30.5	15.2/15.6	332/348	5/4	0.76/0.62	0/0	5/5	0.76/0.78	65/62	9.88/9.67	5/9	0.76/1.4	20/20	3.04/3.12
	101/104	5.87/6.15	26.8/29.8	12.2/11.6	259/321	18/12	2.19/1.39	0/0	0/0	0/0	52/54	6.34/6.26	8/6	0.97/0.69	22/28	2.68/3.24
	98/99	5.72/5.94	27.1/28.5	10.5/10.4	330/268	9/10	0.94/1.04	0/0	1/0	0.1/0	38/37	3.99/3.84	6/7	0.63/0.72	46/46	4.83/4.78
	120/118	6.40/6.41	32.6/32.9	14.2/13.9	452/412	12/11	1.7/1.52	1/0	0/0	0/0	48/50	6.8/6.95	2/4	0.28/0.55	37/35	5.25/4.86
	106/104	6.41/5.94	31.5/28.5	16.3/15.8	321/345	9/15	1.46/2.37	0/0	4/3	0.65/0.47	49/57	7.98/9.0	1/8	0.16/1.26	37/17	6.03/2.68
	106/89	6.84/6.13	28.6/26.1	10.5/9.6	215/118	14/7	1.47/0.67	0/0	0/0	0/0	33/29	3.46/2.78	3/5	0.31/0.48	50/59	5.25/5.66
M±m	107.9±7.5/ 100.8±8.7	6.25±0.3/ 6.24±0.5	29.6±2.2 29.01±1.8	11.93±2.9 10.76±3.4	336.6±97/ 268.6±101	11.3±4.5/ 9.5±3.1	1.28±0.48/ 1.0±0.6	0.1±0.3/0	1.0±1.8/ 1.0±1.7	0.15±029/ 0.15±0.27	42.8±11.5/ 46.2±10.7* Differences from the experimental group P≤0.05	5.36±2.5/ 5.21±2.6* Differences from the experimental group P≤0.01	5.2±2.7/ 6.7±2.6	0.59±0.3/ 0.72±0.3	39.6±11.3/ 36.6±12.8* Differences from the experimental group P≤0.05	4.5±1.15/ 3.6±1.06* Differences from the experimental group P≤0.01

At the beginning of the experiment, the peripheral blood indices of the cows in both experimental groups did not differ significantly. After fourteen days of betulin administration, the cows in the experimental group exhibited a significant decrease in the leukocyte count within the physiological norm, from 12.0 ± 3.1 × 10⁹/L at the start of the experiment to 9.53 ± 1.5 × 10⁹/L at the end ($P \leq 0.01$). An analysis of the leukogram in the cows of the experimental group revealed a reduction in both the relative (from 43.9 ± 6.8% to 28.5 ± 5.6%) and absolute (from 5.28 ± 2.0 × 10⁹/L to 2.7 ± 0.6 × 10⁹/L) numbers of segmented neutrophils by the end of the experiment. These differences were statistically significant ($P \leq 0.05$). Additionally, a decrease in both the absolute and relative numbers of monocytes was observed by the end of the experiment. Conversely, when analyzing the lymphocyte counts, a significant increase in both relative and absolute values was noted. The relative number of lymphocytes in the peripheral blood of the experimental group increased by 16.8% by the end of the experiment, reaching 53.5 ± 9.3%, compared to 36.7 ± 10.7% at the start ($P \leq 0.05$). The absolute lymphocyte counts also increased, from 4.2 ± 1.3 to 5.08 ± 1.1 × 10⁹/L.

Table 7. Comparative evaluation of hematological indicators in the experimental and control groups of highly productive cows before and after Betulin administration.

Indicators	**Norm**	**At the Beginning of the Experiment**		**At the End of the Experiment**	
		Experimental Group (n=10) (M±m)	Control Group (n=10) (M±m)	Experimental Group (n=10) (M±m)	Control Group (n=10) (M±m)
Hemoglobin (g/L)	90-120	107.0±6.9	107.9±7.5	102.3±8.8	100.8±8.7
Erythrocytes (10^{12}/L)	5-7.5	6.34±0.45	6.25±0.3	6.28±0.37	6.24±0.5
Leukocytes (10⁹/L)	4.5-12.0	12.0±3.1*	11.93±2.9	9.53±1.5 P≤0.01	10.76±3.4
Segmented Neutrophils (%)	20-35	43.9±6.8*	42.8±11.5	28.5±5.6* P≤0.05	46.2±10.7* Differences from the experimental group P≤0.05
Segmented Neutrophils (10⁹/L)		5.28±2.0*	5.36±2.5	2.7±0.6* P≤0.05	5.21±2.6* Differences from the experimental group P≤0.01
Monocytes (%)	2-7	5.1±3.03	5.2±2.7	4.5±2.6	6.7±2.6
Monocytes (10⁹/L)		0.64±0.4	0.59±0.3	0.43±0.2	0.72±0.3
Lymphocytes (%)	40-75	36.7±10.7*	39.6±11.3	53.5±9.3* P≤0.05	36.6±12.8* Differences from the experimental group P≤0.05
Lymphocytes (10⁹/L)		4.2±1.3	4.5±1.15	5.08±1.1	3.6±1.06* Differences from the experimental group P≤0.01

*Note: * - Significant differences at P ≤ 0.05 and P ≤ 0.01*

A comparison of the results obtained from the experimental group with those of the control group showed significant differences in the numbers of neutrophils and lymphocytes. The relative number of segmented neutrophils in the control group was 17.7% higher, amounting to 46.2 ± 10.7% compared to 28.5 ± 5.6% in the experimental group ($P \leq 0.05$). The absolute values in the control group were also higher, at 5.21 ± 2.6 × 10⁹/L, compared to 2.7 ± 0.6 × 10⁹/L in the experimental group ($P \leq 0.01$).
In contrast, the number of lymphocytes at the end of the experiment was significantly higher in the experimental group, amounting to 53.5 ± 9.3%, compared to 36.6 ± 12.8% in the control group, a difference of 16.9% ($P \leq 0.05$). The absolute lymphocyte count correlated with the relative values, being 5.08 ± 1.1 × 10⁹/L in the experimental group, compared to 3.6 ± 1.06 × 10⁹/L in the control group. These differences were also statistically significant ($P \leq 0.01$).

CONCLUSION

- The results of the study on the effects of betulin on the hematological indices of five-month-old and ten-month-old breeding calves, as well as high-yielding cows, demonstrated that the preparation significantly increases the number of peripheral blood lymphocytes and eliminates neutrophilic leukocytosis by reliably reducing the number of neutrophils, monocytes, and the total leukocyte count within physiological norms. Thus, it can be inferred that the preparation "Betulin" stimulates active lymphocyte reproduction and enhances phagocytosis, leading to the resolution of inflammatory processes, a reduction in neutrophil and monocyte counts, and an increase in lymphocyte numbers.
- The observed decrease in erythrocyte count and hemoglobin levels by the end of the experiment across all experimental groups appears to be associated with weather conditions. On the first day of the experiment, intense heat was recorded, which may have led to polycythemia. By contrast, the final day of the experiment was markedly cooler.

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Conflict of Interest Statement. The authors declare no conflict of interest.

REFERENCES

1. Ahn JY, Kim J, Cheong DH, Hong H, Jeong JY, Kim BG, (2022). An in vitro study on the efficacy of mycotoxin sequestering agents for aflatoxin B1, deoxynivalenol, and zearalenone, Animals 12(3), 333. https://doi.org/10.3390/ani12030333;
2. Gao Y, Li S, Wang J, Luo C, Zhao S, Zheng N, (2018). Modulation of intestinal epithelial permeability in differentiated Caco-2 cells exposed to aflatoxin M1 and ochratoxin A individually or collectively, Toxins 10(1), 13. https://doi.org/10.3390/toxins10010013;
3. Gnezdilova LA, Fedotov SV, Muradyan ZhYu, Rozinsky SM, (2023). Effect of mycotoxins on reproductive and production performance of lactating cows under conditions of intensive production, Veterinary, Animal Science and Biotechnology 4, 70-80. https://doi.org/10.36871/vet.zoo.bio.202304007;
4. Gnezdilova LA, Fedotov SV, Muradyan ZhYu, Rozinsky SM, (2024a). The influence of mycotoxins on the quality indicators of milk in cows in a large livestock complex, Bulletin of Peoples' Friendship University of Russia. Series: Agronomy and Animal Husbandry 19(1), 30-38. https://doi.org/10.22363/2312-797X-2024-19-1-30-38;
5. Gnezdilova LA, Fedotov SV, Muradyan ZhYu, Rozinsky SM, (2024b). Mycotoxins influence on cows homeostasis under intensive animal husbandry conditions, Bulletin of KrasSAU (4), 78–87. https://doi.org/10.36718/1819-4036-2024-4-78-87;

6. Kemboi DC, Antonissen G, Ochieng PE, Croubels S, Okoth S, Kangethe EK, Faas J, Lindahl JF, Gathumbi JK, (2020). A review of the impact of mycotoxins on dairy cattle health: Challenges for food safety and dairy production in sub-Saharan Africa, Toxins 12(4), 222. https://doi.org/10.3390/toxins12040222;
7. Kosolapova VG, Khalifa MM, Ishmuratov HG, (2021). The impact of mycotoxins on dairy cow health and milk production, Kormoproizvodstvo 9, 38-46;
8. Makau CM, Matofari JW, Muliro PS, Bebe BO, (2016). Aflatoxin B1 and deoxynivalenol contamination of dairy feeds and presence of aflatoxin M1 contamination in milk from smallholder dairy systems in Nakuru, Kenya, International Journal of Food Contamination 3(1), 6. https://doi.org/10.1186/s40550-016-0033-7;
9. Muradyan ZhYu, Rogov RV, Kruglova YuS, (2021). Effect of probiotic drug "Mucinolextra" on the physiological state of the body of young cattle, Agrarian Science 344(5), 11-13. https://doi.org/10.32634/0869-8155-2021-349-5-11-13;
10. Popova SA, Skoptsova TI, Losyakova EV, (2017). Mikotoksiny v kormakh: Prichiny, posledstviya, profilaktika [Mycotoxins in feed: Causes, consequences, prevention], Izvestiya Velikolukskoy gosudarstvennoy sel'skokhozyaystvennoy akademii 1, 16-23;
11. Soldatenko NA, Drobin YuD, Bokun EA, Aliev AYu, (2020). The presence of mycotoxins in the organs of young animals and poultry at feeding by contaminated with mycotoxins feed, Problems of Veterinary Sanitation, Hygiene and Ecology 4, 439-442. https://doi.org/10.36871/vet.san.hyg.ecol.202004005;
12. Sulzberger SA, Melnichenko S, Cardoso FC, (2017). Effects of clay after an aflatoxin challenge on aflatoxin clearance, milk production, and metabolism of Holstein cows, Journal of Dairy Science 100(3), 1856-1869. https://doi.org/10.3168/jds.2016-11612;
13. Valgaeren B, Théron L, Croubels S, Devreese M, De Baere S, Van Pamel E, Daeseleire E, De Boevre M, De Saeger S, Vidal A, Di Mavungu JD, Fruhmann P, Adam G, Callebaut A, Bayrou C, Frisée V, Rao AS, Knapp E, Sartelet A, Pardon B, Deprez P, Antonissen G, (2019). The role of roughage provision on the absorption and disposition of the mycotoxin deoxynivalenol and its acetylated derivatives in calves: From field observations to toxicokinetics, Archives of Toxicology 93(2), 293-310. https://doi.org/10.1007/s00204-018-2368-8;
14. Xiong JL, Wang YM, Nennich TD, Li Y, Liu JX, (2015). Transfer of dietary aflatoxin B1 to milk aflatoxin M1 and effect of inclusion of adsorbent in the diet of dairy cows, Journal of Dairy Science 98(4), 2545-2554. https://doi.org/10.3168/jds.2013-7842;
15. Zhang F, Liu L, Ni S, Deng J, Liu G-J, Middleton R, Inglis DW, Wang S, Liu G, (2019). Turn-on fluorescence aptasensor on magnetic nanobeads for aflatoxin M1 detection based on an exonuclease III-assisted signal amplification strategy, Nanomaterials 9(1), 104. https://doi.org/10.3390/nano9010104;