ASSESSING THE ECOLOGICAL IMPACT OF BETULIN-CONTAINING FEED ADDITIVES: INSIGHTS FROM BIOCHEMICAL PARAMETERS IN BREEDING CALVES AND DAIRY COWS

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ABSTRACT

The study aimed to examine the effect of the feed additive betulin on the biochemical parameters of blood serum in calves and cows. The experiment was conducted in the Tver region and at the K.I. Skryabin Moscow State Academy of Veterinary Medicine and Biotechnology with two experimental and two control groups of animals. Betulin was administered orally at a dose of 10 mg/kg body weight for 14 days, followed by biochemical analyses. The EOS BRAVO v.200 analyzer was used to measure protein, bilirubin, AST, ALT, and other parameters. Statistical analysis was performed using Microsoft Excel with biometric methods, and significance was assessed at $P < 0.05$, 0.01, and 0.001. The results showed that betulin normalized bilirubin levels, which, before the experiment, were below 2.5 μmol/L in 100% of experimental animals. This indicated tissue hypoxia, which was mitigated after using the additive. Analyzing the obtained data on the use of betulin on cows, we can conclude that the studied biochemical parameters of cow serum at the beginning of the experiment did not have significant differences between the groups. By the end of the experiment, the cows of the experimental group showed an increase within the physiological norm of bilirubin and total protein and a decrease in aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase, albumin, lactate dehydrogenase (LDH) and cholesterol.

Keywords: betulin, breeding farm, blood test, therapeutic dose, biochemical parameters, oral administration, live weight.

INTRODUCTION

The productivity and high reproductive performance of cows are directly influenced by production-related stressors. Conditions that impose additional stress on animals, such as an imbalanced diet, infectious and parasitic diseases, or obstetric-gynecological disorders, exacerbate the (Ashenbrenner, 2023; Vorobyova, 2019; Gnezdilova, 2023). When analyzing feed quality, attention should be paid to natural contaminants, including mycotoxins, which continue to have a significant impact on animal health (Gnezdilova, 2024; Gnezdilova, 2024; Demidovich, 2019). The positive experience of using plant-based triterpenoids in veterinary practice as immunostimulants for various pathological conditions in animals of different species is noteworthy. Lupan triterpenoids, specifically betulinic acid and

betulonic acid, are highly promising targets for the development of new pharmaceutical, including veterinary, drugs (Gnezdilova, 2024; Demidovich, 2019). A new approach in veterinary medicine addressing the issue of infectious diseases involves the development and application of environmentally friendly, plant-derived preparations. These preparations exhibit bactericidal, bacteriostatic, virucidal, and immunomodulatory effects on the affected organism (Zemlyanitsyna, 2013; Kosolapova, 2021; Kulikova, 2017). For a practicing veterinarian, it is crucial to have therapeutic agents with high efficacy, broad-spectrum effects, and low production costs (Muradyan, 2021; Popova, 2017; Soboleva, 2008). Such agents can provide a multifaceted impact on disease pathogens, the stages of pathological processes, and stimulate recovery mechanisms. Alongside drugs targeting specific pathological links, substances that enhance the organism's defense mechanisms are increasingly being utilized (Soldatenko et al., 2020; Shukshina & Shiryaeva, 2015). Betulin is a natural pentacyclic triterpenoid of the lupane series. It is found in numerous plants (hazel, calendula, licorice, etc.), but on an industrial scale, it is extracted from birch bark—the outer layer of the white birch (Betula alba) and silver birch (Betula pendula) (Khvostova, 2004; Yurchenko, 2005; Kemboi et al., 2020). In its free form, betulin does not occur naturally. Extensive studies conducted in over 40 international and Russian research centers have demonstrated the effectiveness of triterpenoid compounds as direct regulators of enzymatic activity in the organism (Makau et al., 2016; Sulzberger et al., 2017; Valgaeren et al., 2019). The immunostimulatory activity of betulin is evident in its ability to induce endogenous interferon production in the organism, enhance both cellular and humoral immunity, and increase the activity of certain immunocompetent cells. This includes boosting all phagocytosis indicators (the capacity of phagocytes to destroy viruses and bacterial cells) (Zhang et al., 2019; Gao et al., 2018; Ahn et al., 2022; Xiong et al., 2015).
Research Objective: To study the effect of a betulin-based feed additive on the biochemical parameters of blood serum in breeding calves and dairy cows.

MATERIAL AND METHOD

Research Method

The study was conducted at the dairy complexes of the pedigree farm SPP Kolkhoz "Soznatelny," Zubtsovsky district, Tver region, as well as at the Department of Diagnosis of Diseases, Therapy, Obstetrics, and Animal Reproduction, and the Diagnostic and Treatment Center of the Moscow State Academy of Veterinary Medicine and Biotechnology named after K.I. Skryabin.
To study the effect of betulin on the biochemical parameters of blood serum, the following groups were formed:

- ✓ Two groups of Sychev breed calves (experimental and control), with 10 animals in each group, aged 5 months, and a live weight of 150-165 kg.
- ✓ Two groups of lactating healthy Sychev breed cows in their 2nd-3rd lactation (experimental and control), with 10 animals in each group, a live weight of 550-600 kg, and an annual milk yield of 7-8 thousand liters.
- ✓ Routine diagnostic measures were carried out on the experimental animals (the farm is free from leukemia, tuberculosis, and brucellosis).
- ✓ The betulin-containing feed supplement was administered orally to each animal in the experimental group at a dose of 10 mg/kg body weight, mixed with water, once daily for 14 days.

To evaluate the effect of betulin on the animals' organisms and to rule out any concurrent diseases, all animals underwent clinical examinations and biochemical analyses at the start and end of the experiment. For biochemical studies of blood serum, an automatic biochemical analyzer EOS BRAVO v.200 (Russia) was used. The following parameters were determined: total protein, albumin, globulin, creatinine, urea, bilirubin, AST, ALT, LDH, alkaline phosphatase, glucose, cholesterol, phosphorus, and total calcium.
Statistical Analysis. Experimental data were processed using biometric methods (Lakin GF, 1990; Makarova NV, Trofimets VYa, 2002) and the Microsoft Office Excel "Data Analysis" package. The significance of differences between groups was indicated using the following designations: * for comparisons of the indicators of the first, second, and third groups with the control group. Significance levels were marked as follows: * – $P < 0.05$; ** – $P < 0.01$; *** – $P < 0.001$.

Data analysis

Biochemical Parameters

The results of the biochemical analysis of blood serum in five-month-old calves from the experimental group, before and after the administration of the betulin-containing feed additive, are presented in Table 1.

Table 1. Biochemical parameters of blood serum in five-month-old calves before and after oral administration of a betulin-containing feed additive at a dose of 10 mg/kg body weight with water, once daily for 14 days.

n/n	Total Bilirubin (µmol/L)	AST (U/L)	ALT (U/L)	Alkaline Phosphatase (U/L)	Urea (mmol/L)	Creatinine (µmol/L)	Total Protein (g/L)	Albumin (g/L)	Glucose (mmol/L)	LDH (U/L)	Cholesterol (mmol/L)	Phosphorus (mmol/L)	Total Calcium (mmol/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
	5-month-old calves, n=10. Experimental group. Before and 14 days after oral administration of the betulin-containing feed additive												
1	1,8/2,9	77/89,2	29,2/28,.9	103/100	2,84/2,64	82/90	61,84/65,07	34,1/32,3	4,41/4,1 1	1229/1211	3,22/2,49	2,57/2,64	2,27/2,17
2	1,8/3,87	78,2/91,7	32,4/30,9	229/147	2,2/1,99	92/90	59,1/61,45	37,1/35,6	4,48/4,4 1	1345/1215	2,9/2,66	2,68/2,71	2,08/2,14
3	1,8/4,12	74,9/80,5	30,1/28,1	136/105	2,86/2,62	85/87	64,65/65,23	37,9/34,6	4,59/4,5 1	1129/1107	1,89/1,6	2,83/2,59	2,21/2,24
4	1,8/2,91	99,7/85	26,5/27,5	153/127	2,66/2,05	85/99	68,27/69,10	39,6/35,9	3,97/3,5 4	1190/1024	3,1/1,69	2,8/3,01	2,36/2,01
5	2,4/3,85	96,2/97,6	30,9/27,6	129/112	2,23/2,46	85/93	58,73/62,59	35,4/34,8	3,94/3,8 8	1642/1398	3,06/2,75	2,5/2,45	2,17/2,2
6	1,8/2,8	69,3/79,3	29,9/29,6	147/130	3,86/3,79	99/115	68,45/66,25	41,7/39,3	5,04/4,4 3	1195/946	2,55/1,91	2,58/2,63	2,3/2,29
7	1,8/3,4	94,5/82,2	32,2/30,2	186/162	3,26/3,65	83/85	62,15/66,12	37,2/37,5	4,62/4,6 1	1324/1229	3,15/2,98	2,56/2,61	2,19/2,20
8	1,8/2,81	86,3/81,5	28,6/27,1	148/132	4,36/3,61	87/86	63,51/66,44	38,4/38,1	4,25/4,3 4	1548/1369	2,08/2,88	2,75/2,81	2,18/2,21
9	2,2/3,48	78,3/81,2	32,3/29,1	126/120	2,74/2,27	95/90	60,22/63,43	36,3/34,5	4,59/4,5 2	1285/1147	2,96/2,77	2,63/2,51	2,16/2,15
10	1,8/2,68	75,6/71,1	30,5/27,3	133/115	3,26/3,12	88/90	59,23/64,12	39,5/36,7	3,88/3,7 6	1328/1112	3,12/2,92	2,66/2,62	2,11/2,16
M±m	1,84±0,12*/ 3,28±0,53 (p≤0,05)	83,0±10,4/ 83,93±7,3	30,26±1,8*/ 28,63±1,3 (p≤0,01)	149,0±35,3/ 125,0±19,0	3,02±0,6 8/ 2,82±0,6 7	88,1±5,5/ 92,5±8,8	62,6±3,59* */ 65,3±1,81 (p≤0,01)	37,72±2,2 / 35,93±2,0 4	4,37±0,3 / 4,21±0,3	1321,5±161* / 1175,8±140 (p≤0,01)	2,80±0,4 7/ 2,46±0,5 2	2,65±0,1 0/ 2,65±0,1 5	2,20±0,0 8/ 2,17±0,0 7
	5-month-old calves, n=10. Control group												

1	1,8/1,8	76,8/91,5	27,3/28,4	146/144	3,15/3,25	88/86	64,17/63,02	37,2/35,2	3,28/3,52	1268/1287	3,26/3,52	2,54/2,56	2,12/2,15
2	1,8/2,22	95,6/98,3	32,4/32,4	135/138	2,43/2,65	96/103	59,66/59,12	36,4/34,3	4,52/4,68	1359/1299	3,44/3,21	2,66/2,69	2,11/2,18
3	1,8/2,45	72,5/81,6	28,2/30,2	137/142	2,76/3,48	89/88	62,55/63,31	36,8/37,8	4,84/5,02	1452/1652	3,69/3,14	2,78/2,82	2,26/2,17
4	1,8/1,8	77,2/88	30,6/30,4	138/136	2,64/2,66	86/85	67,82/69,13	40,2/40,1	4,12/4,16	1122/1325	2,64/1,95	2,73/2,71	2,23/2,27
5	1,8/3,2	82,3/98	29,5/32,5	143/139	3,32/3,23	94/88	62,47/62,85	34,1/34,8	4,15/4,28	1297/1356	3,54/3,96	2,81/2,86	2,31/2,25
6	2,4/2,1	84,4/96,6	27,2/28,6	125/128	2,79/3,15	102/100	64,35/69,87	37,6/37,8	4,21/4,11	1589/1687	2,68/3,33	2,54/2,58	2,18/2,22
7	2,92/3,1	89,7/96	30,3/30,1	135/130	4,12/3,95	93/90	66,12/67,16	38,2/37,6	3,99/4,06	1127/1269	2,22/2,45	2,32/2,62	2,26/2,28
8	3,2/3,65	86,2/90,4	32,8/32,9	152/149	2,99/2,82	88/87	69,48/64,23	39,6/37,5	4,56/4,55	1134/1195	2,86/2,95	2,9/2,85	2,21/2,19
9	1,8/1,9	78,6/93,1	28,2/31,5	152/143	3,45/3,75	86/90	62,85/62,23	36,9/37,3	5,38/4,42	1245/1296	3,06/3,11	2,78/2,82	2,24/2,21
10	1,8/2,12	74/88,9	29,8/28,9	166/174	4,08/4,22	92/89	64,57/63,49	37,8/38,2	3,96/4,15	1158/1169	1,95/2,24	2,63/2,67	2,15/2,19
M±m	2,11±0,53/ 2,43±0,65* Differences from the experimental group (P ≤ 0.05)	81,73±7,3*/ 92,24±5,2* (p≤0,05) Differences from the experimental group (P ≤ 0.05)	29,63±1,9/ 30,59±1,6* Differences from the experimental group (P ≤ 0.05)	142,9±11,6 142,3±12,8** Differences from the experimental group (p≤0,01)	3,17±0,57/ 3,31±0,53	91,4±5,06/ 90,6±6,0	64,04±2,83/ 64,44±3,31	37,48±1,7/ 37,06±1,77	4,30±0,5/ 4,29±0,4	1275,1±155/ 1353,5±175** Differences from the experimental group (p≤0,01)	2,93±0,57/ 2,98±0,61	2,66±0,16/ 2,71±0,11	2,20±0,06/ 2,21±0,04
Reference Values	0-27,4	60-125	5-40	18-153	2-8	44-194	67-75	25-38	2,2-5,6	308,6-938,1	1,6-5,0	1,8-2,6	2,0-2,8

*Note: *- differences are significant at P ≤ 0.05; ** - differences are significant at P ≤ 0.01.*

Analyzing the data presented in Table 1, it can be concluded that at the beginning of the experiment, the biochemical parameters of blood serum in five-month-old calves from both the experimental and control groups showed no significant differences. After 14 days of administering the betulin-containing feed additive, the experimental group of calves demonstrated an increase in total bilirubin and total protein levels within the physiological norm, as well as a decrease in alanine aminotransferase (ALT), alkaline phosphatase, and lactate dehydrogenase (LDH). At the start of the experiment, the total bilirubin level in the blood serum was relatively low, measuring 1.84 ± 0.12 µmol/L in the experimental group and 2.11 ± 0.53 µmol/L in the control group, with a reference range of 0–27.4 µmol/L. After 14 days, the total bilirubin level in the experimental group increased by 78% (P ≤ 0.05), reaching 3.28 ± 0.53 µmol/L, while the control group showed no changes, maintaining a level of 2.43 ± 0.65 µmol/L. A comparison of the final results between the experimental and control groups revealed a statistically significant difference (P ≤ 0.05). At the beginning of the experiment, blood serum aspartate aminotransferase (AST) levels in calves were within the physiological norm. By the end of the experiment, AST levels in the experimental group of calves remained unchanged. Meanwhile, in the control group, AST levels increased by 12.8% (P ≤ 0.05), from 81.73±7.3 to 92.24±5.2 U/L. A comparison of the final results between the experimental and control groups showed that AST levels in the control group were 9.9% higher ($P \leq 0.05$) than in the experimental group (92.24±5.2 U/L vs. 83.93±7.3 U/L, respectively).

The alanine aminotransferase (ALT) levels in the blood serum of calves in both the experimental and control groups were also within the physiological norm at the start of the experiment. By the end of the experiment, ALT levels in the experimental group decreased by 5.4% ($P \leq 0.01$), while the control group showed a slight increase from 29.63±1.9 to 30.59±1.6 U/L. The final results indicated significant differences between the experimental and control groups ($P \leq 0.05$).

The alkaline phosphatase (ALP) levels in the blood serum of some animals in both groups exceeded the reference range (18 to 153 U/L) at the start of the experiment. The mean ALP (M±m) in the experimental group was 149.0±35.3 U/L, and in the control group, it was 142.9±11.6 U/L. After 14 days of administering the betulin-containing feed additive, ALP levels in the experimental group decreased by 17%, from 149.0±35.3 to 125.0±19.0 U/L, while the ALP levels in the control group remained unchanged. By the end of the experiment, ALP levels in the experimental group were 12% lower ($P \leq 0.01$) than those in the control group (125.0±19.0 U/L vs. 142.3±12.8 U/L, respectively).

The total protein levels in the blood serum of five-month-old calves were below the reference range (67–75 g/L) at the start of the experiment, measuring 62.6±3.59 and 64.04±2.83 g/L in the experimental and control groups, respectively. By the 14th day of the experiment, total protein levels in the experimental group increased by 4.3% to 65.3±1.81 g/L ($P \leq 0.01$). In the control group, the total protein levels remained almost unchanged at 64.44±3.31 g/L compared to 64.04±2.83 g/L at the start.
Significant changes were observed in the levels of lactate dehydrogenase (LDH) in the blood serum. At the start of the experiment, LDH levels exceeded the reference values by 40.86% in the experimental group and 35.92% in the control group, measuring 1321.5±161 and 1275.1±155 U/L, respectively. After 14 days of administering the betulin-containing feed additive, LDH levels in the experimental group decreased by 11%, reaching 1175.8±140 U/L ($P \leq 0.01$). In contrast, LDH levels in the control group increased by 6% to 1353.5±175 U/L, compared to 1275.1±155 U/L at the start. By the end of the experiment, LDH levels in the experimental group were 13% lower than in the control group ($P \leq 0.01$).

The levels of urea, creatinine, albumin, glucose, cholesterol, phosphorus, and total calcium in the blood serum showed no significant differences between the experimental and control groups, either at the start or at the end of the experiment.

Table 2: Biochemical Parameters of Blood Serum in Cows Before and After Oral Administration of a Betulin-Containing Feed Additive at a Dose of 10 mg/kg Body Weight with Water Once Daily for 14 Days.

n/n	Total Bilirubin (μmol/L)	AST (U/L)	ALT (U/L)	Alkaline Phosphatase (U/L)	Urea (mmol/L)	Creatinine (μmol/L)	Total Protein (g/L)	Albumin (g/L)	Glucose (mmol/L)	LDH (U/L)	Cholesterol (mmol/L)	Phosphorus (mmol/L)	Total Calcium (mmol/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
	Experimental Group – Cows (n=10). Before and 14 days after oral administration of the betulin-containing feed additive												
1	1,8/5,68	107/104,8	31,1/29,3	70/68	6,51/5,56	100/103	68,58/74,49	38,9/33,4	3,06/2,92	1337/1107	4,73/4,29	2,07/1,91	2,35/2,25
2	1,8/5,59	110,9/102,7	40,9/38	65/67	4,93/5,26	99/105	75,93/80,52	36,8/33,8	2,87/2,82	1265/1170	5,64/4,92	1,9/1,87	2,25/2,36
3	2,34/2,9	137,8/89,2	40,2/32,9	92/80	7,81/5,04	121/90	61,84/67,07	41,6/32,3	2,8/4,11	1326/1211	4,36/4,49	1,73/2,43	2,18/2,17
4	4/6,52	118/101,6	44,3/37,1	69/64	6,83/6,4	84/98	69,53/72,82	37,4/37,2	3,34/3,69	1287/1015	5,98/5,87	2,14/1,88	2,16/2,05
5	1,8/5,91	118,2/96,9	34,2/37,6	62/55	5,36/4,55	99/93	78,51/82,11	37,2/36,6	2,88/2,94	1307/1224	6,05/5,82	1,69/1,55	2,07/2,16
6	5,24/6,74	103,2/98,1	44/42,5	68/56	6,58/7,25	108/110	71,86/75,81	39,8/38,4	3,08/3,33	1389/1166	6,29/5,06	2,1/2,53	2,25/2,19
7	2,44/4,89	82,4/84,6	41,9/41,4	102/49	5,02/4,8	106/106	67,69/73,76	40,3/37,2	3,84/3,46	1330/1321	5,89/3,57	2,17/2,21	2,21/2,12

8	1,8/6,15	98,3/98,8	32,4/27,6	41/38	4,57/5,01	101/104	78,36/79,31	42,4/39,6	2,85/3,31	1248/1189	7,13/5,44	1,75/1,78	2,14/2,23
9	1,8/5,73	123/112,1	33,2/32	57/50	5,54/6,75	123/117	76,55/84,1	41,2/43,5	3,37/3,73	1928/1567	5,02/4,77	1,64/2,35	2,44/2,27
10	1,8/5,11	139,3/96,7	38,9/36,3	99/80	5,39/5,11	91/92	71,22/78,32	37,9/34,9	3,12/2,92	1365/1117	5,14/4,52	2,15/1,97	2,31/2,29
M±m	2,48±1,18*/ 5,52±1,08 (p≤0,05)	113,81±17,4**/ 98,55±7,7 (p≤0,01)	38,11±4,9/ 35,47±4,9	72,5±19,4/ 60,7±13,6	5,85±1,02/ 5,57±0,9	103,2±12/ 101,8±8,5	71,8±5,2**/ 76,8±5,05 (p≤0,01)	39,35±2,0**/ 36,69±3,33 (p≤0,01)	3,12±0,3/ 3,32±0,4	1378,2±197*/ 1208,7±149 (p≤0,01)	5,62±0,8**/ 4,87±0,7 (p≤0,01)	1,93±0,2/ 2,04±0,3	2,23±0,1/ 2,2±0,09
	Control Group – Cows (n=10)												
1	1,8/2,4	88/86,5	40,3/41,5	99/103	6,81/7,02	92/96	72,52/74,23	37,5/36,5	3,16/3,22	1233/1240	6,33/6,29	2,14/2,09	2,52/2,36
2	2,45/2,1	114,3/118,3	33,4/33,2	102/116	5,26/5,66	73/75	66,84/68,34	40,1/39,6	2,28/3,41	1237/1288	5,15/5,28	2,1/1,89	2,32/2,38
3	2,42/3,18	132,6/133,2	38,7/39,6	105/110	4,29/5,02	115/116	64,25/69,15	38,9/40,2	3,27/2,95	1347/1355	5,02/5,15	1,56/1,62	2,17/2,16
4	3,4/3,22	96,4/98,6	43,4/41,7	63/58	6,43/6,28	82/84	77,44/74,22	42,5/44,4	3,11/3,25	1316/1334	4,44/4,23	2,17/2,06	2,34/2,25
5	2,2/1,8	114,4/110,5	33,2/30,5	92/105	5,12/5,24	96/99	69,45/68,22	37,5/38,4	2,75/2,95	1356/1326	5,78/5,76	1,85/1,89	2,16/2,14
6	4,3/3,2	122/124,5	36,8/39,8	92/106	4,58/4,42	109/107	71,85/72,12	41,1/41,3	3,06/3,12	1325/1345	6,43/6,62	1,78/1,62	2,19/2,19
7	1,8/2,9	134,2/128,3	37,1/38,5	67/64	7,29/7,25	94/104	67,89/66,21	37,6/36,6	2,79/3,89	1283/1273	4,48/4,34	2,6/2,7	2,33/2,22
8	2,54/3,24	80,4/82,2	32,6/32,9	40/39	5,49/5,11	87/82	78,12/75,24	42,2/41,9	3,11/2,88	1284/1295	6,59/6,25	2,12/2,14	2,38/2,25
9	4,6/5,15	96,6/94,3	31,5/28,5	72/75	6,79/7,15	106/112	70,58/70,11	34,5/36,3	3,25/3,37	1394/1375	5,89/5,49	1,71/1,65	2,13/2,16
10	1,9/2,8	106,2/102,2	38,6/36,1	65/69	4,14/4,77	104/106	75,44/73,65	40,1/42,1	2,97/2,96	1235/1292	3,22/3,52	2,3/2,4	2,24/2,28
M±m	2,74±1,01/ 2,99±0,9* Differences from the experimental group (p≤0,05)	108,51±18,2/ 107,86±17,8	36,56±3,8 36,23±4,7	79,7±21,3 85,5±28,0** Differences from the experimental group (p≤0,01)	5,62±1,13/ 5,79±1,05	95,8±12,9/ 98,1±13,6	71,4±4,57/ 71,1±3,14* Differences from the experimental group (p≤0,05)	39,2±2,48/ 39,73±2,76** Differences from the experimental group (p≤0,01)	2,97±0,3/ 3,2±0,3	1301,0±56/ 1312,3±41** Differences from the experimental group (p≤0,01)	5,33±1,07/ 5,29±1,0	2,03±0,3/ 2,0±0,3	2,27±0,1/ 2,23±0,08
Reference Values	0-27,4	60-125	5-40	18-153	2-8	44-194	67-75	25-38	2,2-5,6	308,6-938,1	1,6-5,0	1,8-2,6	2,0-2,8

*Note: *- differences are significant at P ≤ 0.05; ** - differences are significant at P ≤ 0.01.*

Analyzing the data from Table 2, it can be concluded that the studied biochemical parameters of cow serum did not show significant differences between the groups at the beginning of the experiment. By the end of the experiment, cows in the experimental group exhibited increases in bilirubin and total protein levels within the physiological norm, along with decreases in aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase, albumin, lactate dehydrogenase (LDH), and cholesterol levels.

At the start of the experiment, total bilirubin levels in both the experimental and control groups were at the lower boundary of the norm, measuring 2.48±1.18 µmol/L in the experimental group and 2.74±1.01 µmol/L in the control group (reference range: 0–27.4 µmol/L). After 14 days, bilirubin levels in the experimental group significantly increased to 5.52±1.08 µmol/L, which is 2.2 times higher than at the beginning of the experiment ($P \leq 0.05$). In contrast, the control group's bilirubin levels remained unchanged at 2.99±0.9 µmol/L, which is 1.84 times lower than the experimental group ($P \leq 0.05$).

According to the table, by the end of the experiment, AST levels in the experimental group significantly decreased from 113.81±17.4 U/L to 98.55±7.7 U/L ($P \leq 0.01$). Meanwhile, in the control group, AST levels remained virtually unchanged, measuring 108.51±18.2 U/L at the beginning and 107.86±17.8 U/L at the end of the experiment.

A similar trend was observed for ALT levels. In the control group, ALT levels remained stable, measuring 36.56±3.8 U/L at the beginning and 36.23±4.7 U/L at the end of the experiment. In the experimental group, ALT levels decreased significantly from 38.11±4.9 U/L at the start to 35.47±4.9 U/L at the end.

Alkaline phosphatase levels in the blood serum of the experimental group also decreased by the end of the experiment, from 72.5±19.4 U/L to 60.7±13.6 U/L. Conversely, in the control group, alkaline phosphatase levels increased from 79.7±21.3 U/L to 85.5±28.0 U/L. A comparison of the final results between the groups revealed significant differences ($P \leq 0.01$).

In the experimental group, total protein levels in blood serum significantly increased from 71.8±5.2 g/L to 76.8±5.05 g/L ($P \leq 0.01$). In contrast, no significant changes were observed in the control group, where total protein levels measured 71.4±4.57 g/L at the start and 71.1±3.14 g/L at the end. Comparing the final results, the total protein level in the experimental group was significantly higher ($P \leq 0.05$) than in the control group (76.8±5.05 g/L vs. 71.1±3.14 g/L).

Analysis of albumin levels in blood serum revealed that values exceeded the reference range (25–38 g/L) at the start of the experiment in both groups. After 14 days, albumin levels in the experimental group decreased to the physiological norm, from 39.35±2.0 g/L to 36.69±3.33 g/L ($P \leq 0.01$). In the control group, albumin levels remained unchanged, measuring 39.2±2.48 g/L at the start and 39.73±2.76 g/L at the end. The final comparison showed that albumin levels in the experimental group were significantly lower by the end of the experiment ($P \leq 0.01$), corresponding to the physiological norm (36.69±3.33 g/L), whereas the control group still exceeded the norm (39.73±2.76 g/L).

Both groups exhibited significantly elevated LDH levels above the physiological norm at the start of the experiment. In the experimental group, LDH levels measured 1378.2±197 U/L, and in the control group, 1301.0±56 U/L (reference range: 308.6–938.1 U/L). By the 14th day of the experiment, LDH levels in the experimental group significantly decreased from 1378.2±197 U/L to 1208.7±149 U/L ($P \leq 0.01$). In the control group, LDH levels remained unchanged at 1312.3±41 U/L, which was significantly higher than in the experimental group (1208.7±149 U/L, $P \leq 0.01$).

The average cholesterol levels in blood serum at the beginning of the experiment exceeded the reference range (from 1.6 to 5.0 μmol/L) in both the experimental and control groups. However, by the end of the experiment, this parameter in the experimental group decreased from 5.62±0.8 μmol/L to 4.87±0.7 μmol/L ($P \leq 0.01$), aligning with the physiological norm. In the control group, the serum cholesterol level remained unchanged by the end of the experiment at 5.29±1.0 μmol/L compared to 5.33±1.07 μmol/L at the start of the study.

Analysis of the data presented in the table showed no significant changes in urea, creatinine, glucose, phosphorus, or total calcium levels in the blood serum of animals in both experimental groups.

The results of the study demonstrated that the betulin-containing feed additive had a noticeable effect on the experimental animals. For example, it was observed that the additive normalized bilirubin levels in blood serum. According to Kulikova N.A. (2017), a bilirubin level in blood below 2.5 μmol/L is critically low. In this experiment, 100% of animals in the experimental group and 80% in the control group had bilirubin levels below 2.5 μmol/L before receiving the additive. Bilirubin levels at the lower limit of the norm may indicate tissue hypoxia.

After using the betulin-containing feed additive, the bilirubin level in the blood serum of animals in the experimental group rose above critical values from 1.84±0.12 μmol/L to 3.28±0.53 μmol/L ($P \leq 0.05$).

In the analysis of blood serum biochemical parameters, aspartate aminotransferase (AST) and alanine aminotransferase (ALT) levels are of significant importance. These enzymes are localized in the cytosol of cells in many organs, with the highest concentrations in liver and myocardial cells. Cellular damage increases the enzyme activity in the blood (Shukshina S.S., 2015). At the beginning of the experiment, AST levels in both groups were almost identical, within the physiological norm, at 83.0±10.4 U/L and 81.73±7.3 U/L, respectively ($P \leq 0.05$). By the end of the experiment, AST levels in the experimental group remained unchanged, while in the control group, AST increased by 12.8%. According to Aschenbrenner E.I. (2023), animals with fatty liver dystrophy show significant increases in AST enzymatic activity. ALT levels in the experimental group showed a slight but significant decrease by the end of the study ($P \leq 0.01$).

At the start of the experiment, 50% of cows in the experimental group had ALT levels exceeding the reference range, while the remaining 50% were at the upper limit of normal. A similar pattern was observed in the control group. This is likely related to liver disease, as ALT is located in the cytoplasm of liver cells. Although AST levels did not exceed the physiological norm, they were at the upper limit in both groups. After 14 days of using the betulin-containing feed additive, AST and ALT levels in the blood of experimental cows decreased by 14% ($P \leq 0.01$) and 7%, respectively. This suggests that the betulin-containing feed additive has anti-inflammatory, cardioprotective, and hepatoprotective effects.

The positive effect of the betulin-containing feed additive on liver and gallbladder function is also supported by changes in alkaline phosphatase (ALP) levels in blood serum. ALP is an enzyme found in many tissues of the animal body, particularly in growing bones, liver parenchyma, and bile duct walls. Increased ALP activity is possible in cases of cholestasis, obstructive liver diseases, toxic hepatitis, and hepatotoxin poisoning (Soboleva Y.G., 2008). At the beginning of the experiment, ALP levels in the blood serum of calves exceeded the reference range in 30% of animals, while 70% were at the upper limit of normal. By the end of the experiment, ALP levels in the experimental group decreased from 149.0±35.3 U/L to 125.0±19.0 U/L. Meanwhile, in the control group, ALP levels did not change.

In cows, ALP levels in both groups were not significantly different at the start of the experiment. However, by the end of the study, ALP levels in the experimental group decreased by 16.3%, while in the control group, they increased by 7.2%. By the end of the experiment, the differences between the experimental and control groups were significant ($P \leq 0.01$). This effect can be explained by the pronounced choleretic and hepatoprotective properties of betulin (Yurchenko I.V., 2005).

The anti-inflammatory and hepatoprotective effects of the betulin-containing feed additive are also supported by the results of lactate dehydrogenase (LDH) levels in the blood serum of five-month-old calves. Like ALT, LDH is a cytoplasmic enzyme in blood serum. According to Khvostova O.V. (2004), LDH levels in animals with liver pathologies are higher than in clinically healthy animals. At the start of the study, LDH levels exceeded the upper limit of the norm in both experimental groups. After using the betulin-containing feed additive, LDH levels in the blood serum of calves in the experimental group significantly decreased and approached normal levels ($P \leq 0.01$). Meanwhile, LDH levels in the control group increased further.

LDH levels in cows at the start of the experiment exceeded the upper norm by 46.9% in the experimental group and 38.7% in the control group. After using the betulin-containing feed additive, LDH levels in the blood of cows in the experimental group approached normal levels, decreasing by 18% ($P \leq 0.01$), while LDH levels in the control group still significantly exceeded the upper reference range.

The analysis of total protein levels revealed lower values at the start of the study. The increase in total protein levels in the experimental group calves by the end of the experiment ($P \leq 0.01$) indicates that the betulin-containing feed additive enhances compensatory protein synthesis in the globulin fraction (Demidovich A.P., 2019). According to Zemlyanitsyna I.R. (2013), betulin use increases post-vaccine antibody levels by four times.

An analysis of total protein and albumin levels in cows showed a 6.9% increase in total protein ($P \leq 0.01$) and a 6.8% decrease in albumin in the experimental group by the end of the study. According to Demidovich A.P. (2019), the increase in total protein may be due to compensatory enhancement of globulin protein synthesis with a concurrent decrease in albumin production.

CONCLUSIONS

- The authors conclude that the use of the betulin-containing feed additive contributed to the normalization of blood serum cholesterol levels in cows. Cholesterol is a precursor to hormones and bile acids. At the start of the experiment, cholesterol levels in cows of both groups exceeded the upper limit of the norm by 12% and 5%, respectively. By the end of the experiment, cholesterol levels in the experimental group had aligned with the reference values, unlike in the control group.
- Thus, it can be stated that the betulin-containing feed additive increases the globulin protein fraction while providing hepatoprotective, choleretic, and anti-inflammatory effects.

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REFERENCES

1. Ahn J.Y., Kim J., Cheong D.H., Hong H., Jeong J.Y., Kim B.G., 2022. An in vitro study on the efficacy of mycotoxin sequestering agents for aflatoxin B1, deoxynivalenol, and zearalenone. Animals, 12: 333;
2. Ashenbrenner A.I., Belyaeva N.Yu., Chekunkova Yu.A., Khapersky Yu.A., 2023. Biochemical markers of hepatobiliary system dysfunction in fresh cows. Vestnik Krasnoyarsk SAU, 10 (199): 202-207;
3. Demidovich A.P., 2019. Diagnostic value of biochemical parameters of blood: a teaching aid for students majoring in 1-740302 "Veterinary Medicine". Vitebsk: VGAVM. 36 p;
4. 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: 13;
5. Gnezdilova L.A., Fedotov S.V., Muradyan Zh.Yu., Rozinsky S.M., 2023. The effect of mycotoxins on reproductive and production indicators of lactating cows under intensive production conditions. Veterinary Science, Zootechnics and Biotechnology, 4: 70-80. https://doi.org/10.36871/vet.zoo.bio.202304007;
6. Gnezdilova L.A., Fedotov S.V., Muradyan Zh.Yu., Rozinsky S.M., 2024. The influence of mycotoxins on the quality indicators of milk in cows in a large livestock complex. Bulletin of the Peoples' Friendship University of Russia. Series: Agronomy and Animal Husbandry, 19 (1): 30-38;
7. Gnezdilova L.A., Fedotov S.V., Muradyan Zh.Yu., Rozinsky S.M., 2024. The influence of mycotoxins on hemostasis of cows in conditions of intensive animal husbandry. Bulletin of KrasSAU, 4 (205): 78-87;
8. Kemboi D.C., Antonissen G., Ochieng P.E., Croubels S., Okoth S., Kangethe E.K., Faas J., Lindahl J.F., Gathumbi J.K., 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;
9. Khvostova O.V., 2004. Biochemical blood parameters in various functional states of the liver in cattle. Bulletin of the Vitebsk State Medical University, 3: 3;
10. Kosolapova V.G., Khalifa M.M., Ishmuratov H.G., 2021. The influence of mycotoxins on the health and productivity of dairy cattle. Feed Production, 9: 38-46;
11. Kulikova N.A., 2017. Study of bilirubin content in the blood of cattle. International Student Scientific Bulletin, 4(5): 616-618;
12. Makau C.M., Matofari J.W., Muliro P.S., Bebe B.O., 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): 1–10. https://doi.org/10.1186/s40550-016-0033-7;
13. Muradyan Zh.Yu., Rogov R.V., Kruglova Yu.S., 2021. The influence of the probiotic drug "Mucinol-Extra" on hematological parameters of the blood of young cattle. Agrarian Science, 5: 11-13;
14. Popova S.A., Skoptsova T.I., Losyakova E.V., 2017. Mycotoxins in feed: causes, consequences, prevention. Bulletin of the Velikolukskaya State Agricultural Academy, 1: 16-23;
15. Shukshina S.S., Shiryaeva O.Yu., 2015. Dynamics of transaminase activity depending on the physiological state. Fundamental and Applied Research: Problems and Results, 17: 35-38;
16. Soboleva Yu.G., 2008. Age and physiological features of alkaline phosphatase activity in the blood serum of cattle. In: Youth and science in the 21st century. Collection of articles by young scientists. Vitebsk. pp: 74-78;
17. Soldatenko N.A., Drobin Yu.D., Bokun E.A., Aliev A.Yu., 2020. The presence of mycotoxins in the organs of young animals and birds when feeding feed contaminated with mycotoxins. Problems of Veterinary Sanitation, Hygiene and Ecology, 4(36): 439-442. https://doi.org/10.36871/vet.san.hyg.ecol.202004005;
18. Sulzberger S.A., 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;
19. Valgaeren B., De Baere S., Devreese M., Antonissen G., Duchateau L., Vanhaecke L., Chiers K., De Saeger S., Gholami S., 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;

20. Vorobyova O.A., Malygina D.S., Grubova E.V., Melnikova N.B., 2019. Betulin derivatives. Biological activity and increased solubility. Chemistry of Plant Raw Materials, 4: 407-430;
21. Xiong J.L., Wang Y.M., Nennich T.D., Li Y., Liu J.X., 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;
22. Yurchenko I.V., 2005. Experimental study of the hepatoprotective and choleretic properties of betulin. Dissertation for the degree of candidate of medical sciences. State Educational Institution of Higher Professional Education "Military Medical Academy"; St. Petersburg. 200 p;
23. Zemlyanitsyna I.R., 2013. Use of Betulin-PG to increase the intensity of post-vaccination immunity against erysipelas and porcine circovirus infection. Scientific Notes of the Kazan State Academy of Veterinary Medicine named after N.E. Bauman, 214: 185-190;
24. Zhang F., Liu L., Ni S., Deng J., Liu G.-J., Middleton R., Inglis D.W., 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: 104;