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Monitoring in biochemical parameters of blood serum of calves against the background of the use of betulin-containing feed additive

Larisa A. Gnezdilova ✉, **Zhora Yu. Muradyan**, **Yulia S. Kruglova**, **Serafim M. Rozinsky**

Moscow State Academy of Veterinary Medicine and Biotechnology — MBA named after K.I. Skryabin, *Moscow, Russian Federation*
✉ lag22004@mail.ru

Abstract. The studies were conducted at the dairy complexes of the breeding farm of the Agricultural Enterprise Kolkhoz "Soznatelny", Zubtsovsky District, Tver Region, by the Department of Disease Diagnostics, Therapy, Obstetrics and Reproduction of Animals and the Medical and Diagnostic Center of the Federal State Budgetary Educational Institution of Higher Education Moscow State Academy of Veterinary Medicine and Biotechnology — Moscow State Academy of Veterinary Medicine named after K.I. Skryabin. Betulin-containing feed additive was given to five- and ten-month-old calves of the experimental groups orally at a dose of 10 mg/kg of weight with water individually once a day for 14 days. To assess the effect of betulin on the body of calves, as well as to exclude concomitant diseases, a clinical study of all experimental animals and a biochemical analysis of blood serum were conducted at the beginning and end of the experiment. The study presents the results of clinical trials of a feed additive containing the natural adaptogen betulin. The effect of betulin-containing feed additive on the biochemical parameters of the blood serum of breeding calves and highly productive dairy cattle was studied. It was established that the use of betulin-containing feed additive to calves of five and ten months of age orally at a dose of 10 mg/kg of weight with water individually 1 time per day for 14 days, normalizes the level of bilirubin in the blood serum, alanine aminotransferase and aspartate aminotransferase, alkaline phosphatase lactate dehydrogenase. By the end of the experiment, the calves of the experimental group also showed an increase in the level of total serum protein within the physiological norm. The results obtained indicate that the use of betulin-containing feed additive according to the specified scheme has an anti-inflammatory, cardio- and hepatoprotective effect. An increase in the amount of total protein in calves after using the feed additive indicates a compensatory increase in the synthesis of globulin fraction proteins.

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

Author contribution: Gnezdilova L.A. — idea generation, formulation or development of key goals and objectives; Muradyan Zh. Yu., Kruglova Yu.S., Rozinsky S.M. — experiments, analysis, and interpretation of the obtained data. All authors have read and agreed to the published version of the manuscript.

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Introduction

Productivity and high reproductive performance of cows directly depend on production stress. Conditions that create additional stress for animals, such as an improperly balanced diet, infectious and invasive diseases, or obstetric and gynecological diseases, aggravate the situation [1–3]. When analyzing the quality of feed, it is necessary to pay attention to natural pollutants, including mycotoxins, which continue to have a serious impact on the health of animals [4–6].

The positive experience of using plant triterpenoids in veterinary practice as immunostimulants for various pathological conditions in animals of different species is of a great interest. Very promising objects for the development of new drugs, including veterinary ones, are lupane triterpenoids, namely betulonic and betulinic acids [5, 6]. In the field of veterinary medicine, a new direction for solving the problem of infectious diseases is actively developing — the creation and use of environmentally friendly medicines of plant origin that can have a bactericidal, bacteriostatic, virucidal, and immunomodulatory effect on a sick organism [7–9]. A practicing veterinarian needs to have in his arsenal drugs with good therapeutic activity, possessing a polytropic effect and low cost [10–12]. The use of one drug will allow to have a multifaceted effect on pathogens, stages of development of the pathological process, and also to stimulate the healing processes. Drugs that stimulate the body's defenses are added to the drugs that suppress individual links of the pathology [13, 14]. Betulin is a natural pentacyclic triterpenoid of the lupane series. It is found in a large number of plants (hazel, calendula, licorice, etc.), but on an industrial scale it is obtained by extraction from birch bark — the outer layer of the bark of white birch (*betula alba*), drooping birch (*betula pendula*) [15–17]. The substance is not found in free form. Numerous studies conducted in more than 40 foreign and Russian research centers have demonstrated the effectiveness of using triterpene compounds as direct regulators of the activity of the body's enzymatic systems [18–20]. The immunostimulating activity of betulin is manifested in the ability to induce the production of endogenous interferon in the body, as well as to increase cellular and humoral immunity, enhance the activity of some immunocompetent cells, in particular, activating all indicators of phagocytosis (the ability of phagocytes to destroy viruses and bacterial cells) [21–24].

The aim of the study was to study the effect of the natural adaptogen betulin on the biochemical status of young cattle in a breeding herd.

Materials and methods of research

The studies were carried out at the dairy complexes of the breeding farm of the agricultural enterprise Kolkhoz "Soznatelny" in the Zubtsovsky district of the Tver region, by the Department of Disease Diagnostics, Therapy, Obstetrics and Reproduction of Animals and the Medical and Diagnostic Center of the Moscow State Academy of Veterinary Medicine and Biotechnology — MVA named after K.I. Skryabin. To study the effect of betulin on the biochemical parameters of blood serum, the following were formed:

– 2 groups of Sychevka calves (experimental and control), 10 capita each, 5 months old, with a live weight of 150…165 kg;

– 2 groups of Sychevka calves (experimental and control), 10 capita each, 10 months old, with a live weight of 286…316 kg.

All scheduled diagnostic measures were carried out on the experimental animals (the farm is safe for leukemia, tuberculosis, and brucellosis).

Betulin-containing feed additive was given to each animal of the group orally at a dose of 10 mg/kg of weight with water individually once a day for 14 days.

To assess the effect of betulin on the body of the experimental animals, as well as to exclude concomitant diseases, a clinical study of all animals and a biochemical analysis were carried out at the beginning and end of the experiment.

To conduct biochemical studies of blood serum, an automatic biochemical analyzer EOS BRAVO v.200, manufactured in Russia, was used (Fig. 1). In this case, the amount of total protein, albumins, globulins, creatinine, urea, bilirubin, AST, ALT, LDH, alkaline phosphatase, glucose, cholesterol, phosphorus, and total calcium were determined.

Statistical processing of the obtained results. The experimental data were processed using standard Microsoft Office Excel programs of the Data Analysis package. The reliability of the difference in indicators between groups of animals was shown using the * symbol — the ratio of indicators of groups I, II, and III to the control group; at different probability levels: * — $p < 0.05$; ** — $p < 0.01$; *** — $p < 0.001$.

Fig. 1. Automatic biochemical analyzer EOS BRAVO v.200
Source: compiled by L.A. Gnezdilova, Zh. Yu. Muradyan, Yu.S. Kruglova, S.M. Rozinsky.

Results and discussion

The results of the biochemical study of the blood serum of five-month-old calves of the experimental group before and after the use of the betulin-containing feed additive are shown in Table. 1.

Analyzing the obtained data (see Table 1), we can conclude that at the beginning of the experiment, the biochemical parameters of the blood serum of five-month-old calves, both in the experimental and in the control groups, did not have significant differences. After 14 days of using the betulin-containing feed additive, the calves of the experimental group showed an increase within the physiological norm in the amount of total bilirubin and total protein and a decrease in alanine aminotransferase (ALT), alkaline phosphatase, and lactate dehydrogenase (LDH). At the beginning of the experiment, the amount of total bilirubin in the blood serum was at a low level and amounted to 1.84 ± 0.12 in the experimental and 2.11 ± 0.53 μmol/l in the control group with a norm of 0 to 27.4 μmol/l (Fig. 2). After 14 days, the amount of total bilirubin in the experimental group increased by 78% ($p \leq 0.05$) and amounted to 3.28 ± 0.53 μmol/l. In the control group, at the end of the experiment, the values of total bilirubin remained unchanged and amounted to 2.43 ± 0.65 μmol/l. A comparison of the final results of the experimental and control groups showed a reliable difference between the groups ($p \leq 0.05$).

At the beginning of the experiment, the aspartate aminotransferase (AST) values in the blood serum of calves were within the physiological norm. By the end of the experiment, the AST value in the calves of the experimental group remained unchanged. At the same time, in the animals of the control group, the value increased by 12.8% ($p \leq 0.05$) from 81.73 ± 7.3 to 92.24 ± 5.2 U/L. Comparison of the final results of the experimental and control groups showed that in the control group, the AST value was higher by 9.9% ($p \leq 0.05$) than in the animals of the experimental group (92.24 ± 5.2 and 83.93 ± 7.3 U/L, respectively) (Fig. 3).

Table 1

Biochemical parameters of blood serum of five-month-old calves before and after the use of betulin supplement orally at a dose of 10 mg/kg of weight orally with water individually once a day for 14 day

n/n	Bilirubin total, mcmol/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
	Calves aged 5 months, n = 10. Experimental group. Before and 14 days after oral administration of 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	4,41/4,11	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	4,48/4,41	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	4,59/4,51	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	3,97/3,54	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	3,94/3,88	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	5,04/4,43	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	4,62/4,61	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	4,25/4,34	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	4,59/4,52	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	3,88/3,76	1328/1112	3,12/2,92	2,66/2,62	2,11/2,16
M ± *m*	1,84 ± 0,12*/ 3,28 ± 0,53 ($p \leq 0{,}05$)	83,0 ± 10,4/ 83,93 ± 7,3	30,26 ± 1,8*/ 28,63 ± 1,3 ($p \leq 0{,}01$)	149,0 ± 35,3/ 125,0 ± 19,0	3,02 ± 0,68/ 2,82 ± 0,67	88,1 ± 5,5/ 92,5 ± 8,8	62,6 ± 3,59**/ 65,3 ± 1,81 ($p \leq 0{,}01$)	37,72 ± 2,2/ 35,93 ± 2,04	4,37 ± 0,3/ 4,21 ± 0,3	1321,5 ± 161*/ 1175,8 ± 140 ($p \leq 0{,}01$)	2,80 ± 0,47/ 2,46 ± 0,52	2,65 ± 0,10/ 2,65 ± 0,15	2,20 ± 0,08/ 2,17 ± 0,07
	Calves aged 5 months, 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 with the experimental group ($p \leq 0{,}05$)	81,73 ± 7,3*/ 92,24 ± 5,2* Differences with the experimental group ($p \leq 0{,}05$)	29,63 ± 1,9/ 30,59 ± 1,6* Differences with the experimental group ($p \leq 0{,}05$)	142,9 ± 11,6 142,3 ± 12,8** Differences with the experimental group ($p \leq 0{,}01$)	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** Различия с опытной группой ($p \leq 0{,}01$)	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 $p \leq 0.05$; ** — differences are significant at $p \leq 0.01$.

Source: compiled by Yu.S. Kruglova, Zh.Yu. Muradyan based on the results of the experiment.

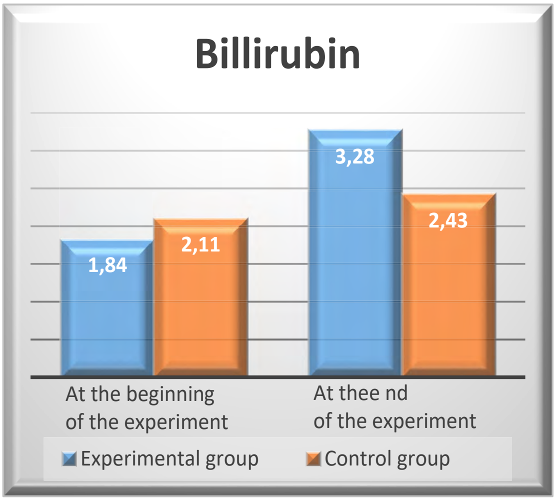

Fig. 2. Bilirubin levels in the blood serum of five-month-old calves

Source: compiled by S.M. Rozinsky.

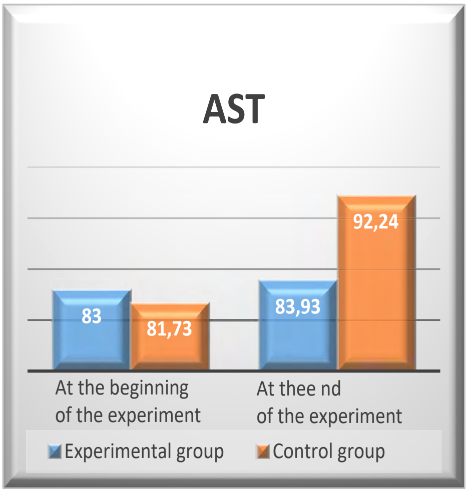

Fig. 3. Aspartate aminotransferase (AST) levels in the blood serum of five-month-old calves

Source: compiled by S.M. Rozinsky.

The ALT values in the blood serum of the calves of the experimental and control groups were also within the physiological norm at the beginning of the experiment. When analyzing the changes in the ALT value in the calves of the experimental group, a decrease in the value by 5.4% ($p \leq 0.01$) was noted by the end of the experiment. In the animals of the control group, a slight increase in the value was observed from 29.63 ± 1.9 to 30.59 ± 1.6 U/l. The final result showed reliable differences between the experimental and control groups ($p \leq 0.05$) (Fig. 4).

The level of alkaline phosphatase (ALP) in the blood serum at the beginning of the experiment in some animals of both the experimental and control groups exceeded the reference values (from 18 to 153 U/l). The average alkaline phosphatase level in the experimental group at the beginning of the experiment was 149.0 ± 35.3 U/L, in the control group it was 142.9 ± 11.6 U/L. Fourteen days after the use of the betulin-containing feed additive, the alkaline phosphatase level in the calves of the experimental group decreased by 17% — from 149.0 ± 35.3 to 125.0 ± 19.0 U/L, while in the animals of the experimental group the alkaline phosphatase value remained the same (Fig. 5).

When analyzing the serum alkaline phosphatase level at the end of the experiment, it was noted that in the animals of the experimental group this indicator was 125.0 ± 19.0 U/L, which is 12% lower ($p \leq 0.01$) than in the control (142.3 ± 12.8 U/L).

The values of total serum protein in five-month-old calves at the beginning of the experiment were below the reference values and, with a norm of 67 to 75 g/L, were 62.6 ± 3.59 and 64.04 ± 2.83 g/L, respectively.

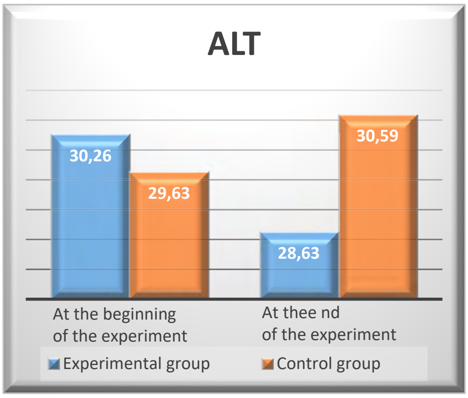

Fig. 4. Alanine Minotransferase (ALT) levels in the blood serum of five-month-old calves

Source: compiled by S.M. Rozinsky.

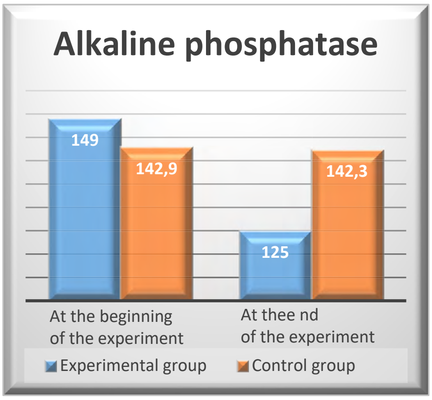

Fig. 5. Alkaline phosphatase levels in the blood serum of five-month-old calves

Source: compiled by S.M. Rozinsky.

By the 14th day of the experiment, the amount of total protein in animals of the experimental group increased by 4.3% and was 6.53 ± 1.81 g/L ($p \leq 0.01$). In the control group, the indicator remained virtually unchanged and amounted to 64.44 ± 3.31 g/l compared to 64.04 ± 2.83 g/l at the beginning of the experiment (Fig. 6).

Significant changes were noted in the analysis of the amount of LDH in the blood serum (Fig. 7). At the beginning of the experiment, the LDH values in animals of both groups exceeded the reference values by 40.86% in the experimental group and by 35.92% in the control group and amounted to 1321.5 ± 161 U/l and 1275.1 ± 155 U/l, respectively. After 14 days of using the betulin-containing feed additive, the amount of LDH in the blood serum of calves in the experimental group decreased by 11% and amounted to 1175.8 ± 140 U/l ($p \leq 0.01$). In animals of the control group, by the 14th day of the experiment, the LDH level, on the contrary, increased by 6% and amounted to 1353.5 ± 175 U/L compared to 1275.1 ± 155 U/L at the beginning of the experiment.

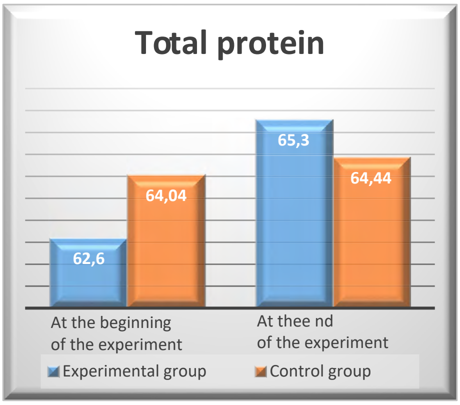

Fig. 6. Total serum protein levels in five-month-old calves

Source: compiled by S.M. Rozinsky.

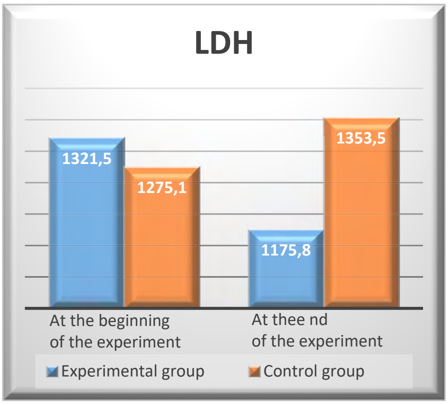

Fig. 7. Lactate dehydrogenase (LDH) levels in the blood serum of five-month-old calves

Source: compiled by S.M. Rozinsky.

The analysis of the final result of the LDH level of the experimental and control groups showed that the LDH value in the calves of the experimental group was 13% lower than in the animals of the control group ($p \leq 0.01$).

The values of urea, creatinine, albumin, glucose, cholesterol, phosphorus, and total calcium in the blood serum did not differ significantly either at the beginning or at the end of the experiment.

The results of the biochemical study of the blood serum of ten-month-old calves of the experimental group before and after the use of the betulin-containing feed additive are given in Table 2.

Analyzing the obtained data in Table 2, we can conclude that the studied biochemical parameters of the blood serum of ten-month-old calves at the beginning of the experiment did not have significant differences between the groups. By the end of the experiment, the parameters of the experimental and control groups differed significantly from each other in the values of total bilirubin, ALT, total protein, and LDH.

At the beginning of the experiment, the amount of total bilirubin in the blood serum of 10-month-old calves of both groups, as well as in 5-month-old calves in a similar experiment, was at a low level and amounted to 1.8 ± 0 μmol/l in the experimental group and 1.87 ± 0.14 μmol/l in the control group (normal from 0 to 27.4 μmol/l). 14 days after the use of the betulin-containing feed additive in calves of the experimental group, this indicator increased by 66% ($p \leq 0.05$) — from 1.8 ± 0 to 3.0 ± 0.62 μmol/l, while in the control group the changes were insignificant (Fig. 8). Comparison of the final result of the experimental and control groups showed a reliable difference between the groups ($p \leq 0.05$). The AST values in calves of the experimental and control groups decreased slightly by the end of the experiment from 97.6 ± 8.2 to 94.3 ± 12.6 U/L. In the control group, the AST value remained virtually unchanged and was 94.3 ± 12.6 U/L at the beginning and 93.2 ± 6.5 U/L at the end of the experiment (Fig. 9).

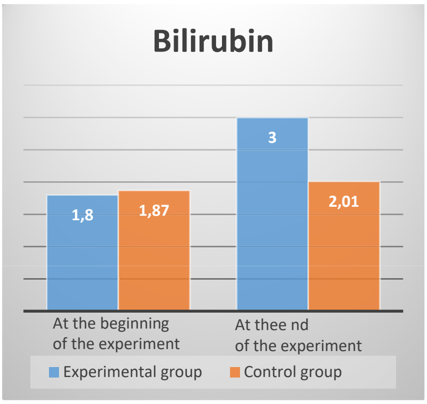

Fig. 8. Bilirubin levels in blood serum of ten-month-old calves

Source: compiled by S.M. Rozinsky.

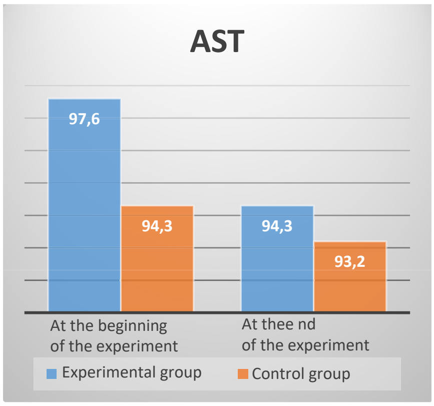

Fig. 9. Aspartate aminotransferase (AST) levels in the blood serum of ten-month-old calves

Source: compiled by S.M. Rozinsky.

Table 2

Biochemical parameters of the blood serum of ten-month-old calves before and after the use of the betulin-containing feed additive orally at a dose of 10 mg/kg of weight orally with water individually 1 time per day for 14 days

n/n	Bilirubin total, mcmol/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
	Calves aged 10 months, n = 10. Experimental group. Before and 14 days after oral administration of betulin-containing feed additive												
1	1,8/2,63	111,9/84,7	25,2/21,9	119/120	3,22/3,47	92/94	70,47/70,01	35,4/33,5	3,71/3,86	1207/1015	2,13/1,84	2,24/2,47	2,35/2,31
2	1,8/3,41	94,8/96,9	30,4/25,7	114/96	4,6/2,25	109/110	64,89/69,18	30,8/29,1	3,93/4,01	1304/1019	2,5/1,94	2,34/2,45	2,2/2,27
3	1,8/4,26	88,5/127,1	30,6/24,2	101/101	3,01/2,62	91/87	73,98/76,14	35,9/32,5	3,64/3,47	1304/915	1,44/1,33	2,29/2,19	2,11/2,08
4	1,8/3,54	96,5/88,4	28,2/26,2	108/104	3,15/3,02	96/95	72,12/76,45	34,2/32,8	3,66/3,52	1112/856	2,4/2,56	2,21/2,35	2,15/2,12
5	1,8/2,86	89,7/86,2	25,1/23,7	102/96	2,32/3,45	89/92	68,22/71,23	33,5/31,4	3,85/3,75	1256/960	2,6/2,4	2,24/2,25	2,26/2,24
6	1,8/2,26	106,2/98,4	29,6/24,3	103/100	4,2/3,74	102/100	65,68/69,45	30,5/29,5	3,74/3,86	1302/1023	1,88/1,7	2,23/2,38	2,29/2,12
7	1,8/3,13	88,2/84,1	27,5/22,3	99/95	2,78/2,95	94/96	70,23/74,54	33,9/32,4	3,52/3,56	1250/850	2,25/2,45	2,31/2,33	2,22/2,23
8	1,8/2,18	95,7/92,6	24,2/22,4	100/88	3,45/3,26	88/92	72,12/75,46	35,2/31,6	3,75/3,77	1307/1115	2,6/2,5	2,28/2,26	2,26/2,25
9	1,8/2,84	106,8/88,3	30,1/29,5	103/102	3,65/3,26	93/95	67,45/72,55	35,4/32,9	3,62/3,65	1228/845	1,89/2,07	2,23/2,24	2,31/2,36
10	1,8/2,92	98,3/96,5	24,6/24,1	101/100	3,56/3,12	87/83	63,57/66,54	32,5/31,1	3,95/3,96	1209/964	2,5/2,47	2,24/2,25	2,3/2,26
M ± *m*	1,8 ± 0*/ 3,0 ± 0,62 ($p \leq 0,05$)	97,6 ± 8,2/ 94,3 ± 12,6	27,55 ± 2,5**/ 24,43 ± 2,2 ($p \leq 0,01$)	105,0 ± 6,63/ 100,2 ± 8,31	3,39 ± 0,66/ 3,11 ± 0,43	94,1 ± 6,8/ 94,4 ± 7,2	68,8 ± 3,4**/ 72,1 ± 3,4 ($p \leq 0,01$)	33,73 ± 1,9**/ 31,68 ± 1,4 ($p \leq 0,01$)	3,73 ± 0,1/ 3,74 ± 0,1	1247,9 ± 62*/ 956,2 ± 89 ($p \leq 0,05$)	2,21 ± 0,38/ 2,12 ± 0,41	2,26 ± 0,04/ 2,31 ± 0,09	2,24 ± 0,07/ 2,22 ± 0,09
	Calves aged 10 months, n = 10. Control group												
1	1,8/1,9	97,4/96,3	24,8/24,3	102/100	3,17/3,25	93/92	64,18/64,26	32,8/32,7	3,61/3,64	1263/1254	2,14/2,05	2,28/2,36	2,31/2,33
2	1,8/1,8	95,2/95,1	28,7/25,9	107/108	3,54/3,62	100/95	65,24/63,15	30,5/30,8	3,63/3,72	1237/1354	2,5/2,31	2,33/2,35	2,26/2,28
3	1,8/2,12	87,3/86,5	29,6/31,2	112/114	2,88/2,95	88/85	75,55/64,52	33,5/31,4	3,73/3,68	1425/1387	1,95/1,87	2,26/2,25	2,31/2,28
4	1,8/1,8	96,5/93,2	28,5/28,4	96/98	3,47/3,12	96/100	78,22/78,24	36,5/34,5	3,58/3,64	1154/1231	2,36/2,4	2,32/2,33	2,16/2,14
5	1,8/1,8	89,4/95,3	24,6/23,4	99/99	3,62/4,15	98/101	68,56/64,51	33,7/32,4	3,67/3,52	1287/1267	2,12/1,95	2,34/2,41	2,24/2,18
6	1,9/2,3	103,5/100,1	30,3/29,8	100/99	4,5/4,1	103/104	63,57/62,45	30,5/32,4	3,75/3,82	1132/1214	1,55/1,7	2,29/2,27	2,12/2,06
7	2,1/1,9	86,2/85,1	27,6/27,4	108/104	2,48/2,54	95/92	65,86/64,87	32,6/34,1	3,83/3,94	1269/1257	2,16/1,95	2,27/2,31	2,36/2,35
8	1,8/2,15	96,8/96,3	28,3/27,6	116/115	3,54/3,24	94/96	70,32/71,45	32,2/32,1	3,87/3,85	1312/1295	2,5/2,2	2,26/2,25	2,3/2,25
9	1,8/1,8	83,9/82,4	31,3/31,2	111/105	2,87/2,88	91/88	72,54/73,45	35,2/33,6	3,68/3,66	1128/1116	1,64/1,52	2,34/2,48	2,24/2,22
10	2,2/2,6	106,3/102,4	25,2/24,6	110/112	3,12/3,26	106/108	72,65/70,25	35,4/35,2	3,91/3,95	985/962	2,23/2,33	2,31/2,42	2,26/2,23
M ± *m*	1,87 ± 0,14/ 2,01 ± 0,27* Differences with the experimental group ($p \leq 0,05$)	94,3 ± 12,6/ 93,2 ± 6,5	27,89 ± 2,8/ 27,55 ± 2,5** Differences with the experimental group ($p \leq 0,01$)	106,1 ± 6,5 105,4 ± 6,5	3,31 ± 0,55/ 3,31 ± 0,51	96,4 ± 5,4/ 96,1 ± 7,2	69,6 ± 5,0/ 67,7 ± 5,3** Differences with the experimental group ($p \leq 0,01$)	33,29 ± 2,0/ 32,92 ± 1,4	3,72 ± 0,1/ 3,74 ± 0,1	1219,2 ± 122/ 1233,7 ± 121* Differences with the experimental group ($p \leq 0,05$)	2,11 ± 0,32/ 2,02 ± 0,28	2,3 ± 0,03/ 2,34 ± 0,07	2,25 ± 0,07/ 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 $p \leq 0.05$; ** — differences are significant at $p \leq 0.01$.

Source: compiled by Yu.S. Kruglova, Zh.Yu. Muradyan based on the results of the experiment.

The ALT value in the calves of the experimental group decreased by 12% by the end of the experiment, from 27.55 ± 2.5 to 24.43 ± 2.2 U/L ($p \leq 0.01$), remaining within the physiological norm. In the calves of the control group, the indicator remained unchanged (Fig. 10).

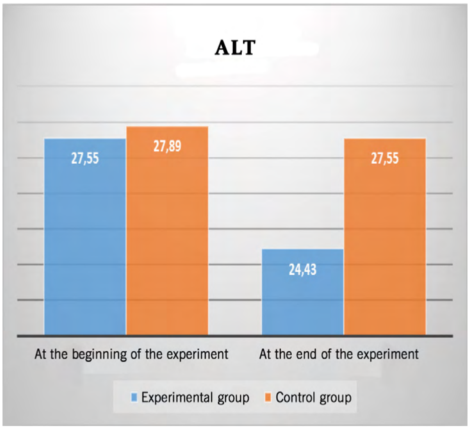

Fig. 10. Alanine Minotransferase (ALT) levels in the blood serum of ten-month-old calves
Source: compiled by S.M. Rozinsky.

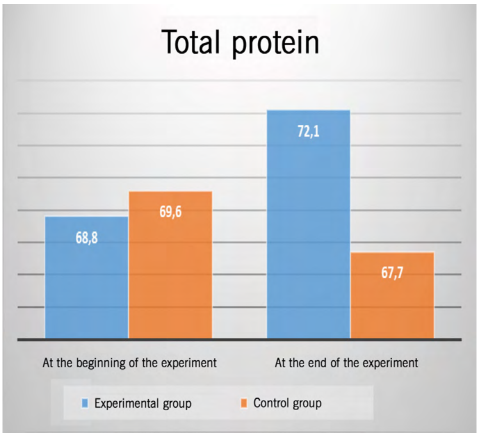

Fig. 11. Total serum protein levels in ten-month-old calves
Source: compiled by S.M. Rozinsky.

Fourteen days after oral administration of the betulin-containing feed additive, a decrease in alkaline phosphatase by 4.6% and urea by 8.3% was noted in the calves of the experimental group. The differences were insignificant and were within the physiological values. In the calves of the control group, the indicators remained the same (Fig. 13). When analyzing the content of total protein in the blood serum, a reliable increase of 4.8% was noted by the end of the experiment in calves of the experimental group — from 68.8 ± 3.4 to 72.1 ± 3.4 g/l ($p \leq 0.01$). In calves of the control group, on the contrary, the level of total protein slightly decreased. Comparison of the final result of the experimental and control groups showed a reliable difference between the groups ($p \leq 0.01$) (Fig. 11). In addition, in calves of the experimental group, a decrease within the physiological norm in the amount of albumin by 6% was observed by the end of the experiment — from 33.73 ± 1.9 to 31.68 ± 1.4 g/l ($p \leq 0.01$). In the calves of the control group, the indicator remained the same. At the beginning of the experiment, calves of both the experimental and control groups had very high LDH levels: 1247.9 ± 62 U/L in the experimental calves and 1219.2 ± 122 U/L in the control calves, with reference values ranging from 308.6 to 938.1 U/L. By the 14th day after oral administration of the betulin-containing feed additive, the LDH level in calves of the experimental group had noticeably decreased, almost approaching the upper physiological norm and amounting to 956.2 ± 89 U/L ($p \leq 0.05$). In the control group, the indicator remained the same and amounted to 1233.7 ± 121 U/L. A comparison of the final results of the experimental and control groups showed a reliable difference between the groups ($p \leq 0.05$) (Fig. 12).

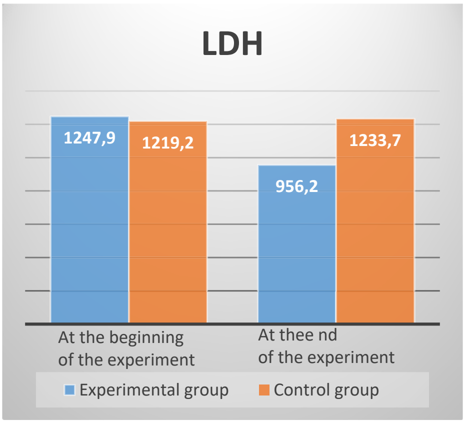

Fig. 12. Lactate dehydrogenase (LDH) levels in blood serum of ten-month-old calves

Source: compiled by S.M. Rozinsky.

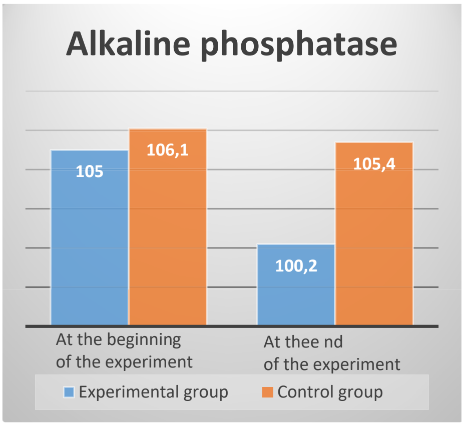

Fig. 13. Alkaline phosphatase levels in the blood serum of ten-month-old calves

Source: compiled by S.M. Rozinsky.

No changes were found in the analysis of creatinine, glucose, cholesterol, phosphorus, and total calcium in the blood serum.

The analysis of the obtained results showed that the use of betulin-containing feed additives has a noticeable effect on the body of the experimental animals. Thus, it was noted that the betulin-containing feed additive normalizes the bilirubin level in the blood serum. According to N.A. Kulikova [12], the amount of bilirubin in the blood less than 2.5 μmol/l is critically low. In our experiment, before the use of betulin-containing feed additive, the bilirubin level was less than 2.5 μmol/l in 100% of the calves of the experimental groups and 80% of the animals of the control group of 5-month-old calves and 100% of the calves of the control group of 10-month-old calves. Bilirubin values at the lower limit of the norm may indicate tissue hypoxia.

After oral administration of a betulin-containing feed additive, the bilirubin level in the blood serum of animals in the experimental groups rose above critical values in 5-month-old calves from 1.84 ± 0.12 to 3.28 ± 0.53 μmol/l ($p \leq 0.05$), and in 10-month-old calves from 1.8 ± 0 to 3.0 ± 0.62 μmol/l ($p \leq 0.05$).

When analyzing the biochemical parameters of blood serum, the AST and ALT parameters are of great importance. These enzymes are localized in the cytosol of multiple organ cells, but their greatest quantities are found in liver and myocardial cells. When cells are damaged, the activity of enzymes in the blood increases. At the beginning of the experiment, the AST values in the blood serum in both 5-month-old calves' groups were practically identical within the physiological norm, and amounted to 83.0 ± 10.4 and 81.73 ± 7.3, respectively ($p \leq 0.05$). By the end of the experiment, the AST amount in the calves of the experimental group remained at the same level, whereas in the calves of the control group it increased by 12.8%. According to E.I. Aschenbrenner et al. [14], a significant increase in the enzymatic activity of

AST is observed in animals with fatty liver dystrophy. The alanine aminotransferase values in the experimental group of 5-month-old calves showed a slight but reliable decrease in this indicator by the end of the experiment ($p \leq 0.01$). In the experimental group of 10-month-old calves, we observed a 12% decrease in ALT compared to the beginning of the experiment ($p \leq 0.01$), while no changes were noted in the control group. Thus, it can be said that the betulin-containing feed additive has an anti-inflammatory, cardio- and hepatoprotective effect. The positive effect of the betulin-containing feed additive on the function of the liver and gallbladder is also evidenced by changes in the content of alkaline phosphatase (ALP) in the blood serum. Alkaline phosphatase is an enzyme contained in many tissues of the animal organism, especially in growing bones, liver parenchyma, and bile duct walls. An increase in ALP activity is possible with cholestasis, obstructive liver diseases, toxic hepatitis, and hepatotoxin poisoning [16].

Conclusion

It was found that at the beginning of the experiment the amount of ALP in the blood serum of 5-month-old calves was higher than the reference values in 30% of animals, and in 70% it was at the upper limit of the norm, then by the end of the experiment in calves of the experimental group the level of ALP decreased from 149.0 ± 35.3 to 125.0 ± 19.0. At the same time, in the control group no changes in the ALP value were noted. Similar, but less pronounced changes were observed in groups of 10-month-old calves. This effect can be explained by the pronounced choleretic and hepatoprotective properties of betulin.

The anti-inflammatory and hepatoprotective effect of the betulin-containing feed additive is also evidenced by the results of studying the level of LDH in the blood serum of calves. Lactate dehydrogenase, like alanine aminotransferase, is a cytoplasmic enzyme of blood serum. The LDH level in animals with liver pathology is higher than in clinically healthy animals. The presence of problems was indicated by the fact that the LDH level at the beginning of the experiment exceeded the upper limit in all experimental groups. After the use of a betulin-containing feed additive, the LDH value in the blood serum of calves in the experimental groups significantly decreased and approached the normal refence level ($p \leq 0.01$ in 5-month-old and $p \leq 0.05$ in 10-month-old calves). At the same time, this indicator became even higher in the calves of the control groups. When analyzing the level of total protein, a decreased level was observed at the beginning of the experiment in 5-month-old calves and the lower limit of the physiological norm in 10-month-old calves. The increased range in calves of the experimental group by the end of the experiment ($p \leq 0.01$) indicates that the betulin-containing feed additive promotes compensatory enhancement of the synthesis of proteins of the globulin fraction. Thus, it can be stated that the betulin-containing feed additive increases the globulin fraction of protein, and has a hepatoprotective, choleretic, and anti-inflammatory effect.

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About authors:
Gnezdilova Larisa Aleksandrovna — Doctor of Veterinary Sciences, Professor, Head of the Department of Disease Diagnostics, Therapy, Obstetrics and Reproduction of Animals, Moscow State Academy of Veterinary Medicine and Biotechnology — MBA named after K.I. Skryabin, 23 Academician Skryabin st., Moscow, 109472, Russian Federation; e-mail: lag22004@mail.ru
ORCID: 0000-0003-1007-3441 SPIN-code: 2376-1425
Muradyan Zhora Yurikovich — Candidate of Biological Sciences, Associate Professor of the Department of Disease Diagnostics, Therapy, Obstetrics and Reproduction of Animals, Moscow State Academy of Veterinary Medicine and Biotechnology — MBA named after K.I. Skryabin, 23 Academician Skryabin st., Moscow, 109472, Russian Federation; e-mail: zh_muradyan@mail.ru
ORCID: 0000-0003-2516-7627 SPIN-code: 4892-9182
Kruglova Yulia Sabirovna — Candidate of Veterinary Sciences, Associate Professor of the Department of Disease Diagnostics, Therapy, Obstetrics and Reproduction of Animals, Moscow State Academy of Veterinary Medicine and Biotechnology — MBA named after K.I. Skryabin, 23 Academician Skryabin st., Moscow, 109472, Russian Federation; e-mail: y7272@mail.ru
ORCID: 0000-0003-2953-0745 SPIN-code: 7902-6183
Rozinsky Serafim Mikhailovich — assistant of the department of diagnostics of diseases, therapy, obstetrics and reproduction of animals, Moscow State Academy of Veterinary Medicine and Biotechnology — MBA named after K.I. Skryabin, 23 Academician Skryabin st., Moscow, 109472, Russian Federation; e-mail: ser.roz1@yandex.ru
ORCID: 0009-0001-1937-6919 SPIN-code: 8705-3851

Мониторинг биохимических показателей сыворотки крови телят на фоне применения бетулиносодержащей кормовой добавки

Л.А. Гнездилова ✉, Ж.Ю. Мурадян, Ю.С. Круглова, С.М. Розинский

Московская государственная академия ветеринарной медицины и биотехнологии — МВА им. К.И. Скрябина, *г. Москва, Российская Федерация*
✉ lag22004@mail.ru

Аннотация. Исследования проводили в молочных комплексах племенного хозяйства СХП Колхоз «Сознательный» Зубцовского района Тверской области, на базе кафедры диагностики болезней, терапии, акушерства и репродукции животных и лечебно-диагностического центра Московской государственной академии ветеринарной медицины и биотехнологии — МВА им. К.И. Скрябина. Бетулиносодержащую кормовую добавку давали пяти- и десятимесячным телятам опытных групп перорально в дозе 10 мг/кг веса с водой индивидуально 1 раз в день в течение 14 дней. Для оценки влияния бетулина на организм телят, а также для исключения сопутствующих заболеваний в начале и конце эксперимента проведены клиническое исследование всех опытных животных, биохимический анализ сыворотки крови. Приведены результаты клинических испытаний кормовой добавки, содержащей природный адаптоген — бетулин. Изучено ее действие на биохимические показатели сыворотки крови племенных телят и молочного высокопродуктивного крупного рогатого скота. Установлено, что применение бетулиносодержащей кормовой добавки телятам пяти- и десятимесячного возраста перорально в дозе 10 мг/кг веса с водой индивидуально 1 раз в день в течение 14 дней нормализует уровень билирубина в сыворотке крови, аланинаминотрансферазы и аспартатаминнинотрансферазы, щелочной фосфатазы лактатдегидрогеназы. К концу эксперимента у телят опытной группы также отмечено повышение в пределах физиологической нормы уровня общего белка сыворотки крови. Полученные результаты свидетельствуют о том, что применение бетулиносодержащей кормовой добавки по указанной схеме дает противовоспалительный, кардио- и гепатопротекторный эффект. Повышение количества общего белка у телят после применения кормовой добавки свидетельствует о компенсаторном усилении синтеза белков глобулиновой фракции.

Ключевые слова: бетулин, племенное хозяйство, анализ крови, терапевтическая доза, пероральное введение, живая масса

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