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Use of DIABAX feed additive and a biogenic stimulant in calves during their rehabilitation after gastrointestinal infections

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ABSTRACT

The results of the use of DIABAX feed additive alone and in combination with a biogenic stimulant for the correction of biochemical, hematological blood parameters in young cattle after gastrointestinal infections taking into account the disease and survival rates, as well as their performance indicators are presented. Three groups of calves at the age of less than 30 days old, two test groups and one control group, were formed for analogous pairs-based trial. Calves of control group were subcutaneously injected with saline solution at a dose of 8 mL on day 1, 5, 10 of the trial; calves of test group 1 (T-1) were fed with DIABAX feed additive with milk at a dose of 3.0 mL once a day; calves of test group 2 (T-2) were intramuscularly injected with the biogenic stimulant at a dose of 0.5 mL/10 kg of body weight on day 1, 5, 10 of the trial and also received DIABAX at a dose of 3.0 mL once a day during 15 days. The tests showed that co-administration of the biogenic stimulant and DIABAX feed additive (in T-2 group) contributed to 100% survival rate in calves, as well as significant increase in calcium and magnesium levels in animal sera by 14.5–23.8 and 61.2–79.5%, respectively, as compared with the initial levels and the levels in control group; increase in albumin and *α*-globulin protein fraction concentrations by 10.1 and 43.2% ($p \leq 0.05$), respectively, albumin/globulin ratio – by 17.5%, color index – by 1.1%, increase in the total immunoglobulin G amount by 2.7 times as compared to the initial values. Daily administration of DIABAX feed additive to calves of T-1 group for 15 days reduced recurrent disease rate in the calves by 14.4%, resulted in significant increase in calcium and magnesium levels in sera by 10.1 and 75.0% ($p \leq 0.05$), respectively, as well increase in immunoglobulin G level by 2.3 times, erythrocyte level – by 3.8%, hemoglobin level – by 8.0%, leukocyte level – by 21.8%, albumin/globulin ratio – by 35.1% in sera as compared to initial values.

Keywords: calves, disease rate, survival rate, morphological, biochemical blood parameters, biogenic stimulant

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Применение кормовой добавки «Диабакс» и биогенного препарата телятам, переболевшим желудочно-кишечными инфекциями, в восстановительный период

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РЕЗЮМЕ

Представлены результаты применения кормовой добавки «Диабакс» в отдельности и в сочетании с биогенным препаратом для коррекции биохимических, гематологических показателей крови молодняка крупного рогатого скота, переболевшего желудочно-кишечными инфекциями, с учетом

заболеваемости, сохранности, продуктивности животных. Для проведения опыта по принципу пар-аналогов были сформированы 3 группы телят до 30-суточного возраста: две опытные и одна контрольная. Телятам контрольной группы подкожно вводили физиологический раствор в дозе 8 мл в 1, 5, 10-й дни опыта; животным 1-й опытной группы (Т-1) в течение 15 дней с молоком задавали кормовую добавку «Диабакс» в дозе 3,0 мл 1 раз в сутки; телятам 2-й опытной группы (Т-2) внутримышечно инъецировали биогенный препарат в дозе 0,5 мл на 10 кг массы тела в 1, 5, 10-й дни опыта и выпаивали 15 дней подряд «Диабакс» в дозе 3,0 мл 1 раз в сутки. На основании проведенных исследований установлено, что совместное применение биогенного препарата и добавки «Диабакс» (в группе Т-2) способствует 100%-й сохранности телят, достоверному увеличению кальция и магния в сыворотке крови животных на 14,5–23,8 и 61,2–79,5% соответственно по сравнению с исходными показателями и показателями контрольной группы, повышению в сравнении с контрольной группой альбуминовой и *α*-глобулиновой фракций белка на 10,1 и 43,2% ($p \leq 0,05$) соответственно, альбумин-глобулинового коэффициента – на 17,5%, цветного показателя – на 1,1%, увеличению общего количества иммуноглобулина класса G в 2,7 раза по сравнению с исходными данными. Ежедневное выпаивание добавки «Диабакс» в течение 15 дней (в группе Т-1) приводит к снижению количества повторных заболеваний телят на 14,4%, достоверному увеличению в сыворотке крови кальция и магния на 10,1 и 75,0% ($p \leq 0,05$) соответственно, повышению уровня иммуноглобулина класса G в 2,3 раза, эритроцитов – на 3,8%, гемоглобина – на 8,0%, лейкоцитов – на 21,8%, альбумин-глобулинового коэффициента – на 35,1% относительно исходных значений.

Ключевые слова: телята, заболеваемость, сохранность, морфологические, биохимические показатели крови, биогенный препарат

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INTRODUCTION

Livestock survivability and performance improvement is one of the important challenges facing the Russian Federation agro-industrial complex [1, 2]. Calf rearing are challenging since the newborns' bodies are poorly adapted to adverse environmental conditions due to the morphofunctional immaturity of immune system and gastrointestinal tract during their first days of life [3]. This, in turn, results in the various gastrointestinal diseases in calves and their deaths [4, 5, 6], as well as the disease recurrence [7, 8].

Therewith, complex rehabilitation procedures including use of various biological stimulants enabling vital component deficiency filling and contributing to the metabolic normalization and full-calf body resistance enhancement are required to maintain the metabolic status and homeostasis of the calves during their recovery [9, 10, 11, 12, 13].

The mechanism of biogenic stimulant action includes changes in activity of some enzymes due to the biogenic stimulant attachment to the enzyme protein. Changes in the enzyme activity results in endocrine restructuring, an increase in tropic hormones secreted by pituitary gland that stimulate adrenal gland, thyroid and pancreas functions, etc. They have a favorable impact on trophic function of the nervous system, enhance thyroid tissue and adrenal gland functions, stimulate corticosteroid hormone secretion and pancreatic gland function, regulate gastrointestinal tract secretory and motor functions, gas and phosphorus metabolism, intermediary metabolism, reticuloendothelial system and regenerative processes, improve body state, appetite, assimilation processes that contribute to higher weight gains [14, 15].

The study was aimed at testing of DIABAX feed additive and a biogenic stimulant for their effectiveness for correction of biochemical, hematological blood parameters in young cattle after gastrointestinal infections.

Test tasks:

1. To test used feed additive and biogenic stimulant for their effects on morphological and biochemical blood parameters in calves during their rehabilitation after gastrointestinal infections.

2. To test the feed additive and biogenic stimulant for their effects on disease rate and survival rate in calves, calf performance during their rehabilitation period.

MATERIALS AND METHODS

Bacteriological tests of calf feces were carried out for the purpose of diagnosis. Isolated microorganisms were tested for their sensitivity to antimicrobials with disk diffusion test [16].

A pilot batch of biogenic stimulant was prepared from raw materials (slaughter waste, category II offal) kept in a refrigerator at temperature of +2 up to +4 °C for 5–7 days, ground and then mixed with an extraction agent at the Department of the All-Russian Institute of Antler Reindeer Breeding, Altai Scientific Center for Agrobiotechnologies. The ultrasonic-assisted extraction was carried out; the final product was filtered, packed, and sterilized in an autoclave [17]. Administration of the biogenic stimulant enhances metabolism, body's resistance and stimulates body's functions.

DIABAX, a new feed additive, developed by the GK KONSTANTA (Saransk) [18] is a viscous fluid of light-brown color with slight odor, has a bactericidal,

Table 1
Scheme of tested product administration to calves of test and control groups

Group of animals	Number of animals	Administered product
C	6	Saline solution administered at a dose of 8.0 mL subcutaneously on day 1, 5, 10 of the trial
T-1	7	DIABAX administered daily at a dose of 3.0 mL once a day for 15 days
T-2	5	Biogenic stimulant administered intramuscularly at a dose of 0.5 mL/10 kg of body weight on day 1, 5, 10 of the trial + DIABAX administered daily at a dose of 3.0 mL once a day for 15 days

bacteriostatic effect on a wide range of microorganisms and pathogenic fungi owing to potassium iodide and electrostatically charged polyelectrolyte polydimethyldiallylammonium chloride contained in it. DIABAX feed additive is highly soluble in water, does not change medium pH level, remains active in acidic and alkaline media, as well as in protein and fatty media. The principle feature of the additive is inhibition of pathogenic microflora based on its physical, but not chemical effect, while its components has no adverse effect on normal gastrointestinal cells of animals. Polyelectrolyte has an electrostatic charge opposite to the charge of cells of pathogenic bacteria, fungi and other microorganisms. When Diabax feed additive interacts with the pathogen surface. This interaction leads to microorganism cell membrane is encapsulation that slows down and then completely inhibits microorganism breathing, nutrition and reproducibility.

For the trial aimed at testing of the restorative treatment for its effectiveness in calves during their rehabilitation after gastrointestinal infections, 3 groups of 10–30 day-old calves were formed according to the analogous pairs principle: two test (T-1, T-2) groups and one control (C) group. Tested products were administered to the calves according to the scheme presented in Table 1.

All procedures involving animals were carried out in accordance with ethical standards laid down by European Convention ETS No. 123.

The feed additive and biogenic stimulant were tested for their effectiveness based on the following: morphological tests of blood (total erythrocyte counts, total leukocyte counts, hemoglobin level) using conventional methods [19]; biochemical tests of sera: refractometric determination (IRF-22, Russia) of serum total protein, nephelometric determination of protein fractions [20]; ELISA determination of total immunoglobulin G using appropriate ELISA test-kit; determination of serum mineral content by unified method using Vital Diagnostics SPb kits (Russia) and Stat Fax® 1904+ biochemical photometer (Awareness Technology, Inc., USA); bacteriological tests of biological material samples in accordance with the Methodological Guidelines[1, 2].

[1] MG 4.2.2723-10 Laboratory diagnosis of salmonellosis, *Salmonella* detection in food products and environmental samples: methodical guidelines (approved by Chief Medical Officer of the Russian Federation on 13 August 2010). https://docs.cntd.ru/document/1200083950?ysclid=lvgmjzwhv062935169

[2] Methodical Guidelines for laboratory diagnosis of animal and avian pasteurellosis: approved by the Veterinary Department of the Ministry of Agriculture of the USSR No. 22-7/82 on 20 August 1992. https://docs.cntd.ru/document/456071306?ysclid=lvgn11uqfc818248150

Blood samples were collected before the trial and 10 days after the trial completion. Mean values was assessed for their reliability of using Student's – Fisher's test.

RESULTS AND DISCUSSION

The gastrointestinal disease incidence in calves kept on the farm periodically rises due to violation of zootechnical and veterinary rules for animal keeping, feeding and handling during mass calving. The etiological causes are: diseased animals, convalescent animals, dams – pathogenic microorganism strain carriers, infected environment. The following bacteria were detected in feces from diseased calves subjected to bacteriological tests: *Salmonella Dublin, Mannheimia haemolytica*, all isolated bacteria strains were pathogenic for white mice. Levofloxacin, marfloxin, enrofloxacin, norfloxacin, ofloxacin, polymyxin, kanamycin were found to be effective antimicrobials. At the final stage of the trial, no pathogenic microorganism strains were detected during the bacteriological tests of fecal samples from calves of test and control groups.

Tests of the blood samples collected before the trial aimed at testing DIABAX feed additive and biogenic stimulant for their effectiveness for correction of biochemical, hematological blood parameters in calves during their rehabilitation after gastrointestinal infections showed decrease in phosphorous level by 19.1%, calcium level by 9.2%, magnesium level by 46.3% as compared with the physiological norm and simultaneous increase in zinc level by 3.5% as compared to the physiological norm (Table 2).

Calves of both test groups demonstrated normalization of serum phosphorus level, a significant increase in serum calcium level by 19.0% in the T-1 group and by 23.8% in the T-2 group ($p < 0.05$) as compared to that ones in sera from control group calves, in serum magnesium level – by 57.1% in T-1 group and by 61.2% in T-2 group ($p < 0.05$), serum potassium level – by 2.2% in T-1 group and by 4.3% in T-2 group 10 days after completion of tested feed additive and biogenic stimulant administration. There was a positive dynamics of zinc level decrease by 0.8% in sera from control group calves, by 5.5% in sera from T-1 test group calves, by 6.3% in sera from T-2 test group calves as compared to initial values during the rehabilitation period.

Biochemical tests of calf sera collected before the trial showed slight decrease in albumin protein fraction by 4.7%, and α-globulin protein fraction by 23.3%. Decrease in albumin-globulin ratio by 31.3% is indicative of imbalance between the protein fractions, 14.0% decrease in the amount of immunoglobulins G mainly responsible for humoral immunity is indicative of body protective function suppression in animals used for the trial (Table 3).

Table 2
Micro- and macroelement levels in sera from calves used for the trial

Mean value for group	P, mmol/L	Ca, mmol/L	Mg, mmol/L	K, mmol/L	Zn, μmol/L
Normal range	1.78–2.42	2.50–3.00	0.82–1.23	4.10–4.86	15.40–23.00
I	1.44 ± 0.17	2.27 ± 0.442	0.44 ± 0.14	4.90 ± 1.99	23.80 ± 1.56
C	2.10 ± 0.161	2.10 ± 0.111	0.49 ± 0.09	4.60 ± 0.38	23.60 ± 1.08
± to/from I, %	+ 45.80	– 7.50	+ 11.40	– 6.10	– 0.80
T-1	1.78 ± 0.121	2.50 ± 0.120*	0.77 ± 0.02*	4.70 ± 0.18	22.50 ± 1.52
± to/from I, %	+ 23.60	+ 10.10	+ 75.00	– 4.10	– 5.50
± to/from C, %	– 15.20	+ 19.00	+ 57.10	+ 2.20	– 4.70
T-2	1.79 ± 0.152	2.60 ± 0.110*	0.79 ± 0.03*	4.8 ± 0.67	22.3 ± 1.27
± to/from I, %	+ 24.30	+ 14.50	+ 79.50	– 2.00	– 6.30
± to/from C, %	– 14.80	+ 23.80	+ 61.20	+ 4.30	– 5.50

* $p < 0.05$; I – initial values, C – values in control group.

Table 3
Concentrations of total protein, protein fractions in sera from the calves used for the trial

Group of animals	Total protein, g/L	Albumins, %	Globulins, %			A/G ratio, units	Immunoglobulin G, mg/mL
			α	*β*	*γ*		
Normal range	56.9–65.0	38–50	12–20	10–16	25–40	0.83–1.19	> 10
I	66.4 ± 8.42	36.2 ± 8.91	9.2 ± 4.34	15.8 ± 7.57	39.0 ± 5.09	0.57 ± 0.22	8.6 ± 3.39
C	60.0 ± 1.71	38.5 ± 1.21	12.5 ± 1.53	21.2 ± 1.72	27.8 ± 1.92	0.63 ± 0.114	14.8 ± 3.45
± to/from I, %	– 9.6	+ 6.4	+ 35.9	+ 34.2	– 28.7	+ 10.5	+ 72.1
T-1	56.9 ± 2.88	43.5 ± 0.82*	12.7 ± 0.76	13.6 ± 1.15*	30.2 ± 1.28	0.77 ± 0.010	19.7 ± 1.78
± to/from I, %	– 14.3	+ 20.2	+ 38.0	– 13.9	– 22.6	+ 35.1	2.3-fold increase
± to/from C, %	– 5.2	+ 13.0	+ 1.6	– 35.8	+ 8.6	+ 22.2	+ 33.1
T-2	60.1 ± 3.66	42.4 ± 1.06*	17.9 ± 1.27*	15.0 ± 1.18*	24.7 ± 2.05	0.74 ± 0.052	23.0 ± 2.16
± to/from I, %	– 9.5	+ 17.1	+ 94.6	– 5.1	– 36.7	+ 29.8	2.7-fold increase
± to/from C, %	0	+ 10.1	+ 43.2	– 29.2	– 11.2	+ 17.5	+ 55.4

* $p < 0,05$; A/G – albumin/globulin ratio, I – initial values, C – values in control group.

At the final stage of the trial, albumin and *α*-globulin protein fractions in the calf sera were found to normalize to the physiological norm with a significant increase ($p \leq 0.05$) in albumin levels in T-1 and T-2 groups and *α*-globulin fraction in T-2 group. In calves of control group, serum *β*-globulin level increased by 34.2% as compared to the initial values and significantly differed ($p \leq 0.05$) from the test group values. Albumin-globulin ratio in animals of control group and test groups increased by 10.5% and 29.8–35.1% during the period of rehabilitation of the calves after gastrointestinal infections.

Immunoglobulin G amount reached its physiological norm in sera from test group calves. Immunoglobulin G amount increased by 72.1% in calves of control group and by 2.3–2.7 times in calves of test groups as compared to the initial values.

Analysis of hematological blood parameters in calves at the beginning and at the end of the trial showed no significant differences between the control and test groups. There was a positive trend towards an increase in the tested blood parameters within the physiological norm at the final stage of rehabilitation of calves in test groups as compared to the initial values and blood parameters of calves in control group (Table 4).

In test groups all calves survived (100% survival rate) during rehabilitation period when the tested feed additive and biogenic stimulant were administered to the calves and then during 60-day clinical observation period (Table 5). Disease rate was 66,7% in control group calves that was higher by 14.4 and 40.0% than that one in T-1 and T-2 groups, respectively.

Increase in average daily weight gains by 33.5% in T-1 group and by 27.9% in T-2 group as compared

Table 4
Hematological blood parameters in the calves used for the trial

Group of animals	Erythrocytes, 10^{12}/L	Hemoglobin, g/L	Leukocytes, 10^{9}/L	Color index, units
Normal range	7.40–8.60	99.00–128.00	4.50–12.00	0.70–1.10
I	8.00 ± 1.36	98.00 ± 17.40	5.50 ± 1.16	0.87 ± 0.14
C	8.20 ± 0.33	104.00 ± 4.67	6.00 ± 0.50	0.90 ± 0.04
± to/from I, %	+ 2.5	+ 6.1	+ 9.1	+ 3.4
T-1	8.30 ± 0.60	105.80 ± 4.33	6.70 ± 0.63	0.90 ± 0.04
± to/from I, %	+ 3.8	+ 8.0	+ 21.8	+ 3.4
± to/from C, %	+ 1.2	+ 1.7	+ 11.7	0
T-2	8.40 ± 0.42	108.00 ± 4.84	6.90 ± 0.59	0.91 ± 0.06
± to/from I, %	+ 5.0	+ 10.2	+ 25.5	+ 4.6
± to/from C, %	+ 2.4	+ 3.8	+ 15.0	+ 1.1

I – initial values, C – values in control group.

Table 5
Disease and survival rates in the calves used for the trial

Group of animals	Number of animals	Diseased		Died		Decrease in proportion of diseased animals in test group as compared to control group, %
		Number of animals	%	Number of animals	%	
C	6	4	66.7	1	16.7	–
T-1	7	4	57.1	–	–	14.4
T-2	5	2	40.0	–	–	40.0

to daily weight gains in control group was recorded during the first control weighing of the calves used for the trail (30 days after the trial start). During the second control weighing of the calves preformed 60 days after the trial start, 55.5–67.7% increase was recorded. Average daily weigh gain in T-1 group calves and in T-2 group calves was by 10.6% and 4.3% higher than that one in control group calves during the whole calf raising period (Table 6).

The test results show that administration of the biogenic stimulant of animal origin by injections and DIABAX feed additive (in T-2 group) with milk contribute to 100% survival rate in calves, significant increase in calcium and magnesium levels in the animal sera by 14.5–23.8% and 61.2–79.5%, respectively, as compared to the initial levels and levels in control group, an increase in albumin and α-globulin protein fractions by 10.1 and 43.2% ($p \leq 0.05$), respectively, as compared to that ones in control group, increase in albumin/globulin ratio by 17.5%, color index – by 1.1% as compared to that ones in control group, the total immunoglobulin G amount was 2.7 times higher than the initial value. Daily administration of DIABAX feed additive to calves of T-1 group for 15 days reduced recurrent disease rate in the calves by 14.4%, resulted in significant increase in serum calcium and magnesium levels by 10.1 and 75.0% ($p \leq 0.05$), respectively, as well as in increase in immunoglobulin G level by 2.3 times, erythrocyte level – by 3.8%, hemoglobin level – by 8.0%, leukocyte level – by 21.8%, albumin/globulin ratio – by 35.1% as compared to the initial values.

CONCLUSIONS

1. Daily administration of DIABAX feed additive to calves for 15 days contributed to significant increase

Table 6
Average body weight of the calves by group

Group of animals	Birth weight	Control weighing, kg			Observation period, days	Average daily weight gain, g	
		at the beginning of the trial	30 days after the trial start	60 days after the trial start		during 30/60 days of observation	during the whole raising period
C	39.00 ± 0.61	89.20 ± 10.34	105.30 ± 5.59	126.50 ± 12.05	113	537/622	774 ± 87.6
T-1	38.60 ± 0.46	85.00 ± 5.23	106.50 ± 8.89	143.00 ± 8.78	122	717/967	856 ± 67.7
T-2	37.20 ± 0.27	73.80 ± 3.84	94.40 ± 8.40	136.40 ± 8.59	123	687/1,043	807 ± 70.0

in serum calcium, magnesium, albumin, β-globulin levels ($p \leq 0.05$) in T-1 group calves, decrease in number of diseased animals by 14.4% and 100% survivability in T-2 group calves as compared to control group calves, increase in average daily weigh gain by 55.5% as compared to that ones in control group calves during 60-day observation period.

2. Combination of the biogenic stimulant injections to calves on day 1, 5, 10 of the trial and adding DIABAX feed additive to the calf diet for 15 consecutive days contributed to a significant increase in calcium, magnesium, albumin levels, α-, β-globulin protein fractions ($p \leq 0.05$), decrease in disease rates by 40.0 and 29.9%, as well as an increase in average daily weight gain by 67.7 and 7.9% as compared to that ones in control and T-1 groups, respectively, during 60-day observation period with 100.0% survival rate in T-2 test group calves.

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