



Stimulating Cellular and Humoral Natural Resistance Factors in Calves with Bronchopneumonia Using Glycyrrhizic Acid

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Abstract | This paper studied the effect of glycyrrhizic acid on the growth and development of calves with an acute form of bronchopneumonia. The research was performed at the Department of Morphology, Pathology, Pharmacy, and Non-Communicable Diseases of the Federal State Budgetary Educational Institution of Higher Vocational Education “Bashkir State Agrarian University.” The research’s scientific and practical parts were carried out in the environment of the State Unitary Agricultural Enterprise “Alekseevsky” Collective Farm in the Republic of Bashkortostan. When conducting research, clinical, biochemical, and immunological research methods were used. A beneficial effect of glycyrrhizic acid on the body of sick animals was observed during research. It was reflected in their growth and development, the animals’ improved clinical and physiological parameters. It was also noted that the calf growth and development rate depended on the dose of glycyrrhizic acid. Glycyrrhizic acid was given to animals in the form of aqueous solutions. When applying it at a dose of 50 mg/kg, a high efficiency in calves growth was obtained. The data obtained on the serotonin and histamine contents indicate that the calf body responded with a set of adaptive reactions. Therefore, glycyrrhizic acid activates biochemical and immunological processes aimed at coordinating the use of energy resources, which are a presage of a favorable outcome of the disease due to increased metabolism and have a positive effect on the calf growth and development.

Keywords | Bronchopneumonia, Calves, Glycyrrhizic acid, Resistance, Acid

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INTRODUCTION

About 75% of non-communicable diseases (e.g., diseases of the digestive and respiratory systems, metabolic disorders, ovarian and uterine diseases) are registered in these farms (Gabitov *et al.*, 2018, 2019; Skovorodin *et al.*, 2020). Things go from bad to worse for the young animals due to secondary intrafarm auto-, re-, and superinfections (i.e., factor superinfections) such as colibacillosis, salmonellosis, pasteurellosis, and anaerobic enterotoxemia, which are accompanied by pneumonia (Skovorodin *et al.*, 2018).

According to literature data, bronchopneumonia occurs in 30–40% of calves in Russia, ranking second in prevalence after the digestive system’s diseases. Respiratory tract diseases in farm animals make up 19.5% of all diseases in the Netherlands, 64.8% in Denmark, and 10.7% in Germany (Mandigers *et al.*, 2006).

Bronchopneumonia should not be considered a local pathological process in the respiratory system but as a complex animal reaction, which is proved by changes in the blood vascular system (Bertagnon *et al.*, 2019). Combination treatment of diseases should include both

the introduction of antibiotics and the use of feed additives and biologically active physical factors (aeroionization, ozonation, bacterial purification) (Dementyev et al., 2018).

The first three to six months of calves' life is when active immunity is established. During this period, the more significant morbidity and mortality are caused by bronchopneumonia because of the pulmonary immune system of the exaggerated cytotoxic response at subsequent infection (Bertagnon et al., 2019).

In the work of Nishi et al. (2019), the relationship between endotoxin activity in blood plasma and bronchoalveolar lavage fluid was studied. This indicator can be used as a diagnostic criterion for identifying calves that may soon die (Nishi et al., 2019).

The success of veterinary medicine is mostly dependent on the development and use of new drugs.

To date, veterinary medicine has offered a wide range of drugs for non-specific immune status stimulation. Many drugs used in veterinary medicine have undesirable effects: allergenicity, high cost, high toxicity, pyrogenicity, etc. (Buttros et al., 2009).

A derivative of triterpene glycoside glycyrrhizic acid, which is the primary biologically active substance of licorice root extract has established itself as a promising compound for increasing the animal immune status. The primary substance in licorice root is glycyrrhizic acid. Baltina et al. (2001) demonstrated in her paper that the maximum value for potassium-calcium-magnesium salt is $K=0.6$; $Mg=0.5$; $Ca=0.5$ of glycyrrhizic acid. 15% of licorice root is glycyrrhizin (Ming and Yin, 2013). Chemical modification of glycyrrhizic acid proved to be a promising method to develop new highly active antiviral drugs to prevent and treat HIV, hepatitis B, C, coronavirus, and herpes virus infections (Baltina et al., 2006, 2009). Possible mechanisms of antioxidant activity of glycyrrhizic acid have been studied (Beskina et al., 2006). It is known from the literature that glycyrrhizic acid has antiarrhythmic, antiallergic, antiviral, and anti-inflammatory effects (Baltina et al., 2009, 2010; Jitesh and Geetha, 2017; Safonov, 2020).

The ability of various glycyrrhizic acid derivatives to increase antibiotic therapy effectiveness for experimental infections has been shown by several authors. Many of them have observed an increase in non-specific resistance to infection with stimulation of the mononuclear phagocytic system (Skovorodin et al., 2018).

Such a high interest in glycyrrhizic acid is primarily due to its high and diverse biological activity with low toxicity compared to synthetic analogues. It enhances the humoral

immune response in animals primed with a thymus-dependent antigen; it has anti-ulcerogenic and anti-inflammatory, hepatoprotective, and antioxidant properties (Bazekin and Ismagilova, 2010, 2015, 2012a, 2012b; Beskina et al., 2006; Tolstikova et al., 2009).

In view of the above, the research aimed to study the effect of glycyrrhizic acid on the calf growth and development, the immunological parameters and biochemical status of animals with an acute form of bronchopneumonia.

MATERIAL AND METHODS

Research involving Animals. All experiments were conducted under the legislation (European Convention for the protection of vertebrates; European Convention for the protection of vertebrates used for experimental and other scientific purposes; guidelines for the care and use of laboratory animals and based on the report of the Committee on ethics in the field of animal research (no.9 of 23.02.2015). The research was performed at the Department of Morphology, Pathology, Pharmacy, and Non-Communicable Diseases of the Federal State Budgetary Educational Institution of Higher Vocational Education, Bashkir State Agrarian University. The research's scientific and practical parts were carried out in the environment of the State Unitary Agricultural Enterprise "Aleksievsky" Collective Farm in the Republic of Bashkortostan.

Object and substance of research. The research object was 24 calves of the white-and-black breed with an acute form of bronchopneumonia.

As the primary pharmacological substance studied, we used the main licorice root triterpene glycoside-glycyrrhizic acid. The studied calves were given glycyrrhizic acid in the form of aqueous solutions in doses of 50 mg/kg, individually, using catheters and rubber bottles once a day.

It is advisable to study the natural resistance of calves with bronchopneumonia taking into account the properties of the glycyrrhizic acid components.

At the initial stage, the calves were subjected to examination. The examination revealed that 24 calves had an acute form of bronchopneumonia. For the purpose of the experiment, 4 groups of calves were formed. Sick animals were divided into three experimental groups and one control group of 6 calves in each. During the experiment, animals were kept in the same conditions.

Biochemical blood tests, serum bactericidal test, and statistical analysis. Venous blood samples were taken on an empty stomach, in the morning, in Vacuette vacuum

tubes: for biochemical tests of blood serum with a clot activator. Blood samples selected for biochemical tests were centrifuged in an EBA 20 centrifuge (Hettich, Germany) at 3,500 rpm for 10 minutes.

Biochemical blood tests were performed using a StatFax 1904+ semi-automatic biochemical blood analyzer (Awareness Technology Inc.) using standardized reagents by Vital Diagnostics Spb and the following reaction conditions: Total bilirubin: According to the Jendrassik-Gróf method (incubation for 20 minutes at a temperature of 20 °C, $\lambda=545$ nm, the optical path length: 10 mm, the lower limit of linearity: 8 $\mu\text{mol/L}$, the upper limit of linearity: 410 $\mu\text{mol/L}$, in comparison with the blank and calibrated bilirubin samples: 171 $\mu\text{mol/L}$); the total protein: according to the biuret method (incubation for 30 at a temperature of 20 °C, $\lambda=545$ nm, the optical path length: 10 mm, the lower limit of linearity: 0 g/L, the upper limit of linearity: 120 g/L, in comparison with blank and calibrated blood protein samples: 70 g/L); albumin: according to the method of determining the albumin concentration in serum by reaction with bromocresol green (without incubation, at the temperature of the reaction mixture of 37 °C, $\lambda=630$ nm, the optical path length: 10 mm, the lower limit of linearity: 20 g/L, the upper limit of linearity: 60 g/L, in comparison with blank and calibrated albumin samples in serum: 60 g/L).

Calibration and verification of the biochemical analyzer were carried out as part of laboratory control with the test design for standardized samples of artificial blood serum with normal and pathological indicators by Vital Diagnostics Spb.

The lysozyme activity of calf blood serum was measured in cuvettes with a working length of 10 mm.

2 mL of blood serum that had been diluted in 0.5% sodium chloride solution with pH = 7.2 in the ratio of 1:1 (1 mL of 0.5% of sodium chloride + 1 mL of serum tested) were added to 2 mL of one-day culture of *Micrococcus Lysodecticus* prepared as follows: A one-day culture of *Micrococcus Lysodecticus* was washed off with a 0.5% sodium chloride solution; then, the resulting suspension was standardized to a content of 1 bln microbial bodies in 1 mL. Standardization was carried out using a photoelectrocolorimeter (FEC 56M) until the extinction of 0.320.

Since, with such dilution, the serum's optical density affects the results of the test, an additional control with serum (1 mL of serum + 3 mL of 0.5% of sodium chloride solution) was made. The obtained extinction test values of the serum control were subtracted from the experimental cuvettes' extinction test results.

As a control, we used 2 mL of 0.5% sodium chloride solution + 2 ml of the same suspension (as in experimental cuvettes). The method for determining calf blood serum's bactericidal activity is based on the blood serum ability to exert a bacteriostatic effect on microorganisms.

The bactericidal activity was characterized by a delay in the test microbe biomass growth in meat-peptone broth (MPB) under the influence of the tested blood serum, expressed as a percentage. As a test microbe, we used a one-day culture of *Staphylococcus aureus* strain No. 209. MPB was dispensed into standard microbiological tubes, 4.5 mL in each. The tested blood serum was added to the test tubes, 1 mL in each, and 1 mL of MPB was added to the control tube. 0.1 mL of test microbe was added to all tubes (a wash of 16-hour agar culture that had been twice washed with 0.85% sodium chloride solution and brought to the concentration of 2.5 bln microbial bodies as per the turbidity standard).

Then, the tubes were shaken, and 2 mL of fluid was taken from each tube with a sterile pipette for measuring the optical density using the FEC-56M, i.e., to determine D_{11} . The measurements were carried out in cuvettes with a working length of 5 mm with a green light filter against distilled water. Next, all tubes were closed with plugs and placed in a thermostat for 3 hours. After incubation, the optical density was again measured using the FEC-56M. Statistical processing of experimental data was carried out using the statistical analysis package for Microsoft Excel. The significance of differences between the groups in quantitative terms was evaluated using Student's t-criterion. Differences were considered as statistically significant when $P < 0.05$.

RESULTS

The tests revealed that bronchopneumonia in animals was acute. Sick animals have decreased appetite; their mucous membranes were pale and cyanotic; animals used to lie; they had a high temperature, refused feed and drink, showed a high pulse rate, and rapid breathing. On the first day of the disease, rapid breathing, coughing, wheezing, and nasal discharge were observed. Purulent nasal discharge appeared in sick calves on the 3rd to 5th day. During the treatment, sick animals of experimental group I (6 animals) received glycyrrhizic acid at a dose of 50 mg/kg, whereas animals of group II served as a control group (6 animals).

The tests showed that in calves with bronchopneumonia, the enzymatic-metabolic process was disturbed in peripheral blood leukocytes with a simultaneous decrease in the lysozyme activity. In the control group, the lysozyme activity was within physiological norms, whereas in the experimental group before treatment, as compared with

the control group, it was decreased. 10 days after using glycyrrhizic acid, the lysozyme activity increased 2 times. The bactericidal activity in the control group before treatment was lower than that in the experimental groups; 10 days after treatment, the bactericidal activity increased 2 times. These clinical studies are the most crucial link in the bronchopneumonia development pattern (Table 1).

The use of glycyrrhizic acid positively affected cellular and humoral parameters of non-specific resistance in calves with bronchopneumonia, which reduced treatment time. Biologically active substances, among which an important link is a histamine, play an active role in the bronchopneumonia development pattern. We found that histamine in the calves' blood of the control group was $3.75 \pm 0.39 \mu\text{g/mL}$. The average histamine concentration in the calf blood exceeded 3 times that in the control group and amounted to $12.0 \pm 1.02 \mu\text{g/mL}$ in the experimental group. Elevated histamine levels in calves were observed during complications and disease attacks. 5 days after treatment, the histamine concentration in the calf blood reached $6.02 \pm 0.81 \mu\text{g/ml}$. It means that histamine in the calf blood increased due to its release by mast cells. 10 days after treatment, the histamine content in the blood of

experimental calves was within physiological norms. Table 2 shows biochemical parameters of blood serum in calves before the experiment; 5 and 10 days after the experiment.

Table 2 shows that, before using glycyrrhizic acid, at the start of the experiment, the contents of total protein, albumin, and alkaline reserve in the blood serum of sick calves were reduced. The contents of α -globulins, β -globulins, γ -globulins, total bilirubin, and the erythrocyte sedimentation rate were increased. On the fifth day after using glycyrrhizic acid, the levels of total protein, alkaline reserve, and albumin in the blood of calves with bronchopneumonia were reduced 0.9 times. Besides, the percentage of globulins was significantly reduced, α -globulins, β -globulins 0.9 times; γ -globulins, 0.1 times; the erythrocyte sedimentation rate also decreased 0.8 times. The total bilirubin also decreased by 0.9 times.

10 days after using glycyrrhizic acid, the amount of total protein increased 1.04 times; albumin, 1.3 times; alkaline reserve, 1.04 times. The levels of globulins decreased: α -globulins, 0.9 times; β -globulins, 0.7 times, γ -globulins, 0.8 times; the erythrocyte sedimentation rate decreased 1.4 times.

Table 1: Immunological and biochemical blood parameters in sick calves when using glycyrrhizic acid (n=6).

Parameter name	Unit of measure	Control group	Experimental group		
			before the use of GA	after 5 days of using GA	after 10 days of using GA
Alkaline phosphatase	units/L	1.39±0.32	2.32±0.09	1.76±0.21	1.31±0.23
Acid phosphatase	units/L	0.25±0.23	0.37±0.13	0.34±0.06	0.27±0.16
Glycogen	mg%	2.09±0.09	2.13±0.08	2.08±0.09	2.20±0.08
Myeloperoxidase	units/L	2.35±0.15	1.89±0.12	2.08±0.09	2.31±0.07
Nitro blue tetrazolium reduction test	%	9.56±0.23	23.11±1.09	12.03±0.55	8.23±0.21
Lysozyme	μg/mL	8.43±0.24	1.84±0.06	15.50±0.61	17.71±0.76
Bactericidal activity	%	76.16±3.56	38.10±1.98	56.50±2.35	61.3±2.98
Acid activity of lymphocyte phosphatase	IE/mL	0.37±0.04	0.54±0.03	0.43±0.03	0.37±0.03
Histamine	μg/mL	3.75±0.39	12.0±1.02	6.02±0.80	4.33±0.29

Note: $P \leq 0.05$.

Table 2: Biochemical parameters of blood serum in sick calves when using glycyrrhizic acid (n=6).

Parameter name	Unit of measure	Control group	Experimental group		
			before the use of GA	after 5 days of using GA	after 10 days of using GA
Total protein	g%	7.66±0.55	6.9±0.78	8.01±0.54	8.09±0.65
Albumins	%	30.6±2.03	30.5±1.29	41.3±2.03	43.4±2.45
α -globulins	%	22.3±1.27	23.9±1.32	20.4±1.32	17.34±1.34
β -globulins	%	22.4±1.22	23.7±1.43	17.3±1.24	17.9±1.32
γ -globulins	%	24.8±1.34	24.9±1.34	19.8±1.32	18.2±1.43
Total bilirubin	%	5.31±0.36	5.09±0.24	4.79±0.37	4.61±0.36
Alkaline reserve	vol.% CO ₂	37.6±3.00	35.4±2.07	39.2±2.12	46.7±2.67
ESR	Min	12.1±1.11	13.5±1.15	8.23±0.11	7.63±0.56

Note: $P \leq 0.05$.

The beneficial effect of glycyrrhizic acid on sick animals' body was observed during the research, which was reflected in their growth and development, the animals' improved clinical and physiological parameters. It was also noted that the calf growth and development rate depended on the dose of glycyrrhizic acid. Glycyrrhizic acid was given to animals in the form of aqueous solutions. It ensured high efficiency in the calf and calves growth at a dose of 50 mg/kg. With a gradual increase in the dose of the aqueous glycyrrhizic acid solution, the average daily gains in calf body weight also increased. The daily increase in live weight of calves in the experimental group I at the age of one and a half months exceeded their counterparts in the control group by 278.45 g, i.e., by 67.51%; at the age of three months, by 178.1 g, i.e., by 38.5%; at the age of four months, by 234.32 g, i.e., by 48.35%.

Table 3: Average daily gain in live weight of the calves in the experimental groups.

Experimental groups	Age	Average daily gain in live weight of calves
Experimental group I	1.5 months	67.51%;
	3 months	38.5%;
	4 months	48.35%.
Experimental group II	1.5 months	71.1 %
	3 months	59.23 %.
Experimental group III	1.5 months	35.12%;
	3 months	23.54%;
	4 months	37.14 %.

Calves in the experimental group II, which received glycyrrhizic acid at a dose of 50 mg/kg, featured a higher daily weight gain than the control group; on the 30th day of the experiment, by 278.36 g, i.e., by 71.1 %; on the 90th day, by 287.3 g, i.e., by 59.23 %. In all age groups, calves' live weight in the experimental groups was significantly higher than in control one. As compared to other groups, calves in the third experimental group gained more live weight, thereby exceeding the data in the control group: on the 30th day of the experiment, the live weight increased by 35.12%; on the 60th day, by 23.54%; on the 90th day, by 37.14 %. The absolute weight gain of calves in groups II and III exceeded that of calves in the control group: on the 30th day of the experiment, 1.54 and 1.8 times, i.e., by 8.13 and 8.67 kg; on the 60th day, 1.29 and 1.39 times, i.e., by 11.34 and 13.0 kg. The relative growth rate of calves in the experimental group II at the age of two months exceeded the values of groups IV and V 1.06 and 1.07 times; at the age of three months, 2.45 and 1.12 times (Table 3).

DISCUSSION

In scientific research, the positive effect of glycyrrhizic

acid on cellular and humoral natural resistance factors in calves with bronchopneumonia was established. As shown by studies, glycyrrhizic acid's preventive use enhances the production of serotonin and histamine (Gao et al., 2011, 2015; Gatiyatullin et al., 2018).

When analyzing the biochemical tests, it can be noted that, before using glycyrrhizic acid, at the start of the experiment, the contents of total protein, albumin, and alkaline reserve in the blood serum of sick calves were reduced. The contents of α -globulins, β -globulins, γ -globulins, the total bilirubin, the erythrocyte sedimentation rate were increased.

On the fifth day after using glycyrrhizic acid, the total protein, alkaline reserve, and albumin in the blood of calves with bronchopneumonia were reduced 0.9 times. Besides, the percentage of globulins was significantly reduced: α -globulins, β -globulins, 0.9 times; γ -globulins, 0.1 times; the erythrocyte sedimentation rate also decreased 0.8 times. The total bilirubin decreased by 0.9 times.

10 days after using glycyrrhizic acid, the total protein increased 1.04 times; albumin, 1.3 times; alkaline reserve, 1.04 times. The number of globulins decreased: α -globulins, 0.9 times; β -globulins, 0.7 times, γ -globulins, 0.8 times; the erythrocyte sedimentation rate decreased 1.4 times.

When analyzing immunological tests, it can be noted that the enzymatic metabolic process was disturbed in calves with bronchopneumonia in peripheral blood leukocytes with a simultaneous decrease in lysozyme activity. In the control group, the lysozyme activity was within its physiological norms, whereas in the experimental group before treatment, it was decreased compared with the control group.

10 days after using glycyrrhizic acid, the lysozyme activity increased 2 times. The bactericidal activity in the control group before treatment was lower than that in the experimental groups; 10 days after treatment, the bactericidal activity increased 2 times. These clinical studies are the most crucial link in the bronchopneumonia development pattern.

CONCLUSIONS

The use of glycyrrhizic acid positively affected cellular and humoral parameters of non-specific resistance in calves with bronchopneumonia, which reduced the treatment time. The data obtained on the serotonin and histamine contents indicate that the calf body responded with a set of adaptive reactions. Therefore, glycyrrhizic acid activates biochemical and immunological processes aimed at coordinating the use of energy resources, which are a presage of a favorable

outcome of the disease due to metabolism activation and has a positive effect on the calf growth and development.

Research prospects. Scientific research can be recommended in the future for the further development of new methods for specific prevention and treatment of respiratory diseases in young farm animals, as well as for the development of new medicines and treatment complexes.

Further studies on the research topic will be aimed at studying the structural and functional state of the calves lungs (at different stages of ontogenesis) when using glycyrrhizic acid. The material is recommended for giving lectures, laboratory and practical classes to veterinary medicine students.

AUTHOR'S CONTRIBUTION

The idea for creating this manuscript was the multifaceted use of glycyrrhizic acid in the field of veterinary medicine. Georgy Bazekin developed and led the research project. Evgeny Skovorodin, Ildar Gatiyatullin studied the work of domestic and foreign authors devoted to the study of diseases of the respiratory system of farm animals, prepared a literature review. Ildar Gatiyatullin, Almaz Sharipov and Ilgiz Dolinin conducted research on calves in the conditions of the Alekseevsky state farm of the Republic of Bashkortostan. Biochemical and immunological studies of calf blood were carried out. Further, analytical calculations, statistical processing of the obtained material were carried out, the obtained data were interpreted. All authors discussed the results and contributed to the final manuscript.

CONFLICT OF INTERESTS

The authors have declared no conflict of interests.

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