One of the most widespread and dangerous helminthiases of large and small cattle is fasciolosis, which causes significant economic losses to animal husbandry: reduction of milk and meat productivity, deterioration of quality, and expenditure of funds for medical and preventive measures (Kulyaba et al., 2016; 2019). Despite the significant successes of foreign and domestic scientists in the study of fasciolosis, the question of the pathogenic effect of fascioles on the protective systems of the animal body is relevant (Freiuk & Stybel, 2020). The analysis of literary sources indicates that helminths are strong immunosuppressants and about the annual spread of fasciolosis on the territory of Ukraine, especially in its western, eastern and southern regions (Sobol’ta, & Gutyj, 2016; Kotelevich, 2017; Avramenko et al., 2019).

Reports in domestic and foreign literature also confirm that, along with bovine fasciolosis, mycobacteriosis has also become widespread. The causative agents of mycobacteriosis in animals are the so-called potentially pathogenic mycobacteria (atypical, anonymous, or unclassified), which are characterized by a

The effect of butaselmevit and closaverm A on the immune status of cows with experimental fasciolosis sensitized by atypical mycobacteria

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There are many reports in the literature about the critical role of the immune system in protecting the body against foreign agents. However, the role of immune status in the association of mycobacteriosis and fasciolosis has not been studied. The work aimed to investigate the effect of butaselmevit and closaverm A on the immune status of cows with experimental fasciolosis sensitized by atypical mycobacteria. For the trial, 15 cows of 4–5 years of age, black and spotted breed, were selected, from which 3 groups were formed, five animals in each. Cows of the first research group for experimental fasciolosis were intramuscularly injected with closaverm A at a dose of 0.5 ml of the drug per 10 kg of body weight. The animals of the second experimental group, for experimental fasciolosis, were intramuscularly injected with closaverm A at a dose of 0.5 ml of the drug per 10 kg of body weight and butaselmevit at a dose of 10 ml of the drug per animal. The obtained results indicate that the drugs “Klosaverm A” and “Butaselmevit” drugs in experimental groups of animals helped reduce the level of CIC to physiological values starting from the 21st day of the experiment. It should be noted that according to the indicators of the immune system in cows with experimental fasciolosis, sensitized by atypical mycobacteria, the combined use of closaver A and butaselmevit shows a better therapeutic effect than the use of only closaver A alone.

**Key words:** fasciolosis, mycobacteriosis, immune system, butaselmevit, closaverm A.

**Introduction**

One of the most widespread and dangerous helminthiases of large and small cattle is fasciolosis, which causes significant economic losses to animal husbandry: reduction of milk and meat productivity, deterioration of quality, and expenditure of funds for medical and preventive measures (Kulyaba et al., 2016; 2019). Despite the significant successes of foreign and domestic scientists in the study of fasciolosis, the question of the pathogenic effect of fascioles on the protective systems of the animal body is relevant (Freiuk & Stybel, 2020). The analysis of literary sources indicates that helminths are strong immunosuppressants and about the annual spread of fasciolosis on the territory of Ukraine, especially in its western, eastern and southern regions (Sobol’ta, & Gutyj, 2016; Kotelevich, 2017; Avramenko et al., 2019).

Reports in domestic and foreign literature also confirm that, along with bovine fasciolosis, mycobacteriosis has also become widespread. The causative agents of mycobacteriosis in animals are the so-called potentially pathogenic mycobacteria (atypical, anonymous, or unclassified), which are characterized by a
wide range of natural drug resistance (Kulyaba et al., 2017). In addition, mycobacteriosis usually develops only in the weakened organism of animals that have been adversely affected by the environment or the development of various diseases, including parasitic ones (Shevtsov, 2015). Despite a significant number of studies devoted to the study of fasciolosis in cattle, issues such as pathogenetic features of fasciolosis in cows sensitized by atypical mycobacteria, and their adequate therapy and prevention of the disease require in-depth scientific approaches.

In the literature (Martyshuk & Hutyi, 2021; Zhelavskyi et al., 2022; Chechet et al., 2022) there are many reports about the important role of the immune system in protecting the body against the effects of foreign agents. However, the role of immune status in the association of mycobacteriosis and fasciolosis has not been studied.

The aim of the study

To study the effect of butaselmevit and closaverm A on the immune status of cows with experimental fasciolosis sensitized by atypical mycobacteria

Material and methods

For the experiments, 15 cows of 4–5 years of age, black and spotted breed, were selected, from which 3 groups were formed, five animals in each. Cows of the control group (C) were experimentally infected with Adoleascaria. Cows of the first research group (E1) for experimental fasciolosis were injected intramuscularly with closaverm A at a dose of 0.5 ml of the drug per 10 kg of body weight. The animals of the second research group (E2) for experimental fasciolosis were intramuscularly injected with closaverm A at a dose of 0.5 ml of the drug per 10 kg body weight and butaselmevit (Martyshuk et al., 2018; 2022) at a dose of 10 ml of the drug per animal.

When conducting the research, the rules were followed, which are mandatory for conducting tests - the selection and maintenance of analogous animals in groups. The diet of the cows was balanced in terms of nutrients and minerals.

The cellular factor of resistance was determined by the total number of T-lymphocytes (E-RUK) – by the method of spontaneous rosette formation with ram erythrocytes according to M. Jondal et al., the total number of B-lymphocytes – according to N.F. Mendes et al. (1973). Phagocytic activity (FA), intensity (index) of phagocytosis (FI) using generally accepted methods in modification (Chumachenko V. E. et al., 1990). Among the humoral indicators of resistance, the bactericidal activity of blood serum (BABS) was studied according to the method of O. V. Smirnova, T. A. Kuzmina (1966), the lysozyme activity of blood serum (LABS) – by the photoelectrocolorimetric method (Vlizlo, 2012). Blood for analysis was taken from the jugular vein before infection and on the 7th, 14th, 21st, and 28th days of the experiment.

Experimental studies were conducted following the Law of Ukraine “On the Protection of Animals from Cruelty Treatment” dated 03/28/2006 and the rules of the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes dated 11/13/1987.

The analysis of research results was carried out using the Statistica 6.0 software package. The probability of differences was assessed by Student's t-test. The results of average values were considered statistically significant when * – P < 0.05, ** – P < 0.001 (ANOVA).

Results and discussion

As a result of the conducted immunological studies, it was established that in the control group of cows that were exposed to experimental fasciolosis and sensitized by atypical mycobacteria, there were certain shifts in cellular indicators of immunity. The specified immune disorders were characterized by a probable decrease in the number of T- and B-lymphocytes in the blood of cows of group K (Table 1).

The use of butaselmevit and closaverm A drugs for the treatment of cows with experimental fasciolosis sensitized by atypical mycobacteria made it possible to significantly increase the cellular link of the immune system. Thus, on the 14th day, the number of T-lymphocytes in the blood of E1 and E2 groups increased by 2.17 and 3.94 % compared to the C group of cows. On the 21st day, the T-lymphocytes number in the blood of cows treated with closaverm A, grew up to 39.20 ± 1.40 %, and in the blood of cows treated with closaverm A and butaselmevit – to 42.53 ± 1.22 %. On the 28th day, the T-lymphocytes number in the experimental groups of animals was the highest.

Table 1

| Indicator          | Groups of animals | Before infection | Term of research (days) |
|--------------------|-------------------|------------------|-------------------------|
|                    |                   |                  | 7          | 14          | 21          | 28          |
| T-lymphocytes, %   | C                 | 44.20 ± 1.25     | 40.14 ± 1.20         | 38.43 ± 1.22 | 36.75 ± 1.24 | 39.52 ± 1.26 | 39.52 ± 1.26 |
|                    | E1                | 44.25 ± 1.20     | 41.53 ± 1.30         | 40.60 ± 1.35 | 39.20 ± 1.40 | 40.15 ± 1.30 | 40.15 ± 1.30 |
|                    | E2                | 44.29 ± 1.17     | 42.11 ± 1.35         | 42.37 ± 1.21* | 42.53 ± 1.22* | 44.26 ± 1.28* | 44.26 ± 1.28* |
| B-lymphocytes, %   | C                 | 18.07 ± 0.60     | 16.52 ± 0.50         | 15.41 ± 0.55 | 14.15 ± 0.55 | 14.75 ± 0.50 | 14.75 ± 0.50 |
|                    | E1                | 18.05 ± 0.55     | 17.14 ± 0.65         | 16.85 ± 0.60 | 16.12 ± 0.55* | 16.43 ± 0.60* | 16.43 ± 0.60* |
|                    | E2                | 18.08 ± 0.52     | 17.78 ± 0.60         | 17.61 ± 0.65* | 17.56 ± 0.62** | 18.08 ± 0.60** | 18.08 ± 0.60** |
We also studied the effect of drugs on the B-lymphocytes number in the blood of cows with experimental fasciolosis sensitized by atypical mycobacteria. The stimulatory effect of closaverm A and butaselmevit on the cellular immunity of cows, namely on the number of B-lymphocytes, was established. Since this indicator in E1 and E2 groups gradually increased throughout the trial, it was the highest on the 21st and 28th days. It should be noted that the use of butaselmevit contributed to a faster increase in the B-lymphocytes number in the E2 than the use of only closaverm A.

Therefore, the obtained results indicate that the drugs “Klosaverm A” and “Butaselmevit” when administered intramuscularly to cows with experimental fasciolosis, sensitized by atypical mycobacteria, contribute to the activation of T- and B-cell links of immunity.

Table 2 presents the influence of butaselmevit and closaverm A on indicators of non-specific immunity of cows in association with mycobacteriosis and fasciolosis.

### Table 2

| Indicator                          | Groups animals | Before infection | Term of research (days) | 7   | 14  | 21  | 28  |
|-----------------------------------|---------------|-----------------|-------------------------|-----|-----|-----|-----|
| Phagocytic activity, %            |               |                 |                         | 51.3±1.95 | 50.4±1.60 | 49.2±1.65 | 49.9±1.80 |
|                                   | C             | 54.3±1.72       | 51.3±1.95               | 50.4±1.60 | 49.2±1.65 | 49.9±1.80 |
|                                   | E1            | 54.5±1.81       | 52.1±2.00               | 52.5±1.90 | 53.1±1.75 | 53.5±1.75 |
|                                   | E2            | 54.4±1.63       | 52.8±1.75               | 53.4±1.88 | 54.2±1.68*| 54.5±1.75 |
| Phagocytic index, units           |               |                 |                         | 8.66±0.23 | 8.35±0.26 | 7.85±0.25 | 8.06±0.20 |
|                                   | C             | 9.61±0.30       | 8.66±0.23               | 8.35±0.26 | 7.85±0.25 | 8.06±0.20 |
|                                   | E1            | 9.60±0.30       | 9.30±0.35               | 9.15±0.30*| 8.95±0.30*| 9.45±0.25**|
|                                   | E2            | 9.62±0.22       | 9.44±0.28*              | 9.37±0.25*| 9.58±0.32**| 9.66±0.21**|

As the research results showed, intramuscular administration of closaverm A and butaselmevit to cows increases the non-specific immunity of cows with experimental fasciolosis sensitized by atypical mycobacteria. Thus, on the 14th day, the phagocytic activity of neutrophil granulocytes in group E1 increased by 2.1%, and in the blood of cows of group E2 by 3.0% compared to the C group. On the 21st day, the phagocytic activity of neutrophil granulocytes in the blood of group E1 was 53.1±1.75%, and in group E2 – 54.2±1.68%, while in the C group this indicator was 49.2±1.65%. On the 28th day, the highest phagocytic activity was established in group E2, which was administered the drugs “Klosaverm A” and “Butaselmevit” in combination, compared to the C group, it increased by 4.6%.

The growth of phagocytic index was also established in animals of research E1 and E2 groups. Compared with the C group of cows, this indicator on the 21st day increased by 14 and 22%, respectively.

### Table 3

| Indicator                          | Groups animals | Before infection | Term of research (days) | 58.3±0.55 | 56.6±0.50 | 55.1±0.52 | 56.8±0.60 |
|-----------------------------------|---------------|-----------------|-------------------------|-----------|-----------|-----------|-----------|
| Bactericidal activity, %          |               |                 |                         | 59.8±0.65 | 59.1±0.70*| 59.7±0.55***| 60.1±0.65***|
|                                   | C             | 62.2±0.55       | 58.3±0.55               | 59.8±0.65 | 59.1±0.70*| 59.7±0.55***| 60.1±0.65***|
|                                   | E1            | 62.4±0.60       | 60.4±0.50*              | 61.4±0.56***| 62.2±0.45***| 62.7±0.62***|
|                                   | E2            | 62.6±0.65       | 60.4±0.50*              | 61.4±0.56***| 62.2±0.45***| 62.7±0.62***|
| Lysozyme activity, %              |               |                 |                         | 21.1±0.50 | 20.6±0.50 | 19.5±0.50 | 20.5±0.51 |
|                                   | C             | 23.5±0.45       | 21.1±0.50               | 20.6±0.50 | 19.5±0.50 | 20.5±0.51 |
|                                   | E1            | 23.2±0.55       | 21.7±0.60               | 21.2±0.65 | 21.6±0.50*| 22.1±0.65*|
|                                   | E2            | 23.3±0.52       | 22.0±0.66               | 22.8±0.50*| 23.0±0.58**| 23.7±0.62**|
| CIC, mol/l                        |               |                 |                         | 70.67±3.5 | 73.21±4.0 | 78.51±4.0 | 76.45±3.5 |
|                                   | C             | 64.74±4.0       | 70.67±3.5               | 73.21±4.0 | 78.51±4.0 | 76.45±3.5 |
|                                   | E1            | 64.70±4.2       | 69.10±3.5               | 70.15±4.1 | 70.56±4.2 | 69.42±3.6 |
|                                   | E2            | 64.72±3.7       | 67.86±4.0               | 68.12±3.6 | 67.67±3.5*| 65.55±3.9*|

The obtained research results indicate that after the application of butaselmevit and closaverm A to cows in appropriate doses, a non-specific link of the immune system is activated. After intramuscular injections of drugs, for 28 days, indicators of non-specific immunity remained at a high level, which ensures the high phagocytic activity of leukocytes.

After researching the values of humoral immunity indicators in cows treated with closaverm A and butaselmevit, high bactericidal and lysozyme activity of blood serum was established (Table 3). The lysozyme activity of the blood serum of cows of group E2 on the 14th and 21st days increased by 2.2 and 3.5%, respectively. The bactericidal activity of blood serum for the specified periods was up by 4.8 and 7.1%, respectively.

During the study of circulating immune complexes in cows of the C group, their level was found to be elevated, where on the 21st day of the test, the level of CIC reached 78.51±4.0 mol/l. A high level of CIC in the blood serum of cows with experimental fasciolosis, sensitized by atypical mycobacteria, indicates suppression of the body's immunoreactive system due to the attachment of specific immunoreactive substances.
antibodies to the metabolic products of the causative agents of the disease.

The use of clozaverm A and butaselmevit in experimental animals contributed to the reduction of the CIC level to physiological values starting from the 21st day of the experiment. On the 28th day, the CIC level in the blood of experimental groups of animals decreased by 9 and 14.3 %.

In experiments studying the effect of closaverm A and butaselmevit to prevent the negative effects of fasciols and mycobacteria, it was established that they exhibit different immunostimulating properties. It should be noted that according to the indicators of the immune system in cows with experimental fasciolosis, sensitized by atypical mycobacteria, the combined use of closaverm A and butaselmevit shows a better therapeutic effect than the use of only closaverm A alone.

Conclusions

The course of fasciolosis in cows sensitized by atypical mycobacteria in the experimental groups contributed to the suppression of humoral, cellular, and non-specific immunity. The use of closaverm A and butaselmevit contributed to the increase of immune protection of the body of cows in association with mycobacteriosis and fasciolosis.

Conflict of interest

The authors declare that there is no conflict of interest.

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