Investigation of the proteolytic activity of liver trematodes in goats of Khizi-Khachmaz zone of Azerbaijan

Topchiyeva Sh.A., Namazova A.A., Mammadova S.M.

Institute of Zoology of NAS of Azerbaijan, Baku
Address: AZ1073,Baku,Sabail, Abbasgulu Abbaszadeh,115

Abstract— The article presents experimental data on the detection of proteolytic activity of liver trematodes in the goats of Khizi-Khachmaz zone of Azerbaijan in different seasons of the year. Determination of the enzymatic activity was carried out spectrophotometrically using a Folin reagent on a Specol 1500 spectrophotometer (Analitik Jena). The maximum peak of intensity of proteolytic activity of trematodes isolated from goat liver was detected. The maximum value of the enzyme activity was reached in March equal to 170 μg of tyrosine per gram of wet weight of the helminth, and the minimum in June reaching 70 μg of tyrosine per gram of wet weight of the helminth.

Keywords—proteolytic activity, goats, trematodes.

I. INTRODUCTION
Proteolytic enzymes play an important role in the study of nutrition of some trematodes and mainly in the study of feeding tapeworms [6]. One of the important factors determining the degree of spread and intensity of invasions is the time of year and the climatic conditions of farms. In the literature, data are given on the extent of the invasion, depending on climatic conditions. The difference in invasiveness is explained by unequal conditions of keeping, the degree of contamination of keeping and feeding areas of animals. The isolation of invasive elements in their opinion is dependent on the condition of the host organism, feeding, habitat conditions and abiotic factors. All these factors affect the viability of helminthes in the external environment and the host organism [1, 2].

It is noted that the increase in the physiological activity of parasites and the mass maturation of most of them occur in the spring and summer and in a lesser degree in the autumn. In this case, the sexual activity of helminthes in a temperate climate begins 1.5-2 months before the growing season and the pasture of animals on the pasture. It should be noted that the time of the year is an important factor determining the effectiveness of diagnostics and establishing the intensity of infestations. All this is due to the biological cycle of helminthes in the host organism and in the environment, the nature of the feeding of the animal, the phenomena of latent invasion and the increase or suppression of the helminthic sexual activity in the host organism [3, 4, 5]. It should be noted that the pathogenesis of helminthiases is a complex phenomenon and has various aspects. The primary pathogenic factors include mechanical and toxic effects of helminthes on the organs and tissues of hosts. The mechanical action is carried out by various morphophysiological and endoecological features of helminthes, which is manifested by traumas, destruction and tissue rupture in the host by special structural elements of parasites (oral capsule, cones, outgrowths, etc.). The toxic effect on the host's organism turns out to be the products of the vital activity and decay of helminthes, toxins that produce in the process of habitation, as well as larvae and products of their vital activity during migration. In the opinion of the authors, in the case of moniosis of lambs, the increase in body weight is reduced by 1.8-3.0 kg, from which received wool gets less, on average, by 700 g, with low tonnage [7,9,10,11].

Proceeding from the foregoing, the purpose of our studies was to study the dynamics of enzymatic activity of trematodes in biomaterial taken from the liver of killed goats of Khizi-Khachmaz zone of Azerbaijan in different seasons of the year.

II. MATERIAL AND METHODS
The object of the study were goats from the districts of Khizi and Khachmaz. The material for the study was the liver of goats slaughtered in winter (January, February), spring (March, April) and summer periods of the year (June and July). Determination of the enzymatic activity was carried out spectrophotometrically using a Folin reagent on a Specol 1500 spectrophotometer (Analitik Jena). We have developed a modified method for the determination of enzymatic activity, using a casein
substrate, based on the determination of the rate of enzymatic substrate hydrolysis reaction under the influence of the proteolytic enzymes contained in the biomaterial under analysis.

The reaction rate corresponds to the amount of amino acids (tyrosine and tryptophan formed) that were determined spectrophotometrically with Folin reagent. This method was used to determine the studied amino acids in the free and bound state. At the same time, the amount of tyrosine and tryptophan contained in the hydrolyzate was used to determine the amount of protein converted during the enzymatic reaction, based on the protein content of 5% tyrosine and 1.5% tryptophan.

For a unit of proteolytic activity, the amount of enzyme catalyzing 30 min hydrolysis of 1 g of protein not precipitated with trichloroacetic acid was taken. In this case, 1 g was 25% of the protein taken for the enzymatic reaction.

Figure 1 shows a plot of the optical density versus the amount of protein converted during the enzymatic activity.

Figure 2 shows the data of the dependence of the optical density on the number of units of activity of proteolytic enzymes.

![Graph](image1.png)

**Table 1.**

| Proteolytic activity in μkg tyrosine |
|------------------------------------|
| Months                            |
| Winter season  | Spring season | Summer season |
| February   | 140           | April        | 110 | June   | 70    |
| March      | 170           | May          | 120 | July   | 80    |

The seasonal dependence of the proteolytic activity of helminth enzymes in goat liver homogenates (in μg tyrosine per gram wet weight of helminths)

III. THE PROTEIN CONTENT IN MG UNIT OF ACTIVITY

Proteolytic activity is characterized by the number of units of activity of the enzyme contained in the 1-gram of the biomaterial. This method makes possible to determine the enzymatic activity of the substances under study.

**The results of the study and their discussion**

Helminthes were extracted from the liver of slaughtered goats in the winter, spring and summer periods, carefully washed with 0.9% sodium chloride solution, then dried with filter paper, followed by grinding and homogenization with three volumes of 0.025N HCl at room temperature. The homogenizer was placed in an ice vessel. As a substrate, casein was used.

Proteolytic activity was determined by the method of Kunitz and Anson in the modification of Orekhovich [8].

1 ml of homogenate of worms was added to a solution of 1 ml of casein. The mixture was incubated for 1 hour in a thermostat at 37 °C, then 3 ml of a 5% solution of trichloroacetic acid was added. Samples were left for 1 hour to form a precipitate, followed by centrifugation. Further, 1 ml of a centrifuge was taken, 2 ml of 0.5 M NaOH and 0.9 ml of Folin solution were added. Previously, the Folin solution was diluted three times with distilled water. The prepared samples were left for 10 minutes before the development of a stable color.

The extinction measurements were carried out on a spectrophotometer at a wavelength of 750 nm. As controls, samples were taken into which trichloroacetic acid was added together with the filtrate. The activity of proteolytic enzymes was expressed in 1 μg of tyrosine.

The results were recalculated for 1 gram of green worm weight.

The activity of proteolytic enzymes was determined by the calibration curve. To construct a calibration curve, solutions of tyrosine containing from 1 to 100 μg of tyrosine in 1 ml were prepared.

Studying proteolytic activity in homogenates of liver tissues of goats in all experimental groups of samples revealed an increase in the quantitative indices of tyrosine in comparison with the control samples. This indicated the presence of proteolytic activity in the studied homogenates.

Quantitative data on the determination of the proteolytic activity of helminth enzymes isolated from liver tissues of goats in winter, spring and summer are given in Table 1.
According to the results of our organoleptic studies, the main changes in trematodes were detected in the liver in goats. At fascioliasis (medium invasion from 16 to 31 specimens), the liver of infected goats was increased, the capsule tense, of a dense consistency, brownish brown (40% of cases) or light brown in color.

In one animal (20%) at mono-invasion during palpation, the presence of parenchyma heterogeneity, granularity, was palpable. The liver was brown, the body consistency during palpation was uniform, without foci of compaction, the capsule was not strained. Only in one animal (20%) the color of the organ was changed, had a pronounced light brown hue. With an average degree of invasion, the liver of infected goats was light brown in color, was increased, the capsule tense, of a dense consistency.

In the infected animals, the percentage of the liver increased by 0.03-0.77%. That means, there is an increase in the organ, which is a consequence of inflammatory processes and intoxication of the animal's body and is accompanied by hyperfunction and compensatory increase in the size of organs (Fig. 3).

Subordinate to the general physiological patterns, immunity in helminthiases has its own characteristics, which depends on parasitic host relationships, physiological and ecological characteristics. There are no parasites that cause only local reactions in the host's body. The changes occurring in helminthiases in organs and tissues serve as an indicator of metabolic disturbances, the presence of dystrophic processes, allergic and immunomorphological reactions, that means, they are the response of the organism to the pathogenic action of the helminth.

E.S. Leikina [13] analyzed the domestic and foreign literature on the mechanism of immunity in helminthiases, which showed that parasites can have a double effect on the host's organism. So, on the one hand, they stimulate the immune response, as a result of which a number of phenomena of the cellular and humoral response are observed, and on the other hand - inhibit the functional and proliferative activity of cells of the lymphoid tissue, which leads to the development of secondary immune deficiencies. This contributes to a sharp change in the nature of the relationship in the host-parasite system and helps the survival of the host in the host organism [14-20].

Thus, comparing the average values of proteolytic activity in tissue homogenates of non-modems isolated from goat liver in different seasons of the year, it should be noted that their difference is significant. In conclusion, it should be noted that proteolytic activity is non-modal, in goat liver tissues reaches its maximum value in the spring season and is characterized by the highest rates in March, and the lowest in June reaching 170 and 70 μkg, respectively, in terms of μg tyrosine per gram of green worm weight.

![Proteolytic activity tyrosine in μg](Fig.3. Diagram of seasonal dependence of proteolytic activity of helminth enzymes in goat liver homogenates (in μkg of tyrosine per gram of wet weight of helminths))

At trematodes, the decrease in the quality and nutritional value of meat, especially protein, is recorded to varying degrees, which is accompanied by a decrease in calorie content by 6.7-21.9%. At the same time, the protein-to-fat ratio for mono-invasions is significantly lower than the control group. This may be due to intoxication of the animal's organism and violation of protein and fat metabolism [12].
REFERENCES

[1] Akbaev M.Sh., Vasilevich F.I. 1992. Parasitology and invasive diseases of animals. M.: Agropromizdat, pp. 37-49.

[2] Akilzhanov R.R. 1987. Association of helminthes and the simplest digestive tract of sheep of specialized farms of the North-West zone of the RSFSR and the development of rational measures of struggle. Actual problems of veterinary medicine: collection of scientific works. L.: Vet.institut. p.16.

[3] Aleshina I.M. 2000. Sheep breeding Velikobretania.Sel'skoe economy abroad.1982, 8, p.39.

[4] Abdullaev V.M., Gudkov A.Yu., Sorokina I.B.1995. Dynamics of pathogenicity of bacteria in the gastrointestinal tract of cattle with helminthiasis. Innovative methods in veterinary medicine, math. International scientific conference. Ivanovo State Agricultural Academy. Ivanovo. 2, pp. 99-101.

[5] Akilzhanov R.R. 1999., Features of clinical treatment of associative bumostomose-eimeriosis invasion in sheep. L., pp. 11-17.

[6] Dubovskaya A.Ya. 1973. Study of proteolytic activity in some species of cestodes. J. Parazitologiya, VII, 2, pp. 154-158.

[7] Terentyeva Z.H. 2012. Dynamics of allocation of helminth eggs and oocysts of coccidia from the organism of sheep and goats during associative invasions in Orenburg region. 3,pp. 102-109.

[8] Orekhovich V.N. 1968. Modern methods of isolating, characterizing and studying the properties of proteins, Medgiz, M .: p.135. http://www.dissercat.com/content/ekologo-epizooticheskie-osobennosti-moniezioza-ovets-v-regione-tsentralnogo-kavkaza-i-razrab#ixzz4oTGt2JSt

[9] Daugalieva E.H. 1991. Immune status in case of goitreosis of goats // Thesis. Transcaucasian Conference Yerevan. pp. 31-32.

[10] Daugalieva E.Kh., Filippov V.V., Kurochkina K.G. 1991. Helminthiases of cattle / Herald of agricultural science. M., pp. 32-38.

[11] Muromtsev, A.B. 2008b. The main helminthiases of ruminant animals in the Kaliningrad region: author's abstract. Diss. ... Doct. wind. Sciences: 03.00.19 / Muromtsev Alexander Borisovich. - St. Petersburg, p. 41.

[12] Muromtsev, A.B. 2008b. The main helminthiases of ruminant animals in the Kaliningrad region: author's abstract. Diss. ... Doct. wind. Sciences: 03.00.19 / Muromtsev Alexander Borisovich. - St. Petersburg, p. 41.

[13] Leikina, E.S. 1976. Immunity with helminthiases / E.S. Leykina // In: The fundamentals of general helminthology. - Moscow: Nauka, pp. 89-168.

[14] Saveliev A.A. 2005. Exchange processes and quality of meat in animals spontaneously infected with trematodes and against the background of dehelminthization Proceedings of the All-Russian Institute of Helminthology. - Moscow, 41, pp. 312-317.

[15] Kozharov A.Z. 2013. Biohelminthoses (echinococcosis, fascioliasis) of cattle of different genotypes in the Kabardino-Balkarian Republic and quality, safety of meat products: the author's abstract. Diss. ... cand. Biol. Sciences: 03.02.11 / Kjarov Alim Zabitovich. - M.,p. 24 .

[16] Kryazhev, A.L. 2012. Helminth fauna of cattle in the Vologda region. // ALKryazhev // Actual questions of veterinary biology, 4, p. 28-32.

[17] Lyapanov B.K. 2001. Helminthiases of sheep in the North Caucasus: Epizootology, measures of struggle and prevention, veterinary and sanitary assessment of the products of their slaughter: the author's abstract. Diss. Doct. Vet. Sciences: 03.00.19. Leypanov Boris Kazievich. - M., p. 55.

[18] Latypov D.G. 2007. Helminthiases of cattle in the Republic of Tatarstan. Mater. All-Russian scientific. - Pract. Conf. - Kazan, pp. 30-20.

[19] Latypov D.G. 2010. Helminthiases of cattle in the Republic of Tatarstan (epizootology, diagnostics and therapy): author's abstract. Dis. Doct. wind. Sciences: 03.02.11, M., p. 41.