

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

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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 helminthiasis 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 morpho-physiological 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.

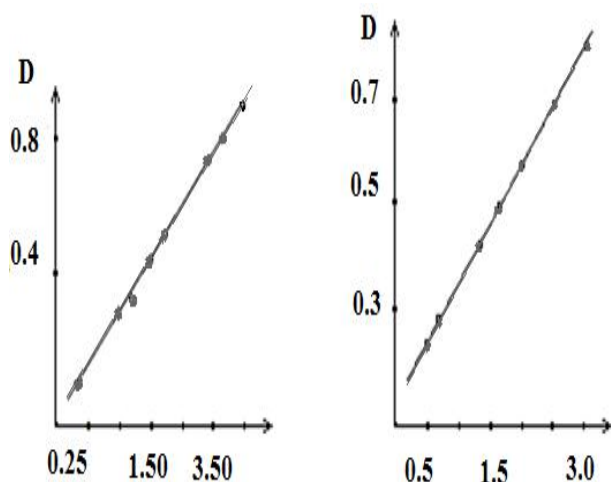


Fig.1. Dependence of the optical density of the test substance on the amount of protein converted during the enzymatic activity	Fig.2. Dependence of the optical density of the test substance on the number of units of activity of proteolytic enzymes.
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III. 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.

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)

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 monoinvasion 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) 1

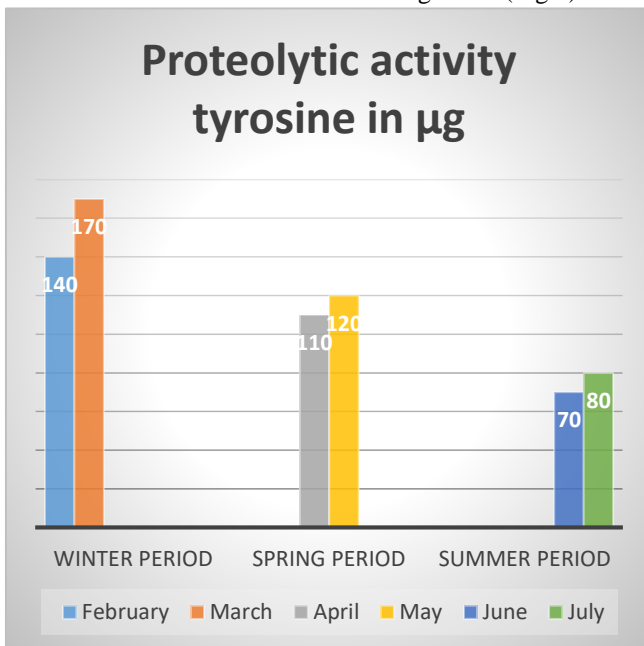


Fig.3. Diagram of seasonal dependence of proteolytic activity of helminth enzymes in goat liver homogenates (in µg of tyrosine per gram of wet weight of helminths)

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 monoinvasions 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].

Subordinate to the general physiological patterns, immunity in helminthiasis 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 helminthiasis 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 helminthiasis, 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 µg, respectively, in terms of µg tyrosine per gram of green worm weight.

IV. CONCLUSIONS

Thus, the carried out experimental studies revealed the presence of goat proteolytic activity in the homogenates of the liver studied.

Based on the obtained data, it can be stated that the season of the year has a significant effect on the enzymatic activity of trematodes in goat homogenates.

The maximum peak intensity of proteolytic activity of trematodes isolated from goat liver was detected. The maximum value of the enzyme activity was reached in spring in March equal to 170 µg of tyrosine per gram of wet weight of the helminth, and minimal in the summer season in June reaching 70 µg of tyrosine per gram of wet weight of the helminth.

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