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Dynamics of Proteolytic Activity of Trematodes in Livers of Sheeps and Cows of Guba - Gusar Zone of Azerbaijan

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Abstract— *The article presents the data of experimental studies of the proteolytic activity of trematodes in livers of sheeps and cows of Guba – Gusar zone of Azerbaijan. Determination of the enzymatic activity was carried out spectrophotometrically using a Folin reagent on a Specol 1500 spectrophotometer (Analitik Jena).*

As a result of the experimental work, maximum peaks in the intensity of proteolytic activity of nematodes isolated from liver of sheep and cows were detected. The maximum enzyme activity was reached in March, which corresponded to 180 and 140 μg of tyrosine per gram of wet weight of the helminth, and the minimum was reached in June reaching a value of 55 and 75 μg of tyrosine per gram of green weight of the helminth isolated from the liver of sheep and cows, respectively

Keywords— proteolytic activity, trematodes, sheeps, cows.

I. INTRODUCTION

Parasitic diseases represent a serious social and economic problem for the community. Multidimensional studies prove that the extent of the spread of invasions throughout the world is increasing, despite all attempts to conduct a a complex of activities for their elimination with the use of modern antiparasitic drugs [15, 16, 17, 18].

Around the world, diseases of farm animals have a negative impact on the production of livestock products. Parasitic diseases cause enormous economic damage.

In recent years, significant progress has been made in the therapy and prevention of invasive diseases. However, the damage caused to livestock by parasitosis continues to be significant.

High concentration of animals in limited areas, the use of pastures in lowland, marshy areas create optimal conditions for intensive infection of cattle with various helminthes. Often they cause associative invasive diseases in the organism of animals [7-10].

Internal parasites or 'worms' impact significantly on the production efficiency of Victorian cattle herds, causing disease, reducing growth rates and sometimes causing death. Internal parasites include tapeworms (cestodes), roundworms (nematodes) and flukes (trematodes). Liver fluke and roundworms cause the biggest problems for Victorian producers [15]. It has been established that effective control of animal helminthiasis is most effective at the knowledge of their epizootology in each climatic and geographical area with the compilation of epizootic maps [11]. It is noted that the development of epizootic process is affected by ecological components such as: conditions of pastures and water reservoirs, weather and climate especially in current pasture season; therefore it is necessary to conduct the antiparasitic treatment. Nematodes are pathogenic parasites, causing disease in the host. Usually they live in the digestive system of the host. Haemonchus contortus attaches to the wall of the abomasums in sheep and goats, feeding on the host's blood, causing aneamia. Other nematodes usurp the nutrients eaten by the host, causing weight loss [16].

One of the important factors determining the degree of spread and intensity of invasions is the season 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 detention, 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 [13, 14]

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. [2, 3, 4].

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Proteolytic enzymes play an important role in the study of nutrition of some nematodes and mainly in the study of feeding tapeworms [12].

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 sheep and cows of the Guba-Gusar zone of Azerbaijan in the winter, spring and summer seasons.

II. MATERIAL AND METHODS

The object of the study was sheep and cows of Cuba - the Hussar zone of Azerbaijan.

The material for the study was the liver of sheep and cows slaughtered in winter (January, February), spring (March, April) and summer seasona (June and July).

Determination of the enzymatic activity was carried out spectrophotometrically using a Folin reagent on a Specol 1500 spectrophotometer (Analitik Jena).

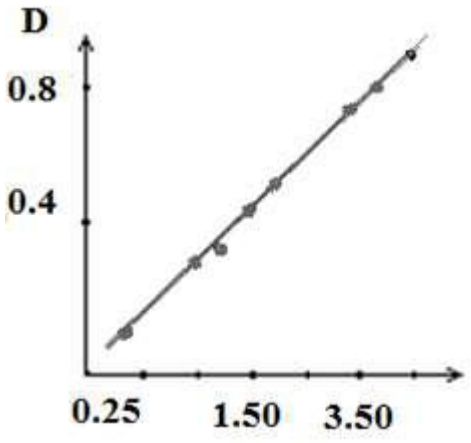
We have developed a modified method for the determination of enzymatic activity, using a casein substrate, based on the determination of the rate of enzymatic substrate hydrolysis reaction under the influence of the proteolytic enzymes contained in the biomaterial under analysis.

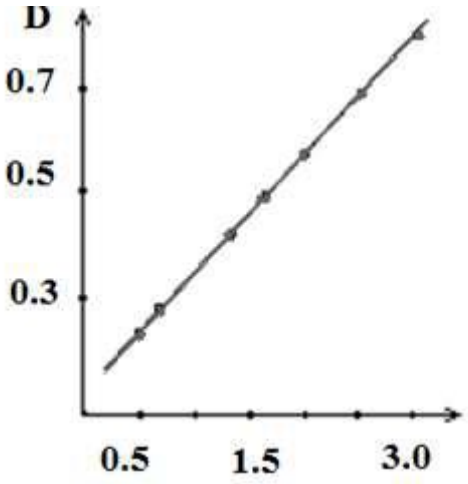
The reaction rate corresponded to the number of amino acids which were determined spectrophotometrically with the Folin reagent. This method was used to determine studied the amino acids in the free and bound state. The amount of tyrosine and tryptophan contained in the hydrolyzate was used to determine 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.





The protein content in mg unit of activity

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.

III. RESULTS AND ITS DISCUSSION

We have extracted worm helminthes of sheeps and cows after slaughter in different seasons of time: in winter, spring and summer periods. The helminthes extracted from the liver of sheep and cows were thoroughly washed with 0.9% sodium chloride solution, dried with filter paper, crushed and homogenized with three volumes of 0.025N HCl at room temperature. As a substrate, casein was used.

Proteolytic activity was determined by the method of Kunitz and Anson in the modification of Orekhovich [1].

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 370 ° C. 3 ml of a 5% solution of trichloroacetic acid was then 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. Beforehand, the Folin solution was diluted three times

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with distilled water. The prepared samples were left for 15 minutes before the development of a stable color.

The extinction measurements were carried out on a spectrophotometer at a wavelength of 750 nm. As a control, samples, in which trichloroacetic acid was added together with the filtrate, were taken . 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 a calibration curve. In order 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 sheep and cows, showed an increase in the quantitative indices of tyrosine in comparison with the control samples, which indicated the presence of enzymes in the examined samples of proteolytic activity

Data of proteolytic activity of helminth enzymes isolated from liver tissues of sheeps and cows in different seasons of the year are given in Tables 1, 2 and fig.1,2.

Table.1: Seasonal data of the proteolytic activity of helminth enzymes in sheep liver homogenates (in µkg tyrosine per gram of alive worm weight)

Proteolytic activity in µkg tyrosine					
Months					
Winter season		Spring season		Summer season	
February	115	April	90	June	55
March	140	May	110	July	70

Comparing the obtained data of seasonal dependence of proteolytic activity of enzymes in homogenates of helminths extracted from sheep liver with activity in homogenates of liver tissues of cows, it should be noted that their difference is reliable (P>0.97 and P >0.96).

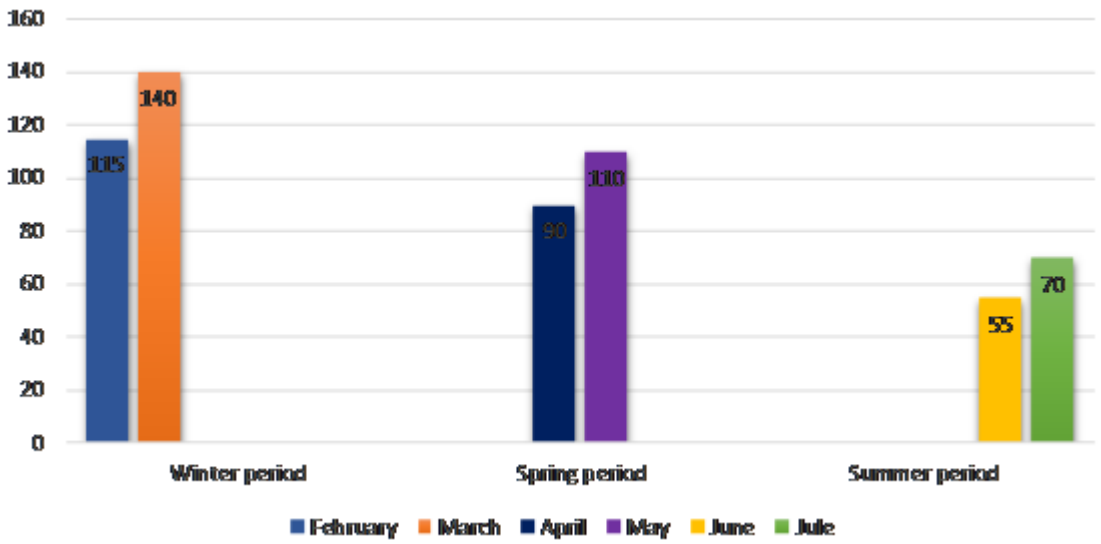

Fig.1: Diagramma of Seasonal data of the proteolytic activity of helminth enzymes in sheep liver homogenates (in µkg tyrosine per gram of alive worm weight)

Table.2: The seasonal dependence of the proteolytic activity of helminth enzymes in the liver homogenates of cows (in µkg of tyrosine per gram of wet alive of helminthes)

Proteolytic activity in µkg tyrosine					
Months					
Winter season		Spring season		Summer season	
February	150	April	120	June	75
March	180	May	140	July	90

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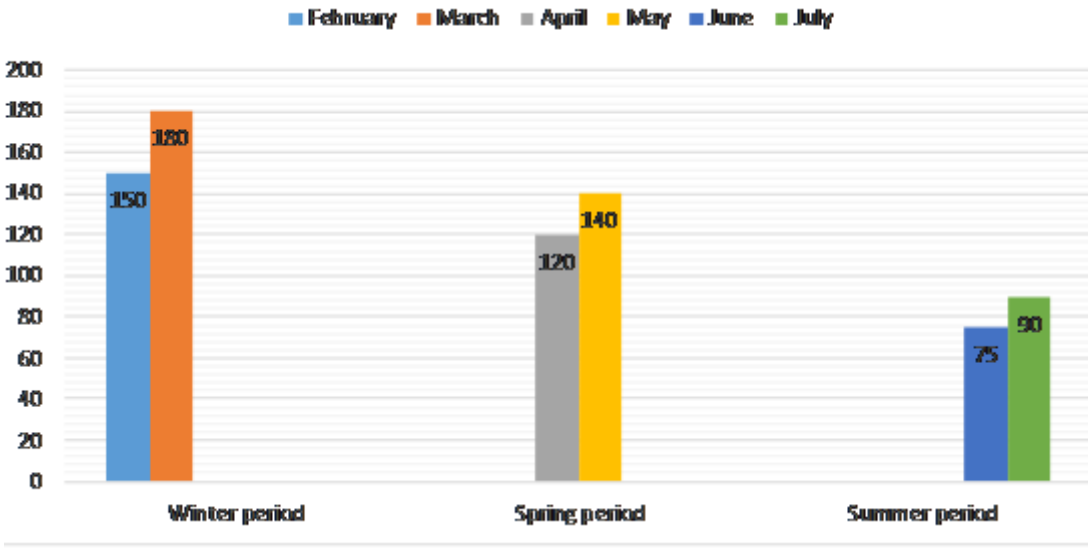

Fig.2: Diagramma of the seasonal dependence of the proteolytic activity of helminth enzymes in the liver homogenates of cows (in μkg of tyrosine per gram of alive of helminthes

Based on the data in Table 1, it can be seen that the minimum level of enzymatic activity detected in homogenates of helminths extracted from the liver of sheep is observed in June -55 μg, the maximum was observed in March - 140 μg, that is to say, the indicator increased by 2.6 times.

At the same time, comparing the activity of the enzyme in the liver homogenates of cows, it is necessary to note the tendency of decreasing enzymatic activity in March from 180 μg to 90 μg (in μg of tyrosine per gram of wet weight of helminths) while in June the indicator decreased by 2.0 times.

Thus, the dynamics of proteolytic activity of enzymes is largely influenced by the season of the year.

An analysis of the seasonal tendency of the dependence of the enzymatic activity of homogenates of parasites of the liver of sheeps and cows indicates that in farm animals a significant decrease in the activity of the enzyme is expressed by the beginning of summer.

IV. CONCLUSIONS

Thus, as a result of the carried out experimental studies, the presence of proteolytic activity in the liver samples of sheep and cows was revealed

Based on the data obtained, it follows that the season of the year influences the enzymatic activity of nematodes in livers of sheeps and cows liver. The reliability of differences in the proteolytic activity of enzymes was revealed (P>0.97 and P >0.96).

The maximum peak intensity of the proteolytic activity of nematodes isolated from liver of sheep and cows was detected in the spring in March, reaching 180 and 140 μg of tyrosine per gram of wet weight of the helminth, and minimal in the summer in June, reaching 75 and 55 μg of tyrosine per gram of wet weight of the helminth for sheeps and cows, respectively.

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