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POSSIBILITIES FOR INCREASING PROGNOSTIC VALUE OF ENZYME ASSAYS IN RAINBOW TROUT (*ONCORHYNCHUS MYKISS* WALBAUM, 1792) SERUM

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Abstract

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This investigation has the purpose of determining the cellular levels of fluctuation of serum malic dehydrogenase (MDH) of healthy trout, bred in fish farms, which have been supplied by Karst and river water sources. For the purpose of enzyme assays, blood samples have been taken from 990 healthy trout fish, bred in Karst water, with different age and sex. The limits of fluctuation of malic dehydrogenase activity in healthy rainbow trout, bred in fish farms, supplied with karst and fresh water, as well as its dependence on age, sex and season, has been established. The values of enzyme assays in healthy rainbow trout may provide a basis for monitoring over the physiology of fish intensive breeding in fish farms.

Key words: rainbow trout, malic dehydrogenase (MDH), enzyme assays, blood serum

Introduction

The enzymes are constructive components of cellular structures. They are synthesized in the cells and have a catalytic effect on the processes of metabolism. The activity of serum enzymes at normal conditions remains at a definite steady level. Maintaining this relatively steady level is determined by enzymes biosynthesis intensity, the range of irradiation in blood, the activation and inhibition processes, the speed of degradation and elimination (Amelung, 1964; Gaudet et al., 1975; McCarthy et al., 1975). The unfavorable effects, which refer to some of these processes, lead to change of the normal enzyme activity of serum. Usually, at such cellular damage, caused by the action of different agents (toxic, infectious, nutrition disturbances, etc.) cellular enzymes activity increases. These changes in enzyme activity transmit early and reliable information for the general physiological and health status of rainbow trout, bred in fish farms (Boyadjiev, 1987; Prearo, et al., 2006). We cannot indicate an enzyme, which will be specific for one organ only. However, there are enzymes, whose concentration in a given organ is exceptionally high, while in the remaining organs it is almost missing. These enzymes fulfill functions of specific indicators for the cellular damage of the given organ.

This investigation has the purpose of determining the cellular levels of fluctuation of serum malic dehydrogenase (MDH) of healthy trout, bred in fish farms,

which have been supplied by Karst and river water sources.

Malic dehydrogenase is contained in the liver, muscles, kidneys, heart, brain and other tissues of fish. There are two forms of MDH – mitohondrial and cytoplasmic, which determine the enzyme activity in the different organs. A considerable increase of its activity has been observed in the acute parenchymal damages of liver, kidneys and heart muscles of fish, in which the ferment has a predominating cytoplasmic concentration (Georgiev, 1971; Georgiev, 1973; Kolb and Kamijshnikov, 1976). The increase of its serum level is connected most of all with deterioration cell membrane permeability. According to the level of enzyme activity increase, we can prognosticate the seriousness at the very beginning of a pathologic process, still from the first light, morphologically intangible cellular damages.

The improvement of technologies in trout breeding has been accompanied by manifestation of diseases, very often of an alimentary nature. The determination of the general enzyme activity of serum malic dehydrogenase is a valuable method in paraclinical assays and the early diagnosis of some diseases (Prearo et al., 2006). Because of the small number of studies published, concerning enzyme assays of rainbow trout serum and the great importance of this information, the authors have investigated this aspect of fish biology.

Material and Methods

For the purpose of enzyme assays, blood samples have been taken from 990 healthy trout fish, bred in Karst water, with different age and sex, as follows: one-summer-old (S_0) – 1 to 11 months – 310; one-year-old (S_1) – 12 to 23 months – 254; two- years-old (S_2) – 24 to 35 months – 331; three-years-old (S_3) – 36 to 47 months – 60; four-years-old (S_4) – 48 to 59 months – 36.

The fish, the object of the enzyme assays, bred in river water have been 1345 in number and have been distributed in the following age groups: S_0 – 620; S_1 – 321; S_2 –96; and S_3 – 46.

The blood samples have been taken from the caudal blood vessel by cutting and have been preserved in test tubes treated with heparin.

The general activity of serum malic dehydrogenase has been determined by means of spectrophotometrical methods and a test of the firm "Boehringer" and spectrophotometer "Lambda-5". The data obtained have been processed variation-statistically.

Results and Discussion

From them, it is evident that the average value of malic dehydrogenase activity in the one-year old fish has been 303 UI (from 238 to 347 UI). It has been 343 UI in two-years-old, and in three-years-old female fish – 303 (from 248 to 340 UI). The highest MDH activity in blood plasma has been established in the two-years-old male fish – 409 UI, and the lowest in female fish of the same age – 227 UI.

The seasonal changes in MDH activity can be seen on Figure 1. In the spring, MDH activity in one-year old fish has been comparatively low – 283 UI, while in the summer it has reached its highest value of 347 UI. Until the autumn, the index level has slightly changed – 345 UI, and after that it has sharply decreased and reached its minimum in the winter – 238 UI.

In two-years-old male fish the changes in the spring and in the summer have been analogical to those of the one-year-old ones. After this period, the enzyme activity has sharply decreased until the autumn (320 UI), and in the winter the activity has increased.

In the two-years-old female fish the enzyme activity has been the highest in the spring. It has remained unchanged in the summer (305 UI), after which it has decreased in the autumn reaching its lowest level of 225 UI. In the winter, the activity has been higher as compared to the autumn – 272 UI. The three-years-old fish have reached a comparatively high index value in the spring – 328.3 UI. In contrast to the other age groups investigated, the MDH activity has been the lowest in the summer concerning the three-years-old fish (248 UI), and then it has increased until winter (340 UI).

Table 1
Malic dehydrogenase (MDH) activity in blood plasma of the rainbow trout bred in karst water (international units, UI)

Age, months	Gender	Season, month	Samples number	Average ± standard deviation	min - max
S14	-	Spring	6	283.33 ± 11.54	250.0 - 300.0
S 26	♂	April	6	393.33±38.01	300.0 - 490.0
S26	♀	April	6	305.0±33.79	200.0 - 390.0
S38	♀	April	6	328.33±31.29	250.0 - 390.0
S50	♀	April	6	345.0±22.04	300.0 - 390.0
S 16	-	Summer	6	346.67±10.66	300.0 - 364.0
S 28	♂	June	6	494.5±5.24	482.0 - 512.0
S 28	♀	June	6	305.33±40.4	227.0 - 482.0
S 40	♀	June	6	248.5±5.79	227.0 - 266.0
S 52	♀	June	6	373.67±19.39	344.0 - 453.0
S 8	-	Autumn	6	345.0±0.06	200.0 - 490.0
S 19	♀	September	6	233.33±33.66	100.0 - 300.0
S 19	♂	September	6	207.5±6.53	50.0 - 345.0
S 31	♂	September	6	320.0±42.14	150.0 - 390.0
S 31	♀	September	6	225.0±30.82	150.0 - 300.0
S 43	♀	September	6	296.67±35.28	200.0 - 390.0
S 11	-	Winter	6	238.0±11.34	195.0 - 266.0
S 23	♂	February	6	430.5±41.05	291.0 - 551.0
S 23	♀	February	6	295.0±32.61	227.0 - 433.0
S 35	♀	February	6	271.83±32.39	212.0 - 403.0
S 47	♀	February	6	340.17±33.20	281.0 - 467.0

The results from the blood samples assayed from fish bred in ponds supplied with river water are presented in Table 2. The MDH activity in the young one-summer-old rainbow trout fish has been at an average of 257 UI. This index has reached its highest value in one-year-old fish – 272 UI. From data in Table 2 it can be seen that the activity has decreased in the two-years-old (248) and three-years-old fish (239).

Dependence has been observed between sex and index dynamics. This can be seen from Figure 2. In the one-summer-old fish, MDH activity in the spring has been the highest one – 289 UI, in the summer it has decreased up to its minimum (223 UI), and after that it has gradually increased until winter – 262 UI.

In the one-year-old male fish, the spring level of the index has been low (191 UI) and has remained constant until summer (191 UI). In this group the minimum enzyme activity has been in the autumn (185 UI), and the maximum in the winter – 471 UI.

MDH activity in the blood of one-year-old female fish in the spring has been 225 UI. In the summer it has slightly decreased (206 UI), and in the autumn it

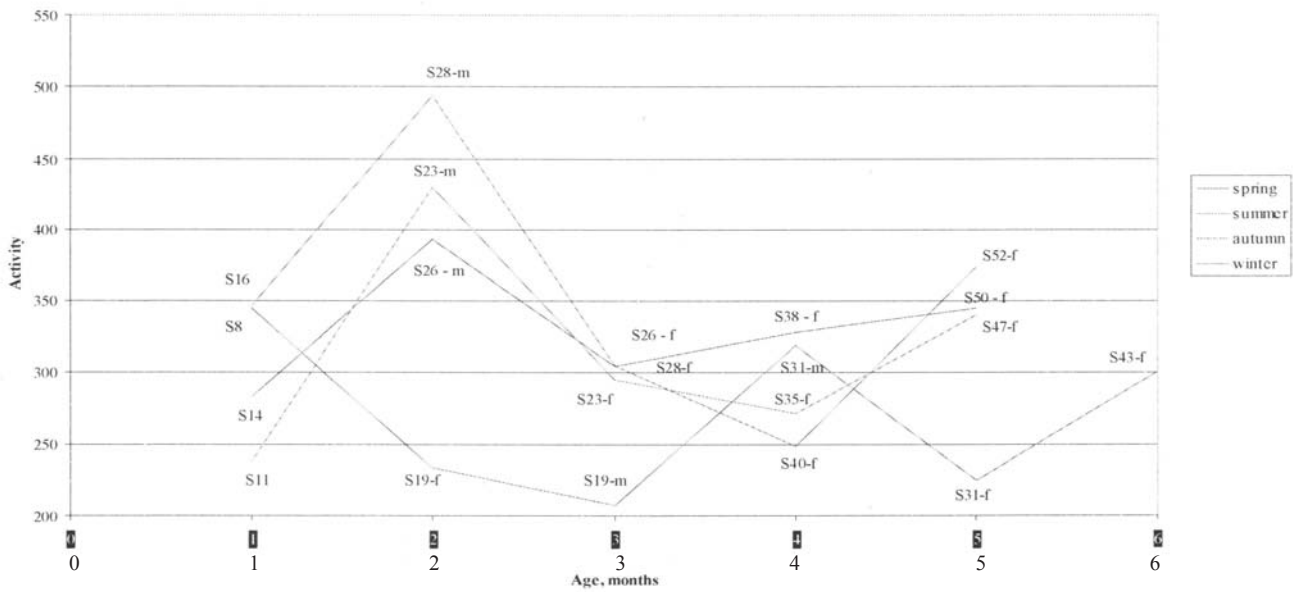

Fig. 1. Malic dehydrogenase activity in blood plasma of the rainbow trout bred in karst water (international units, UI)

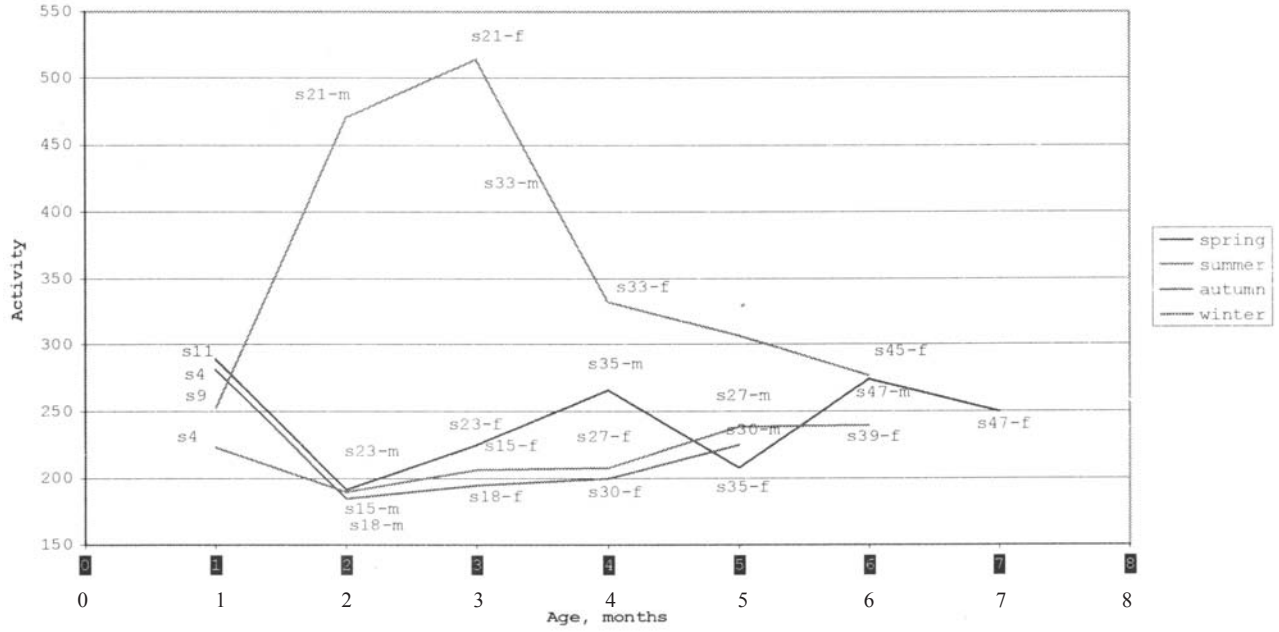

Fig. 2. Malic dehydrogenase (MDH) activity in blood plasma of the rainbow trout bred in fresh water (international units, UI)

has been the lowest – 195 UI. In the winter, it has sharply increased and has reached its highest value – 514 UI.

MDH activity in two-years-old male fish has decreased from the spring (266 UI) until the autumn (225 UI) and during the winter period it has considerably

Table 2
Malic dehydrogenase (MDH) activity in blood plasma of the rainbow trout bred in fresh water (international units, UI)

Age, months	Gender	Season, month	Samples number	Average ± standard deviation	min - max
S 11	-	Spring	6	289.17±31.25	200.0 - 390.0
S 23	♂	April	6	191.67±26.13	150.0 - 300.0
S 23	♀	April	6	225.0±12.24	200.0 - 250.0
S 35	♂	April	6	265.83±22.37	200.0 - 345.0
S 35	♀	April	6	208.33±26.13	150.9 - 300.0
S 47	♂	April	6	274.66±22.82	200.0 - 345.0
S 47	♀	April	6	250.0±24.49	200.0 - 300.0
S 4	-	Summer	6	223.33±25.55	200.0 - 340.0
S 15	♂	August	6	190.0±39.49	100.0 - 340.0
S 15	♀	August	6	206.67±44.42	100.0 - 340.0
S 27	♀	August	6	208.33±21.98	150.9 - 300.0
S 27	♂	August	6	238.33±10.73	100.0 - 390.0
S 39	♀	August	6	240.0±39.99	100.0 - 340.0
S 6	-	Autumn	6	281.67±42.93	100.0 - 390.0
S 18	♂	September	10	185.0±24.90	50.0 - 300.0
S 18	♀	September	10	195.0±19.95	100.0 - 300.0
S 30	♀	September	6	200.0±31.62	100.0 - 300.0
S 30	♂	September	6	225.0±30.82	150.0 - 300.0
S 9	-	Winter	6	252.67±12.82	222.0 - 305.0
S 21	♂	February	6	470.08±15.67	222.0 - 624.0
S 21	♀	February	6	514.0±34.98	433.0 - 644.0
S 33	♂	February	6	332.17±38.16	246.0 - 443.0
S 33	♀	February	6	306.67±19.29	246.0 - 364.0
S 45	♀	February	6	277.17±17.55	246.0 - 344.0

increased – up to 332 UI. The changes in the enzyme activity in two- and three years-old female fish have been similar. From spring until summer the index values have slightly increased, they have decreased in the autumn and reached the highest value in the winter.

Conclusions

The limits of fluctuation of malic dehydrogenase activity in healthy rainbow trout, bred in fish farms, supplied with karst and fresh water, as well as its dependence on age, sex and season, has been established.

The values of enzyme assays in healthy rainbow trout may provide a basis for monitoring over the physiology of fish intensive breeding in fish farms.

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