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Aspartate Aminotransferase Activities in Various Tissues of *Tilapia nilotica*

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Abstracts

The changes in aspartate aminotransferase activities in heart, liver, brain, intestine and spleen of *Tilapia nilotica* with various environmental conditions have been examined. The heart shows the highest activity in these organs.

The organs of *T. nilotica* show higher activity of aspartate aminotransferase in summer and lower in winter. The activity is directly related to the temperature of the cultural water.

The sample fishes have been transferred from fresh water to sea water, and the effect on their aspartate aminotransferase activity have been studied. The activity increases while the first few days after releasing the fishes into sea water due to the physiological upset, which occurs in their body, but the following days, the activity does not change significantly. A reason for which the fishes could endure the sea water-culture is explained as follows; the fishes have been cultured in a pond having 3.6–6.4‰ salinity for several years, and have got a tolerance to drastic change on osmotic pressure.

The inhibitory action of divalent metal ions on aspartate aminotransferase have been tested, Ba^{2+} and Hg^{2+} show high inhibition on the enzyme activity.

Partially purification have been studied with the liver-enzyme and the activity becomes three times stronger than the crude enzyme.

Tilapia, a tropical freshwater fish can survive at 13 to 33°C and can adapt itself to a wide variation in environmental conditions. It grows well at 23 to 30°C having an optimum at 27°C. Therefore, tilapia is a very prospective fish and a good source of food in tropical areas[1].

Tlapia can also be reared in sea water having a salinity of 30‰ and seldomly fall in disease. Most tipalia are known as mouth breeder fish and spawn several times a year. Each time it spawns about 500 to 2,000 eggs and the rate of hatching is about 90%. The growth rate of tilapia is two times higher than that of carp. It grows to 30 cm in a year and 50 cm in two years[2].

Mainly three species of tilapia are being cultured in Japan. These are *T. mossambica*, *T. nilotica* and *T. zillii*. The former one is not so much palatable and does not grow to a bigger size. So, the other two species are commonly cultured in which *T. nilotica* has become more popular because of its palatable meat and is much stable to freezing denaturation[3,4,5].

The aspartate aminotransferase (abbreviated to GOT, E.C.: 2·6·1·1) activity is closely related to the physiological conditions of the animals. The activity of this

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enzyme increases when the animal get sick[6]. The increase in activity during illness has been observed in pig[7,8], rat[9] and catfish[10]. There is no report available on tilapia. GOT is an important enzyme in the metabolism of amino acids. This study includes the relationship of GOT activity to variable environmental conditions.

Materials and Methods

Experimental fish: *Tilapia nilotica* having an approximate size of 20 to 30 cm were collected from a experimental pond of the Faculty of Fisheries, Kagoshima University. The water temperature in the pond ranged from 23–24°C in summer to 14–16°C in winter. The salinity was about 3.6‰.

Reagents: α-Ketoglutaric acid, pyridoxal-5′-phosphate and oxaloacetic acid were obtained from Nakarai Chemical Co. Ltd. L-Aspartic acid and L-glutamic acid were obtained from Wako Pure Chemical Co. Ltd.

Preparation of crude enzyme solution from various tissues of tilapia[7,8,11,12]: After killing the fish, the heart, liver and intestine were removed and washed with cold water. The excess water was blotted with filter paper and weighed individually. The sample was then finely chopped with scissors in cold water at 4°C and homogenized in chilling condition with iced water. The homogenized sample was extracted for 15 hr in cold water at 4°C. The homogenate was filtered and the filtrate was centrifuged at 2,000 G for 15 min at 0°C. The supernatant was added with equal volume of 0.01 M potassium phosphate buffer, pH 7.4. The solution was dialyzed against the buffer solution for 12–18 hr at 4°C and thus obtained the crude enzyme solution.

Enzyme assay[7,13–18]: All spectrophotometric measurements were performed with Hitachi Model 101 spectrophotometer with the cell housing regulated at 30°C. One m*l* assay solution was added with 1 m*l* pyridoxal phosphate solution (30 μg/m*l*) and incubated for 20 min at 38°C. Then 1 m*l* of aspartic acid solution (20 μM/m*l*) was added and the mixture was further incubated for 10 min at the same temperature. The reaction was initiated by addition of 0.2 m*l* of α-ketoglutaric acid solution (100 μM/m*l*) and decrease in O. D. was measured at 280 nm. All the solution were prepared in 0.05 M phosphate buffer, pH 7.4. Blank value measurment was carried out without addition of aspartic acid and α-ketoglutaric acid. The O. D. was recorded every three minutes for 30 min and plotted against time. The best straight line was drawn through the points and the value of dD/dt, the rate of change of optical density with time was determined from the slope of the curve. The enzyme activity is calculated and expressed as $Q_{(T)}$ value.

$$Q_{(T)} = \text{formed oxaloacetic acid } (\mu l)/\text{enzyme protein (mg)/hr}$$

The measurement of protein concentration of internal organs[19]: Protein of the enzyme solution was added with 6% trichloroacetic acid. The precipitated protein was centrifuged and its concentration was measured by micro-Kjeldahl

method. From the remaining protein of the sample, a series of dilution was prepared and the protein concentration was determined by measuring optical dencity at 280 nm.

Methods of separation and purification of GOT prepared from liver of tilapia[13,20]**:** The liver of tilapia was homogenized in 0.05 M phosphate buffer (pH 6.0). One volume of the homogenate was poured into four volumes of 0.05 M malate buffer containing 0.005 M ethylene dinitrylotetraacetate. The mixture was heated to 75°C for 10 min on a water bath. The addition of 2 m*l* of 0.04 M α-ketoglutaric acid at 65°C to the liver homogenate increases its activity. The mixture was continued heating for 20 min at 82°C and then cooled in iced water. Denatured protein was removed by centrifugation at 1,200 G for half an hour. Ammonium sulfate precipitation was performed by adding 50–67% ammonium sulfate to the supernatant. In the second treatment, ammonium sulfate was added at a concentration of 312 g/*l* and thus globulin was precipitated. The solution containing globulin stirred for 10 min and centrifuged at 1,200 G for 45 min. The precipitate was dissolved in 10 m*l* of 0.3 M potassium maleate buffer (pH 6) and dialyzed overnight against deionized water at 4°C. Two M potassium phosphate buffer (pH 6.8) was added to the above dialyzed enzyme solution till the final concentration became 0.02 M. Hydroxyl apatite[21] was packed in 4.5ϕ×40 cm column. The enzyme solution in 0.02 M phosphate buffer was added in the column and stirred the top of the column to absorb protein. The column was developed by 2 *l* of 0.04 M phsophate buffer (pH 6.8). The procedure was carried out below 8°C. After development for 2 hr, yellow colored enzyme protein band appeared in contamination with orange. The yellow band was eluted by 0.08 M phosphate buffer (pH 6.8). The elution curve is shown in Fig. 1. Each 5 m*l* fraction was collected in fraction collector. The fractions containing enzyme were taken together and 60–67% satu-

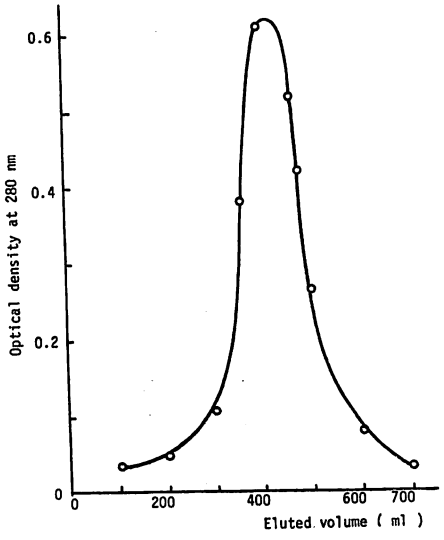

Fig. 1. Elution Pattern of Partially Purified Enzyme on a Column of Hydroxyl Apatite.

rated ammonium sulfate (0.04 M α-ketoglutarate was added to become a final concentration of about 0.025 M) was added slowly until precipitation appeared. The precipitate was removed and ammonium sulfate at a concentration of 55 g/l was added again. The precipitate was dissolved in 0.3 M maleate buffer and 0.04 M α-ketoglutarate and dialyzed for 36 hr against deionized water. By this process the partially purified enzyme was obtained.

Results

The activity of GOT in various tissues of tilapia, carp and pig: The transamination $Q_{(T)}$ values of liver, heart, intestine and spleen of tilapia are shown in Table 1. The $Q_{(T)}$ values of several organs of carp and pig were also measured and

Table 1. GOT Activities of Various Tissues of Tilapia, Carp and Pig.

Enzyme source	GOT activity (Q_T)		
	Tilapia	Carp	Pig
Heart	237.66	202.19	1568.
Liver	22.43	15.12	296.
Intestine	16.54	10.22	—
Spleen	10.35	—	—

compared with those of tilapia. In tilapia, the $Q_{(T)}$ value was found to be the highest in heart followed by liver, intestine and spleen. The value of heart was more than ten times higher than that of liver. The higher value in heart was also found in carp and pig. It was found as 1,568 in pig's heart and 202 in heart of carp. The heart of tilapia showed a $Q_{(T)}$ value of 237 and was higher than that of carp.

The effect of pH value on GOT activity: The enzyme prepared from tilapia liver was added with reagents specified for enzyme assay and adjusted at pH 5, 6, 7, 8 and 9. The $Q_{(T)}$ values at each of the above pH levels were measured and thus the optimum pH value for GOT activity was identified. The results are shown in Fig. 2. The results showed that the GOT activity increased with increase in pH value up to 8, but rapidly decreased at the pH level over 9. Therefore, it may be assumed that the optimum pH value for GOT activity lied between pH 7 and 8.

The effect of temperature on GOT activity: The enzyme samples were adjusted at pH 7.4 and incubated at various temperatures of 25, 30, 35, 40, and 45°C. The activities were measured at each temperature to determine the optimum. According to the results as presented in Fig. 3, the GOT activity value was slightly increased at 25–30°C and rapidly afterwards up to 38°C. The activity then sharply decreased with further rise in temperature. Therefore, it may be considered that the optimum temperature for GOT activity stands between 35 and 40°C.

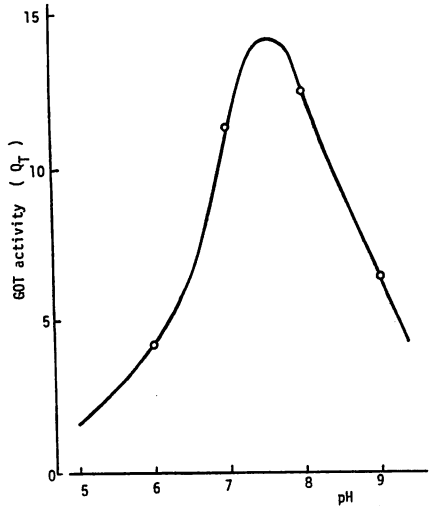

Fig. 2. Effect of pH Values on GOT Prepared from Liver of Tilapia.

The stability of crude enzyme solution prepared from tilapia: After preparing crude enzyme solution from tilapia, it was poured into small plastic container and kept frozen at −15°C. The changes in GOT activities were studied at a strage period of 7 and 15 days. A slight decrease in $Q_{(T)}$ value was observed during 15 days of storage (Fig. 4). It has been reported that the $Q_{(T)}$ value decreased slightly during the first three weeks of storage at 4°C and after that rapidly reduced to about one fifth of its original value and same was continued for storage period of about six months[14]. The trend of slight fall in GOT activity during first few days of storage was also observed with tilapia in this experiment. As the effects of longer

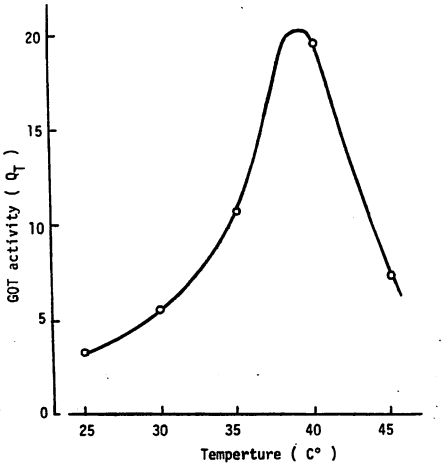

Fig. 3. Effect of Temperature on GOT Prepared from Liver of Tilapia.

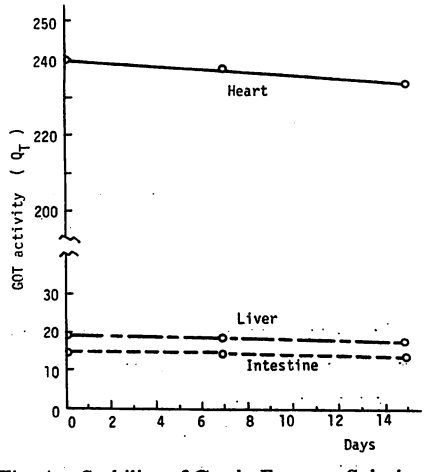

Fig. 4. Stability of Crude Enzyme Solution Stored at −15°C.

storage period on GOT were not investigated in this experiment, the trend in changes of $Q_{(T)}$ value after two weeks of storage could not be ascertained.

The effect of cultural water temperature on GOT activity in tilapia: Tilapia grows to adult in three months when cultured at optimum temperature and spawns 4–6 times a year[1]. Therefore, temperature plays an important role on the overall performance of this fish. In order to investigate the effect of various environmental temperature on GOT, the fishes were reared throughout an year at a seasonal temperature range of 13–24°C. The GOT activity was measured once in February, May, June, July, August, September, October and November. The results are recorded in Fig. 5. Higher activity was obtained in each of heart, liver and intestine during July through October when the water temperature was above 20°C. The highest activity in heart was recorded in September when the temperature of the water was 24°C and in liver and intestine in October when the water temperature was 22°C. The highest GOT activity in heart at maximum temperature (24°C) arises the possibility that the heart is very sensitive to change in water temperature. On the other hand, the liver and intestine seem less sensitive to change in water temperature as their GOT activities are not arrested when the temperature fall in October.

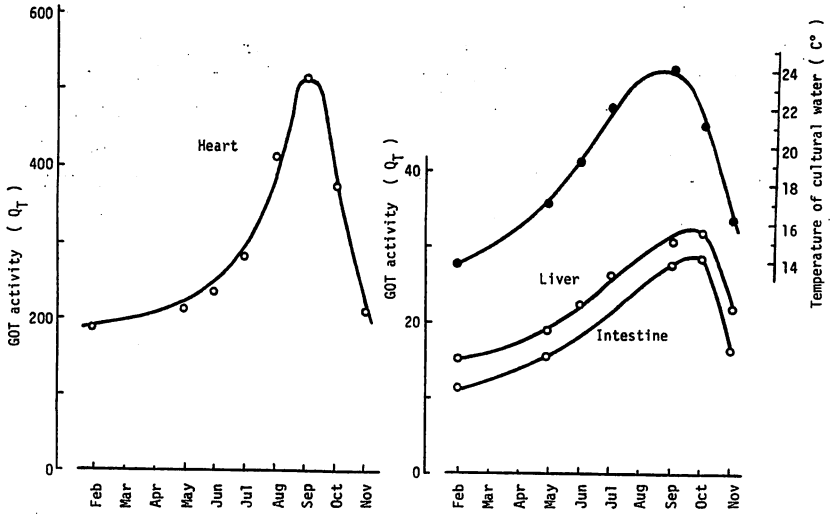

Fig. 5. Monthly Variation of GOT Activities in Various Tissues of Tilapia.

The weak activity in each of the samples were recorded in February when the water temperature went down to 14°C. The activities in winter were found to be reduced to half of those recorded in summer. Therefore, it can be said that the GOT activity of tilapia is directly related to the temperature of the water in which the fish is reared and hence it is decreased with the decrease and increased with the increase in water temperature. Tilapia are less tolerant to cold water and do not feed well during

winter and its movement slow down. It is widely tolerant to high water temperature. The optimum temperature for tilapia has been reported to be 27°C[1)] and it has been said that tilapia cultured at optimum water temperature showed higher GOT activity.

The changes in GOT activity in tilapia reared in sea water: Most tilapia species are tolerant to various salinities. It can grow well at a salinity of 30‰. In this experiment, telapia were transferred from fresh water to sea water and the effects of salinity on GOT activity were examined. The temperature of sea water was 16°C and the salinity was 32–33‰. The feeding trail was continued for 0, 5, 10, 22 and 30 days for five groups. All tilapia transferred in sea water died within 31–35 days. The changes in GOT activity in tilapia reared in sea water with respect to rearing times are presented in Fig. 6. In all the dietary groups, the activities of GOT in all

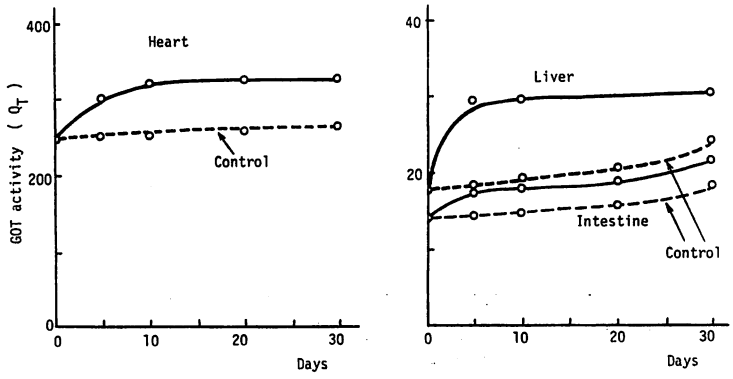

Fig. 6. Variation of GOT Activities of Tilapia Cultured in Sea Water.

heart, liver and intestine were higher in fishes reared in sea water compared to those reared in fresh water. The activity increased remarkably in all the organs within five days after the fish was transferred to the sea water. After five days, this increase occurred at a lower rate. The GOT activity was expected to have been went down since the feed intake of the fish immediately after transferring into the sea water was considerably reduced. On the contrary, the activity went up. It has been reported that the GOT activity is closely related to the physiological status of the animals[12)] and the activity of enzyme in serum increases when the animal fall in illness[22)]. Therefore, GOT value is effective in finding disease. In this experiment, tilapia kept in sea water died completely over one month rearing. The blood color in sea water reared fish was less deep than in fresh water reared fish. This indicate that the physiological conditions of the fish were changed to the worse after the fish were transferred to the sea water and eventually the GOT was affected.

The inhibitory effects of metal ions and amino oxyacetate on the activity of GOT in tilapia: Generally, the enzyme reactions are influenced by metal ions. The reactions of crude enzyme upon addition of inhibitors were tested in this studies. The crude enzyme solution prepared from the heart of tilapia was used. The incu-

bation temperature was 38°C. The enzyme reaction was initiated by addition of following reagents; α-ketoglutaric acid (0.3 M)=0.2 m*l*, L-aspartic acid (0.24 M) =1.0 m*l*, pyridoxal phosphate=1.0 m*l*, crude enzyme solution=1.0 m*l*, metal ions (33 mM)=0.1 m*l*.

Three different molarities of amino oxyacetate (23.8 mM, 1.48 mM, 0.74 mM)

Table 2. Inhibitory Effects of Metal Ions and Amino Oxyacetate on GOT Activity.

Metal ions or Amino oxyacetate	GOT activity (Q_T)	Activity compare with control (%)
Na^+ 33 mM	223.69	92.5
Sr^{2+} 33 mM	186.14	77.0
Ba^{2+} 33 mM	99.67	41.2
Hg^{2+} 33 mM	107.35	44.4
Amino oxyacetate		
0.74 mM	109.46	45.3
1.48 mM	53.15	22.0
Control	241.84	100.0

were tested. The metal ions tested were Na^+, Hg^{2+}, Sr^{2+} and Ba^{2+}. The results of the effects of inhibitors are shown in Table 2. Mainly, the divalent metal ions showed inhibitory effects. The effect of Na^+ was tested to ascertain whether it had accelerated activity when the fish were reared in sea water. The inhibitory effects were

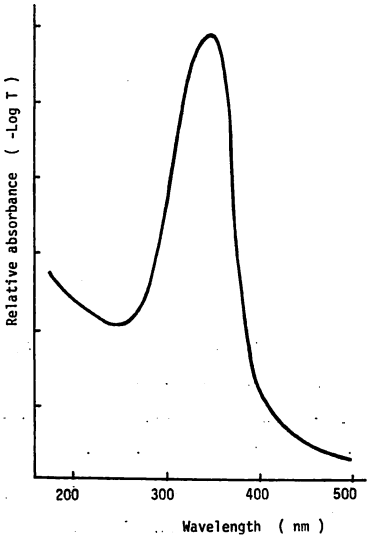

Fig. 7. Absorption Spectrum of Partially Purified GOT in Liver of Tilapia.

found to be in the order of 7% for Na^+, 60% for Hg^{2+} and Ba^{2+} and 23% for Sr^{2+}. In case of amino oxyacetate, the inhibitory effect was 55% at 0.74 mM solution and 80% at 1.48 mM solution. Thus it can be assumed that the inhibition would have been about 100% at over 3 mM solution of amino oxyacetate.

Separation and purification of GOT prepared from liver of tilapia: The separation and purification of heart enzyme could not have been tried due to non-availability of enough sample. The heart from a fish of 20 cm body length weight only about 0.5 g. Therefore, the liver of tilapia whose weight was 1–2 g on a same size fish was used in the study of separation and purification of GOT. The procedure was carried out on 81.5 g of liver homogenate similarly as already described. The absorption spectrum of partially purified enzyme is graphically presented in Fig. 7.

Discussion

The results presented in Table 1 indicate that the quantitative GOT activity in tilapia significantly differs in various tissues. The trend in variation in different tissues in tilapia is identical to that in carp. In both fishes, the heart shows the highest activity. The liver shows intermediate and the intestine shows the lowest activity. The activities in liver and intestine are far less than the activity in heart. Similar results are also obtained in pig whose GOT activity in heart is measured as 1,568 and in liver as 131. The activities in heart and liver of tilapia is found to be 237 and 22, respectively. These values of tilapia are slightly higher than the corresponding values of carp. Comparatively higher activities in tilapia than in carp is explainable in its higher rate of functioning of the body.

The optimum pH for GOT activity in tilapia lies between pH 7 and 8 and optimum temperature between 35 and 40°C. These results are similar to report on pure enzyme[14]. The author found optimum pH as 7.2–8.0 and optimum temperature as 38–40°C for the activity of GOT of tilapia.

The fish had been reared in well-water having a salinity of 3.6–6.4‰. The temperature of the water had been kept at 14°C in winter. When the cultural water was changed well water to the other fresh water in winter at temperatures below 10°C, all fish died although its tolerancy to extreme environmental conditions is excellent. It indicates that tilapia is less tolerant to cold water temperature while its tolerancy to high temperature is very wide. If the fish in this experiment were reared at optimum temperature, its growth and activity would have been excellent. The activity of GOT in tilapia decreases with the decrease in water temperature.

Any changes in natural habitat of tilapia have profound effects on GOT. This is reflected in higher GOT value in fish reared in sea water. When the fish release in sea water, physiological upset occurs and thus affects the GOT as it is directly related to the physiological conditions of the fish[6,22]. Although tilapia can live under 30‰ salinity, such salinity is not suitable for normal growth[2].

The crude enzyme solution prepared from tilapia remains almost stable for about

15 days at −15°C. The activity of GOT undergo a little change within three weeks of storage at 4°C and after that is susceptible to rapid changes[14]. However, the effect of long time storage on GOT was not tested in this experiment.

Divalent metal ions actively inhibit GOT activity[14]. Similar results are also obtained in this study with divalent metal ions such as Ba^{2+}, Hg^{2+} and Sr^{2+}. The rate of inhibition is higher in Ba^{2+} and Hg^{2+}. It is also reported that the inhibitory effect of 0.01 M chloromercuric benzoate as 49% and aryl arsine as 20%[14]. But, the activity recovered completely with the addition of glutathione. Therefore, the –SH group seems to play an important role in transamination function.

Tilapia can withstand adverse environmental conditions and can live under high densities without having any deleterious effects on its growth and reproduction. It seldomly fall in disease if it is cultured in suitable conditions. The flesh is palatable and is not susceptible to quick freezing denaturation[3,4,5]. Presently, the developing countries are facing a serious problem of protein deficiency. Tilapia can be a very important source of protein in those areas if extensively cultured and efficiently utilized.

Literature shows no previous information on GOT activity in tilapia. This study is a preliminary work on the activities of GOT in tilapia in relation to the variable environmental conditions. The nature and activity of GOT in heart could not have been studied in detail owing to the availability of lesser quantities of sample. If substantial amount of sample would be obtained, an elaborate study on the properties of GOT of heart have been made.

Summary

The enzyme involve in the metabolism of proteins and amino acids in fishes is an important field of research. But very few studies seem to have been conducted on enzyme activities in tilapia. The GOT activities in heart, liver, intestine and spleen of *Tilapia nilotica* have been studied in this paper. The changes in activities in these organs with variable environmental conditions have also been examined. The results of GOT activities in various tissues of tilapia show the following points:

1. In *T. nilotica*, the heart shows the highest GOT activity follows in decreasing order by liver, intestine and spleen. The activity in heart is more than ten times higher than the activities in other tissues. The pig and carp also show the same order in their activities of heart, liver and intestine.

2. A pH value of 7–8 and a temperature of 35–40°C is the optimum for the GOT activity of the crude enzyme prepared from *T. nilotica*. The activity goes down if the pH or the temperature exceeds the optimum.

3. The GOT shows higher activity in summer and lower in winter. The activity increases with the rise and decreases with the fall in water temperature. Therefore, GOT activity is directly related to the temperature of the water for culturing fish.

4. The fish have been transferred from fresh water (3.6‰ salinity) to sea water

(32–33‰ salinity) and the effects on GOT activity have been studied. GOT activity increases during the first five days after releasing the fish into sea water due to the physiological upset occurs in the fish body. After five days, the activity does not change significantly as the fish getting acclimatized to the new environment.

5. Among divalent metal ions, Ba^{2+} and Hg^{2+} at 33 mM show an inhibitory action to the extent of 60% and Sr^{2+} to the extent of 23%. The inhibition of Na^{+} is much less to and is only about 7%. In case of amino oxyacetate, the rate of inhibition is 5% at 0.74 mM and 80% at 1.48 mM solution.

6. The separation and purification of GOT have been studied with liver. The heart sample was too inadequate to be purified. Thus only the liver sample has been used for purification. After purifying liver enzyme, the activity becomes three times stronger.

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