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The effects of starvation on the concetration of total and L-ascorbic acid in the organs of juvenile sea bream (*Sparus aurata* L.)

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Juvenile gilthead sea bream (Sparus aurata, L.) *were kept under starvation for 98 days. Starvation resulted in growth inhibition, and significant decline in weight, condition coefficient and hepatosomatic index, which were also due to the time of sampling. Brain and liver total ascorbic acid content decreased with time whereas kidney content increased. Gill total ascorbic acid significantly increased on the 67th day of the experiment. Significant changes in L-asorbic acid content occurred in the liver and gills, whereas there were no significant changes in the brain, kidney and white muscle.*

The principal component anlysis (PCA) revealed that the most part of variance accounts for the changes in the brain, liver and white muscle total and L-asorbic acid levels which coincide in time and are negatively correlated with the gill and kidney total and L-asorbic acid levels. It has also been shown by the method of principal components that starvation appears to affect ascorbic acid content in juvenile gilthead sea bream from 26th day on.

INTRODUCTION

Most teleosts are unable to synthesize sufficient ascorbic acid for their daily metabolic needs. So they should be fed diets containing this vitamin. Inadequate dietary intake leads to anorexia, behavioural abnormalities (HILTON *et al.*, 1978; MAHAJAN and AGRAWAL, 1980a, b), retarded growth (LIM and LOVELL, 1978), slow wound healing because of diminished collagen synthesis (HALVER, 1972), lordosis and scoliosis at chronic ascorbic acid deficiency (HILTON *et al.*, 1978; MURAI *et al.*, 1978 after MAHAJAN and AGRAWAL, 1980a). Symptoms of ascorbic acid deficiency may become manifest during exposure to some environmental factors. So the changes of temperature, salinity, exposure to pollution by oils, heavy metals and to stress (EDDY, 1981) influence a depletion of ascorbic acid content in fish (THOMAS *et al.*, 1982; THOMAS, 1984; THOMAS *et al.*, 1985; THOMAS, 1987). Vitamin C deficiency occurs in starved fish which consume their body reserves. The aim of the present study was to establish the time upon which starvation begins to influence total and L-ascorbic acid concentrations in juvenile gilthead sea bream (*Sparus aurata* L.) organs.

MATERIALS AND METHODS

The gilthead sea bream (*Sparus aurata* Linn. 1758) specimens, seven months old, were obtained by induced spawing in the hatchery of the Institute of Oceanography and Fisheries, Split (KATAVIĆ, 1984).

The experiment lasted 98 days. The tank of 100 l volume with continuous water flow and aeration and natural photoperiod was used. Fourteen fish were kept under starvation at ambient sea water temperature range from maximum 24.6 °C to minimum 18.2 °C.

Total lenght and weight were measured during the experiment, condition coefficient was calculated after the formula $CF=100xW/L^3$ (where W=fish weight and L=fish lenght), as well as hepatosomatic index which expressing the liver weight to body weight relationship.

Fish sampling for ascorbic acid concentrations in the brain, gills, liver, white muscle and kidney was performed at the beginning of the experiment and on 26th, 67th and 98th days of fish starvation. Sampled fish were immediately frozen to prevent ascorbic acid loss from the tissues.

Frozen tissue was excised from fish, weighed and homogenized in ice-cold 0.25 M perchloric acid ($HClO_4$). Homogenate was centrifuged at 5000 g for 30 min in cooled centrifuge (0°C) and resulting supernatant was assayed for the total ascorbic acid = (dehydroascorbic acid + L-ascorbic acid) and L-ascorbic acid by dinitrophenylhydrazin method of THOMAS *et al.*, (1982) with the sample and reagent volume modification for an assay in the 1 cm cuvette.

The single analysis of variance (SOKAL and ROHLF, 1969) and principal component analysis of previously standardized data (HARMAN, 1976) were employes for statistical evaluation of the experimental results.

RESULTS

Total and L-ascorbic acid levels were highest in the brain and kidney followed by liver, gills and white muscle at the beginning of the experiment (Table 1). The relationship between L-ascorbic acid and total ascorbic acid showed that most of ascorbic acid occurred in reduced form (Table 1).

The analysis of variance (ANOVA) showed no significant differences in fish length.

Table 1. Concentrations ($\mu g\ g^{-1}$ of tissue wet weight) of total (TAA) and L-ascorbic acid (LAA) in different gilthead sea bream organs at the begining of the experiment (SE-standard error)

Organs	UAA ($\bar{x}$ SE)	LAA ($\bar{x}$ SE)	$\frac{LAA}{TAA}$ x 100
Brain	297.8 15.5	193.4 23.0	64.8
Kidney	171.5 19.5	163.0 22.5	95.0
Liver	82.4 6.1	52.2 6.8	63.3
Gills	75.3 8.0	56.0 8.4	61.1
White muscle	27.0 3.1	22.5 1.7	83.3

However, the weight differred significantly, the differences being due not only to starvation but to fish sampling. So mean fish weight of the sample on the day 67 was significantly greater than that on the days 26 and 98 of starvation (which may affect the results if only ANOVA is applied). Condition coefficient is a better indication of starvation, since its changes with time (decrease with the assumed same fish length) show that fish lost weight due to starvation. The highest condition coefficient, 1.59, was recorded at the beginning of the experiment to fall to 1.29 at the end of 98 days of starvation (ANOVA P<0.05). Significant difference in hepatosomatic index with time was also recorded (ANOVA P<0.01) with the highest value on the 67th day of the experiment (Fig. 1).

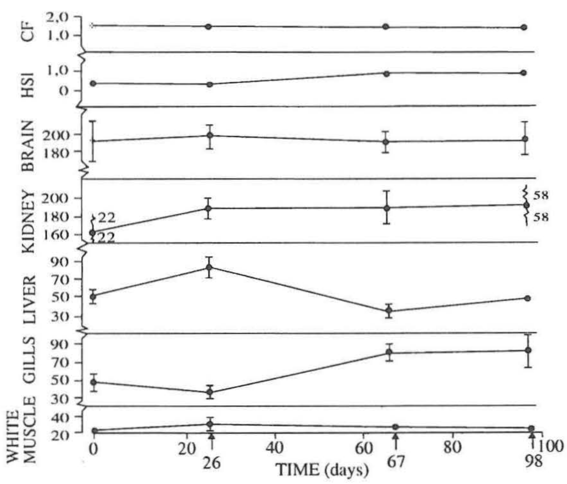

Fig. 1. Fluctutations in condition coefficient, hepatosomatic index and L-ascorbic acid content ($\mu g\ g^{-1}$ wet tissue weight) in the starved gilthead sea bream organs

Fig. 1 shows the changes in L-ascorbic acid levels in different organs and white muscle of starved juvenile gilthead sea bream. It has been shown that 98 days starvation appears not to influence significant changes in L-ascorbic acid content in the brain, kidney and white muscle (ANOVA $P>0.05$). Liver concentrations were significantly higher ($P<0.05$) on day 26 than on other sampling days. Gill levels were equal or significantly higher on days 67 and 98 ($P>0.05$) than those at the beginning and 26th day of the experiment.

Fig. 2 depicts fluctuations in total ascorbic acid content in starved juvenile gilthead sea bream. Brain reserves gradually declined with time (ANOVA $P<0.05$) and kidney reserves increase. Liver levels were significantly higher at the beginning of the experiment and on day 26 than during later samplings. Gill content significantly increased on the 67th day of starvation (ANOVA $P<0.05$). White muscle total ascorbic acid levels were unaltered for the duration of the experiment.

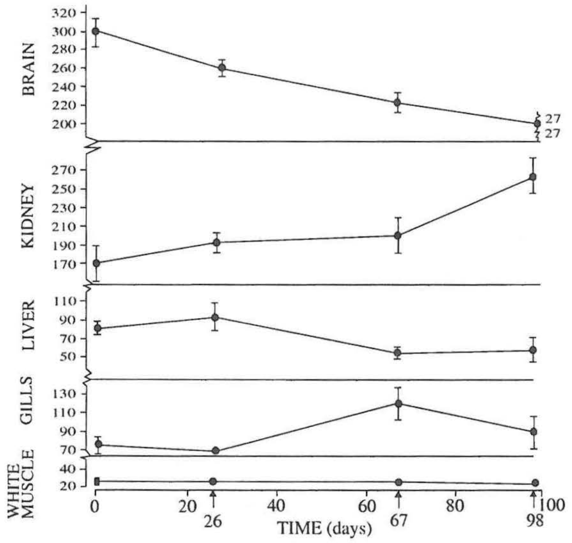

Fig. 2. Fluctutations in total ascorbic acid content (μg g^{-1} of wet tissue weight) in different organs of starved gilthead sea bream

Every fish in the experiment had characteristic content of total and L-ascorbic acid in its organs. These chemical constituents are affected by different environmental factors (THOMAS, 1984; THOMAS *et al.*, 1985), but also by individual differences between fish in the experiment. Therefore, statistical analysis of principal components (PCA) was applied for total and L-ascorbic acid concentrations. This method is very suitable since it reduces the data on a number of variables to a smaller number of principal components, the main property of which is their ortogonality (non correlation).

The decomposition of data on L-ascorbic acid and total ascorbic acid levels in juvenile gilthead sea bream to principal components (PC) gave 45.8% of the total variance for the first principal component, 15.1% for the second principal component, 12.2% for the third. The first principal component alone had weight which were statistically significant, and it extracts about 46% of the total variance. Variance of the remaining principal components is presumably due to wrong sampling and methods of ascorbic acid analysis. Table 2 presents the weight factors of only the first principal component which gave statistically significant correlations between amplitudes of principal components and original variable.

The first principal component (Table 2) of fish in the experiment makes a distinction between fish with: "high brain L-ascorbic acid concentration - high liver total ascorbic acid con-

Table 2. Weight factors of L-ascorbic acid (LAA) and total ascorbic acid (UAA) with statistically significant correlations with the first and second principal components

Variable	Weight factor of the first principal component
LAA Brian	0.32
LAA Kidney	–
LAA Liver	–0.36
LAA Gills	0.33
LAA White muscle	–
UAA Brian	0.36
UAA Kidney	–0.34
UAA Liver	0.37
UAA Gills	–0.31
UAA White muscle	0.29
Eigenvalue	4.58
Explained variance (%)	45.8

centration, low kidney total ascorbic acid concentration - high white muscle total ascorbic acid concentration" and fish with quite the opposite situation with respect to total and L-ascorbic acid concentrations.

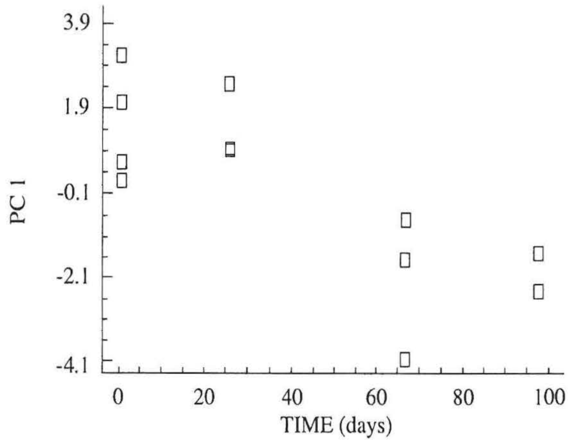

Fig. 3. Amplitude of the first principal component in relation to sampling time of juvenile gilthead sea bream

The first principal component is negatively correlated with the sampling time (r=-0.79) and hepatosomatic index (r=-0.77) and poorly positively correlated with the condition coefficient (r=0.62). Since the condition coefficient and hepatosomatic index are also the functions of time (starvation), the first principal component results from starvation and describes the relationships and concentrations of L-ascorbic acid and total ascorbic acid between different organs of juvenile gilthead sea bream. The amplitude of the first principal component as a function of sampling time of each fish specimen is shown in Fig. 3. The first principal component shows clear contrast on day 67 and 98 to the L-ascorbic acid and total ascorbic acid levels at the beginning of the experiment and on day 26. On days 67 and 98 of starvation fish had "low brain L-ascorbic acid and total ascorbic acid concentrations - low liver L-ascorbic acid and total ascorbic acid concentrations - high kidney total ascorbic acid concentration - low white muscle total ascorbic acid concentration". Starvation influenced the L-ascorbic acid and total ascorbic acid content in juvenile gilthead sea bream not earlier than from the 26th day on.

DISCUSSION

It has been established that ascorbic acid levels in different organs are a good indication of its importance in tissue metabolism. At the beginning of the present experiment the highest concentrations of total and L-ascorbic acid were recorded from the brain and kidney, followed by liver, gills and white muscle (Table 1). Similar succession of L-ascorbic acid and total ascorbic acid concentrations in organs was reported for some other fish species (IKEDA *et al.*, 1963; SOLIMAN *et al.*, 1986; AGRAWAL and MAHAJAN, 1980; HILTON *et al.*, 1979). Ascorbic acid in the fish organs occurs mainly in the reduced form, which was obtained for other fishes as well (IKEDA et al., 1963; HALVER *et al.*, 1975).

Starved juvenile gilthead sea bream lost weight and stopped to grow. No mortality was observed for the duration of the experiment (98 days), since presumably fish try to adapt to starvation conditions by reducing their metabolism. This process is enhanced by temperature decrease from the initial 24.6 to 18.2 °C. It has been shown that standard metabolism is reduced if fish are under starvation. So PHILLIPS *et al.* (1953 after BROWN, 1957) revealed that brook trout had lower metabolism after 63 h starvation than after 15 h starvation. During the first seven days metabolism is reduced by 50% in some fishes, whereas at the end of 300 days it makes up not more than 15-20% of the initial value, that is metabolism reduction appears to be logarithmic (FRY, 1957, after BROWN, 1957). Hepatospomatic index increases with time, and its rise may be accounted for by the fact that fish body weight declines relatively more rapidly than the weight of the liver. SOLIMAN *et al.* (1986) showed the juvenile tilapias *(Oreochromis niloticus)* fed vitamin C free diet exhibited significantly depressed hepatosomatic index compared with fish fed diets containing the vitamin C and its analogues.

Ascorbic acid contents in teleosts may be due to diverse effects of environmental factors (THOMAS, 1984; THOMAS, *et al.*, 1985) and different diets (MAHAJAN and AGRAWAL, 1980a; SOLIMAN *et al.*, 1986). The analysis of principal components showed that the counterphase characteristics of L-ascorbic acid in the brain, liver and white muscles make up the bulk of the total variance on the one hand and those of gill L-ascorbic acid and total ascorbic acid and brain total ascorbic acid on the other (Table 2). The first principal component is in negative correlation with time (Fig. 3) which means that a part of the brain and liver L-ascorbic acid declines with time. THOMAS (1984) reported that mullet *(Mugil cephalus)* liver ascorbic acid content was unaffected by environmental factors, that is salinity and temperature. Ascorbic acid concentrations fell in mullet brains during the exposure to elevatted water temperatures (THOMAS, 1984; THOMAS *et al.*, 1985). Hepatic ascorbic acid concentrations were maximum during the summer months whereas later declined (THOMAS *et al.*, 1985). In the present experiment water temperature was gradually decreased with time. Therefore, the ascorbic acid levels decline in the brain, liver, and white muscle of gilthead sea bream, described by the first principal component, is very likely due to starvation if compared to the earlier reports (THOMAS, 1984; THOMAS *et al.*, 1985).

High brain total and L-ascorbic acid concentrations may be accounted for by the fact that energy needed for the processes, into which ascorbic acid is involved during starvation, brain obtains through blood by acetoacetate and 3-hydroxibutyrate oxidation. Under normal conditions blood acetoacetate and 3-hydroxibutyrate content is very low. However, under starvation fats are extremely mobilized and production of excess ketone bodies occurs (KARLSON, 1988). Some authors like GOLOVATS'KII *et al.* (1963, after JONAS and BILINSKI, 1965) reported ketone bodies concentrations in carp to be similar to those in warm blood animals. Increased concentration of ketone bodies may be recorded during spawning, when fish poorly feed. Their occurrence may also be due to some hormonal changes rather than to starvation.

Euryhaline teleosts, among which the gilthead sea bream also count, are able to adapt to environmental salinity changes by different morphological and biochemical changes in cell membrane. Cell membrane responds immediately to salinity change by changing its permeability. Gill ascorbic acid content also responds rapidly to changes in the external salt concentration and there appears to be an inverse relationship between these two parameters (THOMAS, 1984).

The analysis of principal components showed that a part of total variance of gill L-ascorbic acid and total ascorbic acid concentrations may be accounted for by the positive correlation with time that is the concentrations were lower at the beginning and higher at the end of the experiment. If there is no ascorbic acid synthesis then this increase with time is presumably due to the mobilization of liver ascorbic acid reserves which was pointed to by counterphase characteristics of weight factors of principal factors between the liver and gills (Table 2).

PHILLIPS *et al.* (1953, after LOVE, 1970) reported that vitamin C concentrations declined much slower in starved *Salvelinus fontinalis* than in fish fed vitamin C deficient or vitamin C free diets. Rate of liver metabolic processes are affected, among the other environmental variables, by sea water temperature, as well. It has been found that under laboratory conditions fish do not feed well at lower water temperature. Therefore, decreased hepatic ascorbic acid reserves in fish may be due to the consumption of these reserves for normal metabolic processes. After THOMAS (1984), water temperature causes no persistent alternations in the ascorbic acid status of fish.

Apart from the brain, kidney ascorbic acid levels are very high (HALVER *et al.*, 1975). It appears that normal kidney function under starvation requires high and steady L-ascorbic acid concentrations which may be provided only by elevated total ascorbic acid levels. Therefore, the variance of kidney L-ascorbic acid is almost negligible in the first principal component, whereas a significant part of the variance of total ascorbic acid had counterphase characteristics with total liver and white muscle ascorbic acid (Table 2).

CONCLUSIONS

There were no statistically significant differences in fish length, whereas condition coefficient and hepatosomatic index fell significantly due to starvation and time of sampling.

The 98 day starvation causes no significant changes in the brain, liver and white muscle L-ascorbic acid content. Liver total and L-ascorbic acid concentrations were significantly higher on day 26 of starvation than on days 67 and 98. Gill L-ascorbic acid levels were significantly higher at the beginning and on 26th day of starvation, whereas total ascorbic acid increased on day 67. Brain total ascorbic acid content declined with time and that in the kidney increased.

The analysis of principal components showed that most of the total variance accounts for the changes in the brain, liver and white muscle total and L-ascorbic acid contents, coinciding in time, which are in negative correlation with total and L-ascorbic acid concentrations in the gills and kidney. Principal components method also showed that starvation affected ascorbic acid concentrations in juvenile gilthead sea bream at the end of 26 days of starvation.

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Utjecaj gladovanja na koncentraciju ukupne i l-askorbinske kiseline u organima mlađi komarče (*Sparus aurata* L.)

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KRATKI SADRŽAJ

Mlađ komarče (*Sparus aurata,* L.) je bila u gladovanju 98 dana. Rezultati su pokazali da nije bilo porasta duljine ribe, dok su težina, indeks kondicije i hepatosomaski indeks značajno padali, kao posljedica gladovanja, ali i uzorkovanja ribe. Koncentracija ukupne askorbinske kiseline u mozgu i jetri padala je vremenom, dok je u bubregu rasla. U škrgama dolazi do značajnog porasta ukupne askorbinske kiseline 67. dana eksperimenta. Značajne promjene koncentracije L-askorbinske kiseline izazvane gladovanjem, zapažene su u jetri i škrgama, dok u mozgu, bubregu i bijelom mišiću nema značajnih promjena.

Analizom glavnih komponenti (PCA) pokazano je da najveći dio ukupne varijance objašnjava istovremene promjene ukupne i L-askorbinske kiseline u mozgu, jetri i bijelom mišiću koje su negativno korelirane s ukupnom i L-askorbinskom kiselinom u škrgama i bubregu. Metodom glavnih komponenti je također pokazano da proces gladovanja na askorbinsku kiselinu u mlađi komarče ima utjecaj nakon 26. dana.