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Distribution of ascorbic acid in juvenile gilthead sea bream (*Sparus aurata* L.) organs at different dietary treatments

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Juvenile gilthead sea bream (Sparus aurata *L.*) *were fed commercial diets with 150, 500 and 1000 mg of vitamin C per kg of basal diet. One group was kept in starvation. The experiment lasted 98 days.*

The total and L-ascorbic acid levels were found to be highest in the brain, kidney, liver, gills and white muscle at the beginning of the experiment. Ascorbic acid concentrations in brain, gills, liver and white muscle showed no significant differences due to the different dietary treatments, whereas the diets containing supplemental ascorbic acid of 100, 500 and 1000 mg vit. C kg^{-1} *significantly affected total ascorbic acid level in the kidney. Groups of fish grown on different diets and starved showed no statistically significant differences in L-ascorbic acid levels in the gills and brain, whereas the significantly highest concentrations were recorded in the liver, white muscle and kidney of starved juveniles.*

Sampling time and different diets did not affect the concentrations of the total and L-ascorbic acid in the gills and white muscle, or the total ascorbic acid levels in the kidney which does not hold for the brain and liver.

INTRODUCTION

The development of mariculture and commercial culture of marine fish, in the first place sea bass (*Dicentrarchus labrax* L.) and gilthead sea bream (*Sparus aurata* L.) has shown that the knowledge of fish dietary requirements is essential. This particularly applies to the vitamin component, both because of attaining optimum growth and preventing the occurrence of fish deformities and diseases.

It is well known that dietary supply of defined quantities of vitamins meets the healthy growth and development requirements. Vitamin C (ascorbic acid) appears to be one of rather important vitamins. In fish, like in humans, guinea-pig and some birds, the missing link in the biosynthesis of ascorbic acid is inability to convert L-gulonolactone to 2-keto-L-gulonate due to the lack of L-gulonolactone oxidase enzyme. This shortcoming is explained by the loss of the gene responsible for the synthesis of the enzyme itself. This mutation is not lethal since sufficient quantity of ascorbic acid is supplied by dietary intake (HORNIG, 1975; CHATTERJEE *et al.*, 1975; CHATTERJEE, 1978; GROLLMAN and LEHNINGER, 1957).

Considerations of ascorbic acid chemistry (TOLBERT *et al.*, 1975; KOSTOJANC, 1949) and its physico-chemical properties (HAWK *et al.*, 1949; METH. VIT. ASS., 1947) show its molecule to be very labile and that any change in

its molecule, from the moment of its processing to the moment of administration, may cause complete or partial loss of its biological activity (EVA *et al.*, 1976, after SOLIMAN *et al.*, 1987; HILTON *et al.*, 1977b; SANDNES and UTNE, 1982, after SOLIMAN *et al.*, 1987; SOLIMAN *et al.*, 1987).

It has been attempted for the past 15 years to produce some forms of vitamin C, more stable than the ascorbic acid itself (TUCKER and HALVER, 1984; SANDNES *et al.*, 1984; SOLIMAN *et al.*, 1986; SOLIMAN *et al.*, 1987; ALBREKSTEN *et al.*, 1988; WILSON *et al.*, 1989).

It has also been attempted to determine daily ascorbic acid requirements of fish. Their estimates range from 200 to 500 mg vit C d^{-1} which has been accepted by many industrial producers of pelleted food (MAHAJAN and AGRAWAL, 1980a; HILTON *et al.*, 1978).

Since the gilthead sea bream is one of commercially most interesting fishes for the Mediterranean mariculture, the earlier investigations mainly included their reproductive physiology and culture of early developmental stages under controlled conditions of different ecological factors (KATAVIĆ, 1984). Available literature reveals a lack of knowledge of dietary requirements of fish in general, with the exception of rainbow trout and channel catfish. This particularly applies to the problem of diet microconstituents.

The aim of this paper was to determine vitamin C requirements of the gilthead sea bream, its distribution and temporal changes in different tissues.

MATERIAL AND METHODS

The gilthead sea bream (*Sparus aurata sp.*, Linn. 1758) specimens, seven months old, were obtained by induced spawning in the hatchery of the Institute of Oceanography and Fisheries, Split (KATAVIĆ, 1984). Mean fish length was 12.26 cm and weight 27.44 g at the beginning of the experiment.

Feeding experiment lasted 98 days. The tanks of 100 l volume with constant water flow and aeration and natural photoperiod were used. Water temperature was maintained at minimum 18.2 to maximum 24.6°C. Seven tanks with ten fishes each, with the exception of tank with 14 starved fish, were used.

Fish were fed pelleted commercial food "Gloria Mangimi S.P.A." (Italy) with different declared vitamin C quantities: 150, 500 and 1000 mg kg^{-1}. Daily diet quantity of 5% of fish weight was fed at 8 a.m. and 2 p.m. One fish group was left without food for the duration of the experiment. Fish were sampled and ascorbic acid analyzed at the beginning of the experiment and thereafter at monthly intervals.

Total length and weight were measured during the experiment, condition index was calculated after the formula CF=100xW/L3 (where W=weight and L=lenght), as well as hepatosomatic index, expressing the liver weight to boody weight ratio. Morphological properties of each fish were examined at the beginning of the experiment. For the analysis two fishes were selected from each tank and immediately deep frozen to prevent the loss of ascorbic acid from the tissues. During sampling length and weight of remaining fish were taken. Fish were previously treated by the anesthetic binzocaine-(ethyl-4-aminobenzoate). To prevent bacterial infection during manipulation, "Furazolidon"-11% powder, chemotherapeutic and coccidiostatic, was added to the tanks.

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

The double analysis of variance and Student - Newman - Keuls (SNK) test (SOKAL and ROHLF, 1969) were employed for statistical evaluation of the experimental results.

RESULTS

Intensive growth in length and weight was observed at higher sea water temperatures whe-

reas below 18°C growth was slowed. Fish under starvation showed no growth in length and significant weight loss (Fig. 1).

Significant differences in the condition coefficient were observed between the time of sampling and different feeding treatments (Ta-

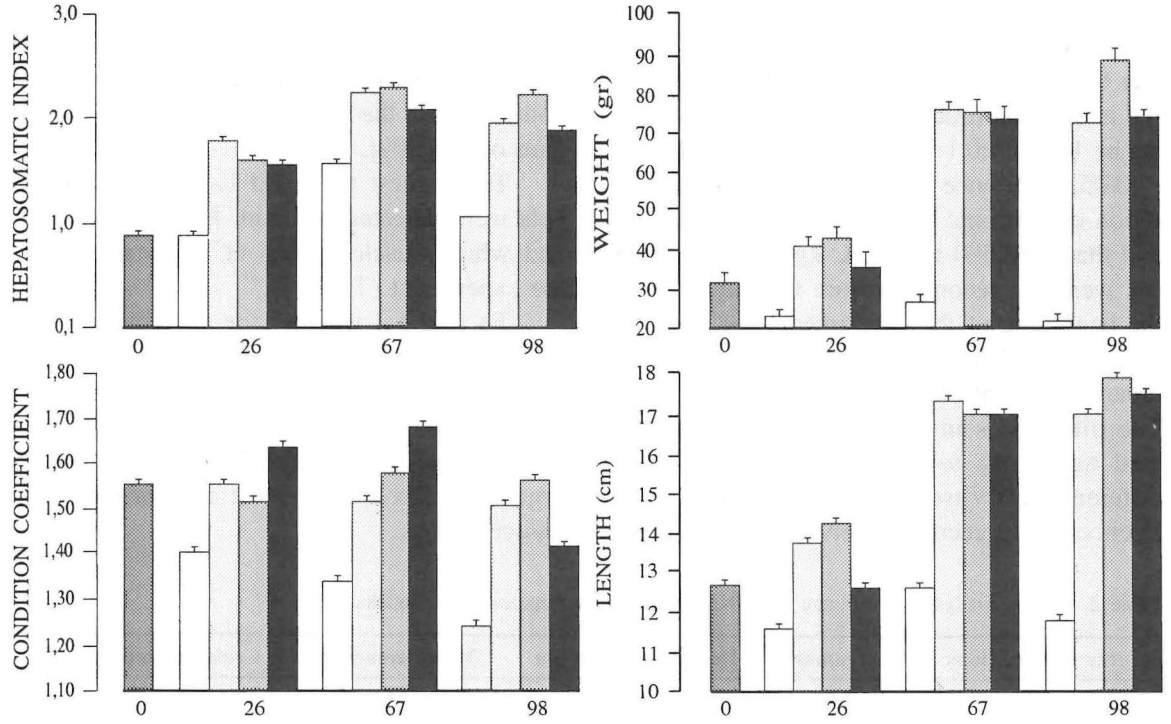

Fig. 1. Length, weight, condition coefficient, hepatosomatic index relationship in juvenile gilthead sea bream between different dietary treatments, 150 ( ), 500 ( ), 1000 ( ) mg vit C kg^{-1} of diet and starvation ( ) and initial sampling ( )

Table 1. Double analysis of variance and SNK-test of mean condition coefficents of gilthead sea bream

Source of variations	**Sum of squares**	**Degrees of freedom**	**Mean square**	**F - relationship**	**P**
Time	0.072	2	0.036	3.585	0.040
Treatment	0.394	3	0.131	13.068	0.001
TxT*	0.137	6	0.023	2.265	0.064
Rest	0.301	30	0.10		
Least significant range (LSR)					
Dietary treatment (mg vit.C kg-1)	Starvation		150	500	1000
Condition coefficient	1.36	*	1.57	1.59	* 1.62
Sampling time (days)	98		67	26	
Condition coeficient	1.49	*	1.56	1.57	

Underline means are not significantly different (P>0.05). TxT* – Treatment.

ble 1). The difference in condition between fish under starvation and those fed with supplemental 1000 mg vit. C kg^{-1} of diet was statistically significant ($P<0.05$). Condition coefficient in starved fish feld during the experiment whereas in fish fed 1000 mg vit. C kg^{-1} increased by the day 67 slightly decreasing thereafter. Therefore the interaction time x treatment was significant at the level $P<0.1$. No statistically significant, $P<0.05$, difference was recorded in mean condition coefficients between treatment with 150 and that with 500 mg vit. C kg^{-1}. The differences were not recorded before the day 98 from the beginning of the experiment (Fig. 1).

The total dietary status of the gilthead sea bream was controlled by hepatosomatic index. The differences in hepatosomatic index appeared to be due to the time of sampling and different dietary treatments (Table 2). The differences were recorded between all the treatments with different vitamin C levels and the starved group. The lowest hepatosomatic index was recorded in starved fish and the highest in fish fed 500 mg vit C kg^{-1}. The differences in hepatosomatic index occurred between the day 26 of the experiment and late samplings (Table 2) and the variations in hepatosomatic index coincide with the length increment and weight gain of fish (Fig. 1).

The highest total and L-ascorbic acid levels were detected in brain, kidney liver, gills and white muscle tissues at the beginning of the experiment (Table 3).

To find out whether the ascorbic acid occurred oxidized or reduced in the fish organs, the relationship between L-ascorbic acid and total ascorbic acid was determined. This relationship showed that ascorbic acid in different organs occurred in greatest concentrations in reduced form.

Table 2. Double analysis of variance and SNK-test of mean hepatosomatic indeks

Source of variations	Sum of squares	Degrees of freedom		Mean square	F - relationship		P
Time	2.646	2		1.323	8.043		0.002
Treatment	3.645	3		1.215	7.386		0.001
TxT*	0.432	6		0.072	0.438		0.848
Rest	4.935	30		0.164			
Least significant range (LSR)							
Dietary treatment (mg vit.C kg-1)		Starvation		1.000	150		500
Hepatosomatic index		1.61	*	1.82	1.94	*	2.04
Sampling time (days)		26		98	67		
Hepatosomatic index		1.43	*	1.92	2.01		

Underline means are not significantly different ($P>0.05$). TxT* – Treatment.

Table 3. Arithmetic mean and S.E.M.standard error of means of total and L-ascorbic acid in tissues and organs of six gilthead sea bream specimens (31 g mean weight and 12.4 cm mean lenght) at the beginning of the experiment

ORGAN	Total ascorbic acid (μgg^{-1} wet weight)	L - ascorbic acid (μgg^{-1} wet weight)	Ratio $\frac{L\text{–}AA}{U\text{–}AA}$ x 100
Brain	297.7 ± 15.4	193.4 ± 22.9	64
Kidney	171.4 ± 13.7	163.0 ± 22.4	95
Liver	82.4 ± 15.4	52.2 ± 6.7	63
Gills	75.2 ± 7.9	46.9 ± 8.7	62
White muscle	27.2 ± 2.5	22.5 ± 1.7	82

Brain

The highest total brain ascorbic acid concentrations were recorded in starved fish on day 26 from the beginning of the experiment, 262 μg g^{-1} wet weight, and in fish fed the diet containing 1000 mg vit. C kg^{-1} of diet, 263 μg g^{-1} wet weight. Thereafter total ascorbic acid stores declined in all the treatments. However, there were no statistically significant differences ($P>0.05$) between different treatments and starved group. Statistically significant difference, ($P<0.05$), occurred between the first sampling (on day 26 from the beginning of the experiment) and later samplings (Fig. 2, Table 4)

Maximum reduced L-ascorbic acid levels were also measured in starved fish. They ranged from 188 to 198 μg g^{-1} wet weight. A decline of L-ascorbic acid levels was recorded in all the treatments, with 150, 500 and 1000 mg vit. C kg^{-1} by day 67 from the beginning of the experiment to rise thereafter. However, there were no statistically significant differences between different dietary treatments and starved fish (Fig. 3, Table 5). Significant diferences ($P<0.05$) were observed only with respect to the sampling time, from the day 26 on.

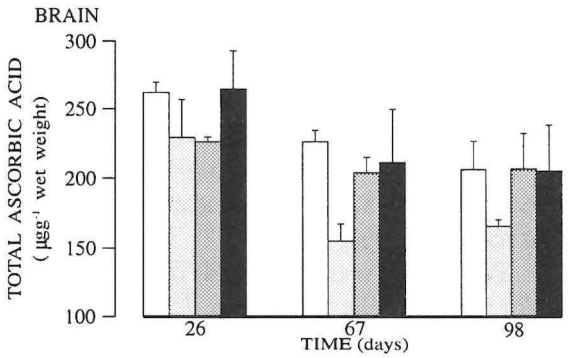

Fig. 2. Brain total ascorbic acid concentration (μg g^{-1} of wet weight) under different dietary treatments, 150 (▭), 500 (▩), 1000 (■) mg vit C kg^{-1} of diet and starvation (▭) for the duration of the experiment (98 days). (A single direction standard error is shown in the columns)

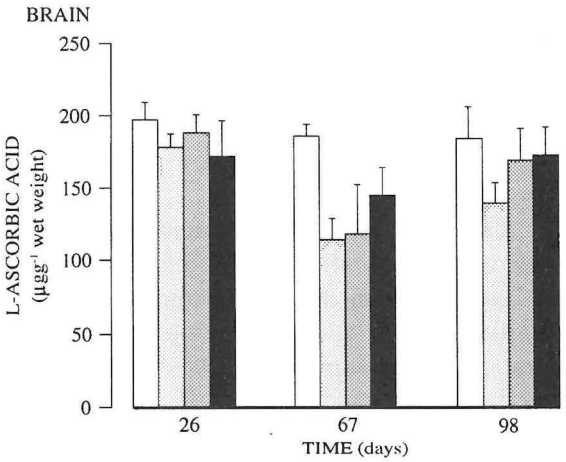

Fig. 3. Brain L-ascorbic acid concentration (μg g^{-1} of wet weight) under different dietary treatments, 150 (▭), 500 (▩), 1000 (■) mg vit C kg^{-1} of diet and starvation (▭) for the duration of the experiment (98 days). (A single direction standard error is shown in the columns)

Table 4. Double analysis of variance and SNK-test mean concentrations of total ascorbic acid in the brain

Source of variations	Sum of squares	Degrees of freedom	Mean square	F - relationship	P
Time	0.525	2	0.262	6.105	0.006
Treatment	0.296	3	0.099	2.297	0.098
TxT*	0.093	6	0.015	0.360	0.898
Rest	1.290	30	0.043		
Least significant range (LSR)					
Dietary treatment (mg vit.C kg-1)	150		500	1000	Starvation
Total ascorbic acid (μgg^{-1})	183.86	*	207.68	216.32	* 230.37
Sampling time (days)	98		67		26
Mean	192.25	*	194.88	*	240.59
Underline means are not significantly different ($P>0.05$). TxT* – Treatment.					

Table 5. Double analysis of variance and SNK-test of mean concentrations of L-ascorbic acid in the brain

Source of variations	Sum of squares	Degrees of freedom	Mean square	F - relationship	P
Time	0.731	2	0.365	3.826	0.033
Treatment	0.415	3	0.138	1.447	0.249
TxT*	0.452	6	0.075	0.789	0.589
Rest	2.865	30	0.095		
Least significant range (LSR)					
Dietary treatment (mg vit.C kg-1)	150		500	1000	Starvation
L-ascorbic acid ($\mu g g^{-1}$)	147.96	*	148.20	160.85	190.87
Sampling time (days)	67		98		26
Mean	135.55	*	168.35	*	181.65

Underline means are not significantly different (P>0.05). TxT* – Treatment.

Gills

Gill total and L-ascorbic acid content showed no statistically significant differences (P>0.05) between dietary treatments and starved fish of due to the sampling time for the duration of the experiment (98 days) (Figs. 4 and 5; Tables 6 and 7).

Liver

The lowest level of the total ascorbic acid of liver was found in fish treated with 150 mg vit C kg^{-1} on 67th day from the beginning of the experiment. It was 39.5 $\mu g\ g^{-1}$ of tissue wet weight. Tissue reserves in starved fish decreased during the experiment so that after days 67 and 98 they declined three-fold from the initial value of 95.5 $\mu g\ g^{-1}$, measured during the first sampling (Fig. 6).

The same was observed for L-ascorbic acid content in the liver (Fig. 7). Fluctuations in total ascorbic acid content of liver showed no significant variations under treatments with 500 and 1000 mg vit. C kg^{-1} of diet (Table 8). This confirms the fact that dietary vitamin C intake is sufficient to keep its liver stores at the

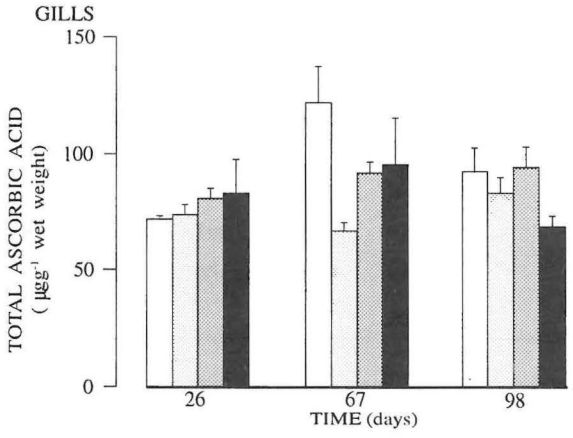

Fig. 4. Gill total ascorbic acid concentration ($\mu g\ g^{-1}$ of wet weght) under different dietary treatments, 150 ( ), 500 ( ), 1000 ( ) mg vit C kg^{-1} of diet and starvation ( ) for the duration of the experiment (98 days). (A single direction standard error is shown in the columns)

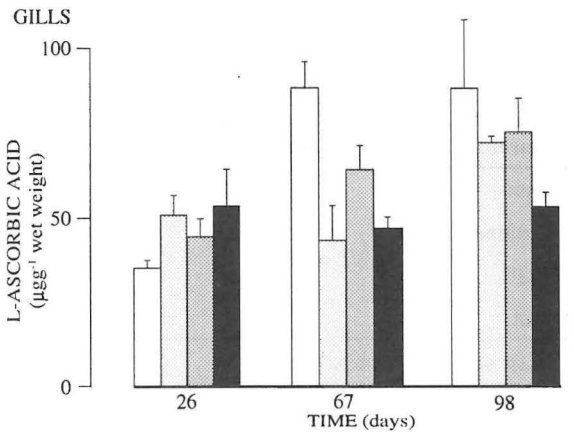

Fig. 5. Gill L-ascorbic acid concentration ($\mu g\ g^{-1}$ of wet weight) under different dietary treatments, 150 ( ), 500 ( ), 1000 ( ) mg vit C kg^{-1} of diet and starvation ( ) for the duration of the experiment (98 days). (A single direction standard error is shown in the columns)

Table 6. Double analysis of variance and SNK-test of mean concentrations of total ascorbic acid in the gills

Source of variations	Sum of squares	Degrees of freedom		Mean square	F - relationship	P
Time	0.182	2		0.091	1.780	0.186
Treatment	0.263	3		0.088	1.710	0.186
TxT*	0.494	6		0.082	1.608	0.179
Rest	1.535	30		0.051		
Least significant range (LSR)						
Dietary treatment (mg vit.C kg-1)		150		500	1000	Starvation
Total ascorbic acid ($\mu g g^{-1}$)		72.74	*	77.75	87.01	93.70
Sampling time (days)		26		98		67
Mean		76.34	*	80.34		90.69
Underline means are not significantly different (P>0.05). TxT* – Treatment.						

Table 7. Double analysis of variance and SNK-test or mean concentrations of L-ascorbic acid in the gills

Source of variations	Sum of squares	Degrees of freedom		Mean square	F - relationship	P
Time	0.731	2		0.365	3.826	0.033
Treatment	0.415	3		0.318	1.447	0.249
TxT*	0.452	6		0.075	0.789	0.586
Rest	2.865	30		0.095		
Least significant range (LSR)						
Dietary treatment (mg vit.C kg-1)		1000		150	500	Starvation
L-ascorbic acid ($\mu g g^{-1}$)		47.98	*	49.34	56.64	63.09
Sampling time (days)		26		67		98
Mean		44.50	*	56.28		63.93
Underline means are not significantly different (P>0.05). TxT* – Treatment.						

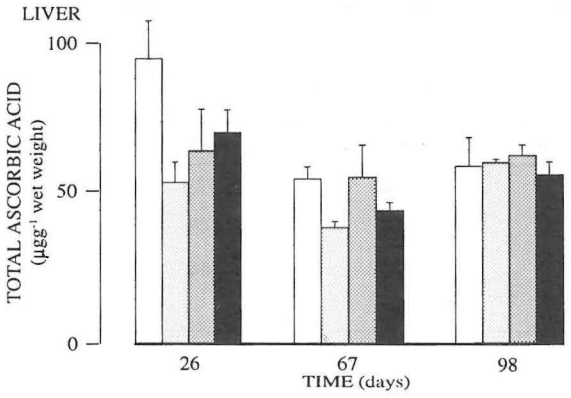

Fig. 6. Liver total ascorbic acid concentration ($\mu g\ g^{-1}$ of wet weight) under different dietary treatments, 150 ( ), 500 ( ), 1000 ( ) mg vit C kg^{-1} of diet and starvation ( ) for the duration of the experiment (98 days). (A single direction standard error is shown in the columns)

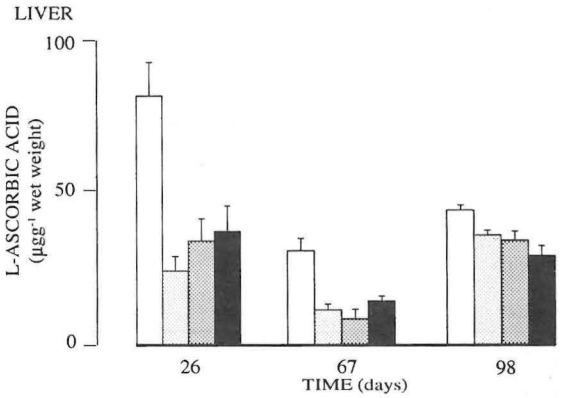

Fig. 7. Liver L-ascorbic acid concentration ($\mu g\ g^{-1}$ of wet weight) under different dietary treatments, 150 ( ), 500 ( ), 1000 ( ) mg vit C kg^{-1} of diet and starvation ( ) for the duration of the experiment (98 days). (A single direction standard error is shown in the columns)

level to meet metabolic requirements. There are significant interactions between sampling time and different treatments, that is total ascorbic acid is time dependent (Table 8).

As to the L-ascorbic acid, this interaction is not pronounced, since there was no difference (P>0.05) between treatments with 150 and 500 mg vit C kg^{-1} and other two treatment. There was no difference due to the time of sampling, that is between the days 67 and 98 (Table 9), either.

White muscle

The highest total ascorbic acid levels in the white muscle, 35 and 42 μg g^{-1} of tissue wet weight (Fig. 8) were obtained for treatments

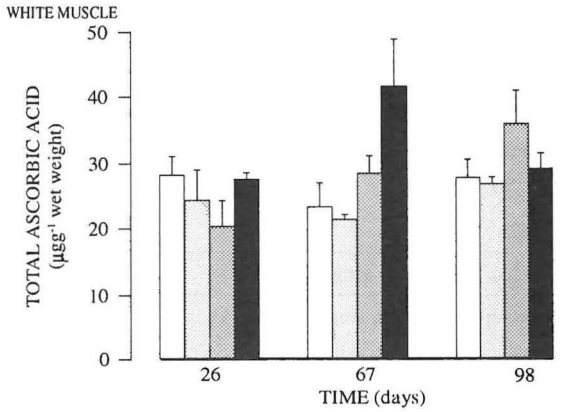

Fig. 8. White muscle total ascorbic acid concentration (μg g^{-1} of wet weight) under different dietary treatments, 150 (▭), 500 (▭), 1000 (▭) mg vit C kg^{-1} of diet and starvation (▭) for the duration of the experiment (98 days). (A single direction standard error is shown in the columns)

Table 8. Double analysis of variance and SNK-test of mean concentrations of total ascorbic acid in the liver

Source of variations	Sum of squares	Degrees of freedom	Mean square	F - relationship	P
Time	0.525	2	0.262	6.105	0.006
Treatment	0.296	3	0.099	2.297	0.098
TxT*	0.093	6	0.015	0.360	0.898
Rest	1.290	30	0.043		
Least significant range (LSR)					
Dietary treatment (mg vit.C kg-1)		150	1000	500	Starvation
Total ascorbic acid (μgg^{-1})		49.10 *	57.46	60.12	66.52
Sampling time (days)		67	98		26
Mean		48.90 *	59.82	*	66.65
Underline means are not significantly different (P>0.05). TxT* – Treatment.					

Table 9. Double analysis of variance and SNK-test of mean concentrations of L-ascorbic acid in the liver

Source of variations	Sum of squares	Degrees of freedom	Mean square	F - relationship	P
Time	8.328	2	4.164	25.739	0.000
Treatment	4.099	3	1.366	8.446	0.000
TxT*	1.713	6	0.285	1.764	0.142
Rest	4.691	29	0.162		
Least significant range (LSR)					
Dietary treatment (mg vit.C kg-1)		500	150	1000	Starvation
L-ascorbic acid (μgg^{-1})		22.10 *	20.52 *	25.45 *	45.29
Sampling time (days)		67	98		26
Mean		14.13 *	33.91		36.03
Underline means are not significantly different (P>0.05). TxT* – Treatment.					

with 500 and 1000 mg vit C kg^{-1} respectively. However, there were no statistically significant differences (P>0.05) due to different treatments or to sampling time (Table 10).

L-ascorbic acid contents were significantly lower in treatments with 150 and 500 mg vit C kg^{-1} than in the treatment with 1000 mg vit C kg^{-1} and in starved fish. There were no statistically significant differences due to the sampling time (Table 11), either.

Table 10. Double analysis of variance and SNK-test of mean concentrations of total ascorbic acid in the white muscle

Source of variations	Sum of squares	Degrees of freedom	Mean square	F - relationship	P
Time	0.176	2	0.088	0.972	0.006
Treatment	0.340	3	0.113	1.257	0.098
TxT*	0.849	6	0.141	1.56	0.898
Rest	2.709	30	0.090		
Least significant range (LSR)					
Dietary treatment (mg vit.C kg-1)		150	500	Starvation	1000
Total ascorbic acid ($\mu g g^{-1}$)		23.36	26.81	60.12	30.52
Sampling time (days)		26	67	98	
Mean		24.27	26.75	29.73	
Underline means are not significantly different (P>0.05). TxT* – Treatment.					

L-ascorbic acid levels during the experiment were shown in Fig. 9.

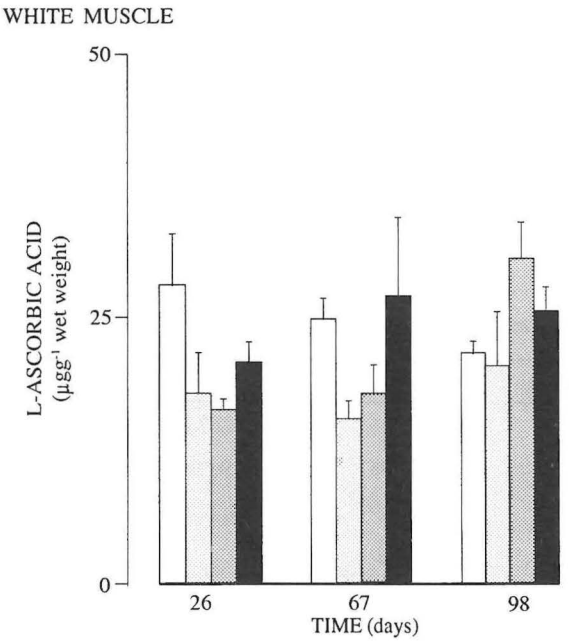

Fig. 9. White muscle L-ascorbic acid concentration (µg g^{-1} of wet weight) under different dietary treatments, 150 (▭), 500 (▨), 1000 (■) mg vit C kg^{-1} of diet and starvation (□) for the duration of the experiment (98 days). (A single direction standard error is shown in the columns)

Kidney

During 98 days of the laboratory experiment the highest kidney total and L-ascorbic acid content was recorded in starved fish. Total ascorbic acid concentrations varied from 194 µg g^{-1} to 268 µg g-1 of tissue wet weight, whereas L-ascorbic acid content remained constant at approximately the level of 188 µg g^{-1} of tissue wet weight (Figs. 10 and 11) for the duration of the experiment.

Total ascorbic acid concentrations declined in fish treated with 150, 500 and 1000 mg vit C kg^{-1}. The highest concentration, however, was recorded in fish under 1000 mg vit C kg^{-1} treatment and in starved fish. Concentrations were lower in other treatments and differences (P<0.05) occurred between all the treatments. However, there were no significant differences (P>0.05) due to the sampling time (Table 12).

L-ascorbic acid concentrations showed the same relationships between different treatments (Fig. 11 and Table 13). The differences due to

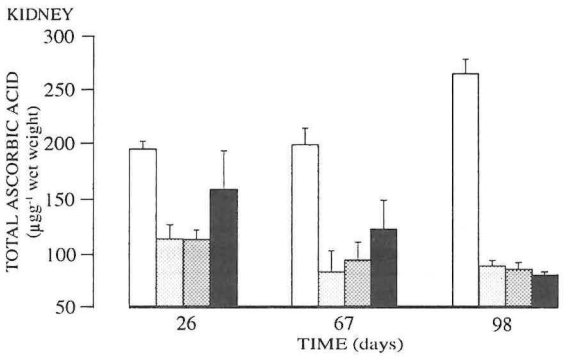

Fig. 10. Kidney total ascorbic acid concentration (µg g^{-1} of wet weight) under different dietary treatments, 150 (▭), 500 (▩), 1000 (■) mg vit C kg^{-1} of diet and starvation (▭) for the duration of the experiment (98 days). (A single direction standard errror is shown in the columns)

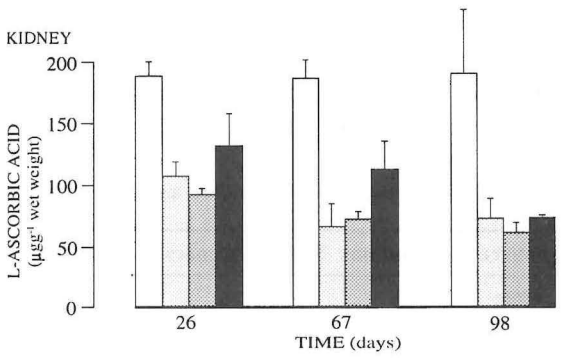

Fig. 11. Kidney L-ascorbic acid concentration (µg g^{-1} of wet weight) under different dietary treatments, 150 (▭), 500 (▩), 1000 (■) mg vit C kg^{-1} of diet and starvation (▭) for the duration of the experiment (98 days). (A single direction standard error is shown in the columns)

Table 11. Double analysis of variance and SNK-test of mean concentrations of L-ascorbic acid in the white muscle

Source of variations	**Sum of squares**	**Degrees of freedom**	**Mean square**	**F - relationship**	**P**
Time	0.223	2	0.111	1.523	0.235
Treatment	0.687	3	0.229	3.128	0.040
TxT*	0.809	6	0.135	1.842	0.124
Rest	2.197	30	0.073		
Least significant range (LSR)					
Dietary treatment (mg vit.C kg-1)	150		500	1000	Starvation
L-ascorbic acid (µgg^{-1})	17.13	*	20.41 *	24.14	24.96
Sampling time (days)	26		67		98
Mean	19.67		20.91		25.02
Underline means are not significantly different (P>0.05). TxT* – Treatment.					

Table 12. Double analysis of variance and SNK-test of mean concentrations of total ascorbic acid in the kidney

Source of variations	**Sum of squares**	**Degrees of freedom**	**Mean square**	**F - relationship**	**P**
Time	0.358	2	0.179	2.634	0.088
Treatment	4.018	3	1.339	19.699	0.000
TxT*	0.705	6	0.117	1.727	0.149
Rest	2.040	30	0.068		
Least significant range (LSR)					
Dietary treatment (mg vit.C kg-1)	150		500	1000	Starvation
Total ascorbic acid (µgg^{-1})	90.67	*	98.36 *	111.38 *	210.67
Sampling time (days)	98		67		26
Mean	104.00	*	116.57		136.04
Underline means are not significantly different (P>0.05). TxT* – Treatment.					

Table 13. Double analysis of variance and SNK-test of mean concentrations of L-ascorbic acid in the kidney

Source of variations	Sum of squares	Degrees of freedom	Mean square	F - relationship	P
Time	0.896	2	0.048	5.606	0.009
Treatment	4.875	3	1.625	20.342	0.000
TxT*	0.414	6	0.069	0.864	0.532
Rest	2.396	30	0.080		
Least significant range (LSR)					
Dietary treatment (mg vit.C kg-1)		500	150	1000	Starvation
L-ascorbic acid ($\mu g g^{-1}$)		72.00 *	80.97 *	96.96 *	184.17
Sampling time (days)		98	67		26
Mean		77.89 *	98.00	*	118.81

Underline means are not significantly different (P>0.05). TxT* – Treatment.

the sampling of time were significant at the level P=0.05.

Ascorbic acid stores decline in treatments with 150, 500 and 1000 mg vit C kg^{-1} could have occurred due to temperature decrease of the sea water from 24.6°C at the beginning of the experiment to 18.2°C at the end of the experiment.

DISCUSION

Growth and development of poikilotherms are affected by a series of factors, particularly by sea water temperature, food quallity and content (KINNE, 1960). Apart from other constituents, food should contain vitamins which are essential for healthy growth and development.

MAHAJAN and AGRAWAL (1979) showed reduced growth and increased mortality in *Channa punctatus* fed vitamin C deficient diet for 210 days compared to diet with 100 mg vit C kg^{-1}. The same authors (1980a) fed hatchlings of Indian major carp *Cirrhina mrigala* graded levels of ascorbic acid. The average weight gain was much better in fish fed supplemental ascorbic acid exceeding 600 mg kg^{-1} of diet.

Juvenile gilthead sea bream fed graded levels of vitamin C for 98 days in the present experiment showed length increment, weight gain and increase of condition coefficient (Fig. 1). Total nutritional status of fish was observed through variations in hepatosomatic index at different dietary treatments (Table 2, Fig. 1).

SOLIMAN *et al.* (1986) showed that hepatosomatic index in juvenile tilapias *(Orechromis niloticus)* fed vitamin C deficient diet was significantly lower than in juveniles fed diet with supplemental vitamin C or its analogs. DOIMI *et al.*, (1985) also showed that hepatosomatic index in juvenile sea bass *(Dicentrarchus labrax)* fed dry synthetic food increased by 33% in relation to controls fed another diet.

Hepatosomatic index may increase if fats are deposited in liver. Better weight gain of liver in relation to body weight gain in gilthead sea bream, expressed as the difference in hepatosomatic index between the day 67 and later samplings (Table 2), presumably indicates fat deposition in fish liver.

Starved gilthead sea bream lost weight and showed break in length growth. However, no mortality occurred. It is very likely that fish try to adapt to starvation reducing their metabolism.

Determination of ascorbic acid distribution in different organs with respect to different dietary treatments and time during 98 day experiment (Tables 20 and 21) is the best way to gauge the involvement of ascorbic acid in the overall metabolism of juvenile gilthead sea bream. The highest tissue content of total and L-ascorbic acid were measured in the brain and kidney, then in liver, gills and white muscle (Table 3). Similar was reported by other authors (Table 14). The total/L-ascorbic acid relationship shows that reduced ascorbic acid form is mainly present in tissues (Table 3). The same was reported by TAKEDA *et al.*, 1963.

Table 14. Content of total ascorbic acid in different organs of some fish species

FISH SPECIES	ORGANS				
	Brain (μgg-1)	Liver (μgg-1)	Kidney (μgg-1)	Gills (μgg-1)	Muscle (μgg-1)
Sparus aurata[1]	297	82.4	171.4	75.2	27.2
Labeo rohita[2]	75.44	272.24	109.73	N.M	N.M
Labeo calbasu[2]	102.62	188.44	64.02	N.M	N.M
Cirrhina mrigala[2]	83.86	147.85	91.07	N.M	N.M
Catla catla[2]	124.60	179.49	104.21	N.M	N.M
Rainbow trout[3]	397	196	232	N.M	43
Carrasius auratus[4]	195	48	101	N.M	21
Carp[4]	258	39*	93	N.M	18
Oreochromis niloticus[5]	167.26	51.36	N.M	92.07	14.31
* - Hepatopankreas	N.M. - not measured				

1) Our data
2) *Agrawal i Mahajan* (1980)
3) *Ikeda i sur.* (1963)
4) *Hilton i sur.* (1979)
5) *Soliman i sur.* (1986)

HILTON *et al.* (1978) measured the ascorbic acid levels in the rainbow trout and reported that ascorbic acid requirements vary with age of the trout in that the requirement is higher in young fish.

Brain

A laboratory study showed that increasing the water temperature from 18 to 30°C caused a decline in brain ascorbic acid content. This temperature effect appeared to be limited to the brain since other organs stores were unaffected (THOMAS, 1984). This may account for relatively high brain content of the total and L-ascorbic acid at sea water temperature range of 24.6-18.2°C in the present experiment (Figs. 2 and 3).

THOMAS *et al.* (1985) reported highest ascorbic acid levels in brain tissues of the mullet *(Mugil cephalus)* which ranged from 156 μg g^{-1} in April to 104 μg g^{-1} 1 month later affected by temperature. Although temperature affecting ascorbic acid mechanism is uncertain, presumably defined ascorbic acid concentrations are essential for maintenance of neuron functions (THOMAS *et al.*, 1982; THOMAS, 1987; AGRAWAL and MAHAJAN, 1980).

Ascorbic acid in the brain tissue is present mainly in reduced form (Table 15). Within brain neurons ascorbic acid is an indispensable factor of dopamin conversion in norpimerin by means of dopamin-hydroxilase (SPECTOR and LORENZO, 1974).

Table 15. Relationship between L-ascorbic acid and total ascorbic acid in the brain (%)

Treatments (mg. vit. C kg-1)				
Sampling (days)	Starvation	150	500	1000
26	75	29	84	66
67	83	77	60	71
98	92	88	85	88

High total and L-ascorbic acid content in the brain of starved gilthead sea bream shows that juveniles meet their requirements by ascorbic acid from other organs. It is well known that standard metabolism declines under starvation conditions. For the first seven days the initial metabolism declined by 50% whereas after 300 days it was only 15 to 20% of the initial

one. Metabolism decrease is logarithmic (FRY, after BROWN, 1957).

Gills

Euryhaline teleosts are able to adapt to environmental salinity changes by different morphological and biochemical changes in cell membrane. In these species, where gilthead sea bream also count, cell membrane responds instantaneously by changing permeability. Ascorbic acid in gills also responds very quickly to salinity changes in its surroundings, which points to an inverse relationship of these two parameters. Ascorbic acid has been shown to inhibit the activity of Na^+, K^+-ATPase - an enzyme responsible for the transport of Na^+ and K^+ ions through cell membrane in mammal tissues, whereas Na^+ - K^+ -ATPase activity decreases in striped mullet *(Mugil cephalus)* upon exposure to diluted sea water whereas ascorbic acid increases by approximately 20 μg g^{-1}. These results point to the fact that ascorbic acid is involved in the regulation of Na^+, K^+ -ATPase activity and osmoregulation in the gills of euryhaline teleosts (THOMAS, 1984). The present experiment revealed no marked effect of sea water temperature on ascorbic acid levels in the gills at temperature change from 24.6°C at the beginning of the experiment to 18.2°C at the termination of the experiment (Figs. 4 and 5). There were no statistically significant differences due to different dietary treatments and sampling time (Tables 6 and 7), either.

Table 16. Relationship between L-ascorbic acid and total ascorbic acid and total ascorbic acid in the gills (%)

Treatments (mg. vit. C kg-1)				
Sampling (days)	Starvation	150	500	1000
26	49	68	55	64
67	72	64	68	47
98	94	84	78	74

As affected by the sampling time and different dietary treatments and starvation, ascorbic acid appears to be present mainly in reduced form (Table 17).

Table 17. Relationship between total and L-ascorbic acid in the liver (%)

Treatments (mg. vit. C kg-1)				
Sampling (days)	Starvation	150	500	1000
26	85	45	52	53
67	56	31	17	32
98	73	59	55	51

The operculum deformities were recorded for the treatment with 150 mg vit C kg^{-1}. This may be due to ascorbic acid deficiency but also to the genetic background of fish and some environmental factors (anoxia, nutrition). The causes of this disease are not lethal for fish but could make them more liable to infections. Vitamin C deficiency results in decreased calcium uptake by the gills, as reported by MAHAJAN and AGRAWAL (1980b) for the species Channa *(= Ophicephalus) punctatus*. They showed that chronic vitamin C deficiency in the diet causes a decrease in calcium uptake by the gills and changes in gill filaments as pointed out by HALVER, 1972.

Liver

Symptoms of vitamin C deficiency in the liver occur when the ascorbic acid content drops below critical level. After HILTON *et al.*, (1977a) levels 20 μg g^{-1} of tissue wet weight and lower are marginal and immediate supplementing is required.

PHILLIPS *et al.* (1953; after LOVE, 1970) reported that vitamin C levels in starved *Salvelinus fontinalis* declined much slower than in fish fed small ascorbic acid quantities or ascorbic acid deficient food. These authors also belieived that vitamin requirements are affected by diet quantity and content, so that food with low supplemental vitamin C levels causes fish to consume body reserves of their own. LIM and LOVELL (1978) showed on the species *Ictalurus punctatus* that the liver ascorbic acid levels are proportional to dietary vitamin uptake, and that all the contents lower than 30 μg g^{-1} of the tissue wet weight are insufficient to meet metabolic requirements which leads to juvenile diseases.

Lowest total ascorbic acid levels in the gilthead sea bream liver, 39.5 μg g^{-1} of tissue wet weight, were recorded for the treatment with 150 mg vit C kg^{-1}. There were no significant differences in ascorbic acid content between other treatments. This confirms the fact that supplemental vitamin C in the fish diet maintains its concentrations at the level which meets metabolic requirements (Figs. 6 and 7).

The relationship of total and L-ascorbic acid is constantly changing, that is reactive balance is constantly shifted in favour of oxidized ascorbic acid form showing that many ascorbic acid dependent physiological reactions are known to occur in the liver (AGRAWAL and MAHAJAN, 1980), (Table 17).

However, the rate of metabolic processes in the liver and some other fish organs is temperature dependent, apart from being affected by some other environmental factors. So ascorbic acid depletion has been observed in the livers of fish which did not feed well at lower temperatures due to the consumption of body reserves for normal metabolism. However, raising of sea water temperature, causes reestablishment of metabolism so that temperature effects are only momentary (THOMAS, 1984).

White muscle

The function and involvement of vitamin C in muscle metabolism is poorly understood. It is only known that there is no difference in vitamin content between the white and red muscle (PORA *et al.*, 1964 after LOVE, 1970). The occurrence of scurvy in fish due to dietary deficiency of vitamin C, results in atrophy, reduced calcification, swelling and hemorrhages (SEBRELL, 1967).

The results of present experiment show (Tables 10 and 11) that there were no statistically significant differences in vitamin C content due to different dietary treatments or sampling time (Figs. 8 and 9). The relationship between the total and L-ascorbic acid showed that (Table 18) ascorbic acid appears to be present mainly in reduced form in the white muscle throughout the experiment. IKEDA *et al.*, (1963) came to the same conclusion measuring the concentration of dihydroascorbic acid in various tissues of *Plecoglossus altivelis, Sparus sarba, Acanthopagrus schlegeli* and *Seriola purpurascens*. Oxidized ascorbic acid form made up less than 10% of the total ascorbic acid and most part was in reduced form.

Table 18. Relationship between L-ascorbic acid and total ascorbic acid in the white muscle (%)

Treatments (mg. vit. C kg-1)				
Sampling (days)	Starvation	150	500	1000
26	100	73	83	75
67	98	72	63	72
98	79	72	86	90

The function of dietary vitamin C in the processes of calcification and contraction of the muscle has already been shown (PAULING, 1989; HUSS, 1988). If fish are given diets containing insufficient vitamin C quantity the disturbances in muscle contraction will occur followed later by atrophy.

Kidney

Kidney total and L-ascorbic acid content of juvenile gilthead sea bream (Figs. 10 and 11) confirmed the report of HALVER *et al.*, (1975) who detected the highest ascorbic acid levels in brain and kidney of rainbow trout *(Salmo gairdneri)*.

High kidney ascorbic acid levels in starved fish show that juvenile gilthead sea bream start to utilize kidney reserves for healthy metabolism not before depleting these reserves in the liver and other organs (MAHAJAN and AGRAWAL, 1979). LIM and LOWELL (1978) showed great variations in *Ictalurus punctatus* kidney ascorbic acid levels due to dietary vitamin C levels with no statistically significant difference between different dietary treatments. The relationship between total and L-ascorbic acid content in the present study shows that most of the ascorbic acid kidney content is in reduced form (Table 19). The ability of ascorbic acid to oxidize and reduce reversibly involves it in a variety

Table 19. Relationship between L-ascorbic acid and total ascorbic acid in the kidney (%)

Treatments (mg. vit. C kg^{-1})				
Sampling (days)	Starvation	150	500	1000
26	94	94	80	83
67	93	79	74	89
98	70	79	67	87

of electron cell transfer reactions. However, the mechanism of these reactions is still poorly understood.

To determine the dietary quantity of ascorbic acid, a series of factors should be taken into consideration, such as the total and L-ascorbic acid status of the fish, age and sex. After a consideration of these factors in the present study it is recommended that fish should be given diets containing not less than 500 mg vit C kg^{-1}. After MAHAJAN and AGRAWAL (1980a), who fed newly hatched Indian major carp, it appers that these fish require a supplement of 650 to 700 mg vit C kg^{-1} of diet. SOLIMAN *et al.* (1986) feeding juvenile tilapias *(Oreochromis niloticus)* varying forms of ascorbic acid added to basal diet, containing no ascorbic acid to supply 125 mg vit C kg^{-1} of dry diet, and vitamin C free diet concluded that the quantity of ascorbic acid in different organs differed as affected by the diet and that 125 mg vit C kg^{-1} is insufficient to meet all fish requirements. This was confirmed by the present study where pathological changes in juvenile fish occurered after 62 days of continued diet containing 150 mg vit C kg^{-1}. So the "jugular line break syndrome", distortion and crystalline deposits in the anterior eye chamber were observed in some fishes and the occurrence of exophthalmia in some others. Exophthalmia was attempted to be accounted for by many earlier authors (DUIJN, 1971; PAPERNA *et al.*, 1977, 1980; PAPERNA, 1987; SARUSIC and LISAC, 1987) suggesting that the vitamin C deficient pelleted food max be one of its causes. To prevent fish diseases many authors tried to determine optimum ascorbic acid quantity (HALVER *et al.*, 1969, 1972 after MAHAJAN and AGRAWAL, 1980a; HILTON *et al.*, 1978; ANDREWS and MURRAY, 1975 after MAHAJAN and AGRAWAL, 1980a; LIM and LOVELL, 1978; MURRAY *et al.*, 1978 after MAHAJAN and AGRAWAL, 1980a; MAHAJAN and AGRAWAL, 1980a; DURVE and LOVELL, 1982).

Actual ascorbic acid requirements of different fish species are affected by some additional factors such as age, size, exposure to stress and the other nutrients present on the diet.

Due to vitamin C instability, its content in the food should be controlled since severe losses occur during its processing, storage and administration (EVA *et al.*, 1976 after SOLIMAN *et al.*, 1987; HILTON, 1977b; SANDNES and UTNE, 1982 after SOLIMAN *et al.*, 1987; LOVELL, 1987; SOLIMAN *et al.*, 1987). Due to significant losses of ascorbic acid which is highly water soluble vitamin it was attempted to improve its stability and more stable forms were proposed: L-ascorbic acid 2-sulphate, sodium ascorbic acid, glyceride doated ascorbic acid, ascorbic acid palmitate, L-ascorbic acid 2-polyphosphate (TUCKER and HALVER, 1984; SANDNES *et al.*, 1982; SOLIMAN *et al.*, 1986; SOLIMAN *et al.*, 1987; ALBREKTSEN *et al.*, 1988; WILSON *et al.*, 1989). However, the best ascorbic acid form, appropriate for fish food, has not yet been determined.

Observing the graded levels of ascorbic acid, 150, 500, 1000 mg kg^{-1} of diet administered in this study, after 98 days, the actual L-ascorbic acid content was by about 20-30% lower than declared. To avoid losses during processing, storage and administration this vitamin should be added to diets in another form.

CONCLUSIONS

At the beginning of the present experiment the highest concentrations of total and L-ascorbic acid were recorded from the brain (298 μg tot. ascorbic acid/g of wet tissue weight and 193 μg L-ascorbic acid/g of wet tissue weight), kidney (171 μg tot. ascorbic acid/g of wet tissue weight and 163 μg L-ascorbic acid/g of wet tissue weight), liver (82 μg tot. ascorbic acid/g of wet tissue weight and 52 μg L-ascorbic acid/g of wet tissue weight), gills (75 μg tot. ascorbic acid/g of wet tissue weight and 47 μg

L-ascorbic acid/g of wet tissue weight) and white muscle (27 μg tot. ascorbic acid/g of wet tissue weight and 23 μg L-ascorbic acid/g of wet tissue weight).

There were no statistically significant differences in ascorbic acid contents in the brain, gills, liver and white muscle between starved fish and different dietary treatments (150, 500, 1000 mg vit C kg^{-1}). Treatments, however, significantly affected total ascorbic acid levels in the kidney. The highest concentrations were recorded in starved fish, followed by those in fish fed 1000, 500 and 150 mg.

Table 20. Concentration of total and L-ascorbic acid in μgg^{-1} in different organs of juvenile gilthead sea bream *(Sparus aurata)* under different dietary treatments during 98 days experiment

TISSUE		TREATMENTS			
		Starvation	150	500	1000
Brain	A:	230.4a	183.9a	207.7a	216.3a
	B:	190.9a	148.0a	148.2a	160.9a
Gills	A:	93.7a	72.7a	77.8a	87.0a
	B:	63.1a	49.3a	56.6a	48.0a
Liver	A:	66.5a	49.1a	57.5a	60.1a
	B:	45.3a	20.5c	20.1c	25.5b
White muscle	A:	28.2a	23.4a	26.8a	30.5a
	B:	25.0a	17.1c	20.4b	24.1a
Kidney	A:	210.0a	90.7d	98.4c	111.4b
	B:	184.2a	81.0c	72.0d	97.0b

A - total ascorbic acid in μgg^{-1}
B - L-ascorbic acid in μgg^{-1}
a, b, c, d, - Statisticaly significant differences $P<0.05$ occurring at different concetrations

There were no statistically significant differences in L-ascorbic acid contents in the brain and gills between starved fish and different treatments, whereas significantly highest concentrations were recorded in liver, white muscle and kidney of starved fish.

Sampling time and dietary treatments do not affect the content of total and L-ascorbic acid in the gills and white muscle or total ascorbic acid in the kidney. Kidney L-ascorbic acid levels decline with time. Brain total and L-ascorbic acid content fell during 67 days of the experiment, remaining approximately unaltered by the end of the experiment (98 days). Lowest ascorbic acid concentration was recorded in gilthead sea bream liver on day 67 to start to increase on day 98 from the beginning of the experiment.

Table 21. Concentration of total and L-ascorbic acid in μgg^{-1} in organs of gilthead sea bream *(Sparus aurata)* at different sampling time

TISSUE		TIME (days)		
		26	67	98
Brain	A:	240.6a	194.9a	192.3b
	B:	181.7a	135.6a	168.4b
Gills	A:	76.3a	90.7a	80.3a
	B:	44.5a	56.3a	65.9a
Liver	A:	66.7a	48.9c	59.8b
	B:	36.0a	14.1b	33.9a
White muscle	A:	24.3a	26.8a	29.7a
	B:	19.7a	20.9a	25.0a
Kidney	A:	136.0a	116.6a	104.0b
	B:	118.8a	98.0b	77.9c

A - total ascorbic acid in μgg^{-1}
B - L-ascorbic acid in μgg^{-1}
a, b, c, d, - Statisticaly significant differences $P<0.05$ occurring at different concetrations

Operculum deformities appeared in 150 mg vit C kg^{-1} group, as well as exophthalmia, presumably due to vitamin C deficient diet.

Dietary ascorbic acid quantity is affected by a series of factors, such as total and L-ascorbic acid status, fish age and sex. Considering these factors in the present experiment, the fish should be given diets containing not less than 500 mg vit C kg^{-1}.

Due to vitamin C instability, its actual content in the food should be controlled since severe losses occur during its processing, storage and administration.

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Distribucija askorbinske kiseline u mlađi komarče (*Sparus aurata* L.) pri različitim tretmanima ishrane

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KRATKI SADRŽAJ

Mlađ komarče (*Sparus aurata,* L.) je hranjena komercijalnom hranom sa 150, 500, 1000 mg vit C kg^{-1} hrane, a jedna grupa je bila u gladovanju. Eksperiment je trajao 98 dana.

Rezultati su pokazali da su najveće koncetracije ukupne i L-askorbinske kiseline izmjerene na početku eksperimenta u mozgu, bubregu, zatim jetri, škrgama i bijelom mišiću. S obzirom na različite tretmanske grupe u mozgu, škrgama, jetri i bijelom mišiću nema značajnih razlika u koncetraciji ukupne askorbinske kiseline, dok ishrana sa 150, 500, 1000 mg vit. C kg^{-1} hrane ima značajan utjecaj na koncetraciju ukupne askorbinske kiseline u bubregu. Prateći promjene u različitim tretmanima ishrane i tretmanu gladovanja nisu utvrđene statistički značajne razlike sadržaja L-askorbinske kiseline u škrgama i mozgu, dok su u jetri, bijelom mišiću i bubregu značajno najviše koncetracije prisutne kod mlađi u gladovanju.

Vrijeme uzorkovanja i tretmani ishrane ribe nemaju utjecaja na koncetraciju ukupne i L-askorbinske kiseline u škrgama i bijelom mišiću, a također i na ukupnu askorbinsku kiselinu u bubregu, dok u mozgu i jetri postoje značajne interakcije.