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PIGMENTATION OF SALMONIDS: FACTORS AFFECTING CAROTENOID DEPOSITION IN RAINBOW TROUT (Salmo gairdneri).

by

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ABSTRACT

Factors affecting carotenoid deposition in rainbow trout (Salmo gairdneri) were investigated in three feeding experiments.

The deposition of astaxanthin in the flesh increased significantly with increasing levels of astaxanthin and fat in the diets. However, increasing levels of astaxanthin in the diet decreased the ratios of astaxanthin in flesh to astaxanthin in diet.

Fish size, dietary fat quality and vitamin E supplementation seemed to have no effect on the astaxanthin deposition.

Depletion of the amount of astaxanthin in the flesh suggests metabolization of deposited astaxanthin.

INTRODUCTION

The pink colour of the flesh of salmonids is one of the factors that distinguishes these fishes, and make a major contribution to their elite image.

Since salmonids are not able to synthesize astaxanthin (Hata and Hata, 1973), and it is of great economical importance to achieve a ´natural´ pigmentation of farmed salmonids, great effort has been put into testing and comparison of available natural and synthetic carotenoid sources. It is shown by several authors that the fish can be pigmented by inclusion of astaxanthin

sources such as crustaceans, crustacean byproducts, and meal and pigment extracts of these (Satio and Regier, 1971; Spinelli et al, 1974; Spinelli and Mahnken, 1978; Torrissen et al, 1981; Allahpichay et al, 1984), by synthetic cantaxanthin (Deufel, 1965; Schmidt and Baker, 1969), by synthetic astaxanthin (Tidemann et al, 1973) and by the red yeast, Phaffia rhodozyma (Johnson et al, 1980).

In spite of the relatively high cost of carotenoids, less work has been done on other factors affecting carotenoid absorption and deposition in the flesh. Johnson et al (1980) found that rupturing the cell wall of the yeast Phaffia rhodozyma increased the availability of the pigment. Torrissen et al (1981) presented similar results by acid silage of shrimp waste. Abdul-Malak et al (1975), Spinelli (1979) and Seurman et al (1979) found that the fat content of the diet promoted deposition of astaxanthin in the flesh, Torrissen and Braekkan (1979) reported that free astaxanthin in the diet was absorbed and deposited better than the ester forms and Torrissen and Nævdal (1984) found genetical differences in the ability to deposit astaxanthin in the flesh by rainbow trout.

The purpose of this study was to investigate the effects of different levels of astaxanthin in the diet, of vitamin E supplementation, of dietary fat quality and of fat level on the deposition of astaxanthin in the flesh of rainbow trout (Salmo gairdneri).

MATERIALS AND METHODS

The fish were cultured in circular fiberglass tanks containing 1.7 m3 sea water (28-30 ppt) flowing through at a rate of 25 l per min. The sea water was filtered to remove zooplankton, mainly Calanus finmarchichus.

The fish were fed ad libitum three times per day. At the start of the experiments and at intervals throughout the experiments the fish were weighed and 4 to 10 fishes were sampled for determination of the astaxanthin level in the flesh. Before weighing, the fish were anaesthetized with 0.03% benzocain (saturated in etanol) added to the sea water.

Astaxanthin was determined by the method described by Lambertsen and Braekkan (1971), free fatty acids by the method of analysis of the Association of Official Analytical Chemists (Horwitz, 1975), crude fat by soxhlet extraction in diethyl ether and dry matter by drying to constant weight at 105°C.

Statistical analysis was carried out by using BMDP statistical software (Dixon, 1981) and RS/1 (Bolt Beranek and Newman, Inc., Cambridge, Massachusetts).

Experiment 1

Shrimp waste such as byproducts (heads, shells) of hand shelled shrimps (Pandalus borealis) were used as the pigment source. Juvenile rainbow trout, weighing 28 g on average, were divided into twelve groups of 110 fish and fed a diet of 5%, 10% and 20% shrimp waste, a diet with and without supplementation of vitamin

E and a diet containing 5.8% and 7.9% free fatty acids. The composition of the experimental diets are shown in Table 1.

Samples of 10 fish were collected 7 times during the 225 days of feeding for flesh astaxanthin determination.

The temperature of the water ranged from 5 to 12°C, dependent on the season.

Experiment 2

Rainbow trout with a mean weight of 230 g were divided into 4 groups of 38 fish. They were fed the same amount of the experimental diet, as consumed by the group with least appetite. The composition of the diets are shown in Table 2. The fat content ranged from 3.5% to 35.9%. 20% of the feed consisted of the pigment source, Red beat, Calanus finmarchicus. This resulted in astaxanthin concentrations (±S. E.M) of 19.9±0.13, whereof 40% was diester, 36% was monoester and 24% was free astaxanthin. Samples for the determination of flesh pigmentation were collected after 21 days and at the end of experiment, after 37 days.

The average temperature during the experiment was 12.6°C.

Experiment 3

Twenty one relatively well pigmented, sexually immature, rainbow trout were fed a diet without astaxanthin supplementation. The development of flesh pigmentation was followed by determining the astaxanthin concentration in samples after 2, 5, 10, 14 and 21 weeks.

The temperature ranged from 7 to 12°C depending on the season.

RESULTS

Experiment 1

There were no significant correlations between the growth rate and vitamin E supplementation ($P=0.87$), fat quality as level of free fatty acids ($P=0.89$) and the level of shrimp waste in the diet ($P=0.93$)

The level of astaxanthin in the flesh increased significantly ($P<0.000$) during the experimental period (Fig.1). The difference between groups fed different levels of shrimp waste in the diet was significant ($P=0.013$), and the pigment level in the flesh ranged according to the astaxanthin level in the diet (Fig. 2). The differences in flesh astaxanthin between groups fed the different dietary levels of shrimpwaste within each period were only significant after 195 days feeding among the groups fed 5% shrimp waste and 20% ($P=0.05$) and after 225 days for the groups fed 10% and 20% ($P=0.039$). The differences between the groups fed 5% and 10% shrimp waste were not significant at any period during the experiment.

The mean ratios of astaxanthin in flesh to astaxanthin in diet were 0.144, 0.096 and 0.057 for the groups fed 5, 10 and 20% shrimp waste respectively. These values were significantly different ($P<0.000$).

The average weight of the fishes increased from about 28g at the start to 232g at the end of the experiment (Fig.1). By dividing the samples into groups of different weights independent of diet concentration of astaxanthin, a linear relation was found between the weight groups and the astaxanthin level in the flesh (Fig.1).

There were no significant effect of vitamin E supplementation ($p=0.88$) or level of free fatty acids ($p=0.89$) on the deposition of astaxanthin in the flesh.

Experiment 2

The average content of astaxanthin in the flesh increased significantly ($P<0.001$) from 0.06 mg/kg at the start of the experiment to 0.71 ± 0.09 after 21 days and 1.5 ± 0.2 after 37 days. The level of astaxanthin in the flesh (Fig. 3.) increased significantly ($P=0.011$) with increasing levels of fat in the diet.

Experiment 3

The level of astaxanthin in the flesh decreased significantly ($P<0.001$) during the 21 weeks of experiment (Fig.4B). The level had been constant during the first 5 weeks but was followed by a rapid decline (Fig. 4). Also the total amount of astaxanthin per fish seemed to decrease, however not significantly.

DISCUSSION

Even though there are some indications of a biological function of astaxanthin in fishes (Tacon, 1981; Torrissen, 1984) the main purpose of carotenoid supplementation in commercial diets of salmonids is to achieve an acceptable pigmentation in the marketable product.

Considering only the market's demand for pigmented flesh, two biological factors should determine the regime of pigmentation: 1) absorption and deposition of ingested carotenoids, and 2) metabolic loss of deposited carotenoids.

Dietary level of astaxanthin

The digestion and rate of absorption depends on the material where the carotenoids are deposited (Kuo et al, 1976; Spinelli et al, 1974; Johnson et al, 1980; Torrissen et al, 1981) and the ester form of the pigment (Torrissen and Braekkan, 1979). The level of carotenoids in the diet, as shown in this experiment, is important both in respect to the deposition and utilization of the astaxanthin (Fig.2). As expected with time, a diet rich in astaxanthin gives a higher pigment level in the flesh than a diet low in astaxanthin. Diet 3 contained 4 times the astaxanthin concentration of Diet 1, but at the end of the experiment the fish fed on Diet 1 contained 62% of the pigment level of fish fed Diet 3 (Fig.2). These results are in agreement with those presented by Kotic et al (1974), who fed three groups of rainbow trout with 10, 20 and 30% krill meal , and found after 4 months that the fish fed 10% krill meal contained 80% of the flesh level of astaxanthin as fish fed 3 times that pigment

level. Therefore, feeding at a relatively low pigment level gives a higher ratio of astaxanthin in flesh to astaxanthin in diet (Fig.2).

Dietary level of lipids

The effect of dietary lipid level on the pigment deposition is not clear. Abdul-Malak (1975), Spinelli (1979) and Seurman et al (1979) reported results indicating an increased pigment deposition with increasing the lipid content in the diet, but Choubert and Luquet (1984) did not observe any clear effect. However, they all used relatively moderate amounts of fat in the diet, 9 to 18%, whereas in the present experiment, the fat content ranged from 3.5 to 35.9% fat. Even with a tenfold increase in the dietary fat content, the effect on flesh pigmentation was limited (Fig. 3). The deposition of astaxanthin increased by about 0.018 mg/kg per percent increase in the dietary lipid.

Dietary fat quality

Choubert and Luquet (1984) reviewed the possible effects of fat and vitamin A on the absorption and deposition of carotenoid pigments. Fat would be favorable to the absorption, but unfavorable to the stability of the carotenoids , as free radicals may oxidize the carotenoids.

In this experiment there was no significant difference in the pigmentation among the fish fed a capelin oil 'high' in free fatty acid and the fish fed a oil lower in free fatty acid. The difference among the two qualities was limited (Table 1) and possibly not big enough to cause any effect.

Depletion of flesh pigment

The depletion of astaxanthin during the 21 weeks on a diet lacking astaxanthin supplementation was significant in experiment 3. Most of this decline can be explained by the growth of the fish but not the whole metabolic loss of astaxanthin during the experiment (Fig. 4).

Effect of vitamin E

Both vitamin E and carotenoids (β-carotin) can act as antioxidants and quench singlet oxygen extremely efficiently (Tacon, 1981). It was therefore thought that exclusion of vitamin E from the diet would decrease the available amount of astaxanthin. However, in the present experiment, no significant effect of exclusion of vitamin E was found.

Effect of fish size

The rate of pigment accumulation in the flesh changed with time during the experiment (Fig.1). However, this change is mainly due to variations in the growth rate resulting from seasonal variations in the rearing temperature. Considering the mean weight of samples, linear relation was found between fish weight and astaxanthin in the flesh. Assuming an equal feed conversion rate among the fish of the same weight, the accumulation is not influenced by fish weight but the amount of feed ingested. Abdul-Malak (1975) found that rainbow trout below 150g deposited little cantaxanthin in the flesh, and similar results are found by the author in other unpublished experiments. It is therefore believed that the ability to deposit pigments is not related to body weight, but more to the physiological status of the fish.

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Table 1. Composition of the experimental diets for experiment 1.

DIET	EXPERIMENTAL DIETS					
COMPOSITION (%)	1&7	2&8	3&9	4&10	5&11	6&12
Capelin	25.0	25.0	25.0	25.0	25.0	25.0
Capelin oil *	5.3	5.2	5.3	5.3	5.2	5.3
Capelin meal	0	0.7	1.4	0	0.7	1.4
Coal fish	54.7	49.1	38.3	54.7	49.1	38.3
Shrimp waste	5.0	10.0	20.0	5.0	10.0	20.0
Dextrinated Wheat-oat meal	9.0	9.0	9.0	9.0	9.0	9.0
CMC and vit 1 **	1.0	1.0	1.0			
CMC and Vit 2 ***				1.0	1.0	1.0

* Capelin oil: Diet 1 to 6 with 5.8% Free fatty acids
Diet 7 to 12 with 7.9% Free fatty acids.

** Carboxymethylcellulose and Vitamins according to Halver's recommendation (1971).

*** Vitamins as Vit 1 minus vitamin E

Astaxanthin:
Diets 1,4,7,10 : 3.4 mg/kg
Diets 2,5,8,11 : 6.0 mg/kg
Diets 3,6,9,12 :12.1 mg/kg

The diets were calculated to contain the same level of protein and fat.

Table 2. Diet composition for Experiment 2.

DIET NR	1	2	3	4
Capelin meal, extracted, Norseamin	52.5	46.8	41.0	30.0
Coal fish muscle (trimmings)	25.5	26.2	27.0	28.0
Calanus finmarchicus	20.0	20.0	20.0	20.0
Macrell-sprat oil	0.0	5.0	10.0	20.0
Vitamins, minerals, alginat *	2.0	2.0	2.0	2.0

*Vitamins according to Halver (1971).

Dry matter g/100g	60.5	59.8	59.1	59.4
Fat g/100g dry weight	3.5	9.9	19.8	35.9

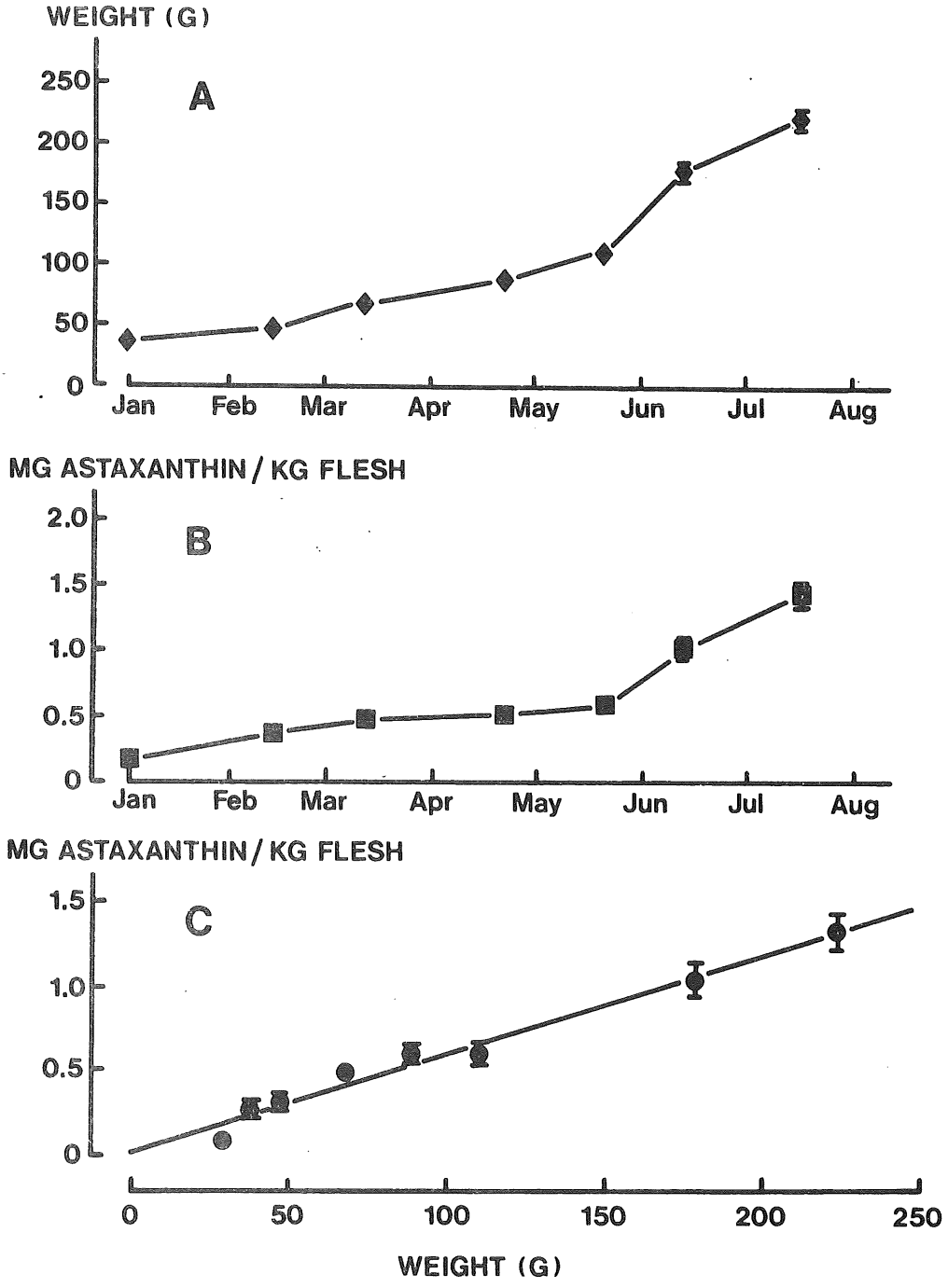

Fig.1. The weight, the average astaxanthin level in flesh and the relation between weight and flesh pigmentation of rainbow trout.

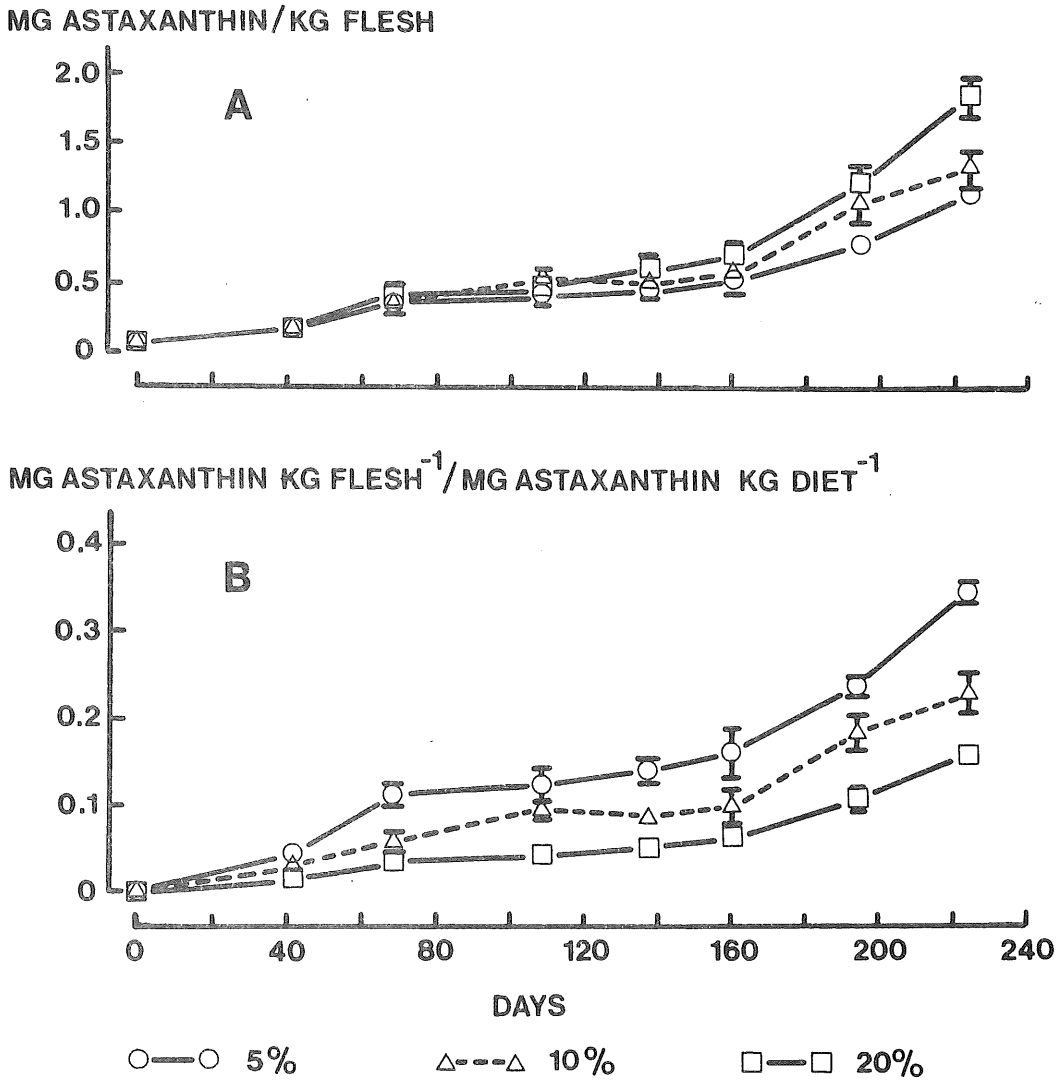

Fig.2. The effect of diet levels of shrimpwaste on astaxanthin deposition in the flesh and the ratio of astaxanthin in the flesh to astaxanthin in the diet.

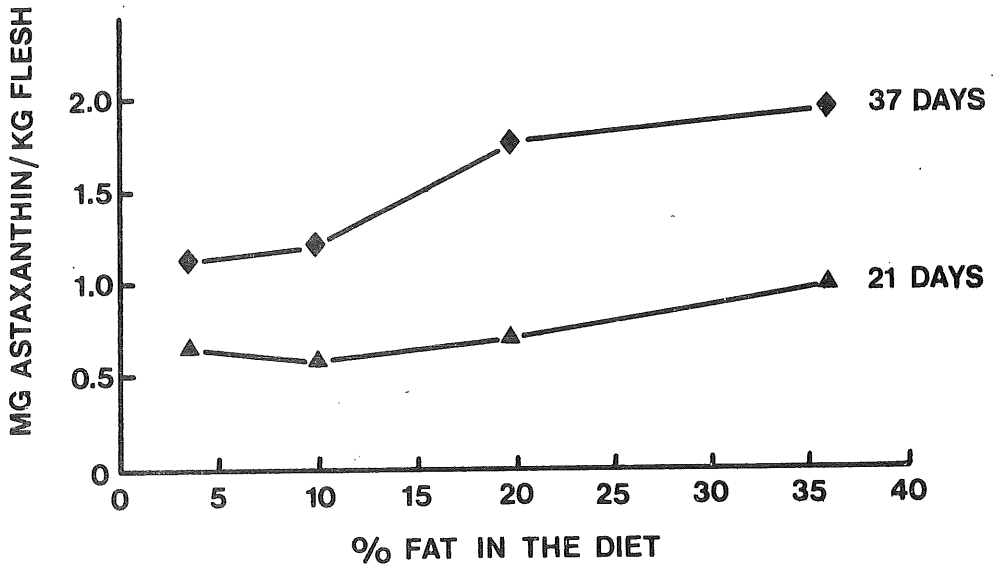

Fig.3. The pigment levels in flesh of rainbow trout after 21 and 37 days in groups fed dietary fat levels from 3.5 to 35.9%.

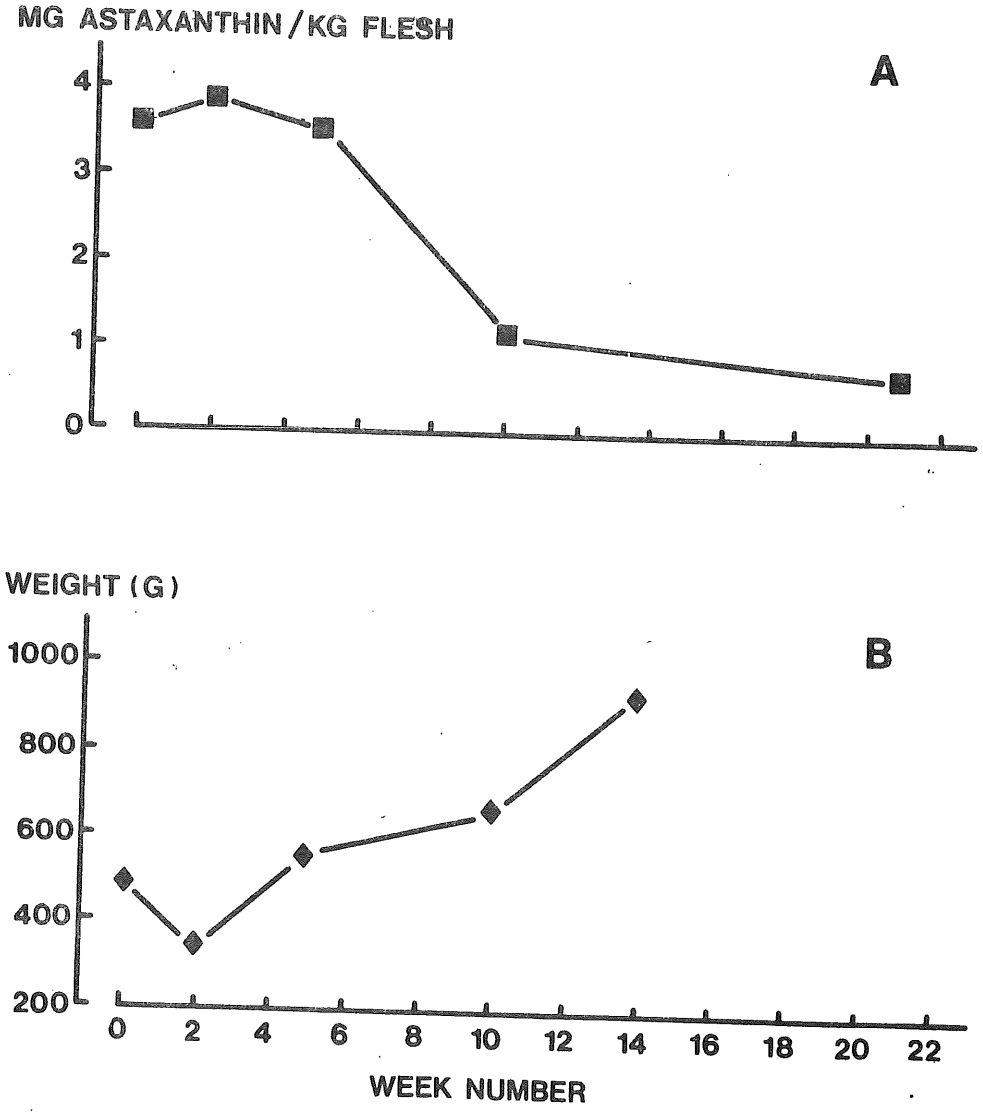

Fig.4. The astaxanthin levels in the flesh and the average weight of rainbow trout during 21 weeks feeding on astaxanthin free diet.