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EFFECT ON GROWTH AND MUSCLE CONTENTS OF TURBOT (*Scophthalmus maximus*) USING DIETS WITH DIFFERENT LEVELS OF FAT, TOCOPHEROL AND L-CARNITINE.

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ABSTRACT.-

This work studies growth in weight of 10 groups of turbot (*S. maximus*) fed on a diet in which fat, Ó-tocopherol or L-Carnitine have been incorporated, or in other cases a combination of the these. Significant differences between the groups were found, which were determined through a MANOVA with a design of repeated measurements, being $p<0.001$ for all groups. Those which presented higher growth ingested a dose of 300 mg of l-Carnitine and those presenting lower growth ingested fat (16% of dry food), without any other factors added.

The protein and lipid content analyses in carcass and liver show a difference of fat accumulation, with values varying from 7 to 20% of humid weight for those treated with l-Carnitine, Ó-tocopherol, and fat respectively according to treatment.

INTRODUCTION.-

For many years, and above all in the field of aquaculture, investigation has moved towards better and faster fattening diets, and has been based on the determination of the most satisfactory diets to attain good growth while incurring low producion costs; one of the most commonly used products for this purpose has been fat, which, although some authors refer to it

as an appropriate element for fattening diets, (Takeuchi & Watanabe, 1978; Watanabe,1982) when used over long periods of time or in elevated doses it produces non-beneficial effects, such as fat accumulation in the tissues and reduced growth rates. This implies a suppression of growth in fish in which it is used (Ringrose, 1971). For this reason, the substitution of protein for lipids as a way of reducing costs of diets and the time necessary for fattening should be examined, given the disadvantages produced by the use of fat. One of the points where fat accumulates most is the liver; an excess of fat can lead to the malfunction of this organ, consequently causing the oxidation of these fats in the fish.

While mammals possess endogenous agents preventing lipidic oxidation and facilitate their digestion and transformation, this does not happen in fish (Farkas, 1967), and so the use of anti-oxidation agents such as vitamin E is recommended (Smith, 1979; Moccia, 1984).

The properties of this vitamin as an anti-oxidant have been referred to by several authors; the National Research Council (Recommended Dietary Allowances, 1964) determined the dose of vitamin E for each gramme of PUFFA in such a way that an increase of this necessitates a greater quantity of this vitamin.

The aim of this experiment has been to compare growth in turbot juveniles using a diet maintaining a constant protein content, to which an agent, l-Carnitine or vitamin Bt, has been added, the effects of which have been cited as a growth factor since 1952 (Carter et al., 1952). It was later discovered that their activity occurs through the mitochondrial wall facilitating the transport of fatty acids; these fatty acids represent the greatest and main source of energy to the muscle (Friedman et al., 1955).

In fish l-Carnitine has been used and its effects have been described by Bilinski and Jonas, 1970. Later, it was also used in bass (*Dicentrarchus labrax*), when the same beneficial effect was reported (Santulli and D'Amelio, 1986,a,b; Santulli et al., 1988).

MATERIAL AND METHODS.-

Ten groups of 200 turbot in each have been used for this experiment, distributed at random, and a different diet has been administered to each group according to the following scheme. Each diet has been supplemented with

Treatment 1: 70g of fat
Treatment 2: 150mg of Carnitine
Treatment 3: base food
Treatment 4: 400mg of Ó-tocopherol and 150mg of Carnitine

Treatment 5: 400mg of Ó-tocopherol and 70g of fat
Treatment 6: 400mg of Ó-tocopherol and 150mg of Carnitine and 70g of fat
Treatment 7: 300mg of Carnitine
Treatment 8: 400mg of Ó-tocopherol
Treatment 9: 150mg of Carnitine and 70g of fat
Treatment 10: 300mg of Carnitine and 70g of fat

The fish were kept on fattening diets in circular polyester tanks of 1000 l capacity in which water flow was 0.5 to 0.8 litres/min./kg., with oxygen saturation of approximately 85%. The temperature and salinity of the water were registered daily; during the experiment illumination was 200 lux with a daylength of 14:10h. (Light/Dark).

The fish were fed "ad libitum" twice daily, and periodically measured and weighed. Samples of live fish and food were analysed to determine protein, fat, carbohydrate and ash content.

The food was made in the laboratory twice a week and stored at 4°C; the composition of this food was: white fish 50%; flour [1] 50%; vitamins and minerals[2]

The quantities of L-Carnitine were added according to live fish weight
The dose of Ó-tocopherol was supplemented per kg. of fat
The 70g of fat was added per kg. of food.

At the time of making the food the treatments were added according to the doses established for each of the groups.

Conversion index and specific growth were calculated periodically. To enable the comparison and determination of possible differences in growth during the experiment a multivariable analysis of variance (MANOVA) was applied with a design of repeated measurements, and Bartlett's test for the homogeneity of the variances.

When the fish were measured and weighed , samples were taken for the analysis of hepatic and muscular content; the composition of each of the diets was also determined. The techniques employed for these analyses were Kjendall methods (Bradstreet, 1965), for contents in protein, and Soxlhet device (Pearson, 1976) for lipid.

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[1]Supplied by DIBAQ S.A.

[2]Supplied by ROCHE S.A.

RESULTS.-

Temperature during the experiment ranged from 10.7 to 18.7°C; salinity varied between values of 24.4 and 34.1 ppt.

The approximate composition of the base food was

Protein	54%
Fat	8%
Carbohydrate	<1%
Ash	8%
Humidity	40%

In treatments supplemented with cod liver oil, the lipid content was 16%.

The conversion index and specific growth are shown in table 1

The muscular and hepatic contents are presented in Tables 2 and 3.

Significant differences were found between all groups in growth in terms of weight gained ($P<0.001$), and those individuals treated with 300mg of L-Carnitine presented significant differences with respect to the other groups.

Groups whose diets were supplemented with 150mg of L-Carnitine, vitamin E, or both attained a slightly lower weight gain than those treated with 300mg of L-Carnitine, and also showed significant differences with respect to the other groups whose diets were supplemented exclusively with fat.

Finally, lowest growth was found in all groups whose diet had been supplemented with fat.

According to the data determined for protein and fat content of the muscle and liver, individuals with diets supplemented with L-Carnitine (300mg) also presented a somewhat higher protein content than the others, a level only found to be higher in the group whose diet was not supplemented (Treatment 3). On the other hand, these show a higher accumulation of fat in the liver and carcass, and lower growth.

DISCUSSION AND CONCLUSIONS.-

Examining all the data, and comparing the growth values with those obtained in the previous experiment (Fernandez-Pato et al., 1991), in which levels of fat in the base food were twice as high, the lowest growth can be seen in individuals fed on fat (in

both experiments.As well in both trials, differences between groups were significant ($p<0.001$).

On the other hand, not only the substitution of lipids for proteins results in an improvement in growth (Andersen and Alsted, 1991), but furthermore, maintaining constant the protein levels,and only with the reduction of fat, improvements in weight gain are obtained.

Robert 1992(verbal comm. in Bordeaux Aquaculture) exposed that the best protein/lipid ratio was 47/12%, to get better growth, in turbot;however, our data desagree with those in terms of fat levels.

Concerning the protein content of muscles the highest levels are found in groups fed with 300mg of Carnitine(group 7), 400mg of Ó-tocopherol and 16% fat(group 5), and that fed only on the base food, nevertheless, the first of these three groups shows better growth,being the other two groups those that showed the higher fat levels in the liver.

Consistent with the previously mentioned authors (Farkas and Friedman), the results obtained in the hepatic analyses of groups 5 and 6, in which the diets of both were supplemented with fat, suggest that L-Carnitine (Treatment 6) facilitates digestion of lipids, as shown by the values of 13.8 and 20.7% fat content for the two groups respectively.

Concluding from the point of view of these data,and agree with Robert 1992, the increase of protein in diet,improve the growth in turbot.

The L-Carnitine produces a better growth,independently the amount of fat contained in food.

The adition of fat in any of levels tested has shown negative effect on growth.The adition of fat could be recommended in those cases that, in terms of market, taste of filets were required more fatty.

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GROUP	CONVERSION INDEX	SPECIFIC GROWTH(%)
1	1.3 - 2.6	1.6 - 0.5
2	1.5 - 2.2	1.1 - 0.7
3	1.6 - 3.2	1.1 - 0.4
4	1.2 - 2.8	1.9 - 0.6
5	1.8 - 2.9	1.3 - 0.7
6	1.5 - 2.5	1.8 - 0.6
7	1.8 - 1.9	2.6 - 9.9
8	2.6 - 2.6	1.8 - 9.6
9	1.3 - 3.6	2.1 - 0.6
10	2.6 - 3.7	2.1 - 0.4

TABLE 1

FOOD CONVERSION INDEX AND SPECIFIC GROWTH

(Begining and ending of experiment)

GROUP	MOISTURE	PROTEIN	FAT	CARBHYD.	ASH
1	80.20	18.30	0.53	1.60	1.20
2	79.90	18.50	0.22	1.20	0.40
3	80.21	19.90	0.35	0.40	1.20
4	79.90	18.30	0.29	0.20	1.10
5	79.00	19.50	0.42	0.10	1.20
6	80.10	18.80	0.40	0.10	1.20
7	79.50	19.40	0.20	0.10	1.20
8	80.00	18,50	0.20	0.20	1.20
9	79.70	18.80	0.50	0.10	1.20
10	80.50	18.00	0.30	0.20	1.20

TABLE 2

MUSCLE COMPOSITION IN WET WEIGHT(%)

GROUP	MOISTURE	PROTEIN	FAT	CARBHYD.	ASH
1	71.3	13,6	8.40	0.6	1.37
2	75.6	14.6	7.30	0.5	1.41
3	76.0	12.2	13,0	1.1	1.28
4	78.5	12.5	9.10	1.0	1.38
5	70.9	11.1	20.7	0.3	1.16
6	74.6	11.9	13.8	0.3	1.28
7	78.9	13.2	9.20	0.4	1.25
8	77.8	13.2	18.6	0.7	1.28
9	73.5	11.8	20.2	<0.01	1.28
10	75.2	11,6	20.5	0.3	1.20

TABLE 3

LIVER CONTENTS IN WET WEIGHT (%)