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Combined effect of lipid level and fish meal quality on liver histology of gilthead seabream (*Sparus aurata*)

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Abstract

Effect of eight diets comparing three different lipid levels (15, 22 and 27%) and two fish meal qualities were studied on growth and liver histology. Fish meal quality was judged by the content of biogenic amines and temperature processing techniques. The experiment included a comparison of pelleted feed with extruded feed for the 22% lipid diet. A total of 1140 gilthead seabream of 70 g average initial body weight were randomly stocked in 500-l fiberglass tanks in duplicate groups of 60 fish. After 2 months of experiment, the fish were transferred to 1-m^3 tanks. Fish were fed twice a day to apparent satiation for 6 months until they reached about 400 g (commercial size). Fish fed diets containing high quality fish meal showed, in general, a higher growth than those fish fed with low quality fish meal. For diets containing high quality fish meal, the fish fed 22 and 27% dietary lipid had significantly higher growth than those fish fed 15% dietary lipid. On the contrary, in diets containing low quality fish meal, only fish fed 27% dietary lipid showed significantly the higher growth rate. Fish fed the pelletized diets showed a lower growth than those fish fed extruded diets. Livers from fish fed diets containing high quality fish meal and 27% lipid showed foci of swelling hepatocytes that were not found for low quality fish meal at the same dietary lipid content. Ultrastructurally, these foci were characterized to present irregular nuclei displaced to periphery of hepatocytes and large lipid droplets in the cytoplasm. Livers from fish fed high and low fish meal qualities with 22% lipid showed similar morphological characters of

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hepatocytes to those that fed 15% lipid, but the difference was observed in the nuclei displacement.

Keywords: Gilthead seabream; Meal quality; Lipids; Growth; Histology; Liver

1. Introduction

In the last years there has been a trend in commercial fish feed formulations to increase dietary lipid levels to improve feed utilization for the optimization of production. The use of fish meal as a major source of dietary protein and lipid is also a common practice in commercial diets (Tacon, 1994), its quality being increasingly considered important for good growth performance. The combined effect of dietary lipid level and fish meal quality is of crucial importance to obtain optimal growth and reduce final production costs. However, the effect of both mentioned parameters on the quality of the produced fish must be also considered.

The use of the liver as an indicator organ of the nutritional and physiological status in fish is well-known (Hibiya, 1982; Storch and Juario, 1983; Segner and Juario, 1986). Several authors have described liver alterations produced by different nutritional factors. Godino et al. (1990) described hepatic disturbances in gilthead seabream fed diets stored at high temperature. Changes in hepatocytes of red drum fed diets containing menhaden oil and soybean oil were observed by Tucker et al. (1997). Other authors have described pathological conditions in livers as result of dietary lipid imbalances (Bautista and De la Cruz, 1988; Watanabe et al., 1989). Bell et al. (1995) observed a high degree of vacuolation due to lipid deposition in livers of turbot fed marine fish oil that was not observed in fish fed diets containing borage oil, suggesting the cause to be due to the higher level of unsaturate lipid in marine fish oil, which may present a greater risk from lipid peroxidation and be the cause of the mortalities observed. In *Clarias gariepinus* larvae, Verreth et al. (1994) showed that lipid volume in the liver of larvae fed high HUFA-enriched *Artemia* was higher than in livers of larvae fed low HUFA-enriched *Artemia*, probably due to different digestibilities. Also, these authors concluded that feeding level can result in an accumulation of lipid in the liver and be a more the most decisive parameter for larval growth and metabolic performance of the liver than feed type.

Histological studies concerning the effects of fish meal quality are scarce. Aksnes and Mundheim (1997) reported a high content of lipid in the hepatocytes with atrophic and pycnotic nuclei of halibut fed fish meal produced from spoiled raw material compared with fresh raw fish. Although, the effect of fish meal quality on growth has been studied by several authors. For salmonids, Pike et al. (1990) found an average of 15% increase in growth of fish fed diets containing high quality fish meal compared with fish fed diets containing fair so average quality fish meal. Aksnes and Mundheim (1997) reported a reduction in growth of Atlantic halibut fed fish meal containing high levels of biogenic amines.

Previous workers have shown that structural alterations of liver can provide information on diet quality, diet metabolism and the nutritional status of the fish (Storch et al.,

1983; Escaffre and Bergot, 1986; Segner and Braunbeck, 1988). Thus, the objective of this work was to study the combined effect of fish meal quality and lipid content on growth and liver histology in gilthead seabream feed these type of diets. This specie is one of the most important marine fish for Mediterranean aquaculture.

2. Materials and methods

2.1. Fish and diets experimental

A total of 1140 gilthead seabream (*Sparus aurata*) of 70 g average initial body weight provided by a local commercial farm (ADSA) were weighed and randomly stocked in 500-l fiberglass tanks in groups of 60 fish. Once the fish reached about 150 g, they were transferred to 1-m^3 tanks. The flow rate of the water in all tanks was increased from 4 to 10 l min^{-1} during the experimental period whereas temperature range from 20.7–24.4°C. Prior to starting the experiment, the fish were acclimated for 1 week with a commercial diet (Mistral 3 mm, Proaqua, Palencia, Spain). Fish were fed twice a day to apparent satiation, 6 days a week during 6 months of experimental period (April–October) until the fish reached a final body weight of about 400 g, which is a standard commercial size for the Mediterranean market. The diets were fed to duplicate groups of fish. The fish were individually weighed and measured once each month and at the end of experiment.

Eight experimental diets were prepared with different dietary lipid content (15, 22 and 27%) combined with two different fish meal qualities, ''good'' (71.6% protein, 8.0% lipid) and ''poor'' (71.5% protein, 10.0% lipid). Diet formulations and compositions are shown in Table 1. The high quality meal contained 0.5 g/kg cadaverine and 0.2 g/kg histamine and was processed at 60°C. The low quality meal contained 1.5 g/kg cadaverine and 0.24 g/kg histamine and was processed at 100°C. All diets were processed by extrusion. In addition, the 22% lipid diets were also pelletized for each of the fish meals assayed.

2.2. Histology sampling

At the end of the experimental period livers from 15 fish per tank were removed for different histological examinations.

2.2.1. Light microscopy

Livers from eight fish per tank were collected. Samples were fixed in 10% buffered formalin, dehydrated in a graded ethanol series and embedded in paraffin. Sections series of 4 μm were stained with hematoxylin and eosin (H&E).

2.2.2. Transmission electron microscopy

Livers from two fish per tank were fixed in 2.5% glutaraldehyde in 0.2 M phosphate buffer (pH 7.2). Fifty-nanometer sections of liver were stained with uranyl acetate and lead citrate and observed with a Philips CM-10 transmission electron microscopy. The diameter of lipid droplet was measured for morphometric analysis on the electron

Table 1
Ingredients and chemical composition of the experimental diets

	Diet number							
	Extruded						Pelletized	
	1	2	3	4	5	6	7	8
Ingredients (g / 100 g)								
FM ''good''	44.6	0	48.6	0	52.6	0	48.6	0
FM ''poor''	0	45.6	0	49.7	0	53.7	0	49.7
Fish oil	7.4	6.4	13.4	12.3	19.4	18.3	13.4	12.3
Soybean meal	22	22	17	17	12	12	17	17
Bread crumbles	22	22	17	17	12	12	17	17
Wheat meal	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0
Soybean lecithin[a]	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Inositol	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03
Betafin	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4
Vitamin mix[b]	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Mineral mix[c]	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Chemical composition (g / 100 g dry matter)								
Crude protein (Nx 6.25)	47.7	51.7	47.5	49.8	48.3	49.7	47.9	48.0
Lipid*	15.1	16.0	22.8	24.1	27.0	29.2	23.7	23.9
Ash	9	7.4	8.5	11.3	9.2	7.7	9	8.0
Carbohydrate[d]	28.2	24.9	21.2	14.8	12.1	13.4	19.4	20.1
Gross energy (MJ/kg)[e]	22	22	24	24	25	25	24	24
Cadaverine	0.50	1.50	0.50	1.50	0.50	1.50	0.50	1.50
Histamine	0.20	0.24	0.20	0.24	0.20	0.24	0.20	0.24

[a] Soybean lecitin obtained from Denota (Norway).
[b] Provides per kilogram feed: vitamin A 3000 IU (Rovimix A 500P); vitamin D_3 1600 IU (Rovimix D_3 500); vitamin E 160 mg (Rovimix E50SD); thiamin 12 mg (thiamin mononitrate); riboflavin 24 mg (Rovimix B280SD); pyridoxine 12 mg (pyrodoxin HCl); vitamin C 60 mg (Rovimix Stay-C25); pantothenic acid 48 mg (Rovimix Calpan); biotin 0.6 mg (Rovimix H2); folic acid 6.0 mg (Rovimix Folic 80SD); niacin 120 mg (Rovimix Niacin); vitamin B_{12} 0.024 mg (B12 1% FG); menadione Na-bisulfite 12 mg. The vitamins were obtained from Hoffman La Roche (Switzerland).
[c] Provides per kilogram feed: $MnSO_4 \cdot H_2O$ 10 mg; $MgHPO_4 \cdot 3H_2O$ 500 mg; $FeSO_4 \cdot 2H_2O$ 50 mg; $ZnSO_4 \cdot H_2O$ 80 mg; $CuSO_4 \cdot 5H_2O$ 5 mg; KH_2PO_4 400 mg; K_2CO_3 400 mg; K_2CO_3 400 mg; $CaCO_3$, 18.89 mg.
[d] Carbohydrate = NFE + fiber = 1000-protein–lipid–ash.
[e] GE = Calculated gross energy content.
* Proximate values (15, 22 or 27%) were used in all the text.

transmission microscopy plates using a video-equipped Hitachi VK-C150DE and Imago® Imagen software. Ten areas per liver were selected from fish fed the different dietary lipid levels. The mean diameter of the lipid droplets was calculated for samples from fish fed each diet.

2.2.3. Lipid identification

Livers from three fish per tank were fixed in liquid nitrogen for 5 min. Frozen sections of 5 μm were cut in cryostat (Reichert-Jung 2800 Frigocut N) and stained with Oil-Red O (70% ethanol).

2.3. Statistical analysis

All data were subjected to one-way analysis of variance (ANOVA) and differences among means were detected with Tukey's test at $P < 0.05$ level (Sokal and Rohlf, 1995).

3. Results

3.1. Growth

Growth results comparing dietary lipid levels for each quality of meal showed in general higher values for the highest dietary lipid content. Thus, for the high quality fish meal, 22 and 27% dietary lipid produced significantly ($P < 0.05$) higher growth rates than 15% dietary lipid (Fig. 1). For the low quality fish meal, 27% dietary lipid produced higher growth compared to 22 and 15% dietary lipid (Fig. 1).

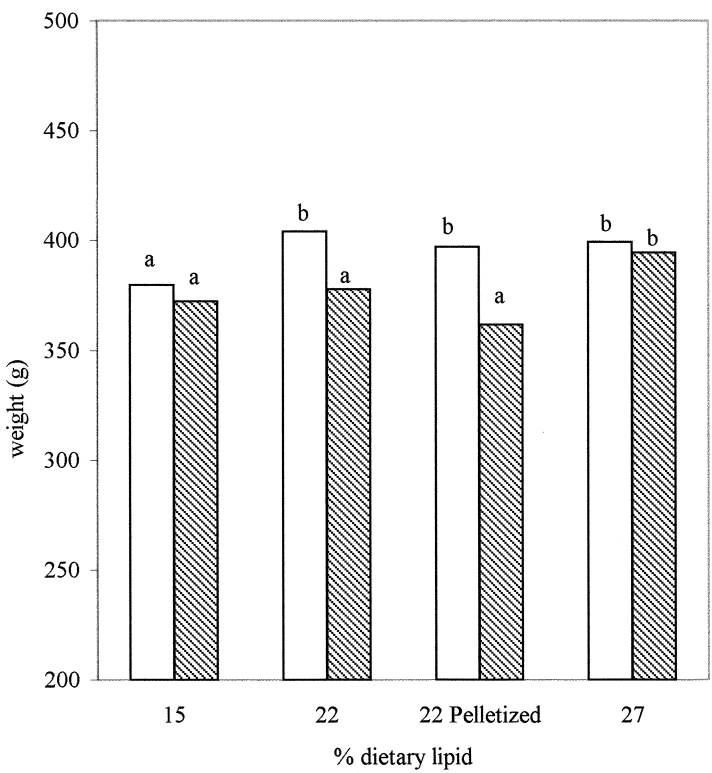

Fig. 1. Effect of dietary lipid content and fish meal quality (□ — high quality fish meal; box with diagonal lines — low quality fish meal) on growth rate of gilthead seabream. Different letters denote significant difference ($P < 0.05$).

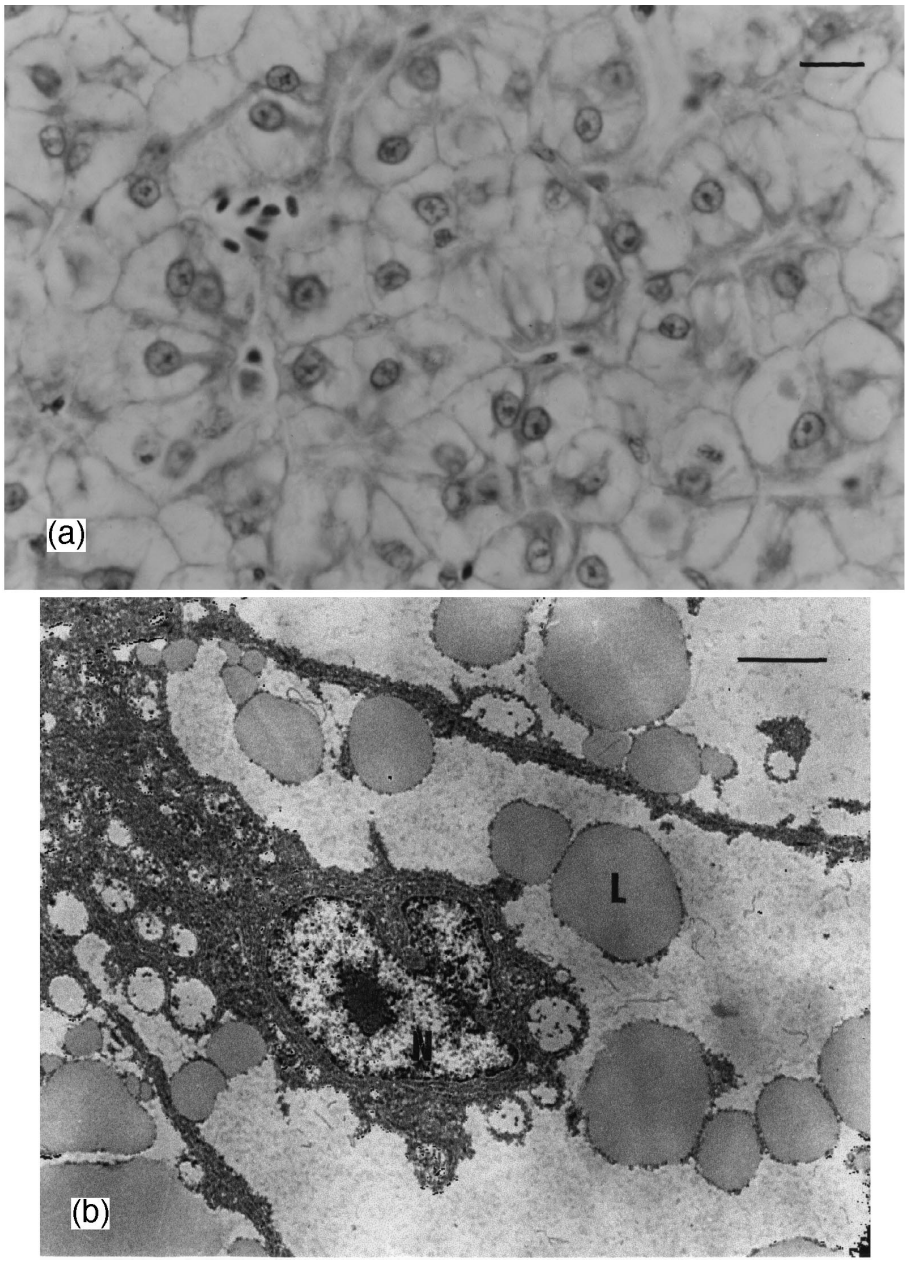

Fig. 2. Hepatocytes from fish fed experimental diets. Diet: 15% dietary lipid content. (a) Large and spherical nucleus centrally located (H&E). Bar = 10 μm. (b) Electron micrograph. Bar = 2.5 μm. N: nucleus; L: lipid droplet.

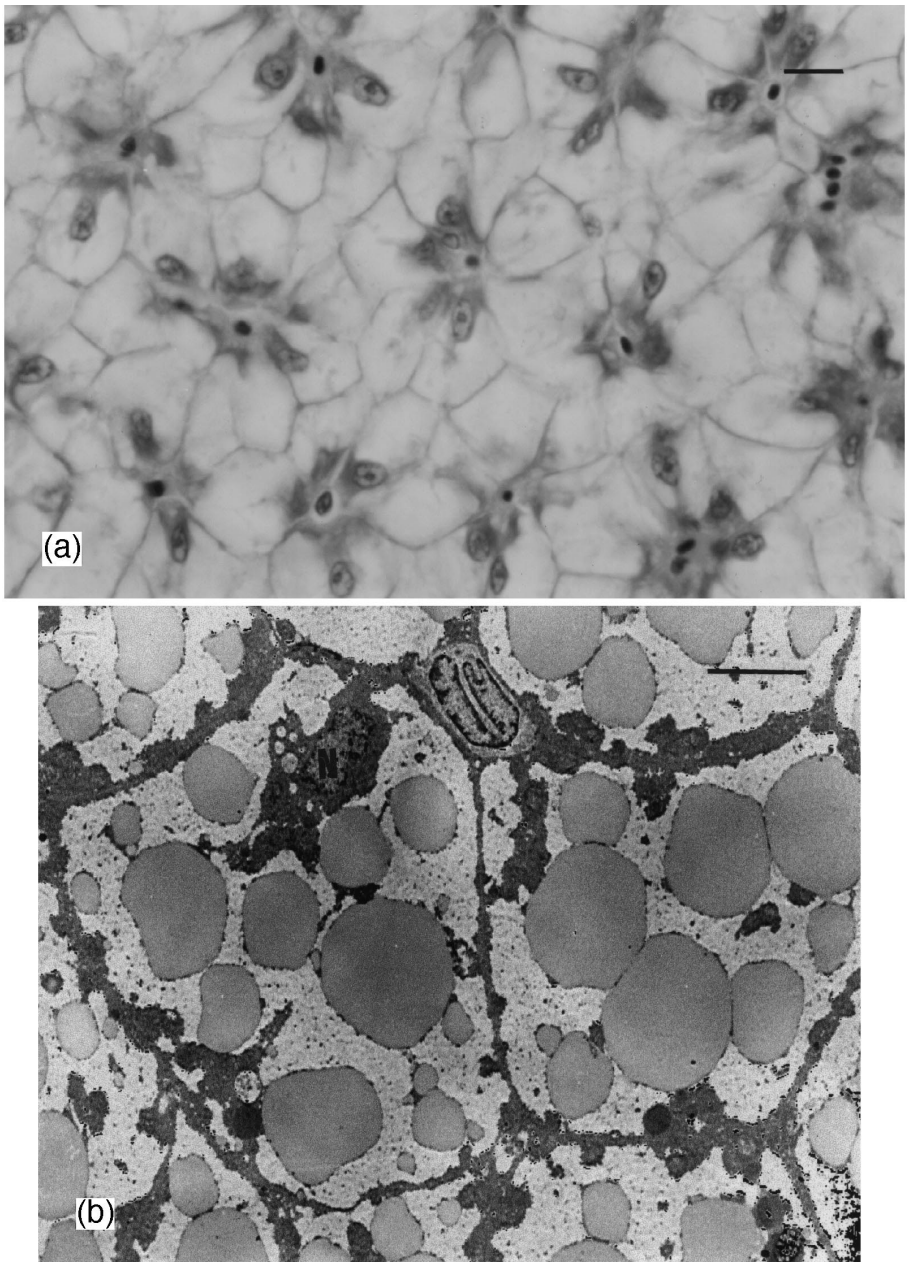

Fig. 3. Hepatocytes from fish fed experimental diets. Diet: 22% dietary lipid content. (a) Nucleus homogeneous with shape regular but displaced to hepatocyte peripheral (H&E). Bar = 10 μm. (b) Electron micrograph. Bar = 5 μm.

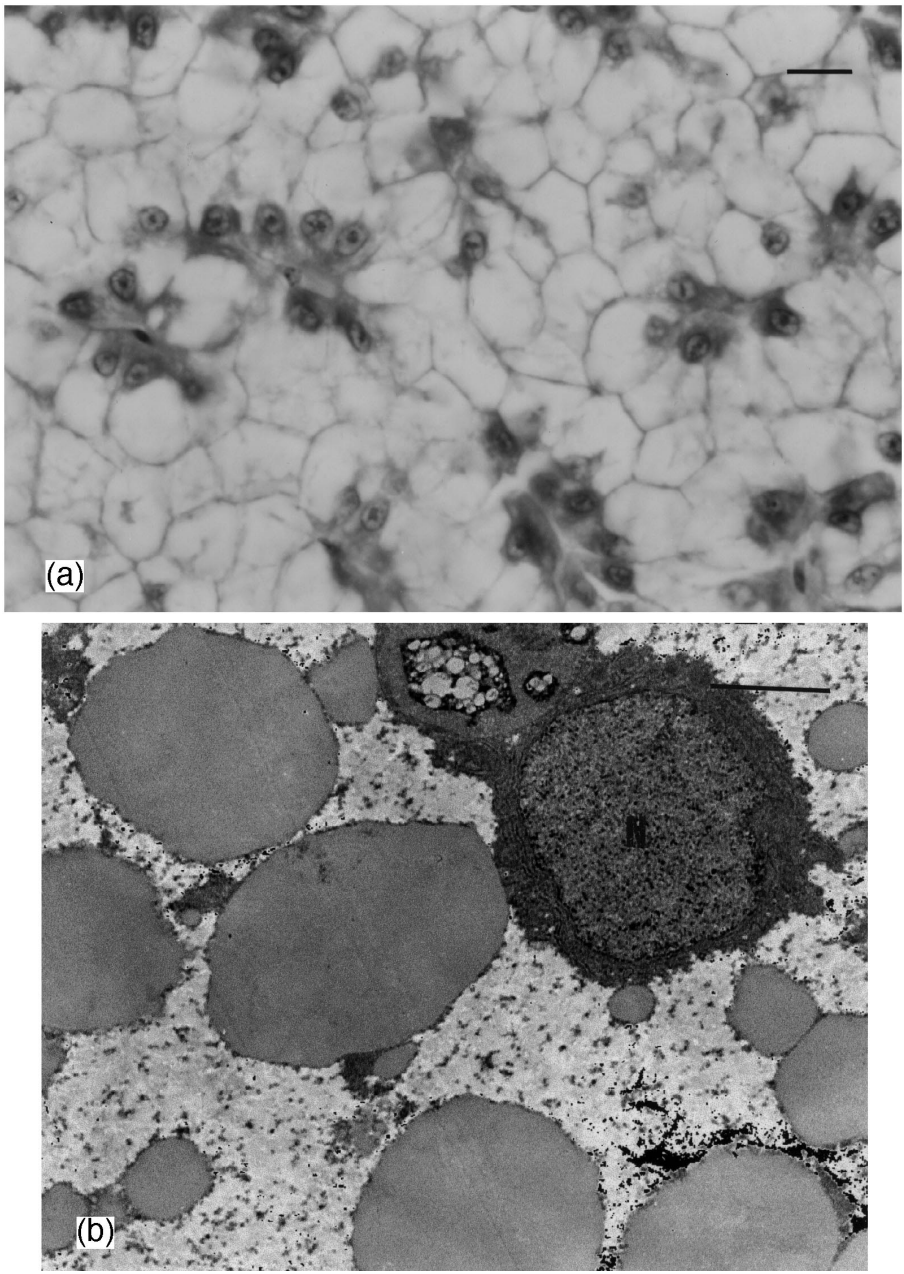

Fig. 4. Hepatocytes from fish fed experimental diets. Diet: 27% dietary lipid content and low quality fish meal. (a) Note hepatocyte with a morphology similar to livers from fish fed diets 22% dietary lipid (see Fig. 3a) (H&E). Bar = 10 μm. (b) Electron micrograph. Note the nucleus spherical. Bar = 2.5 μm.

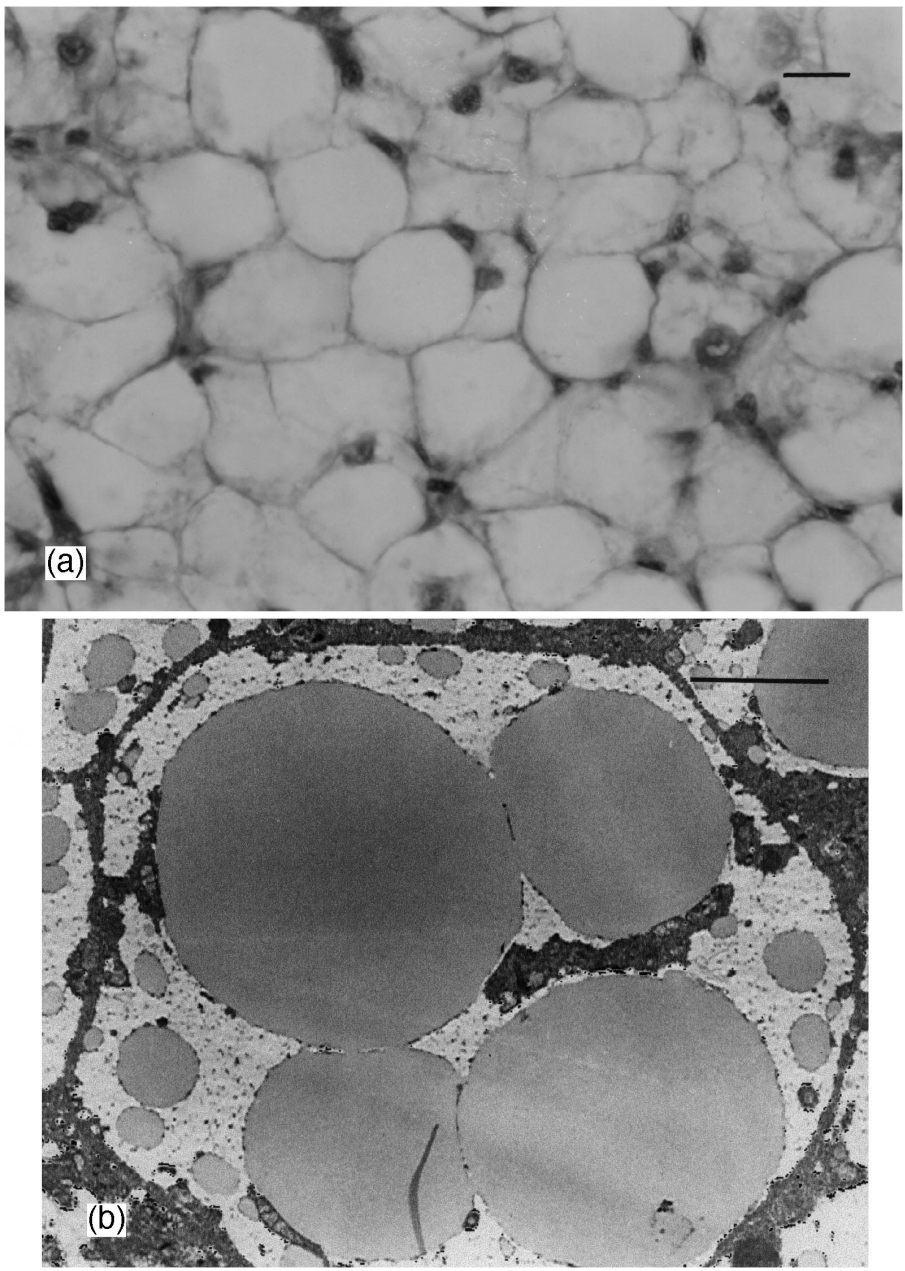

Fig. 5. Hepatocytes from fish fed experimental diets. Diet: 27% dietary lipid content and high quality fish meal. (a) Note swelling hepatocytes with enlarged irregular nucleus located at the periphery of the cell (H&E). Bar = 10 μm. (b) Electron micrograph. Note large lipid droplets. Bar = 5 μm.

Comparing growth results between the two fish meal qualities for each lipid level assayed, no differences in average final weight were found for fish fed diets with 15 and 27% dietary lipid (Fig. 1). On the contrary, for 22% dietary lipid fish fed with high quality fish meal, both pelletized and extruded, resulted in significantly ($P < 0.05$) better growth compared to fish fed diets containing the low quality fish meal (Fig. 1).

3.2. Histological examinations

Livers from fish fed 15% lipid had the same histological characteristics for both meal qualities assayed. Hepatocytes showed large and spherical nuclei centrally located with prominent nucleolus and a moderate eosinophilic cytoplasm (Fig. 2a).

There was no effect of fish meal quality on liver parenchymal morphology in fish fed the 22% lipid diet. Hepatocytes were similar to fish fed 15% lipid, the nuclei homogeneous in shape and chromatin density, but having migrated to the cell periphery (Fig. 3a).

Transmission electron microscopy studies agreed with this observation. Hepatocytes of fish fed the lowest dietary lipid levels showed a greater number of small lipid droplets (1.92 ± 1.83 μm diameter) and large nuclei with a high electron density nucleolus (Fig. 2b). On the contrary, hepatocytes from fish fed the 22% lipid diet showed a significantly ($P < 0.05$) higher lipid droplet diameter (4.35 ± 5.32 μm diameter) (Fig. 3b).

For the 27% dietary lipid level, fish fed the low quality meal showed similar characteristics as fish fed the 22% lipid diet containing the same fish meal (Fig. 4a and b).

On the contrary, fish fed the high quality meal and 27% lipid showed foci of swelling hepatocytes with enlarged irregular nuclei located at the periphery of the cell (Fig. 5a). Observation with transmission electron microscopy revealed large lipid droplets (4.47 ± 5.86 μm diameter) in a small number that coalesced among them, causing nuclei and cellular organella displacement to periphery of hepatocytes (Fig. 5b).

No differences were found in livers from fish fed the extruded and pelletized diets containing 22% dietary lipid.

4. Discussion

The quality of fish meal has been evaluated depending on the processing temperature and content of biogenic amines. Fish meal processing conditions such as temperature significantly affect the biological value of the product (Pike et al., 1990). Recent studies have demonstrated that these parameters influence fish growth (Pike et al., 1990; Moksness et al., 1995; Aksnes and Mundheim, 1997; Aksnes et al., 1997). In this way, temperature processing can affect protein digestibility, influencing in turn growth performance. Thus, Pike et al. (1990) reported that protein digestibility in mink was significantly reduced with increased processing temperature of fish meal. In addition, Aksnes and Mundheim (1997) described a reduction of 4% in protein digestibility for diets processed at high temperatures and, consequently, a reduction in growth for halibut

fed those diets. In the present study, fish growth showed a tendency to decrease when fish were fed diets prepared with low quality fish meal processed at 100°C compared to diets prepared with meals of high quality processed at 60°C. Differences in the processing temperature for the fish meals studied could be responsible for the different growth observed. In this respect, Danielssen et al. (1989) reported better growth rates in turbot fed a diet containing LT (low-temperature-processed) fish meal. Similar results have been observed for rainbow trout, Atlantic salmon (Mundheim and Opstvedt, 1989), and chinook salmon (McCallum and Higgs, 1989). Moksness et al. (1995) concluded that heat treatment may significantly alter the free amino acids in a diet, with negative effects on attractiveness and/or palatability, affecting juvenile wolffish growth.

In relation to dietary lipid level, lower growth rates were obtained in fish fed diets with 15% lipid content in both qualities of fish meal assayed. This could be related to an insufficient lipid level to meet energy needs, leading to a subsequent utilization of dietary protein for energy. This idea is in accordance with histological examinations of liver from fish fed these diets, where large and spherical nuclei centrally located in the hepatocytes and small lipid droplets were observed (Fig. 2a and b). This reflects the utilization of lipid by fish fed the lowest dietary lipid levels to obtain energy in agreement with the growth results. Thus, the low dietary lipid content of these diets may be responsible for the lack of effect of fish meal quality.

Growth of fish fed 22% dietary lipid increased in comparison with fish fed 15% dietary lipid regardless of or fish meal quality employed and indicates a protein sparing effect of dietary lipid. Protein retention is enhanced by the protein-sparing effect of dietary lipid (Kaushik and Cowey, 1991; Kaushik and Médale, 1994). Arzel et al. (1994) showed an increase in protein utilization by brown trout fed diets with high-lipid content. Van der Meer et al. (1997) reported an increase in protein deposition in *Colossoma macropomun* fed high dietary lipid levels. Vergara et al. (1996) reported a protein sparing effect of dietary lipid when increased from 9 to 15%, resulting in improved growth of *S. aurata*. In the present study, the effect of dietary lipid content was evident, but the sparing effect observed for 22% dietary lipid was more evident, for fish fed diets containing high quality fish meal that showed significantly higher growth.

The nuclear displacement to the hepatocyte periphery in livers from fish fed 22% dietary lipid and the larger lipid droplets showed in the ultrastructural study (Fig. 3a and b) may be only a reflection of liver adaptation to an increment of dietary lipid content. This could be in agreement with the results obtained by Mosconi-Bac (1987) who suggested that the presence of numerous and voluminous lipid droplets in hepatocytes may be a physiological response to lipid excess and therefore represents an energy storage but not a pathological situation. Segner and Witt (1990) also found that the increase of lipid in liver of turbot after the start of weaning may only be due to a change of feed and can be considered as an expression of a well-fed status rather than a pathological syndrome. Fontagné et al. (1998) reported that common carp larvae fed *Artemia* showed larger sized enterocytes and voluminous hepatocytes. These authors concluded that this is only a mechanical consequence of fat accumulation indicating a well-fed status and may not be related to nutritional disorders. In most marine fish, high lipid reserves in the liver appears to be an adaptive mechanism to periods of low trophic activities at low water temperatures (Kaushik, 1997).

Elevation of dietary lipid levels from 22 to 27% improved growth of fish fed low quality fish meal. However, the good growth obtained by feeding the fish 22% dietary lipid and high quality fish meal was not further improved when dietary lipid levels were increased up to 27%. This could be due to a possible sparing effect promoted by 27% dietary lipid when low quality fish meal was used, which was evident for high quality fish meal when dietary lipid levels increased from 15 to 22%.

Fish meal quality effects were observed on the hepatocytes' morphology. On the hand, for fish fed low quality fish meal similar hepatic morphology was observed when 27% (Fig. 4a and b) and 22% lipid levels (Fig. 3a) were fed. On the other hand, foci of swelling hepatocytes with enlarged irregular nuclei located at the periphery of the cell (Fig. 5a and b) were observed in fish fed the highest dietary lipid content and high quality fish meal. This observation agrees well with the fact that the increase in dietary lipid levels up to 27% was not able to further promote protein sparing in fish fed high fish meal quality, leading to an increased deposition of dietary fat in hepatocytes which was not observed when low quality fish meal was fed. Aksnes and Mundheim (1997) reported similar morphological characteristics on liver histology in Atlantic halibut fed diets with fish meal from spoiled raw materials and 16.7% lipid. These authors suggested a negative effect caused by the high biogenic amine content in that fish meal. In the present study, the biogenic amine content was lower that those reported by those authors, thus the dietary lipid level could affect liver histology more than the biogenic amine content in the diets.

The structural modifications of nuclei observed within hepatocytes could reflect a nutritional pathology. Several authors have reported that the hepatonuclear size can be used as an indicator of the nutritional condition of fish (Escaffre and Bergot, 1986; Segner and Braunbeck, 1988; Strüssmann and Takashima, 1990). Mosconi-Bac (1987) observed modifications of shape, nuclei chromatin density and the atypical deposition of lipid droplets in livers of European seabass fed artificial diets as an alteration in fatty acid metabolism, thus being signs of true nutritional pathology. Ghittino (1978) also considered this type of lipid deposition as a pathological process and has referred to it as fatty degeneration or steatosis, that can be used as an indicator of hepatic disturbances in the fat metabolism. Spisni et al. (1998) described steatosis as a liver alteration due to an excessive dietary intake of lipid which saturates the physiological capability of the liver leading to lipid droplet accumulation. Similar hepatic lipid deposition due to excessive caloric intake has been described for other vertebrates such as poultry, denoting a pathological situation (Stake et al., 1980).

In conclusion, the hepatic morphology observed in gilthead seabream fed diets containing 22% lipid and high or low quality meal reflects the storage of lipidic reserve without pathological consequences, whereas for diets with 27% lipid and high quality meal the steatosis observed could reflect the non-utilization of dietary lipid which was not observed for diets with low quality fish meal and same lipid level. Hence, these results show that the beneficial effects of dietary lipid level follow a curves and the location of the optimum point depends on the quality of the fish meal.

Finally, this work confirmed that the study of histological alterations due to nutritional imbalances provides an idea of the cell and tissue condition, complementing the information obtained with growth studies to evaluate the nutritional status of fish.

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