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EFFECTS OF DIETARY FISH OIL, SOY-ACID OIL, AND YELLOW GREASE ON GROWTH AND HEPATIC LIPIDOSIS OF HYBRID TILAPIA FRY

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Key words: hybrid tilapia fry, soy-acid oil, yellow grease, hepatic lipidosis

Abstract

The objective of this study was to compare the effects of dietary lipids on growth and liver histopathology of hybrid tilapia, *Oreochromis niloticus x O. aureus*, fry (6.0 g). Fish were fed one of six diets containing 8.4% fish oil (control), 8.4% soy-acid oil, 8.4% yellow grease, 5.6% yellow grease plus 2.8% soy-acid oil, 2.8% yellow grease plus 4.6% soy-acid oil, or 4.2% soy-acid oil plus 4.2% yellow grease for 60 days. Growth was similar in all groups and retarded in comparison to earlier studies. Lipid accumulation as well as microvesicular (foamy degeneration) and macrovesicular degeneration in the liver were histopathologically detected.

Introduction

The demand for fish oil, the most frequently-used oil in the fish feed industry, is predicted to exceed resources within the next decade (Barlow and Pike, 1999). Partial or total replacement of fish-based feeds by vegetable meals and oils is important for the development of aquaculture (Kaushik, 2004). Recent studies demonstrated that, in some tropical fish, up to 90% of the dietary fish oil can be replaced by vegetable oils without causing problems to growth or feed utilization (Ng et al., 2000; Lim et al., 2001).

Soy-acid oil was used as an alternative vegetable lipid source in broiler diets in the

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1980s (Sevgican et al., 1986) and later in rainbow trout diets (Canyurt et al., 1991). In both studies, a supplementary lipid level of approximately 8.0% improved growth parameters. Yellow grease has also been used in broiler diets and consists of vegetable and animal lipid sources. The low cost and year-round availability of soy-acid oil and yellow grease justifies investigating the use the use of these lipid sources in aquafeeds.

As in other vertebrates, the type of lipid affects growth parameters, body composition, and the histological structure of organs in fish (Dossanjh et al., 1984). Lipid metabolism is mainly regulated by the liver. Fat storage affects fat metabolism, uptake of dietary fat, mobilization of fatty deposits as in acute diseases, and synthesis or degradation of fatty acids, triglycerides, cholesterol, and lipoproteins. Thus, when dietary lipid or energy exceeds the capacity of the hepatic cells to oxidize fatty acids, or when protein synthesis is impaired, the result is synthesis and deposition of large amounts of triglycerides in vacuoles, leading to the morphological condition known as steatosis or hepatic lipidosis. Steatosis is associated with nutritional imbalances in cultured fish (Tacon, 1996; Caballero et al., 2004).

The aim of this study was to compare the effects of dietary soy-acid oil and yellow grease on growth parameters and liver morphology in hybrid tilapia, *Oreochromis niloticus x O. aureus*, fry.

Materials and Methods

Six practical diets were formulated (Table 1). For each diet, the major ingredients were ground (<500 µ) and mixed, and warm water (40°C) and the lipid source(s) were added into the blend. The resultant dough was passed through a 2 mm diameter die in a food grinder. The pellets were dried at 45°C and stored at 4.0±1.0°C until use.

Hybrid tilapia fry (*Oreochromis niloticus x O. aureus*; 6.0 g) were obtained from a local fish hatchery (DSI, the VIth regional directorate of the state hydraulic works, Adana, Turkey) and stocked at 15 fish per 96-l glass aquaria in 18 aquaria (triplicates of six treatments). After 10 days acclimization, the experimental diets were given the fish *ad libidum* each day at 10:00-16:00. The daily water exchange rate was 80%. Water remained at the constant temperature of 25±1°C. Oxygen varied 6.2-6.5 mg/l, pH 7.82-8.33, and total alkalinity 250-255 mg $CaCO_3$/l. The feeding trial was conducted for two months.

The proximate compositions of the diets and fish fillets were analyzed according to AOAC (1997) procedures as follows: moisture was determined by oven-drying at 105°C for 24 h, crude protein (N x 6.25) by the Kjeldahl method, and crude ash by combustion in a muffle furnace at 550°C for 16 h. Total lipid concentration was determined by extract with the chloroform-methanol method described by Bligh and Dyer (1959). On completion of the feeding trial, all fish were starved for 48 h, killed, and weighed. All fish were dissected to determine hepatosomatic index values and for histopathological examination. Liver specimens were manually fixed (4% neutral buffered formaldehyde) for histology and embedded in paraffin wax. Sections (5 µ) were cut and mounted on glass slides (Leica) before staining with Mayers Hematoxylin and Eosin (H&E). Stained sections were examined and photographed under a light trinocular (Olympus BX50) microscope (Takashima and Hibiya, 1995). Data were statistically analyzed with one-way ANOVA and Duncan's multiple range tests (SPSS for Windows, version 10.01. Chicago, IL).

Results

There were no significant differences in weight gain, feed conversion ratio, or body indices among the treatment groups (Table 2). No mortality was observed. From highest to lowest, liver degeneration (HSI) was: diet 2>6>5>3>4>1 and lipid degradation of the fish muscles (VSI) was diet 1>2>4>6>3>5, with no significant differences. There were some significant differences in final carcass compositions among groups.

Lipid accumulation as well as microvesicular (foamy degeneration) and macrovesicular degeneration in the liver were histopathologi-

Table 1. Composition (%) of the experimental diets.

	Diet					
	8.4% fish oil	*8.4% soy-acid oil*	*8.4% yellow grease*	*5.6% yellow grease plus 2.8% soy-acid oil*	*2.8% yellow grease plus 5.6% soy-acid oil*	*4.2% yellow grease plus 4.2% soy-acid oil*
Diet no.	1	2	3	4	5	6
Fishmeal	28	28	28	28	28	28
Soybean meal	26	26	26	26	26	26
Corn bran	20	20	20	20	20	20
Cotton seed cake	15.5	15.5	15.5	15.5	15.5	15.5
Fish oil[1]	8.4	-	-	-	-	-
Yellow grease[2]	-	8.4	-	5.6	2.8	4.2
Soy-acid oil[2]	-	-	8.4	2.8	5.6	4.2
Premix[3]	1	1	1	1	1	1
DCP	1	1	1	1	1	1
Protein	36.67	36.67	36.67	36.67	36.67	36.67
Lipid	12.36	12.36	12.36	12.36	12.36	12.36

[1] Fish oil was obtained from a factory in Sinop, Turkey, that processes anchovies for fishmeal.
[2] Yellow grease and soy-acid oil were purchased from a factory in Istanbul that processes the wastes of different oil sources.
[3] Premix (for 1 kg diet): 5,000,000 IU vitamin A; 1,250,000 IU vitamin D; 12,500 mg vitamin E; 1,250 mg vitamin K_3; 750 mg vitamin B_1; 2,000 mg vitamin B_2; 15,000 mg niacin; 5,000 mg calpan; 1,750 mg vitamin B_6; 8 mg vitamin B_{12}; 375 mg folic acid; 25 mg biotin; 50,000 mg vitamin C; 225,000 mg choline chloride; 12,500 carophyll red; 2,500 mg carophyll yellow; 50,000 mg Mn; 50,000 mg Fe; 50,000 mg Zn; 10,000 mg Cu; 150 mg Co; 800 mg I; 150 mg Se.

cally detected (Fig. 1). Severe hepatic lipidosis was especially observed in Diets 2, 3, and 4. Also steatotic cells, large extracellular fat globules, disruption of the hepatic microcirculation, and hepatocyte abnormalities (cytoplasmic clarification) associated with steatosis were found.

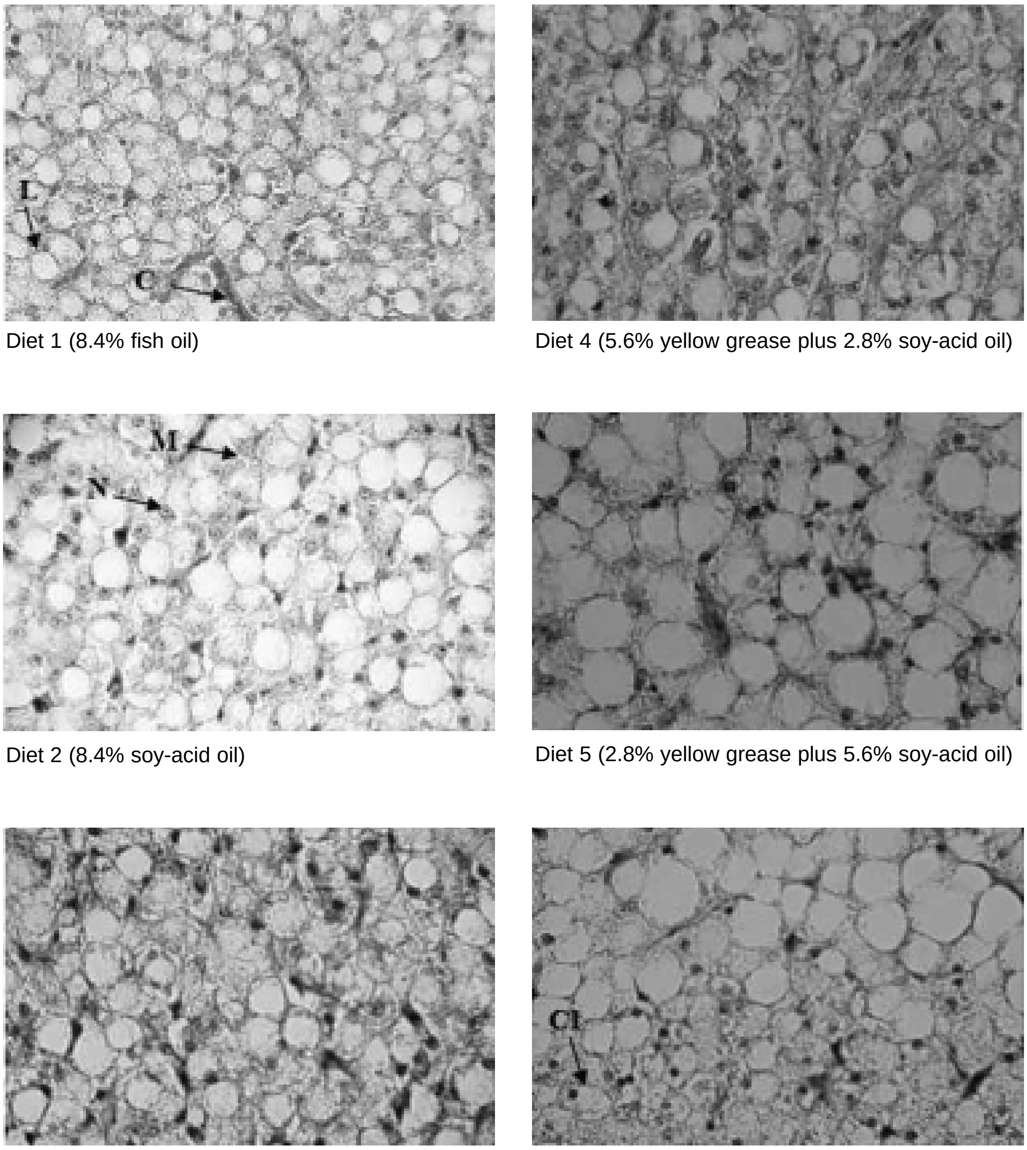
Discussion

Some warm water fish species such as *Tilapia zillii* (El-Sayed and Garling, 1988), African catfish (*Clarias gariepinus*; Lim et al. 2001), and sunshine bass (*Morone chrysops* x *M. saxatilis*; Keembiyehetty and Wilson, 1998) efficiently use dietary lipids up to a cer-

Table 2. Growth, body indices, and carcass composition of *Oreochromis niloticus* x *O. aureus* fry fed one of six experimental diets (means of triplicate groups of five fish).

	Diet					
	8.4% fish oil	*8.4% soy-acid oil*	*8.4% yellow grease*	*5.6% yellow grease plus 2.8% soy-acid oil*	*2.8% yellow grease plus 5.6% soy-acid oil*	*4.2% yellow grease plus 4.2% soy-acid oil*
Diet no.	1	2	3	4	5	6
Initial weight (g)	6.51±0.02	6.40±0.06	6.53±0.03	6.38±0.09	6.40±0.03	6.24±0.11
Final weight (g)	16.13±0.56^{a}	14.98±0.42^{a}	15.37±1.11^{a}	14.64±0.38^{a}	14.35±0.27^{a}	14.35±0.64^{a}
Weight gain (g)	9.62±0.56^{a}	8.58±0.35^{a}	8.83±1.08^{a}	8.27±0.45^{a}	7.95±0.28^{a}	8.11±0.63^{a}
Feed conversion ratio	1.67±0.09^{a}	1.76±0.07^{a}	1.87±0.19^{a}	1.95±0.10^{a}	2.01±0.07^{a}	1.95±0.11^{a}
HSI (%)[1]	1.81±0.13^{a}	2.12±0.14^{a}	1.93±0.14^{a}	1.92±0.10^{a}	2.01±0.09^{a}	2.02±0.10^{a}
VSI (%)[2]	10.08±0.92^{a}	10.05±0.43^{a}	9.94±0.20^{a}	10.04±0.42^{a}	9.87±0.38^{a}	9.97±0.57^{a}
K (%)[3]	1.47±0.02^{b}	1.51±0.04^{ab}	1.52±0.03^{ab}	1.51±0.04^{ab}	1.62±0.04^{a}	1.50±0.03^{ab}
Carcass composition[4]						
Dry matter	24.73±0.16^{b}	27.43±1.85^{a}	26.68±0.95^{a}	26.57±0.06^{a}	27.06±1.55^{a}	27.58±0.69^{a}
Ash	1.62±0.13^{b}	1.52±0.07^{b}	1.81±0.13^{a}	1.46±0.08^{b}	1.45±0.04^{b}	1.39±0.04^{b}
Protein	19.25±0.38^{b}	22.36±1.93^{a}	22.15±1.01^{a}	22.16±0.13^{a}	22.95±1.58^{a}	22.14±0.81^{a}
Lipid	3.85±0.08^{ab}	3.55±0.07^{b}	2.72±0.08^{c}	2.96±0.15^{c}	2.66±0.07^{c}	4.05±0.07^{a}

Means in rows with different superscripts are significantly different ($p<0.05$).
[1] Hepatosomatic index = (liver wt/total body wt) x 100
[2] Visserosomatic index = (viscera wt/total body wt) x 100
[3] Condition factor = (total body wt/length^{3}) x 100
[4] Initial carcass composition was 22.55±0.02% dry matter, 2.81±0.76% ash, 15.63±0.58% protein and 4.11±0.15% lipid.



Diet 1 (8.4% fish oil)

Diet 4 (5.6% yellow grease plus 2.8% soy-acid oil)

Diet 2 (8.4% soy-acid oil)

Diet 5 (2.8% yellow grease plus 5.6% soy-acid oil)

Diet 6 (4.2% yellow grease plus 4.2% soy-acid oil)

Diet 3 (8.4% yellow grease)

Fig. 1. Hepatic lipidosis (hepatocellular vacuolization) in *Oreochromis niloticus* x *O. aureus* fry stained with hematoxylin and eosin. L = large lipid droplet, C = capillary, M = microvesicle, N = hepatocyte nucleus, Cl = cytoplasmic clarification. Diet 1: diffuse micro and macrovesicular degeneration (x 80); diet 2: diffuse macrovesicular, centrilobular degeneration, with diffuse microvesicular vacuolar change (x 100); diet 3: fat in macrovesicles peripheral nuclei, no cellular damage (x 100); diet 4: normal or slightly moderate macro and severe micro vacuolization (x 100); diet 5: fat in microvesicles and fine droplet fatty changes (large clear bubbles or vacuoles within the liver cells; x 100); diet 6: fatty change with macrovesicular fat showing a slight (upper side) predominance and cytoplasmic clarification (hepatocyte abnormalities) associated with steatosis (x 80).

tain level. Other authors found that the muscle lipid content of sea bass and hybrid striped bass is unaffected by the dietary lipid level (Peres and Oliva-Teles, 1999; Gaylord and Gatlin, 2000). The levels of dietary lipids were equal in all treatments in the present study yet the muscle lipid contents were higher in diets 1, 2, and 6 than in diets 3, 4, and 5 and than found in other studies (Samantaray and Mohanty 1997; Mathis et al., 2003). Thus, a direct relationship was not found between the dietary and muscle lipid contents. Slightly higher percentages of muscle lipid and lipid accumulation in the liver were detected in soy-acid oil group, perhaps as a result of different digestibility of the lipid source.

Nutritional and pathological studies of high lipid inclusion or nutritional imbalances in fish diets support our pathological findings (Tacon, 1996; Spisni et al., 1998; Caballero et al., 1999, 2002; Manera, 2003). In fatty liver, lipid accumulates in the cytoplasm of hepatocytes creating large clear vacuolar spaces within the cells that are visible in H&E stained sections. The nuclei of such cells are pressed to the periphery of the cell. These changes occur with various types of liver degeneration in higher vertebrates, including the early stages of cirrhosis (Erikkson et al., 1986; Bacon et al., 1999; Reid, 2001). The effects of lipid in the correct functioning of the liver and possible reversibility are not well understood. Some authors consider steatosis a physiological adaptation to the diet (Segner and Witt, 1990; Caballero et al., 1999) while others stress the pathological significance of steatosis (Mosconi-bac 1990) even if necrosis or cellular damage is not found, arguing that longer periods of feeding would irreversibly damage the tissue (Caballero et al., 2004). In the current study, the lipid level was the same in all treatments yet lipoid degeneration varied. Perhaps the different lipid sources had different levels of digestibility and some accumulated more in the viscera than in the carcass (Murai et al., 1985).

In conclusion, the present study showed that the inclusion of vegetable oils in pelleted feeds did not change the growth performance or feed conversion rate. While inclusion of yellow grease or/and soy-acid oil might be desirable for economic reasons, the histopathological findings show that this is harmful to fish health. Therefore, it is recommended to continue using fish oil in feeds for tilapia fry.

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