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Effects of Alternative Dietary Lipid Sources (Soy-acid oil and Yellow grease) on Growth and Hepatic Lipidosis of Common Carp (*Cyprinus carpio*) Fingerling: A Preliminary Study

Erdal Yılmaz[1], Ercument Genc[1,*]
[1] Dept. of Aquaculture, Faculty of Fisheries and Aquaculture, Mustafa Kemal University, Tayfur Sokmen Campus 31034, Hatay, Turkey.

* Corresponding Author: Tel.: +90. 326 2455845/1302, Fax: +90. 326 2455817;
E-mail: egenc@mku.edu.tr

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Abstract

A 60 days feeding trial was conducted to determine the effect of increasing dietary lipid (alternative lipid source: soy-acid oil, SAO and yellow grease, YG) levels on growth performance, body composition and liver morphology of common carp. Seven isonitrogenous practical diets were formulated to contain 4, 8.5, 13, 18% SAO and 4, 8.5, 13% YG and the control diet without supplementation with dietary oil. Growth performance of fish fed diet containing 8.5% YG showed the best weight gain and was similar to the control group in respect to feed conversion, daily feed intake, protein and, energy consumption. No improvement was found in growth parameter in SAO-fed groups. In addition, liver lipoid degeneration (steatosis) was observed in fish fed as the highest dietary lipid. The preliminary results indicated that common carp did not efficiently utilize SAO and YG as an alternative dietary lipid source.

Key Words: Cyprinus carpio, soy-acid oil, yellow grease, nutritional pathology, steatosis, alternative dietary lipid

Introduction

It has been possible to manufacture high lipid diets with the recent technological advances in the aquafeed industry. Fish oil has been used at higher dietary lipid source as a general practice for its protein sparing effect, but the demand for fish oil by the aquafeed industry has been predicted to exceed the available resources within the next decade (Barlow and Pike, 1999). Therefore, use of different kinds of alternative lipid sources in fish nutrition should be investigated in studies. Alternative oils obviously represent more sustainable sources in most countries. Ng *et al.* (2000) and Lim *et al.* (2001) demonstrated that up to 90% of dietary fish oil can be replaced by vegetable (palm) oils without compromising growth or feed utilization of some fish species such as *Clarias gariepinus* and *Mystus nemurus*. Providing fish with adequate energy through dietary lipids can minimize the use of expensive protein (Watanabe, 1977; Beamish and Medland, 1982; De Silva *et al.*, 1991; Murthy and Naik, 1999).

Although high lipid diets improve growth rate, and feed and protein utilization, these may be associated with rancidity and lowered flesh quality due to lipid oxidation (Scaife *et al.*, 2000). Partial replacement of fish oil by certain vegetable oils has proved feasible in several species without affecting growth negatively (Caballero *et al.*, 2002; Bell *et al.*, 2003; Izquerdo *et al.*, 2003; Regost *et al.*, 2003). However, the effects of vegetable oils on lipid metabolism and health status of fish remain unclear.

Lipid metabolism is mainly regulated by the liver including both the synthesis and degradation of fatty acids. Several enzymes regulating these pathways show varying affinities for the different fatty acids available in the liver, and thus the imbalances in the dietary fatty acids could modify the function and morphology of this organ (Caballero *et al.*, 2004). Liver steatosis (vacuolization) has been observed to be associated with nutritional imbalances, increase in dietary lipid, essential fatty acid deficiency and the use of vegetable oils in cultured fish (Tacon, 1996; Alexis, 1997; Caballero *et al.*, 1999; Montero *et al.*, 2001). The protein-sparing effects of soy-acid oil (SAO) and yellow grease (YG) have not been studied in warmwater fish. The SAO is manufactured by acidulation of soapstock (acidulated fatty acids) with predominance of free fatty acids. In addition, the YG has been used especially in broiler (poultry) diets and consists of both vegetable and animal lipid sources.

Specific aims of this study are to determine the effects of two different lipid sources (YG and SAO) on growth, feed utilization, feed consumption, and body composition and liver histopathology of common carp fingerlings.

Materials and Methods

Fish, Diets and Experimental Design

The feeding trial was conducted with common carp fingerlings obtained from a local fish hatchery (The VIth Regional Directorate of the State Hydraulic Works, DSI, Adana, Turkey). All fish were acclimatized in a 1000 L fiberglass tank upon arrival and, after 10 days, groups of 10 fish averaging 18.6 g were weighed, stocked into 96 L glass aquaria, and assigned one of the eight experimental diets. The aquaria system was housed inside a room and each

aquarium was supplied with continuous aeration. A static system was used, and 80% of the water in each aquarium was changed daily. Water temperature remained constant at 26±1°C throughout the feeding trial. Oxygen (mgL^{-1}), pH and total alkalinity (mgL^{-1} $CaCO_3$) varied between 6.2-6.5, 7.82-8.33, and 250-255, respectively. The feeding trial was conducted for two months. Each diet was fed to triplicate groups of fish ad libitum per day (at 10:00 and 16:00 h).

Eight practical diets containing 35% crude protein, three and four lipid levels of two different sources, SAO and YG were formulated respectively. Diet 1 (control diet) was not supplemented with any lipid source and contained 3.6% lipid (with 2.5% coming from anchovy fish meal and the remainder from other ingredients). Diets 2, 3, 4 and 5 were supplemented with 4%, 8.5%, 13% and 18% SAO, and diets 6, 7, and 8 were supplemented with 4%, 8.5% and 13% YG, respectively. Mixed ingredients passed through a 2 mm diameter die in a food grinder. The pellets were dried at 45ºC and stored frozen (-20±1ºC) until use (Table 1).

Sampling Procedures and Analyses

The proximate composition of diets and fish fillets were analyzed according to the methods described in AOAC (1997). On completion of the feeding trial, all fish were starved for 48 h (to empty the digestive tract), killed and weighed. Liver from five fish per aquarium were used for hepatosomatic index values and histopathological investigation. For histology fixed (4% buffered formaldehyde) liver specimens were processed manually and embedded in paraffin wax. Sections (5 µ) were cut and mounted on glass slides before staining with Mayers Haematoxilen and Eosine. Stained sections were examined and photographed under light trionocular (Olympus BX50) microscopy (Takashima and Hibiya, 1995).

The remaining five fish were pooled and stored frozen for proximate analysis. Data were statistically analyzed with one-way ANOVA and Duncan's multiple range tests. Statistical analyses were performed using SPSS for Windows (Standart Version 9.0.0. SPSS Inc. Illinois).

Results

The best weight gain and feed conversion were obtained in the fish fed diet 7 (8.5% YG). Live weight gain, protein retention and feed conversion of fish fed diets 6, 7 and 8 were significantly higher ($P<0.05$) compared to the fish fed diets 2, 3, 4 and 5 ($P<0.05$), while no significant differences were detected between the control and diet 6, 7 and 8 ($P>0.05$). Daily energy consumption of fish fed diets with increasing level of YG increased, and no significant differences in feed or protein consumption were observed among fish fed with the diet 1 (control) and

Table 1. Ingredient and proximate composition of the experimental diets (g kg^{-1} dry diet)

Ingredients	Diet							
	Control	acid oil	yellow grease					
	1	2	3	4	5	6	7	8
Fish meal[a]	250	250	260	270	280	250	260	270
Soybean[b] meal	250	270	280	300	320	270	280	300
Corn meal[c]	190	180	190	190	150	180	190	190
Barley meal[d]	100	80	60	10	10	80	60	10
Wheat bran[e]	50	30	10	10	0	30	10	10
Cotton seed cake[f]	130	120	95	70	40	120	95	70
Lipid source[g]	0	40	85	130	180	40	85	130
Vit-min mix[h]	10	10	10	10	10	10	10	10
Corn starch	10	10	0	0	0	10	0	0
DCP	10	10	10	10	10	10	10	10
Proximate composition								
Crude protein	356	358	352	357	353	358	352	357
Crude lipid	36	70	110	154	205	70	110	154
CP/DE (g proteinMJDE^{-1} kg^{-1} diet)	32.81	30.28	27.65	26.07	23.43	30.28	27.65	26.07

[a] Anchovy fish meal contained (% dry weight): 69.6 crude protein, 10.7 crude lipid, 10.7 ash.
[b] Solvent extracted contained (% dry weight): 44.29 crude protein, 1.6 crude lipid, 6.5 ash
[c] Obtained from local market and contained (% dry weight): 7.66 crude protein, 2.8 crude lipid, 2.49 ash.
[d] Obtained from local market and contained (% dry weight): 10.28 crude protein, 1.8 crude lipid, 2 ash.
[e] Obtained from local market and contained (% dry weight): 12.29 crude protein, 2.2 crude lipid, 4 ash.
[f] Solvent extracted contained (g/100 g dry weight): 38.87 crude protein, 1.2 crude lipid, and 6.96 ash.
[g] Both lipid sources were purchased from Prometa Inc., Istanbul, Turkey.
[h] 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 Viamin 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.

diet 2 (Table 2). Hepatosomatic indices showed no differences (P>0.05) between the groups. All fish survived and no mortality was observed. The final carcass composition of all groups was not statistically different (P>0.05), except for carcass lipid content. YG groups (diet 6, 7 and 8) had lower carcass lipid content than control (diet 1) and the SAO (diets 2, 3, 4 and 5) groups (Table 3).

Different degrees of lipid accumulations in livers were detected. Fish fed diet 1 was found normal with moderate lipidosis, fish fed diets 2, 3, 4, 6 showed macrovacuolization in their livers. Serious fatty changes -steatosis- in fish liver fed diet 5 (with melano-macrophages centers) and diet 8 (with centrilobular degeneration) were determined (Figure 1).

Discussion

Fish fed diets with elevated levels of YG attained higher live weight and better feed conversions than that of fish fed SAO. These results may be attributable to the lower utilization of dietary SAO compared to YG in common carp fingerlings.

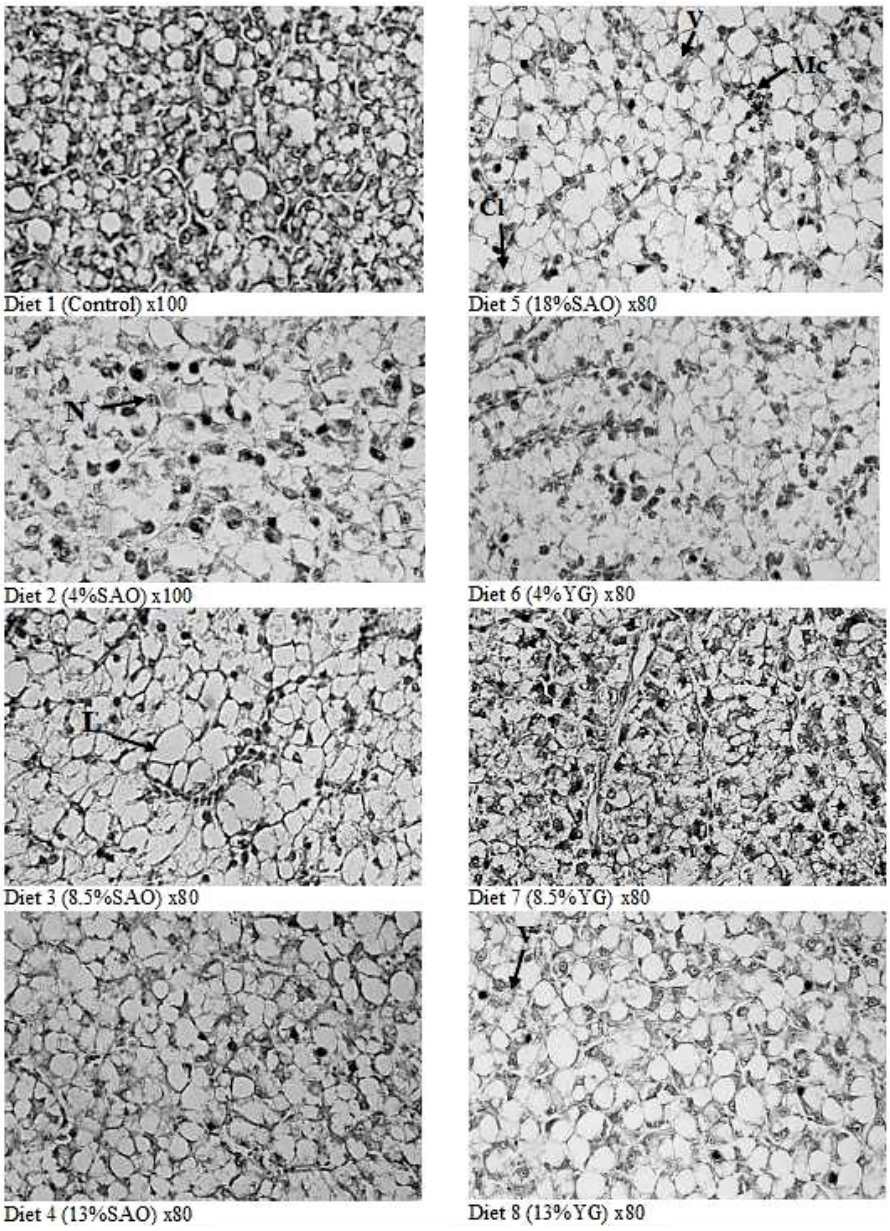

Figure 1. Hepatic lipidosis (steatosis) was found in different diet groups.
Diet 1: Normal or moderate lipid accumulation. Diet 2, 3, 4 and 6: Diffuse macrovacuolization type. Diet 5: Fat in microvesicles and fatty change, slightly cytoplasmic clarification and melano-macrophage focus. Diet 8: Typical macro and slighty microvesicular degeneration with centrilobular change. N: Hepatocyte nucleus, L: Large lipid droplet, Cl: Cytoplasmic clarification V: Microvesicle, Mc: Melano-macrophages centers

Table 2. Growth performance, feed utilization, hepatosomatic index and feed consumptions of common carp fingerlings fed diets containing soy-acid oil (SAO) or yellow grease (YG) for two months[1]

Parameters	Control	SAO	SAO	SAO	SAO	YG	YG	YG	
	1	2	3	4	5	6	7	8	S.E.M
Initial bod weight (g)	18.66±0.03	18.63±0.29	18.76±0.17	18.40±0.26	18.80±0.43	18.60±0.23	18.66±0.33	18.73±0.03	0.08
Final body weight (g)	32.46±0.88^{ab}	29.46±0.63^{b}	25.86±1.38^{c}	25.80±0.23^{c}	23.13±0.29^{c}	32.30±0.18^{ab}	34.16±0.70^{a}	33.03±0.81^{a}	0.85
Weight gain (g)[2]	13.80±0.85^{a}	10.83±0.41^{b}	6.73±1.18^{cd}	7.40±0.40^{c}	4.43±0.53^{d}	13.70±0.50^{a}	15.50±0.91^{a}	14.30±1.80^{a}	0.85
Specific growth rate(%/day)[3]	0.99±0.02^{a}	0.76±0.01^{b}	0.61±0.07^{bc}	0.56±0.03^{c}	0.36±0.04^{d}	0.95±0.04^{a}	1.00±0.03^{a}	0.94±0.09^{a}	0.04
Feed conversion[4]	2.56±0.01^{a}	3.32±0.12^{ab}	4.33±0.47^{b}	4.42±0.17^{b}	5.27±0.33^{c}	2.74±0.04^{a}	2.55±0.14^{a}	2.76±0.34^{a}	0.28
Protein retention (%)[5]	19.74±0.15^{a}	12.74±0.51^{b}	9.11±1.34^{c}	9.84±0.53^{bc}	5.40±1.01^{d}	18.70±0.40^{a}	18.03±1.14^{a}	18.41±0.75^{a}	1.09
Hepatosomatic index[6]	3.09±1.18^{a}	3.13±0.36^{a}	2.63±0.16^{a}	2.61±0.14^{a}	3.05±0.12^{a}	2.71±0.18^{a}	2.97±0.13^{a}	2.78±0.17^{a}	0.08
Daily feed consumption[7]	3.46±0.11^{a}	3.32±0.16^{a}	2.91±0.16^{b}	2.85±0.04^{b}	2.33±0.08^{c}	3.44±0.09^{a}	3.51±0.05^{a}	3.41±0.08^{a}	0.08
Daily protein consumption[8]	1.21±0.03^{a}	1.18±0.04^{a}	1.07±0.03^{b}	1.02±0.02^{b}	0.82±0.02^{c}	1.20±0.03^{a}	1.23±0.01^{a}	1.19±0.03^{a}	0.03
Daily energy consumption[9]	8.98±0.27^{a}	9.05±0.21^{a}	8.89±0.51^{a}	5.77±0.17^{b}	4.93±0.14^{b}	9.74±0.26^{a}	10.69±0.17^{c}	11.16±0.28^{c}	0.44

[1] Values are the mean of triplicate groups of 10 fish. Mean values in rows with different superscript are significantly different (P<0.05).
[2] Weight gain = final weight – initial weight;
[3] Specific growth rate = 100 x [ln (final weight) – ln (initial weight)]/ duration.
[4] Feed conversion ratio = total weight of diet fed (g)/ wet weight gain (g),
[5] Protein retention (percentage protein intake) = (final body protein amount-initial body protein amount /protein intake) x 100,
[6] Hepatosomatic index = (liver weight / total body weight) x 100,
[7] Daily feed consumption = (total feed consumed /day x total live weight) x 100,
[8] Daily protein consumption = (protein consumed /day x total live weight) x 100,
[9] Daily energy consumption (kcal) = (digestible energy consumed /day x total live weight) x 100

Table 3. Carcass composition (wet weight %) of common carp fingerlings fed diets containing different inclusion levels of soy-acid oil and yellow Grease

Diet no	Dry matter	Ash	Protein	Lipid
1	22.04±0.37^{a}	1.33±0.04^{a}	18.25±0.09^{a}	3.06±0.11^{a}
2	19.93±0.11^{b}	1.35±0.08^{a}	17.25±0.13^{a}	2.61±0.57^{ab}
3	21.14±0.14^{ab}	1.43±0.06^{a}	17.00±0.85^{a}	2.35±0.07^{ab}
4	20.13±0.07^{b}	1.29±0.09^{a}	17.60±0.15^{a}	2.61±0.37^{ab}
5	20.68±0.60^{b}	1.16±0.01^{a}	17.25±0.25^{a}	2.55±0.17^{ab}
6	20.30±0.41^{b}	1.39±0.06^{a}	18.53±0.49^{a}	1.44±0.11^{b}
7	20.93±0.52^{ab}	1.30±0.15^{a}	17.45±0.45^{a}	1.77±0.08^{ab}
8	20.27±0.04^{b}	1.20±0.08^{a}	17.48±0.48^{a}	1.67±0.08^{ab}
Pooled S.E.M	0.18	0.03	0.17	0.24

Values are the mean of triplicate composite samples of five fish. Mean values in columns with different superscipt are significantly different (P<0.05). Initial carcass composition was 76.77±0.75% moisture, 1.13±0.04% ash, 18.65±0.33% protein and 4.75±0.96% lipid.

Warmwater omnivorous fish like the channel catfish *Ictalurus punctatus* and tilapia, *Tilapia zillii* have been reported to utilize lipids as effectively as carbohydrate derivates (dextrin) for energy metabolism (Garling and Wilson, 1977; El Sayed and Garling, 1988). Additionally, African catfish, *Clarias gariepinus* (Lim *et al*., 2001) and sunshine bass, *Morone chrysops* x *M. saxatilis* (Keembiyehetty and Wilson, 1998) have been found to utilize dietary lipid, efficiently up to a definite level. Lim *et al.* (2001) have reported that the level of dietary lipid may influence growth performance and protein utilization as in the present experiment. Protein retention is influenced by a variety of factors including the digestibility of feed ingredients, protein content of the diet, amino acid balance and dietary energy to protein ratio (Thoman *et al.*, 1999).

Fatty acid composition of the lipid sources could be another factor influencing protein sparing capabilities. According to Takeuchi and Watanabe (1977), common carp require both n-6 and n-3 fatty acids and a supply of 1% of each of these fatty acids leads to best growth and feed efficiency. Our results showed that partially low values in monoenes might cause impaired protein retention of the experimental diets which contain SAO (Henderson, 1996). Daily energy consumption of fish decreased with increasing rates of SAO. The lower feed or energy intake of high lipid diets has also been shown in most cultured fish species (Kaushik and Luquet, 1983; Keembiyehetty and Wilson, 1998; Hernandez *et al.*, 2001; Lee *et al.*, 2002). In common opinion, feed intake is regulated by the dietary available energy; but the energy consumption rates in our study were irregular. The growth performance of fish fed with diet 1 (control) and diet 7 (8.5% YG) was comparable. The muscle lipid contents of European sea bass (Peres and Oliva-Teles, 1999) and hybrid striped bass (Gaylord and Gatlin, 2000) were not affected by dietary lipid levels. The carcass lipid contents of the all other groups were found lower than the control group. This could be resulting from lipid accumulation in viscera than carcass (Murai *et al.*, 1985) and might be attributed to poor utilization of the SAO and YG compared to the fish fed fish oil (FO). Lipoid accumulations of fish fed diets 4 (13% SAO) and 5 (18% SAO) were more dramatic than that of fish fed diet 1 and 7. Caballero *et al.* (2004) reported that the reduction of the dietary fatty acids due to the inclusion of vegetable oils in the diets tends to promote fat accumulation in the livers of fish. Also, Genc *et al.* (2005) studied the effects of dietary SAO and YG on hepatic lipidosis of hybrid tilapia and their findings supported our histopathological results. Other nutritional and pathological studies on higher lipid inclusion or nutritional imbalances in fish diets and nutritional deficiencies like these cases also supported our pathological findings (Spisni *et al.*, 1998; Manera, 2003; Agius and Roberts, 2003).

In conclusion, the present study showed that the inclusion of YG and SAO in pelleted feeds of common carp did not improve growth performance and feed conversion of fish. Histopathological findings about hepatic tissues supported the present cases. From these preliminary results, YG and SAO are not recommended as an alternative lipid source for common carp fingerlings.

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