

Influence of Dietary Lipid Sources on the Growth Performance, Immune Response and Resistance of Nile Tilapia, *Oreochromis niloticus*, to *Streptococcus iniae* Challenge

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ABSTRACT. This experiment was conducted to determine the effect of dietary lipid sources on growth performance, body proximate composition, hematology, immune response and resistance of Nile tilapia, *Oreochromis niloticus*, to *Streptococcus iniae* infection. Six isocaloric (3.2 kcal/g) and isonitrogenous (34% crude protein) semi-purified diets were supplemented with 7% of various sources of lipid, namely, corn oil (CO), beef tallow (BT), menhaden fish oil (FO), linseed oil (LO), and equal combinations of FO+CO+BT or LO+CO+BT. Diets were fed to tilapia in quadruplicate aquaria to apparent satiation, twice daily for 12 weeks. Fish fed the BT-diet exhibited significantly lowest weight gain,

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diet intake, feed and protein efficiency ratios, apparent protein utilization, and survival. Whole-body protein and ash were significantly ($P < 0.05$) lowest and highest, respectively, for fish fed the beef tallow-diet, but the values of these parameters did not differ among fish fed other diets. No significant differences ($P > 0.05$) were found among hematological values, except for fish fed the FO-diet which had abnormally high red and white blood cell counts. Serum protein concentration, lysozyme activity, and natural hemolytic complement activity were significantly ($P < 0.05$) reduced in fish fed the BT-diet. The values of these parameters did not differ among fish fed other diets. Post-challenge antibody titer was not influenced by dietary lipid sources. Cumulative mortality 15 days post-challenge with *S. iniae* was significantly lower ($P < 0.05$) for fish fed the BT diet compared with those fed FO or FO+CO+BT diets. No significant differences were observed in fish fed other dietary lipid sources. doi:10.1300/J028v19n02_02 [Article copies available for a fee from The Haworth Document Delivery Service: 1-800-HAWORTH. E-mail address: <docdelivery@haworthpress.com> Website: <<http://www.HaworthPress.com>> © 2007 by The Haworth Press, Inc. All rights reserved.]

KEYWORDS. Lipid sources, tilapia, growth performance, immune response, disease resistance, *Oreochromis niloticus*

INTRODUCTION

Tilapia possess a variety of desirable characteristics that make it the most widely and successfully cultured species worldwide. Like other fish species, tilapia utilize dietary lipids as an energy source very efficiently. For juvenile *Oreochromis aurea* × *O. niloticus* hybrid, Chou and Shiau (1996) suggested that a level of 5% dietary lipid appeared to be sufficient to meet the minimum requirement of this tilapia hybrid but a level of about 12% was needed for maximum growth. However, a dietary lipid level in excess of 12% depressed growth and increased carcass lipid accumulation in the same hybrid species (Jauncey and Ross 1982; Jauncey 2000), suggesting that tilapia do not tolerate as high a dietary lipid as salmonids. Commercial diets for grow-out of tilapia usually contain 5-8% of dietary lipid.

Tilapia have been shown to require a dietary source of n-6 series fatty acids (18:2 n-6 and 20:4 n-6) for normal growth (Kanazawa et al. 1980; Takeuchi et al. 1983a) and reproduction (Santiago and Reyes 1993). Improved growth in Nile tilapia, *O. niloticus*, has been reported for fish fed

diets supplemented with soybean oil or corn oil rich in linoleic acid (18:2 n-6), as compared with those fed diets containing fish oil rich in eicosapentaenoic acid (20:5 n-3) and beef tallow rich in oleic acid (18:1 n-9) (Takeuchi et al. 1983b). However, with the broodstock of the same species, Santiago and Reyes (1993) obtained the highest weight gain but the poorest reproductive performance for fish fed the cod liver oil diet. Besides n-6, n-3 fatty acids also have been reported to be a dietary essential for hybrid tilapia, *O. niloticus* × *O. aureus* (Chou and Shiau 1999; Chou et al. 2001). Kanazawa et al. (1980), however, showed that the growth-promoting effect of n-6 series fatty acids (18:2 n-6 and 20:4 n-6) for *Tilapia zillii* were superior to those of the n-3 series (18:3 n-3 and 20:5 n-3). Stickney and Hardy (1989) suggested that the requirement of n-6 fatty acids of blue tilapia, *O. aureus*, can be reduced in the presence of n-3 fatty acids.

There is evidence that dietary lipids influence immune responses and disease resistance of fish (Blazer 1992; Balfry and Higgs 2001). A deficiency of n-3 fatty acids has been shown to adversely affect antibody production and macrophage killing ability in rainbow trout, *Oncorhynchus mykiss* (Kiron et al. 1995) as well as decreasing serum complement activity (Tort et al. 1996; Montero et al. 1998) and agglutinating antibody titers in gilthead seabream, *Sparus aurata* (Tort et al. 1996). On the other hand, excessive levels of n-3 HUFA have also been reported to increase the mortality of rainbow trout (Kiron et al. 1995) and channel catfish (Fracalossi and Lovell 1994) infected with *A. salmonicida* and *Edwardsiella ictaluri*, respectively. As there is limited information with respect to tilapia species, this study was conducted to evaluate the effects of different lipid sources, namely corn oil (CO), beef tallow (BT), menhaden fish oil (FO), linseed oil (LO), and equal combinations of FO+CO+BT or LO+CO+BT on the growth performance, whole-body proximate composition, immune responses and resistance of Nile tilapia to *Streptococcus iniae* infection.

MATERIALS AND METHODS

Experimental Diets and Fish Culture

Six semi-purified diets (Table 1) were formulated to contain equal levels of protein (34%), lipid (7%), and digestible energy (3.2 kcal/g) on an as-fed basis. Digestible energy was calculated based on feedstuff values (NRC 1993). The composition of the diets was the same except for the

TABLE 1. Percentage composition and estimated nutrient content of basal diet.

Ingredients	Diet (%)
Casein	32
Gelatin	8
Corn starch	33
Lipid ¹	7
Vitamin premix ²	1
Mineral premix ³	4
CMC	3
Celufil ⁴	12
Estimated Nutrients (%)	
Crude protein	34
Crude fat	7
D.E. (kcal/g diet)	3.2

¹Lipid sources: corn oil (CO), beef tallow (BT), fish oil (FO), linseed oil (LO), and a combination of equal level of FO+CO+BT and LO+CO+BT.

²The vitamin mix, diluted in cellulose, provided the following in mg/kg diet: vitamin A (500,000 IU/g), 8; vitamin D₃ (1,000,000 IU/g), 2; menadione, 10; dl-alpha tocopherol acetate, 200; thiamin, 10; riboflavin, 20; pyridoxine, 20; d-calcium pantothenate, 200; nicotinic acid, 150; folic acid, 5; vitamin B₁₂, 0.02; biotin, 2; inositol, 400; choline chloride, 2,000; L-ascorbyl-2-polyphosphate (15% vitamin C activity), 100.

³Williams and Briggs mineral mix (U.S. Biochemical Co., Cleveland, Ohio) supplemented in mg/kg diet with aluminum potassium sulfate, 0.7; sodium selenite, 0.08; and cobalt chloride, 1.4.

⁴Non-nutritive filler.

lipid source. The lipid sources, CO, BT, FO, LO, and combination of equal levels of FO+CO+BT and LO+CO+BT were added to the basal diet at a level of 7%. Before the oil was added, the dry ingredients of each diet were mixed thoroughly in a Hobart mixer (Hobart Corporation, Troy, Ohio¹). After the oil was dispersed, approximately 200 mL of deionized water/kg diet was added. Ethoxyquin was blended in the oil and added to each diet at a level of 200 mg/kg as an antioxidant agent. The resulting dough-like mixture was extruded in a Hobart meat grinder into 3-mm diameter pellets. The resulting moist pellets were air-dried at room temperature (23°C) to a moisture content of about 10%. Pellets were ground into small pieces, sieved to obtain appropriate sizes and stored frozen in plastic bags at -8°C until used.

Nile tilapia fry produced at our laboratory and fed on a commercial fry diet were acclimated for 2 weeks by feeding the basal diet containing 7%

1. Use of trade or manufacturer's name does not imply endorsement.

stearic acid (18:0, 98%) as the lipid source. At the end of the acclimation period, fish (average weight of 2.85 ± 0.38 g) were randomly stocked into 24, 55-L aquaria at a density of 35 fish per aquarium. Aquaria were supplied with flow-through dechlorinated municipal water at a rate of 0.5-0.6 L/minute and increased gradually to about 1.0 L/minute by the end of week 10. Water was continuously aerated using air stones. Water temperature and dissolved oxygen in four randomly chosen aquaria were measured once every other day in the morning, using a YSI model 58 Oxygen Meter (Yellow Spring Instrument Co., Inc., Yellow Spring, Ohio). During the trial, water temperature averaged $27.8 \pm 0.79^\circ\text{C}$, and dissolved oxygen averaged 6.5 ± 0.23 mg/L. Photoperiod was maintained at a 12:12 hours light:dark schedule.

Fish in four randomly assigned aquaria were fed one of the six experimental diets twice daily to apparent satiation for 12 weeks. The amount of diet consumed was recorded daily by calculating the differences in weight of diets prior to the first and after the last feeding. Aquaria were cleaned once a week and fish were fed only in the afternoon on the cleaning days. Fish in each aquarium were group-weighted and counted at 2-week intervals to determine the average weight gain and survival. Diet was not offered on sampling days. Feed efficiency ratio (FER), protein efficiency ratio (PER) and apparent protein utilization (APU) were calculated at the end of the feeding trial.

Diet Fatty Acid Composition

Lipids from the experimental diets were extracted following the method of Folch et al. (1957) and were analyzed for fatty acid composition. Lipid fractions were saponified with 1 mL of 0.5 N KOH in methanol (70°C water bath for 30 minutes) and then esterified with 14% of boron trifluoride (BF₃) in methanol (70°C for 45 minutes). The tubes were allowed to cool, and 2 mL hexane and 2 mL of NaCl saturated solution were added. The tubes were vortexed for 1 minute, and the hexane layer was pipetted off into a labeled vial and stored at -70°C. Fatty acid methyl esters were analyzed with a Shimadzu GC-17A gas chromatograph equipped with a flame ionization detector and Omega Wax™ 530 capillary column (30 m × 0.53 mm × 0.5 μm film thickness; Supelco Inc., Bellefonte, Pennsylvania). Helium was used as the carrier gas (3.4 mL/minute) and the oven temperature was programmed for a thermal gradient of 140°C to 260°C at a rate of 3°C/minute. The injector temperature was set at 260°C and the detector at 270°C. Methylene chloride was

used as solvent, and the extract was transferred into 2 mL vials (Supelco, Inc.). The integrated peak areas of the fatty acid methyl esters were identified by comparison with known standards (FAME and PUFA Supelco Inc.). The relative concentration of fatty acids was calculated and expressed as weight percentage of fatty acids identified and a correction factor was applied to convert the percentages of peak areas into mass-percentages of the components. The fatty acid composition of the experimental diets is presented in Table 2.

TABLE 2. Fatty acid composition (% by weight of total fatty acids) of experimental diets. Values reported are means of two determinations per diet.

Fatty acid	Diet containing					
	CO	BT	FO	LO	FO+CO+BT	LO+CO+BT
14:0	0.73	3.80	8.34	1.32	4.03	2.08
16:0	12.70	27.80	21.24	8.22	21.32	17.11
18:0	2.63	30.39	5.54	4.17	14.44	13.00
Saturates	16.06	61.98	35.11	13.71	39.78	32.18
16:1 n-7	0.37	1.36	11.40	0.77	3.59	0.79
18:1 n-7	0.52	4.59	3.69	1.01	3.65	2.19
18:1 n-9	29.06	23.51	10.17	20.07	21.90	25.03
20:1 n-9	0.40	0.18	1.33	0.35	0.62	0.41
Monoenes	30.34	29.63	26.58	22.19	29.76	28.41
18:2 n-6	52.25	4.46	2.74	13.79	20.25	23.15
20:2 n-6	–	–	–	–	–	–
18:3 n-6	–	–	–	–	–	–
20:3 n-6	–	–	–	–	–	–
20:4 n-6	–	–	0.82	–	–	–
Total n-6	52.25	4.46	3.56	13.79	20.25	23.15
18:3 n-3	1.12	3.19	1.61	48.26	1.93	15.30
20:3 n-3	–	–	–	–	–	–
18:4 n-3	–	–	3.09	0.68	0.87	–
20:4 n-3	–	–	1.72	–	0.45	0.03
20:5 n-3	0.07	0.31	11.21	0.65	3.02	0.32
22:5 n-3	0.11	–	2.26	0.16	0.65	–
22:6 n-3	0.08	0.16	12.53	0.76	3.27	0.29
Total n-3	1.37	3.65	32.42	50.50	10.18	15.93

Body Proximate Composition

Fifty fish at the beginning of the study and four fish from each aquarium at the end of the trial were randomly sampled, pooled and stored at -20°C for determination of whole-body proximate composition. Each sample was analyzed in duplicate for whole-body proximate composition following the standard methods (AOAC 1990). Moisture content was determined by drying fish samples in an oven at 105°C until a constant weight was reached. Samples used for dry matter measurements were digested with nitric acid and then incinerated in a muffle furnace at 600°C overnight for ash contents. Protein was measured by combustion method using a FP-2000 Nitrogen Analyzer (Leco Corporation, St. Joseph, Michigan), following the method of AOAC. Lipid content was determined by petroleum ether extraction using a Soxtec System (2055 Soxtec Avanti, Foss Tecator, Höganäs, Sweden) apparatus. Prior to lipid analysis, fish samples were blended in diatomaceous earth and dried in oven for 2 hours at 100°C .

Hematological Assay

At the end of the feeding period, four fish were randomly chosen from each tank and anesthetized with tricaine methanesulfate (MS-222) at 150 mg/L, and blood samples were collected from the caudal vein with heparinized (20 U/L) tuberculin syringes for hematological assays. Red and white blood cell counts were performed in duplicate for each sample by diluting (1:10,000) whole blood and enumerating in a Spencer Bright Line hemacytometer. Hemoglobin was determined using a cyanomethemoglobin method (Sigma Chemical Co., St. Louis, Missouri). Hemoglobin values were adjusted by cyanomethemoglobin correction factor for channel catfish described by Larsen (1964). Hematocrit of each fish was determined in duplicate using the microhematocrit method (Brown 1988).

Serum Total Protein

Serum was collected from four fish from each tank and assayed in duplicate for serum total protein concentrations, using the modified Biuret method. Total protein reagent (Sigma, Chemical Co., St. Louis, Missouri) was added to each well of the microtiter plate at $250\ \mu\text{L}/\text{well}$. Then, $5\ \mu\text{L}$ of serum were added to each well. After incubation at RT for 30 min-

utes, the absorbance of the samples was read at 570 nm. Serum total protein concentrations were calculated using bovine serum albumin as an external standard.

Lysozyme Assay

Serum lysozyme activity was determined by the method of Litwack (1955) as modified by Sankaran and Gurnani (1972). The assay is based on lysis of lysozyme-sensitive Gram positive bacterium *Micrococcus lysodeikticus* (Sigma Chemical Co., St. Louis, Missouri) by the lysozyme present in the serum. Freeze-dried *M. lysodeikticus* suspension (0.25 mg/mL) was prepared immediately before use by dissolving in acetate buffer (0.02 M NaC₂H₃O₂, pH 5.5). Serum (10 µL/well in duplicate) from four fish per tank was placed in a microtiter plate, and 250 µL of bacterial cell suspension was added to each well. Hen egg white lysozyme (HEWL) was used as an external standard. The initial and final (20 minutes incubation at 35°C) absorbance of the samples was measured at 450 nm. The rate of reduction in absorbance of samples was converted to lysozyme concentration (µg/mL) using a standard curve.

Natural Hemolytic Complement Activity

Natural hemolytic complement activity (alternative pathway) was measured in the serum of experimental fish using an assay adapted from Sunyer and Tort (1995) and modified for use in microtiter plates. This assay is based on the hemolysis of sheep erythrocytes (Remel Inc., Lenexa, Kansas) by complements present in fish serum. Sheep erythrocytes were washed four times with gelatin-veronal buffer (GVB²⁺, pH: 7.5) (Sigma Chemical Co., St. Louis, Missouri) and standardized to 5×10^7 cell/mL in GVB²⁺ prior to use. A twofold serial dilution was made in 96-well round-bottom microtiter plates by adding 50 µL of serially diluted serum from four fish per tank into the wells plated with 50 µL of GVB²⁺. The volume in each well was adjusted to 250 µL by adding 200 µL buffer to give final concentrations of 20, 10, 5, 2.5, 1.25, 0.625, 0.313, and 0.157%. Thereafter, 50 µL of sheep erythrocyte cell suspension were added to each well. Positive controls (100% lysis) of distilled water plus sheep erythrocytes (250 µL H₂O and 50 µL erythrocyte), negative controls (spontaneous lysis) of buffers and sheep erythrocytes (250 µL GVB²⁺ and 50 µL erythrocyte) were also processed in each plate. Sam-

ples were incubated in room temperature (22°C) for 1 hour with regular shaking. The reaction was stopped by placing plates on ice. The plates were centrifuged at $800 \times g$ for 10 minutes at 4°C to avoid unlysed cells. Supernatants (250 μ L) were transferred to a flat-bottom 96-well microtiter plate and the absorbance measured at 410 nm using an ELISA plate reader.

Complement hemolytic activity was expressed as ACH₅₀ value which represents the volume of serum necessary to produce lysis of 50% of the target cells under standard conditions and results presented as units/mL. The degree of hemolysis was estimated and the lysis curve for each sample was obtained by plotting $Y/(100 - Y)$ against the volume of serum added (mL) on a log-log scaled graph. The value Y (percentage of hemolytic activity at each dilution with respect to controls) was defined as;

$$Y = 100 \times (\text{Abs (A)} - \text{Abs (B)}) / [\text{Abs (C)} - \text{Abs (B)}];$$

where A = supernatant of the test serum dilution;

B = minimum hemolytic – negative control (spontaneous lysis); and

C = maximum hemolytic – positive control (100% lysis).

Bacterial Challenge

Frozen stock-culture of *S. iniae* (ARS 98-60) from an outbreak of streptococcal disease in Nile tilapia was grown in tryptic soy broth (TSB) at 25°C with shaking at 100 rpm for 24 hours. The concentration of the culture was adjusted to an optical density of 1.2 measured on a Biorad SmartSpec 3000 spectrophotometer (Bio-Rad Laboratories, Hercules, California) at 540 nm to give a *S. iniae* concentration of 1×10^9 colony forming unit (cfu)/mL. The desired bacterial concentrations were prepared in sterile medium by 1:10 serial dilutions.

To determine the optimum bacterial cell concentration to use in the experimental challenge, groups of 20 fish which were held in separate aquaria and fed a diet containing stearic acid for 10 weeks were intraperitoneally (IP) injected with 0.1 mL of 0, 10^5 , 10^6 , 10^7 , 10^8 , and 10^9 *S. iniae* cfu/mL using a tuberculin syringe. Following challenge, each group of fish was kept in a 57-L aquarium equipped with flow-through water. Mortality was recorded twice daily for 15 days. The LC₅₀ (concentration lethal to 50% of exposed fish), which was calculated by the Karber method (Plumb and Browser 1983), was 5×10^5 cfu per fish and was the concentration used for the experimental challenge.

At the end of the 12-week feeding period, fish (15 fish per aquarium) were randomly selected and intra-peritoneally (IP) injected with 0.1 mL of 5×10^6 cfu/mL of *S. iniae* (5×10^5 cfu/fish) using a tuberculin syringe. They continued to receive their respective diets. Fish were monitored and mortality was recorded twice daily for 15 days following injection and dead fish removed.

Agglutination Antibody Titer Assay

Before the bacterial challenge, four fish from each of the four replicate tanks were bled to determine their antibody response against *S. iniae*. At day 15 of the bacterial challenge, blood samples were collected from the surviving fish (7, 9, 11, 8, 4, and 12 fish per treatment from diets containing lipids from CO, BT, FO, LO, FO+CO +BT and LO+CO+BT, respectively) and allowed to clot at 4°C overnight. Serum samples were collected following centrifugation and stored at -80°C until assayed for agglutinating antibody titers against *S. iniae* by modifying the method of Chen and Light (1994). *Streptococcus iniae* (ARS 98-60) was grown in TSB for 24 hours and killed with 10% formalin 3 hours before the assay. The bacterial cell suspension was centrifuged at $1000 \times g$ for 15 minutes and the supernatant was discarded. The resulting pellets were washed twice with 0.85% phosphate buffer saline (PBS) solution and pellets were resuspended in PBS to an optical density of 0.8 at 540 nm. Twofold serial serum dilutions (starting with 30 μ L serum and 30 μ L PBS) were made in 96-well round bottom microtiter plates by adding 30 μ L of diluted serum into the remaining wells plated with 30 μ L of PBS. Thereafter, 10 μ L of bacterial cell suspension were added to each well. The plates were covered with plastic film and incubated at room temperature for 16-18 hours. The agglutination end point was established as the last serum dilution where cell agglutination was visible after incubation. Agglutination titers were reported as \log_2 of the reciprocal of the highest serum dilution showing visible agglutination, as compared with the positive control.

Statistical Analysis

All data were analyzed by one-way analysis of variance using the general linear model. Mortality data at 15 days post-challenge was arc-sin transformed before analysis. Duncan's Multiple Range test was used to compare treatment means. Differences were considered significant at the

0.05 probability level. All analysis was performed using the SAS program (Statistic Analysis Systems, SAS Institute, Inc., Cary, North Carolina).

RESULTS

The average final weight gain (WG), dry matter feed intake (FI), feed efficiency ratio (FER), protein efficiency ratio (PER), apparent protein utilization (APU), and survival rate (SR) are given in Table 3. Fish fed diets containing CO, FO, FO+CO+BT and LO+CO+BT had similar patterns of growth throughout the 12-week feeding period. Fish fed LO and BT diets grew slower and by week 6, the weight gain of fish in these treatment groups became significantly lower ($P < 0.05$) than those of the groups fed other diets. Thereafter, the growth of fish fed the LO diet, although still inferior, followed the same trend as those of fish fed CO, FO, FO+CO+BT and LO+CO+BT diets. For fish fed the BT-diet, however, the growth remained consistently poorer than those in the other treatments.

The mean final weight gain of fish fed this diet was significantly lower ($P < 0.05$) than those of fish fed other diets. No significant differences were found among final weight gains of fish fed CO, FO, LO, FO+CO+BT and LO+CO+BT diets. Numerically, however, fish fed the LO+CO+BT diet performed consistently better than the other diets. Total FI was a reflection of weight gains with fish fed the BT diet having significantly lower FI than those fed the other diets. Fish fed the LO-diet had FI that was significantly lower than those fed CO, FO+CO+BT and LO+CO+BT diets, but did not differ from that of fish fed the FO diet. FER was significantly lowest for fish fed the BT diet. The FER value for the CO diet was significantly lower than that of LO diet, but these were not significantly different for those of FO, FO+CO+BT and LO+CO+BT diets.

PER and APU were significantly lowest for the BT diet but were statistically similar for other diets. The SR was lowest for fish fed BT diet but this was not significantly lower than that of fish fed the FO diet. There were no significant differences among the SR of fish CO, FO, LO, FO+CO+BT and LO+CO+BT diets.

No significant differences ($P > 0.05$) were observed among the moisture and lipid contents of fish receiving various dietary lipid sources (Table 4). Crude protein content was significantly lowest and ash content

TABLE 3. Mean final body weight gain (WG), dry matter feed intake (FI), feed efficiency ratio (FER), protein efficiency ratio (PER), apparent protein utilization (APU) and survival of Nile tilapia fed diets containing various sources of lipid for 12 weeks. Values are means of four replicates per treatment. Means (\pm SE) in the same row with different letters are significantly different ($P < 0.05$).

	Dietary sources of lipid						
	CO	BT	FO	LO	FO+CO+BT	LO+CO+BT	
Weight gain (g)	29.5 \pm 4.1a	11.2 \pm 2.2b	27.2 \pm 2.3a	26.6 \pm 1.4a	31.6 \pm 0.4a	34.6 \pm 3.1a	
Feed intake (g)	27.8 \pm 2.7a	12.9 \pm 1.6c	24.3 \pm 1.8ab	22.2 \pm 1.1b	29.4 \pm 0.7a	29.6 \pm 1.8a	
FER ¹	1.1 \pm 0.1b	0.9 \pm 0.1c	1.1 \pm 0.0ab	1.2 \pm 0.0a	1.1 \pm 0.0ab	1.2 \pm 0.0ab	
PER ²	3.1 \pm 0.2a	2.5 \pm 0.2b	3.3 \pm 0.1a	3.5 \pm 0.1a	3.2 \pm 0.1a	3.4 \pm 0.1a	
APU ³	47.8 \pm 2.3a	37.8 \pm 3.2b	51.0 \pm 1.4a	53.4 \pm 2.3a	48.9 \pm 0.7a	52.6 \pm 1.8a	
Survival	97.1 \pm 2.0a	88.6 \pm 3.5b	93.6 \pm 3.0ab	99.3 \pm 0.7a	97.1 \pm 2.9a	99.3 \pm 0.7a	

¹FER = Weight gain (g)/dry feed fed (g).

²PER = Wet weight gain (g)/crude protein fed (g).

³APU = 100 \times [body protein gain (g)/crude protein fed (g)].

TABLE 4. Whole-body proximate composition of Nile tilapia fed diets containing various sources of lipid for 12 weeks.¹ Values are means of two determinations of pooled samples of four fish per tank and four tanks per treatment. Means (\pm S.E.) in the same row with different letters are significantly different at $P < 0.05$.

Proximate composition	Dietary sources of lipid (% wet weight basis)					
	CO	BT	FO	LO	FO+CO+BT	LO+CO+BT
Moisture	70.6 \pm 0.6a	70.9 \pm 0.8a	70.4 \pm 0.3a	70.9 \pm 0.2a	70.5 \pm 0.6a	70.5 \pm 0.4a
Protein	15.3 \pm 0.1a	14.6 \pm 0.3b	15.3 \pm 0.2a	15.2 \pm 0.2a	15.3 \pm 0.1a	15.3 \pm 0.1a
Lipid	8.5 \pm 0.4a	8.5 \pm 0.5a	8.7 \pm 0.3a	7.8 \pm 0.1a	8.8 \pm 0.3a	8.8 \pm 0.2a
Ash	3.2 \pm 0.1b	3.6 \pm 0.1a	3.2 \pm 0.1b	3.2 \pm 0.1b	3.2 \pm 0.1b	3.3 \pm 0.1b

highest for fish fed the BT-diet but the values of these parameters did not differ among fish fed other diets.

Mean red blood cell (RBC) and white blood cell (WBC) counts were significantly highest in fish fed the FO diet (Table 5). These values did not differ significantly in fish fed the other dietary lipids. The sources of dietary lipid had no effect on hemoglobin and hematocrit. Serum protein, lysozyme activity, and natural hemolytic complement activity (Table 5) were significantly lowest in fish fed the BT diet. The values of these parameters did not significantly differ among fish fed other diets. Data of pre-challenge antibody titer indicated that fish were naive for *S. iniae*. Post-challenge antibody titers were not influenced by dietary source of lipid.

Daily cumulative percent mortality of Nile tilapia 15 days post-challenge with *S. iniae* is presented in Figure 1. Cumulative mortality at day 15 of fish fed the BT diet was significantly lower than those obtained in the groups fed CO and FO+CO+BT diets, but these values did not significantly differ from those of the other treatments.

DISCUSSION

Based on WG, FI and FER, PER, APU, and SR, beef tallow was poorly utilized by juvenile Nile tilapia. Beef tallow has also been reported to be the least effective lipid source in diets for blue tilapia (Stickney and McGeachin 1983) and red-belly tilapia (Takeuchi et al. 1983b) relative to corn oil, soybean oil, catfish oil, and marine fish oil. Poor performance of fish fed the BT-diet could be related to the lack of essential fatty acids. The level of dietary n-6 fatty acid in BT-diet (0.31%) was much lower than the requirement levels of 0.50% and 1.0% of diet reported for Nile tilapia (Takeuchi et al. 1983a) and red-belly tilapia (Kanazawa et al. 1980), respectively. In addition, the presence of high levels of saturated fatty acids in the BT-diet also may have contributed to the low nutritional value of this diet. Takeuchi et al. (1983b) reported that saturated fatty acids with carbon lengths of 8 to 18 are not suitable lipid sources for Nile tilapia since fish fed diets supplemented with medium chain triglycerides or BT grew slower than the group fed the diet containing 18:1 n-9.

Corn oil, FO, LO, FO+CO+BT, and LO+CO+BT were equally effective as dietary lipid sources for juvenile Nile tilapia. Published information on the effect of dietary lipid sources on the growth performance of different tilapia species is contradictory. Takeuchi et al. (1983b) observed significantly slower growth of Nile tilapia fed the pollock liver oil

TABLE 5. Hematological values (red blood cell count, white blood cell count, hemoglobin and hematocrit) and immune responses (serum protein, lysozyme, alternative complement activity and agglutinating antibody titers against *S. iniae*) of Nile tilapia fed diets containing various sources of lipid for 12 weeks. Values (means \pm SE) in the same row with different letters are significantly different ($P < 0.05$).

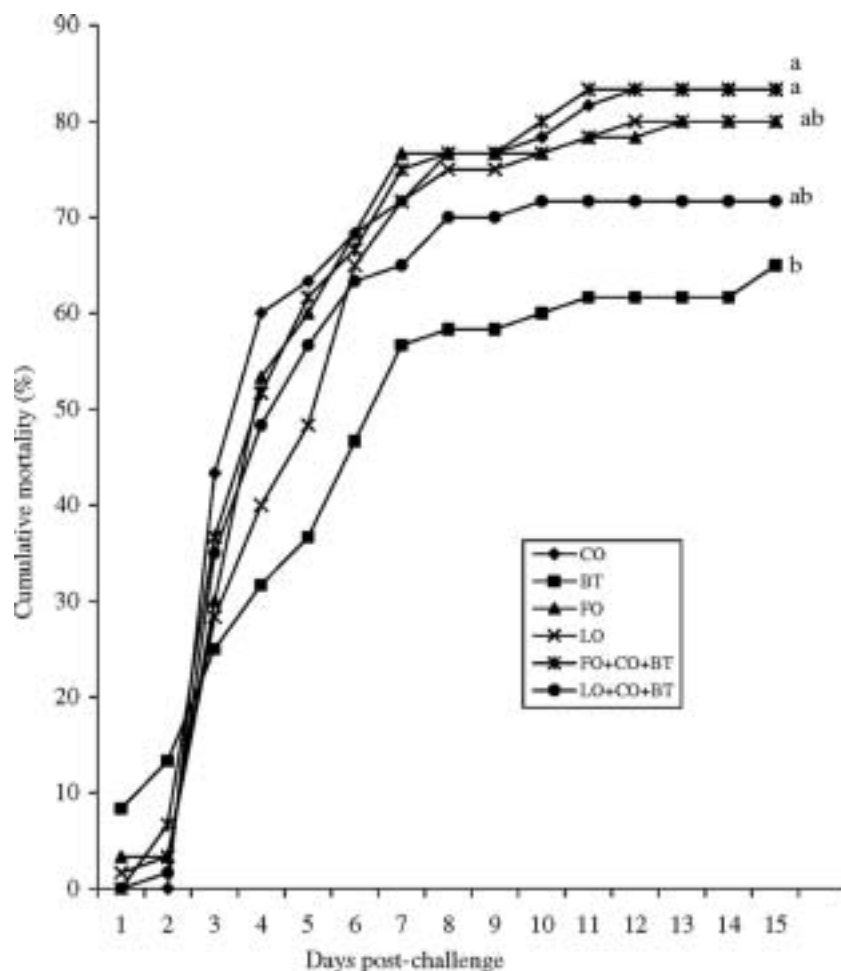
	Dietary sources of lipid				
	CO	BT	FO	LO	LO+CO+BT
Hematological values					
Red blood cell ($\times 10^6/\mu\text{L}$) ¹	2.2 \pm 0.1b	2.4 \pm 0.1b	3.1 \pm 0.2a	2.2 \pm 0.1b	2.3 \pm 0.1b
White blood cell ($\times 10^5/\mu\text{L}$) ¹	4.2 \pm 0.7b	5.2 \pm 0.4b	8.0 \pm 0.6a	4.2 \pm 0.7b	4.8 \pm 0.6b
Hemoglobin (g/dL) ²	10.4 \pm 0.3a	10.8 \pm 0.4a	10.7 \pm 0.2a	10.5 \pm 0.4a	10.8 \pm 0.2a
Hematocrit (%) ¹	33.1 \pm 1.1a	33.5 \pm 1.2a	31.2 \pm 1.3a	32.7 \pm 0.7a	32.0 \pm 2.0a
Immune responses					
Serum protein (mg/mL) ¹	33.7 \pm 1.0ab	30.0 \pm 2.2b	36.9 \pm 1.1a	39.7 \pm 2.7a	38.2 \pm 1.2a
Lysozyme activity ($\mu\text{g}/\text{mL}$) ¹	18.3 \pm 0.6a	13.0 \pm 1.5b	17.0 \pm 1.5a	18.4 \pm 0.4a	18.4 \pm 0.6a
Alternative complement activity (U/mL) ²	105.1 \pm 13.6a	63.7 \pm 2.8b	124.4 \pm 13.7a	116.5 \pm 7.6a	112.0 \pm 7.9a
Antibody titer (Log_2) ³	2.1 \pm 0.3a	2.6 \pm 0.4a	3.4 \pm 0.4a	3.4 \pm 0.5a	3.2 \pm 0.5a

¹Values are means of two determinations per fish, four fish per tank and four tanks per treatment.

²Values are means of four fish per tank and four tanks per treatment.

³Values are means of one determination per surviving fish (7, 9, 1, 1, 8, 4, and 12 fish for treatments fed diets containing CO, BT, FO, LO, FO+CO+BT, and LO+CO+BT, respectively) 15 days post-challenge.

FIGURE 1. Daily percent cumulative mortality of Nile tilapia challenged with *S. iniae* for 15 days. Values are means of four replicates per treatment. Means values at day 15 post-challenge with different letters are significantly different ($P < 0.05$).



diet than those fed corn and soybean oil diets. With red-belly tilapia, Kanazawa et al. (1980) also obtained lower weight gain of fish fed the pollock liver oil diet than those fed the soybean oil diet. In contrast, Santiago and Reyes (1993) reported highest weight gain of Nile tilapia fed the cod liver oil diet, but poorest reproductive performance. Blue tilapia have

been reported to grow equally well on diets containing either soybean or menhaden FO (Stickney and McGeachin 1983). Based on this information and that obtained in our study, both n-6 and n-3 fatty acids may be required in the diet of Nile tilapia as has been reported for hybrid tilapia (Chou and Shiau 1999; Chou et al. 2001). However, because fish fed the CO-diet high in n-6 but deficient in n-3 fatty acids, and the FO-diet deficient in n-6 but high in n-3 HUFA exhibited similar growth as those fed LO, FO+CO+BT and LO+CO+BT diets that contain sufficient or excessive levels of both fatty acids, the presence of high levels of either fatty acid may spare the requirement of the other.

It was observed that during the first two weeks of this feeding study, fish readily consumed the FO diet but reluctantly consumed the LO diet. Fish fed the FO diet, however, had excessive mucus production and were more sensitive to handling stress, as compared with those fed other diets. Mortality of fish in this treatment occurred after sampling. Excessive mucus production and the sensitivity of this group of fish to handling stress, although have not been previously reported, may be the results of synergistic effects of a high level of n-3 HUFA (1.94% of diet) and deficiency of n-6 (0.25% of diet).

There were no significant differences in body composition of tilapia among treatments except crude protein and ash contents of fish fed the BT diet which were significantly higher and lower, respectively, than those of fish fed other diets. This was possibly related to the small size of fish in this treatment since, generally, smaller fish contain a lesser proportion of flesh and higher bone content.

Klinger et al. (1996) reported that channel catfish fed a menhaden oil diet had similar red blood cell count, but significantly lower hematocrit and higher total leukocyte counts, as compared with fish fed corn oil, soy oil, and beef tallow diets. In our study, hematocrit and hemoglobin were not affected by dietary lipid sources. The values of these parameters are in the normal range observed in juvenile channel catfish (Peres et al. 2003; Yildirim-Aksoy et al. 2004). However, in the present study, the abnormally high red blood cell count but similar hematocrit value in tilapia fed the FO diet, as compared to those fed other diets, implies a low mean corpuscular volume which is an indication of the presence of large numbers of small, immature erythrocytes. This abnormality, as well as the significantly higher level of white blood cell and excessive mucus production of fish in this treatment, could be related to stress, infection, or hemolytic disease as has been reported by Brown (1998) for warm-blooded animals.

Published information on the effect of dietary lipid sources and essential fatty acids on immune responses and disease resistance in fish is

inconsistent and is often contradictory. Studies with rainbow trout, *Oncorhynchus mykiss*, showed that a deficiency of n-3 fatty acids adversely affected antibody production and macrophage killing ability (Kiron et al. 1995) as well as decreasing serum complement activity (Tort et al. 1996; Montero et al. 1998) and agglutinating antibody titers (Tort et al. 1996). Decreased activity of head kidney macrophages from channel catfish (Blazer et al. 1989; Sheldon and Blazer 1991) and rainbow trout (Ashton et al. 1994) has also been demonstrated in fish fed diets containing low levels of n-3 fatty acids. Likewise, in the present study, tilapia fed the BT-diet which is deficient in both n-6 (0.31% of diet) and n-3 (0.26% of diet) had significantly reduced serum protein (except fish fed CO-diet), lysozyme and natural hemolytic complement activity. These immune parameters did not differ among fish fed the other dietary lipid sources which contained high levels of n-6 or n-3 fatty acids or their combination.

In contrast, Erdal et al. (1991) reported that Atlantic salmon fed diets high in n-3 HUFA had decreased antibody titers after vaccination and reduced survival after challenge with *Vibrio salmonicida*. Excessive levels of n-3 HUFA have also been reported to increase the mortality of rainbow trout infected with *Aeromonas salmonicida* (Kiron et al. 1995). Fracalossi and Lovell (1994) obtained significantly higher antibody titers after immunization against *E. ictaluri* in catfish fed FO than those fed diets containing CO, LO, or a mixture of FO, CO, and BT. They also found that antibody titer in fish fed the BT diet did not differ significantly from fish fed the other diets. Li et al. (1994) reported no differences in antibody titers of catfish fed diets supplemented with 2% of menhaden oil, catfish oil or beef tallow. In our study, antibody production against *S. iniae* of tilapia 15 days post-challenge was not affected by dietary treatments.

Fracalossi and Lovell (1994) obtained similar mortality of channel catfish fed diets containing BT, CO, or a mixture of FO, CO, and BT. These values, however, were significantly lower than fish fed FO or LO diets. Li et al. (1994) reported that mortality of catfish fed the diet supplemented with BT was intermediate between fish fed menhaden and catfish oil diets. In our study, cumulative mortality following experimental challenge with *S. iniae* was not affected by dietary lipid sources. However, fish fed the BT diet had lower cumulative mortality to *S. iniae* challenge than fish fed CO and FO + CO + BT diets. This unexpected response could not be explained since, prior to experimentally challenged, fish fed this diet exhibited the poorest growth performance and were immunosuppressed (significantly reduced levels of serum protein, lysozyme activity and complement activity). It is commonly believed that immuno-

suppressed animals, caused by a nutrient deficiency, are more susceptible to infection than healthy animals. Whether this was due to factors other than dietary lipid sources or fatty acid composition cannot be ascertained.

The present study appears to indicate that Nile tilapia have a dietary requirement for both n-6 and n-3. Fish fed the BT diet containing low level (0.31% of diet) of n-6 and n-3 (0.26% of diet) exhibited poor growth performance. The presence of high dietary levels of either n-6 or n-3 may spare the requirement of the other. However, excessive levels of n-3 HUFA (FO diet) may lead to abnormally high red and white blood cell counts and excessive mucus production. Deficiency of essential fatty acids leads to decreased immune responses. However, this did not translate into reduced resistance of fish against *S. iniae* challenge. More detailed studies to better understand the relationships between dietary lipid sources, levels and ratios of essential fatty acids, and growth performance, immune responses and disease resistance, are needed.

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