Soybean–Lecithin Supplementation of Practical Diets for Juvenile Goldfish *(Carassius auratus)*

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ABSTRACT: Soybean lecithin, alone or in combination with cod liver or soybean oil, was tested as a supplemental lipid in practical diets for juvenile (0.3 g) goldfish *(Carassius auratus)*. Goldfish were fed one of five diets containing: (i) 4% cod liver oil (CLO); (ii) 2% CLO + 2% lecithin (LEC); (iii) 4% soybean oil (SBO); (iv) 2% SBO + 2% LEC; (v) 4% LEC. After 6 wk, weight gain and feed efficiency of fish fed diets with 4% LEC were significantly higher than those of fish fed diets with 4% CLO or SBO. Gain and feed efficiency of fish fed diets with combinations of LEC and CLO or SBO were intermediate. Survival and whole-body lipid were unaffected by diet. Possible mechanisms of performance enhancement in goldfish fed LEC include provision of myoinositol or phosphatidylcholine to support rapid membrane proliferation, enhanced absorption of dietary lipid, and facilitation of lipid transport.
JAOCS 74, 149–152 (1997).

KEY WORDS: *Carassius auratus,* cod-liver-oil, goldfish, juvenile, practical diets, soybean–lecithin, soybean oil.

Phospholipids (PL) and their derivatives have multiple functions in metabolic processes: sources of energy, vitamins, phosphorus, and essential fatty acids (EFA); emulsification of lipids during digestion and absorption; and structural integrity in lipid transport particles.

Many finfish, particularly immature stages, exhibit improved growth, survival, or feed conversion rates in response to diets supplemented with PL. An early study showed that lecithin (LEC) enhanced growth and feed conversion and reduced mortality in rainbow trout *(Onchorhynchus mykiss)* (1). Subsequent studies on different fish species have documented similar improvements in performance attributable to various forms and sources of LEC (2–4). The primary mechanisms implicated in the enhanced performance of fish by PL or LEC have been provision of EFA or choline, or facilitation of lipid absorption. Nutritional essentiality of PL in fish was not considered initially because they possess enzymes for *de novo* PL synthesis (5). However, studies of larval ayu *(Plecoglossus altivelus)* (6) and larval carp *(Cyprinus carpio)* (7) indicated that PL were dietarily essential because large amounts were needed to support the rapid proliferation of cell membranes associated with their exponential growth rate.

Little information is available on the value of phospholipids in diets for nonfood fish. Ornamental species including goldfish *(Carassius auratus)* are among the most valulable aquaculture exports in the United States (8). Goldfish are marketed also as "feeder" fish for other animals and as bait. Commercial feeds for goldfish vary considerably in composition, depending on the method of fish production. "Ornamentals" raised in closed systems are fed nutritionally complete feeds that contain large amounts of animal proteins such as fish meal. By contrast, "feeders" or baitfish produced in ponds consume natural biota in addition to artificial diets that contain comparatively more plant products. Solvent-extracted fish and soybean meals both contain small quantities of oil consisting of about 20% PL. However, plant feedstuffs contain lower levels of bioavailable nutrients, including PL. Supplemental lipids are often added to commercial fish feeds to enhance palatability, reduce fines, or increase flotation of the pellets. The comparative nutritional advantages of LEC over other lipids have not been widely exploited in the aquaculture industry in the United States.

Recently it was shown that first-feeding larval goldfish suffered high mortality and low growth when fed semipurified diets without PL (9). However, there is no information on the comparative nutritional efficacy of LEC and other lipids as supplements in practical feeds for juvenile goldfish. Therefore, the objective of this study was to establish the efficacy of deoiled soy LEC, soybean oil (SBO), and cod liver oil (CLO) alone or in combination as supplemental lipids in practical diets for goldfish.

EXPERIMENTAL PROCEDURES

Diets. Practical diets for goldfish were formulated from a basal diet to which various lipids were added (Table 1). Diets contained 33% protein from a combination of fish and soybean meals. Prior to supplementation of diets with the test lipids, basal diet ingredients were analyzed for total lipid (10). The fish and soybean meals were solvent-extracted commercially; however, the basal diet contained approximately 1.5% lipid. Therefore, the test lipids were referred to as supplemen-

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TABLE 1
Composition (percentage dry weight) of Practical Diets[a] Supplemented with Different Lipids in a Feeding Trial with Goldfish

Ingredient[b]	Diet number 1	2	3	4	5
Cod liver oil	4.0	2.0	0.0	0.0	0.0
Soybean oil	0.0	0.0	4.0	2.0	0.0
Deoiled soy lecithin[c]	0.0	2.0	0.0	2.0	4.0

[a]Diets contained 33% protein and 5.5% total lipid.
[b]All diets contained 5% menhaden fish meal, 55% soybean meal, 28.5% wheat shorts, 3% vitamin premix (11) [with 3 g/kg choline (from choline chloride) and 150 mg/kg inositol], 2% mineral mixture [with 68 g/kg phosphorus (from calcium phosphate monobasic)], 2.5% carboxymethylcellulose, and 0.0125% ethoxyquin.
[c]Lecithin contained 97% (min) "Acetone Insolubles" consisting of: 26% phosphatidylcholine, 20% phosphatidylethanolamine, 14% phosphatidylinositol, 4% phosphatidylserine, 13% phytoglycolipids, and 14% other phosphatides.

tal lipids. CLO and SBO were in liquid form, and the soy lecithin was a deoiled dry granular form. The PL composition of the LEC is shown in Table 1. A total of 4% lipid was added to diets as CLO (diet 1), equal amounts of CLO and LEC (diet 2), SBO (diet 3), equal amounts of SBO and LEC (diet 4), or LEC (diet 5).

Procedures for diet preparation and storage were as described previously (11,12) except that all diets were supplemented with 125 mg ethoxyquin/kg. Diets for golden shiners were ground to small crumbles in a Wiley mill (Arthur H. Thomas Co., Philadelphia, PA) to obtain appropriate particle sizes.

Culture system and experimental design. Juvenile goldfish were produced in ponds at the University of Arkansas at Pine Bluff (UAPB) for the 6-wk feeding trial conducted in July and August. Fish were maintained in 110-L conical tanks in a semistatic system containing reservoir water with a temperature of 28 ± 3°C. Water quality was maintained within acceptable ranges for goldfish by aeration and weekly water exchanges.

A conditioning period of one week preceded the experiment. Fish were fed a diet containing 4% SBO while acclimating to experimental conditions. Individual initial weights of goldfish averaged 0.3 g. Groups of 40 fish were stocked into three replicate tanks per treatment. Mortalities due to stress effects incurred during stocking were replaced during the first week of each experiment. In addition, 20 mg/L tetracycline hydrochloride (Pfizer Chemical, New York, NY) was added to aquaria to prevent bacterial infections of goldfish immediately following handling procedures.

During the experimental period, goldfish were fed 6% of their body weight daily, divided into two equal feedings. Fish were weighed biweekly, and mortalities were recorded. At termination of the study, final weights were obtained and fish were frozen for subsequent analysis. Weight gain was calculated per replicate as (final group weight + weight of mortalities) − initial group weight (g), where group refers to all fish in the replicate. Feed efficiency was calculated as: weight gain (g)/feed fed (g) per replicate. Whole-body lipid was analyzed (10) for one pooled sample of homogenized fish tissue per replicate. Each pooled sample consisted of 38–40 individual fish.

Statistical analysis. Treatment means for weight gain, feed efficiency, survival, and whole-body lipid were compared using the General Linear Models procedure of the Statistical Analysis System (13). Fisher's protected least significant difference (LSD) test was used to test for differences among treatment means at alpha = 0.05 (14).

RESULTS AND DISCUSSION

Weight gain of goldfish was lowest in fish fed diets with no supplemental LEC, intermediate in fish fed diets with equal amounts of SBO or CLO and LEC, and highest in fish fed the diet with 4% LEC (Table 2). The difference in gain of fish fed diets with no supplemental LEC (diets 1 and 3) vs. those fed a diet with 4% LEC was significant. Feed efficiencies were low, ranging from 0.45 to 0.55, because the feeding rate exceeded consumption slightly (Table 2). However, relative comparisons can be made because feed efficiency followed the same pattern as weight gain. There were no significant differences in survival or whole-body lipid among treatments

TABLE 2
Performance of Juvenile Goldfish Fed Practical Diets Supplemented with Soy Lecithin, Soybean Oil, and/or Cod Liver Oil for Six Weeks[a,b]

Diet number	Supplemental lipid[c] (%)	Initial average weight (g)[d]	Average weight gain (g)[e]	Feed efficiency[f]	Survival (%)	Whole-body lipid (%)
1	CLO (4%)	12.0 ± 0.2	19.9 ± 2.0^{z}	$0.45 \pm 0.04y^{y}$	98.3 ± 2.9	19.5 ± 9.0
2	CLO (2%) + LEC (2%)	11.9 ± 0.1	$23.1 \pm 2.6^{x,y}$	0.51 ± 0.03^{x}	100.0 ± 0.0	22.0 ± 5.3
3	SBO (4%)	11.9 ± 0.2	$22.1 \pm 1.1^{y,z}$	$0.50 \pm 0.03^{x,y}$	98.3 ± 2.9	23.1 ± 2.5
4	SB0 (2%) + LEC (2%)	11.9 ± 0.3	$23.1 \pm 0.9^{x,y}$	0.51 ± 0.02^{x}	100.0 ± 0.0	24.8 ± 1.4
5	LEC (4%)	11.9 ± 0.2	25.2 ± 0.7^{x}	0.55 ± 0.02^{x}	100.0 ± 0.0	21.2 ± 3.3

[a]Values represent means of three groups ± SD.
[b]Means with different superscripts are significantly different ($P < 0.05$) as determined by Fisher's protected least significant difference test.
[c]Key to abbreviations: cod liver oil (CLO), soy lecithin (LEC), and soybean oil (SBO).
[d]Weight of 40 fish. Average individual initial weight was 0.3 g.
[e]Final average group weight + weight of mortalities-initial average group weight (g).
[f]g gain/g dry feed.

($P > 0.05$) (Table 2). Whole-body lipid was variable for unknown reasons.

Both weight gain and survival improved with phospholipid supplementation of semipurified diets for larval goldfish (9) and larval common carp (7,15). Weight gain of Cyprinids in these studies improved consistently with dietary PL supplementation despite differences in fish size, and PL and diet composition between studies. By contrast, PL supplementation was unnecessary for survival of juvenile goldfish fed practical diets in this study, whereas it was critical for larval goldfish and carp (7,9). Enhanced survival of larval carp fed dietary PL was evident only for the first 3 wk of feeding (7,15).

Whole-body lipid of fish generally increases with dietary PL supplementation (2). In this study, whole-body lipid was higher in fish fed diets with CLO + LEC vs. CLO alone, and SBO + LEC vs. SBO alone. However, lipid of fish fed the diet with LEC only was lower than that of fish fed diets with combinations of lipids. Although differences were not significant, the trend in the data might indicate that LEC improved the digestibility of CLO and SBO in fish fed diets containing combinations of lipids. PL are required as emulsifiers during lipid absorption, but dietary or endogenous sources could fulfill the role. Dietary-PL enhancement of lipid absorption has been demonstrated in crustaceans (16) that have a limited ability to synthesize PL, and immature finfish appear to have a similarly limited ability. However, no correlation was found between the emulsification strength of various dietary PL sources and their effects on performance of larval carp (7).

One mechanism of enhanced performance of fish fed dietary PL is provision of EFA. The EFA requirement of goldfish is unknown, but presumably is similar to that of carp which require fewer fatty acids of the n-3 family than marine fish (17). Recent studies with carp larvae (7,18) indicated that trace amounts (0.05–0.1% of the dry diet) of the n-3 fatty acids satisfied their EFA requirements. Diets containing supplemental SBO, SBO + LEC, or LEC alone provided about 0.3% α-linolenic acid (18:3n-3) and 2% linoleic acid (18:2n-6). The fatty acid profiles of these diets were nearly identical, yet goldfish performance increased with the level of dietary LEC. Therefore, the effect did not appear to be a function of EFA. The diet containing supplemental CLO alone provided 1.1% of the n-3 fatty acids and 0.12% of the n-6 fatty acids. The dietary n-3/n-6 ratio declined progressively with partial or complete replacement of fish oil by LEC. However, all of the diets met or exceeded the reported EFA requirements of larval carp (7,18), survival of juvenile goldfish was high and independent of diet, and none of the fish showed external signs of EFA deficiency such as fin erosion or "shock syndrome" (17). Enhancement of fish performance by dietary PL apparently was unrelated to provision of EFA in ayu (6), striped jack *(Longirostris delicatissimus)* larvae (19), and carp larvae (7).

Diets in this study were supplemented with choline and phosphorus in excess of requirements established for common carp and channel catfish (20), and no specific deficiency signs of these nutrients were observed. Myoinositol is synthesized in the liver and intestine of channel catfish, and dietary supplementation is generally unnecessary (21). However, the 150 mg/kg of myosinositol provided by the vitamin premix was below the 440 mg/kg required by common carp (22). The basal diet ingredients contained only 1.5% lipid that provided no more than 130 mg/kg inositol based on published values (2). The supplemental LEC provided 0, 420, or 840 mg/kg myoinositol to diets containing 0, 2, or 4% supplemental LEC, respectively. Deficiency signs of this nutrient such as anorexia, fin erosion, and dark skin coloration reported in other fish (20) were not observed in goldfish in this study, but it is possible that the improved growth and feed efficiency observed in fish fed diets with supplemental LEC were related to dietary inositol content. Quantitative myoinositol requirements for goldfish must be established to confirm this possibility.

The improved weight gain and feed efficiency of goldfish fed diets with LEC could have resulted from increased availability of myoinositol, phosphatidylcholine or other specific PL fraction, enhanced absorption of dietary lipid, facilitation of lipid transport, or some combination of these. Additional nutritional requirements of juvenile goldfish must be established before it can be determined whether or not dietary PL themselves are essential for optimal growth. However, the present study indicated that supplemental PL were not essential for survival of small juvenile goldfish. Ultimately, the nutritional and economic advantages of soy LEC supplementation of practical diets for goldfish of various sizes must be demonstrated in ponds.

ACKNOWLEDGMENTS

This work was supported in part by research grant #ARKO1437. Lecithin and soybean oil were graciously donated by Riceland Foods, Inc.

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[Received May 20, 1996; accepted October 17, 1996]