Use of Soy Protein Concentrates and Lecithin Products in Diets Fed to Coho and Atlantic Salmon[1]

P.B. Brown*, K.A. Wilson, Y. Hodgin, and J.D. Stanley

Purdue University, Department of Forestry and Natural Resources, West Lafayette, Indiana 47907-1159

ABSTRACT: Aquacultural production is increasing in most parts of the world, establishing new and rapidly growing markets for various oil products. One of the more interesting nutritional requirements for aquatic animals is lecithin or phosphatidylcholine. In this paper, lecithin in aquaculture is reviewed with emphasis on freshwater fish and crayfish. Further, new data on use of lecithin and two soy protein concentrates in diets fed to coho and Atlantic salmon are presented. Juvenile coho and Atlantic salmon were fed either solvent-extracted soybean meal (SBM) or Promocalf® at 30% of the diet, Promoveal® at 10, 20 or 30% of the diet, or one of three new lecithin products at a constant level of 3% of the diet. Juvenile coho salmon fed SBM, Promocalf®, or Promoveal® at 30% of the diet exhibited depressed weight gain and an elevated feed conversion ratio (FCR) compared to fish fed a positive control diet. Fish fed 10 or 20% Promoveal® had similar weight gain and FCR compared to fish fed the control diet. Coho salmon fed either of the three lecithin products (Aqualipid®, Blendmax®, or Centrol®) had similar weight gains and FCR values compared to fish fed the control diet. Whole-body proximate components were not as responsive to dietary treatments as weight gain and FCR data. Juvenile Atlantic salmon exhibited depressed weight gain only when fed 30% Promocalf® and all three lecithin products. Further, whole-body crude protein concentrations in fish fed the three lecithin products were depressed.

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Aquacultural production is increasing at rapid rates throughout the world (1). This increase is in response to loss of traditional supplies of fish from the oceans that occurred in the latter half of this century. Even if wild populations of fish returned to pre-1950 levels, harvest would be unlikely to keep pace with increasing population and demand for fish. The increase in fish production places demands on the available feed stocks around the world and has resulted in a new active area of research. The initial focus of that research has been on sources of protein in diets fed to fish. The use of readily available commodities, particularly soy products, is a logical initial step (2–11).

Soybean production and processing are two of the largest agricultural pursuits in the world, and products from those industries serve as potential feedstuffs for the rapidly developing aquacultural industries. Fish require relatively high levels of crude protein in their diets as well as several atypical nutrients. Processed soy products and products from processing offer the potential of supplying both crude protein and several of the atypical nutrients.

Soy protein concentrates (SPC) are some of the new products from soy processing that could find an immediate use in diets fed to fish. A generalized proximate composition of SPC is >65% crude protein, <0.5% fat, <5% fiber, 23–25% nitrogen-free extract (NFE), and 7–8% ash. Fish grow maximally when fed diets that contain 25–45% crude protein, 6–25% fat, less than 7% crude fiber, and 20–30% NFE (12). Thus, SPC have potential as ingredients in diets fed to fish. The essential amino acid composition is also favorable for most species of fish. However, soy products contain antinutritional factors known to limit use of certain types of soy products in diets. Further processing of raw soybeans and soybean meal to SPC may remove some of these compounds and facilitate use of soy products.

Fish and aquatic crustaceans require several nutrients in their diets that are not typically thought of as essential nutrients in terrestrial animals. One of these is lecithin, or phosphatidylcholine (PC) (13–18). Lecithin is the primary phospholipid in most cell membranes and facilitates entry of compounds into cells. Phospholipids are both hydrophilic and lipophilic and are considered the primary compounds that impart viscosity to cell membranes. Fish and crustaceans are apparently the only animal groups that require PC in the diet; terrestrial vertebrates can synthesize sufficient quantities of PC, given sources of choline, methyl donors, and lipid substrates to form phosphatidylethanolamine.

Lecithin is a phospholipid with a three-carbon back-

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*To whom correspondence should be addressed at Purdue University, 1159 Forestry Building, West Lafayette, IN 47907-1159.

bone derived from glycerol-3-phosphate, similar to triacylglycerols (19). Fatty acids are attached to the *sn*-1 and *sn*-2 carbons, similar to triacylglycerols, but a nonfatty-acid compound, typically a nitrogenous base, is attached to the *sn*-3 position. Lecithin contains a choline moiety attached to the *sn*-3 carbon.

The purpose of this paper is to provide a brief review of PC metabolism in aquatic animals and present some new data on use of SPC and lecithin products in diets fed to coho (*Oncorhynchus kisutch*) and Atlantic salmon (*Salmo salar*). The focus of the review portion of the paper will be on those aspects that are different from terrestrial vertebrates and on the known aspects of PC metabolism in freshwater crayfish; crayfish are often overlooked in reviews of crustacean biochemistry. A thorough review on PC metabolism in fish, which can be found elsewhere (20), served as the basis of this discussion.

LECITHIN IN AQUACULTURE

The biochemical role of PC in aquatic animals has fascinated biologists for most of this century. Paul and Sharpe (21) described the mobilization of PC in the crustacean molt cycle in 1919 in marine crabs. That line of research languished until the latter half of the century when it expanded to those marine crustaceans of economic and aquacultural importance (17,18). The role of PC in fish biochemistry is a relatively new line of research, but the results have been interesting.

Poston examined the dietary essentiality of PC in diets fed to trout and salmon (13–15). The studies were designed as factorial experiments with supplemental choline and PC. On the basis of these results, PC appears to be an essential nutrient in diets fed to juvenile rainbow trout (*O. mykiss*) and Atlantic salmon in addition to a dietary source of choline. The need for dietary PC decreased as Atlantic salmon grew from 0.18 to 7.5 g initial weight. There was a significant effect on weight gain, feed conversion ratio, survival and whole-body fat concentrations in juvenile Atlantic salmon that were fed two forms of commercially available lecithin. At dietary incorporation levels at 4 and 8% of the diet, both a food-grade and a feed-grade form of lecithin promoted better response parameters than a control diet with no supplemental lecithin. The food-grade lecithin product contained approximately 16% PC from soy, whereas the feed-grade material contained approximately 50% PC from corn. Hung (22) determined that PC was not required in diets fed to white sturgeon (*Acipenser transmontanus*). However, studies with ayu (*Plecoglossus altivelis*) demonstrated the need for a dietary source of PC, but apparently no choline requirement existed (23). Larval puffer (*Fugu rubripes*) (24) and red sea bream (*Chrysophrys major*) require a dietary source of PC (25). Deficiency signs in these species included poor growth and feed conversion, poor survival, and scoliosis. Thus, there appear to be species differences among the fishes and age-related changes in dietary need for PC. Further work in this area may elucidate some of these differences.

PC is the major phospholipid in membranes of fish, with phosphatidylethanolamine (PE) found in the next highest concentration. Phosphatidylserine, phosphatidylinositol, cardiolipin, and sphingomyelin are found in smaller amounts. The typical fatty acids attached to the *sn*-1 and *sn*-2 carbons are similar to those of terrestrial vertebrates. Palmitic (16:0) and oleic (18:1n9) acids are the fatty acids typically found in the highest concentrations at the *sn*-1 position, and docosahexaenoic (22:6n3) and eicosapentaenoic acids (20:5n3) are the two most commonly attached fatty acids at the *sn*-2 position. The ratio of n-3/n-6 fatty acids in PC is in the range of 10–15:1.

Lecithin is also the predominant phospholipid in crustaceans, with phosphatidylethanolamine the next highest (26). The fatty acids of PC in crayfish are similar to those in fish (27–29), but two atypical classes of fatty acids have been identified associated with the *sn*-1 carbon. Furanoid fatty acids, those containing a furan ring, have been identified in the red swamp crayfish (*Procambarus clarkii*) (30), and branched-chain saturated fatty acids have been characterized from the noble crayfish (*Astacus astacus*) (28). Although the atypical fatty acids associated with PC in crayfish have been an interesting finding, perhaps the most interesting aspect of lipid metabolism in aquatic animals is the change in fatty acid and phospholipid composition with changing temperatures.

Species of fish and crustaceans living in temperate climates experience significant changes in environmental and, therefore, body temperatures with changing seasons. If biological membranes contained relatively high concentrations of saturated or monounsaturated fatty acids, then membrane permeability and viscosity would likely be impaired. Thus, changes occur with changing seasons and environmental temperatures in both fish and crayfish (31,32). The phase-transition temperature (or melting point) of the polyunsaturated fatty acids in PC is generally below 0°C in fish. Also, the concentrations of n-3 polyunsaturated fatty acids typically increase as temperature decreases. This response is partially attributable to synthesis of fatty acids and is associated with changes in prey items in wild populations of fish. In addition to the changes in fatty acid concentrations of PC, the concentrations of phospholipid classes change. Decreasing temperature results in increases in PE and decreases in PC in fish. Relatively little information has been developed on digestion and absorption of PC in aquatic animals.

Phospholipases A_1 and A_2 have been identified in fish, but only the cellular forms. It is probably safe to assume that the action of phospholipase A occurs in the intestine of fish and that absorption of lysolecithin and reesterification of PC follow similar patterns to those seen in higher vertebrates. The site of absorption of PC has been documented and occurs in the anterior ileum in some species and in the cecum to midgut region in others. Verification of the similarities in absorption between fish and terrestrial vertebrates would be a positive step toward understanding the dietary needs of PC.

High-density lipoprotein (HDL) is the predominant carrier of PC in pink salmon (*O. gorbuscha*). HDL was the only transport form of lipids found in that species, whereas rainbow trout has HDL, low-density lipoprotein, and very low-density lipoprotein. Biosynthesis of PC in aquatic animals is largely unstudied, but goldfish and trout synthesize PC by the cytidine diphosphate-choline pathway as in terrestrial vertebrates. This area of investigation is confounded by several nutritional factors, elucidated below, that could serve as points of further study.

One of the more important nutritional studies with new aquacultural species is establishment of the L-methionine requirement and the ability of cyst(e)ine to spare part of the dietary requirement for methionine (33–37). The sulfur amino acids are often first limiting, along with lysine, in diets fed to many species of fish. Catabolism of the essential amino acid methionine yields cyst(e)ine and choline. Thus, there is a potential that choline also spares part of the dietary methionine requirement. However, this has not been explored in diets fed to fish or crustaceans. Another logical study is the ability of PC to spare either the choline or methionine requirement. Again, this has not been explored in aquatic animals. Based on current information, it is difficult to dispute the dietary need for PC in trout and salmon, as many of the essential amino acids and choline requirements have been quantified. In the newer aquacultural species, dietary inadequacies in methionine, choline, triacylglycerols, or other cofactors in PC biosynthesis (38) may have lead to results that only appear supportive of the dietary essentiality of PC in diets. Despite the uncertainties, PC metabolism in aquatic animals deserves further attention, both for comparative purposes and for the apparent need in the developing aquacultural industries.

The above discussion led to the studies presented below, particularly as new products were developed by the major agricultural companies.

EXPERIMENTAL PROCEDURES

Two soy protein concentrates (Promoveal® and Promocalf®), three lecithin products (Centrol®, Blendmax®, and Aqualipid®), and solvent-extracted, toasted soybean meal were supplied by Central Soya (Fort Wayne, IN). Fish meal (Norse LT) was from the U.S. Fish and Wildlife Service, Tunison Laboratory of Fish Nutrition (Cortland, NY). Fish oil was from Zapata Haynie (Reedville, VA). The L-methionine, dextrin, cellulose, all vitamins, and a purified source of lecithin were supplied by U.S. Biochemical (Cleveland, OH). Minerals were of reagent grade and supplied by Sigma Chemical Co. (St. Louis, MO). All protein feedstuffs were supplied with guaranteed analysis from the supplier. All diets were formulated to meet or exceed the known nutritional requirements of salmonids (12,39). Centrol® is a standard grade of soybean lecithin, Blendmax® is an enzyme-hydrolyzed lecithin product, and Aqualipid® is a deoiled lecithin.

TABLE 1
Ingredient Composition (% of the dry diet) of Diets Fed to Coho and Atlantic Salmon

Ingredient[a]	Diet number 1	2	3	4	5	6
Fish meal	48.2	28.7	39.3	30.4	21.4	22.2
Soybean meal	0	30.0	0	0	0	0
Promoveal®[b]	0	0	10	20	30	0
Promocalf®[b]	0	0	0	0	0	30
L-Methionine	0	0.1	0.1	0.2	0.3	0.3

[a]All diets contained by weight 15% fish oil, 8% mineral premix, 0.8% vitamin premix, 3% lecithin, 10% dextrin, and cellufil in varying amounts.
[b]Central Soya (Fort Wayne, IN).

Each diet was mixed and pelleted separately as described elsewhere (40). Dry ingredients were weighed and mixed in a twin-shell V-mixer (Patterson-Kelly, East Stroudsburg, PA), then transferred to a benchtop Hobart mixer (Hobart Corp., Troy, OH). Water and lipid were added and further mixed. Diets were pelleted with the chopping-end attachment of the Hobart mixer. Each diet contained nutritionally complete vitamin and mineral premixes (40) mixed separately from the diets.

The control diet contained fish meal as the sole source of crude protein and purified lecithin (Table 1). One diet contained 30% soybean meal (SBM), one diet contained 30% Promocalf®, and three diets contained either 10, 20 or 30% Promoveal®. All soy products were incorporated at the expense of fish meal on an isonitrogenous basis. Three diets contained the commercial lecithin products each substituted at 3% of the dry diet.

Coho salmon were obtained from the Michigan Department of Natural Resources, and Atlantic salmon were obtained from the U.S. Fish and Wildlife Service. All fish were quarantined prior to conducting the studies. Two separate studies were conducted, one with each species.

Juvenile coho salmon were stocked into 40-L glass aquaria at a density of 15 fish per aquarium. Initial weight of individual fish ranged from 2.8 to 3.1 g. Each dietary treatment was fed to triplicate groups of fish. Water temperature was 15°C throughout the study, and critical water quality parameters (dissolved oxygen, ammonia-N, and nitrite-N) did not exceed concentrations considered dangerous for this species.

Juvenile Atlantic salmon were stocked into 40-L glass aquaria at a density of 10 fish per aquarium. Initial weight of individual fish ranged from 3.8 to 4.1 g. Each dietary treatment was fed to triplicate groups of fish. Water temperature was 18°C throughout the study, and water quality values were acceptable throughout the study.

Fish in both studies were fed twice per day. Coho salmon were fed 3.5% of their wet body weight per day, and Atlantic salmon were fed 4.0% of their wet body weight. Fish in each aquarium were weighed every two weeks for adjustment of food allotment. The duration of each experiment was 56 days. Weight gain was calculated as percentage increase from initial weight over the entire study period. Feed conversion was calculated as dry weight of feed offered/wet weight gain of fish.

At the end of each study, all fish were weighed and samples of fish (three per replicate) collected for whole-body proximate analysis. Nitrogen, ash, and moisture were determined by AOAC methods (41). Moisture was determined by chopping individual fish into 0.5-cm slices and drying at 100°C overnight. Nitrogen was determined by micro-Kjeldahl, and crude protein was estimated as N × 6.25. Ash was determined by combustion in a muffle furnace at 600°C overnight. Lipid concentrations were determined by chloroform:methanol extraction (42).

Final weight gain, feed conversion ratio (FCR), and whole-body proximate composition data were subjected to one-way analysis of variance by using the Statistical Analysis System (43). If significant differences were detected, Duncan's New Multiple Range Test was used to rank treatment means.

RESULTS AND DISCUSSION

Weight gain and FCR values from coho salmon, fed the various soy and lecithin products, are shown in Table 2. Both values were significantly higher in fish fed the control diet and either 10 or 20% Promoveal®. Fish fed 30% of either SPC exhibited significantly lower weight gain and FCR than fish fed the control diet; both response variables were significantly lower in fish fed 30% soybean meal compared to fish fed 30% of either SPC. Weight gain and FCR values for fish, fed any of the three lecithin products, were not significantly different from fish fed the control diet.

Whole-body moisture concentration of coho salmon, fed the various experimental diets, was not significantly different from fish fed the control diet (Table 3). Whole-body crude protein concentrations were significantly higher in fish fed 30% soybean meal and significantly lower in fish fed two of the three lecithin products (Aqualipid® and Centrol®). Fish fed any level of SPC or Blendmax® exhibited the same crude protein concentrations as fish fed the control diet. Whole-body fat concen-

TABLE 2
Mean Weight Gain and Feed Conversion of Coho Salmon Fed Various Soy Products or Lecithin[a]

Diet	Weight gain[b]	Feed conversion ratio[c]
Soy products		
1 (control)	229.1a	1.4c
2 (30% soybean meal)	102.0c	2.5a
3 (10% Promoveal®)	227.3a	1.4c
4 (20% Promoveal®)	218.4a	1.4c
5 (30% Promoveal®)	170.0b	2.0b
6 (30% Promocalf®)	161.1b	2.0b
Lecithin		
7 (Aqualipid®)	251.3a	1.3c
8 (Blendmax®)	219.3a	1.4c
9 (Centrol®)	227.4a	1.4c

[a]Means of three replicate groups of fish. Values in the same column with the same letter designation were not significantly different ($P < 0.05$). See Table 1 for company supplier.
[b]Expressed as the percentage increase from initial weights.
[c]Dry weight of feed/wet weight gain of fish.

TABLE 3
Mean Proximate Composition of Coho Salmon Fed Various Soy and Lecithin Products[a]

Diet	Moisture	Crude protein	Fat	Ash
Soy products				
1 (control)	63.6	42.0b	48.8a	7.3b
2 (30% soybean meal)	66.0	51.6a	41.9b	8.6a
3 (10% Promoveal®)	64.8	45.4b	42.9a,b	8.4a
4 (20% Promoveal®)	64.7	43.4b	49.0a	7.2b
5 (30% Promoveal®)	64.3	39.6b,c	44.0a,b	7.3b
6 (30% Promocalf®)	67.2	45.5b	43.8a,b	8.1a,b
Lecithin				
7 (Aqualipid®)	65.8	38.8c	48.5a	7.0b
8 (Blendmax®)	66.5	45.8b	43.6a,b	7.0b
9 (Centrol®)	65.5	38.4c	44.4a,b	7.8a,b

[a]Crude protein, fat, and ash concentrations expressed as a percentage of dry matter ($n = 9$). Values in the same column with the same letter designation were not significantly different ($P < 0.05$). Moisture concentrations were not significantly different. See Table 1 for company supplier.

TABLE 4
Mean Weight Gain and Feed Conversion of Atlantic Salmon Fed Various Soybean Products and Lecithin[a]

Diet	Weight gain[b]	Feed conversion[c]
Soy products		
1 (control)	386.2a	1.9b
2 (30% soybean meal)	359.6a,b	1.9b
3 (10% Promoveal®)	444.7a	2.1a,b
4 (20% Promoveal®)	352.6a,b	1.8b
5 (30% Promoveal®)	381.1a	1.9b
6 (30% Promocalf®)	331.9b	2.1a,b
Lecithin		
7 (Aqualipid®)	326.7b	2.2a,b
8 (Blendmax®)	276.5b,c	2.3a
9 (Centrol®)	263.9b,c	1.4a

[a]Means of three replicate groups of fish. Values in the same column with the same letter designation were not significantly different ($P < 0.05$). See Table 1 for company supplier.
[b]Expressed as the percentage increase from initial weights.
[c]Dry weight of feed/wet weight gain of fish.

TABLE 5
Mean Proximate Composition of Atlantic Salmon Fed Various Soy and Lecithin Products[a]

Diet	Moisture	Crude protein	Fat	Ash
Soy products				
1 (Control)	68.8	53.0a	39.0	9.0
2 (30% soybean meal)	70.4	52.2a	38.5	8.7
3 (10% Promoveal®)	70.0	52.3a	40.5	8.8
4 (20% Promoveal®)	69.5	51.4a	38.1	8.8
5 (30% Promoveal®)	69.7	53.0a	38.1	8.2
6 (30% Promocalf®)	69.8	55.0a	39.1	8.1
Lecithin				
7 (Aqualipid®)	69.4	47.8b	42.4	9.3
8 (Blendmax®)	68.1	49.6a,b	43.2	8.8
9 (Centrol®)	68.7	46.1b	40.4	8.7

[a]Crude protein, fat, and ash concentrations expressed as a percentage of dry matter (n = 9). Values in the same column with the same letter designation were not significantly different ($P < 0.05$). Moisture, fat, and ash concentrations were not significantly different. See Table 1 for company supplier.

trations in fish fed 30% soybean meal were significantly lower than in fish fed the control diet. Fat concentrations were not significantly different in fish fed other experimental diets. Whole-body ash concentrations were significantly higher in coho salmon fed 30% soybean meal or 10% Promoveal®; other ash concentrations were not significantly different from those in fish fed the control diet.

Weight gain and FCR values of Atlantic salmon, fed the experimental diets, are shown in Table 4. Fish fed 30% Promocalf® and the three lecithin products exhibited significantly lower weight gain than fish fed the control diet; other weight gain values were not significantly different from those for fish fed the control diet. Feed conversion ratios were significantly higher in fish fed two of the three lecithin products (Blendmax® and Centrol®) compared to fish fed the control diet. Other FCR values were not significantly different from fish fed the control diet.

Whole-body moisture, fat, and ash concentrations of fish fed the various diets were not significantly different from fish fed the control diet (Table 5). Whole-body crude protein concentrations of fish, fed two of the lecithin products, were significantly lower than in fish fed the control diet. Other whole-body crude protein concentrations were not significantly different from fish fed the control diet.

Promoveal® appears beneficial as an ingredient in diets fed to coho and Atlantic salmon. The level of incorporation in diets fed to coho salmon should be less than 30%, whereas a concentration that resulted in adverse growth or FCR of Atlantic salmon was not identified. Soybean meal was clearly inferior to the two SPC when fed to coho salmon, but it did not result in reduced weight gain when fed to Atlantic salmon at 30% of the dry diet. These results are similar to those of other researchers for Atlantic salmon (9,10) and rainbow trout (6–8). Coho salmon appear more susceptible to antinutritional factors remaining in soybean meal and possibly SPC. Specific factors that limit use of soy products in diets fed to coho salmon have not been identified.

The three lecithin products evaluated in this study appear beneficial in diets fed to coho salmon. No obvious signs of toxicity or deficiency were apparent. However, feeding any of the three products to Atlantic salmon resulted in reduced weight gain. The cause of this remains unknown. Lecithin is considered an essential nutrient for certain sizes of Atlantic salmon (14), including the initial size of fish used in this study. However, the concentrations used may not have been sufficient to meet the requirements of the fish when incorporated at a constant 3% of the diet. Further studies with Atlantic salmon seem warranted.

Feed conversion ratios in both studies were relatively high and served only for comparative purposes. Attempts were made at the beginning of each study to determine voluntary consumption of experimental diets. It seems clear from the data that the experimentally determined initial consumption values decreased through the course of the study, resulting in elevated FCR values. This factor should not have affected the results because all fish had ample access to feed.

Soy products offer great potential for replacing fish meal in diets fed to cultured fish in the short term. However, those products are only recently receiving sufficient attention that will facilitate acceptance among the world's large suppliers of fish feed. Additional detailed studies are necessary to define the modifications in soy products necessary for routine incorporation into diets fed by the rapidly expanding aquaculture industries. Lecithin products are considered essential in some species, but the detailed interactions of all nutritional components that impact use of that nutrient and the new forms of the nutrient have not been fully elucidated.

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