

Growth Factors in the Fish Meal Component of Catfish Diets¹

JAMES W. ANDREWS AND JIMMY W. PAGE

University of Georgia Agricultural Experiment Station and the
Skidaway Institute of Oceanography, P. O. Box 13687,
Savannah, Georgia 31406

ABSTRACT Four feeding studies were conducted to determine to what extent soybean meal could be used to replace fish meal in diets for channel catfish (*Ictalurus punctatus*). When soybean meal was substituted on an isonitrogenous basis for menhaden meal, growth and feed efficiency were substantially reduced ($P < 0.05$). Gains were not enhanced when synthetic methionine, cystine or lysine, the most limiting amino acids as the results of this substitution, were added to the soy-substituted diets. When diets void of fish meal were supplemented with a lipid extract, nonlipid residue or ashed fraction of menhaden meal, growth and food conversion data revealed that the growth factors not provided by soybean meal were in the nonlipid residue of menhaden meal and were destroyed by ashing. A further study demonstrated that these factors were not polar lipids that could be removed by extraction with chloroform-methanol (2:1). These data suggested that the growth factors were in the protein fraction of menhaden meal but do not explain why growth responses were not obtained when the soy-substituted diets were supplemented with the most limiting amino acids. *J. Nutr.* 104: 1091-1096, 1974.

INDEXING KEY WORDS catfish · *Ictalurus punctatus* · fish meal · soybean meal · unidentified growth factors · methionine · lysine · cystine

Previous studies² have shown that a substantial quantity of fish meal is desirable in diet for high density culture of catfish. In these experiments, a severe reduction in growth was obtained when all the fish meal in the diet was replaced on an isonitrogenous basis by soybean meal. When soybean meal was substituted for one-half of the fish meal protein, a substantial but less dramatic reduction in growth occurred. This indicates that there may be growth factors for catfish in fish meal, which are not present in sufficient quantity in soybean meal.

The amino acid profile of diets in the above study reveals that lysine and methionine appear to be the most limiting essential amino acids (1) as a result of this substitution.

Synthetic methionine is widely used to supplement diets in which soybean meal is the major protein source. Although synthetic methionine has been used in experi-

mental diets for catfish (1-4), it has not been demonstrated to what extent the addition of this amino acid improves performance.

The following study was conducted in order to provide more information on the growth factors for catfish that are present in fish meal, but are not provided by an isonitrogenous substitution of soy protein.

METHODS

Four feeding experiments were conducted with channel catfish (*Ictalurus punctatus*) fingerlings. In experiments 1

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²Andrews, J. W. A study of the nutritional, physiological and economical requirements for the production of channel catfish in an intensive running water culture. Completion report for project 2-84-B, Public Law 88-309, NOAA, National Marine Fisheries Service, Washington, D. C. June 1972.

and 2, graded levels of methionine and cystine were added to diets in which fish meal had been replaced by soybean meal. In experiments 3 and 4, in order to determine if the unidentified growth factors in fish meal were in the fat-soluble, residue or ash fractions, these fractions were added to diets in which soybean meal had been substituted for fish meal.

Description of diets

Experiments 1 and 2. The compositions of the diets used in these experiments are presented in tables 1 and 2.

In experiment 1, soybean meal was substituted on an isonitrogenous basis for one-half (diet 2) and all (diet 4) of the menhaden meal in the control diet (diet 1). In diet 3, 0.35% methionine was added to diet 2 in order to bring the total sulfur amino acid content up to the level of the control diet (3.80% of protein). In diets 5 to 8, graded levels of methionine were added in 0.36% increments to a diet in which all the menhaden meal had been replaced by soybean meal. Two supplemental levels of cystine were added in diets 9 and 10. The total lysine levels of all diets were kept constant (1.9% of diet) by the addition of small amounts of *L*-lysine.

In experiment 2, 75% of the menhaden meal in the control diet, which contained 20% menhaden meal (diet 1), was re-

placed on an isonitrogenous basis by soybean meal (diet 2). Two 0.15% increments of methionine and one 0.15% increment of cystine were added in diets 3 to 5. The lysine level of each diet was brought up to the control level by the addition of *L*-lysine.

Experiments 3 and 4. The diets used in these experiments are summarized in table 3. Diets 1 to 3 contained varied levels of substitutions of soybean meal for fish meal.

In an attempt to determine if the unidentified growth factors in fish meal were in the fat-soluble, water-soluble or mineral fractions, these fractions were added in diets 4 to 7 in lieu of the menhaden meal. The ether extract³ from a portion of menhaden meal equivalent to 20% of the diet was added in diet 4. In diet 5, the nonlipid residue³ from an ether extraction of a portion of the menhaden meal equivalent to 20% of the total diet was substituted in lieu of 20% menhaden meal. In order to test the effects of removing the more polar lipids, the residue from a chloroform-methanol (2:1) extraction³ of fish meal was added in diet 6 (experiment 4 only). In diet 7 the ash³ from a portion of menhaden meal equivalent to 20% of the total diet was added to a diet in which soybean meal was substituted for menhaden meal.

* See footnotes 4 to 7 in table 3.

TABLE 1
Composition of diets¹ in experiment 1

Diet	MM ²	SBOM ³	Cellulose ⁴	Total percentage of protein as			Supplemental	
				Met	Cys	Met + Cys	Met	Cys
	%	%	%	% of diet				
1	30	16	24.6	2.65	1.15	3.80	0	0
2	15	34	18.4	2.15	1.29	3.44	0	0
3	15	34	18.0	2.51	1.29	3.80	0.36	0
4	0	52	12.1	1.66	1.42	3.08	0	0
5	0	52	11.7	2.02	1.42	3.44	0.36	0
6	0	52	11.4	2.38	1.42	3.80	0.72	0
7	0	52	11.0	2.74	1.42	4.16	1.08	0
8	0	52	10.7	3.14	1.42	4.52	1.44	0
9	0	52	11.7	1.66	1.78	3.44	0	0.36
10	0	52	11.4	1.66	2.14	3.80	0	0.72

¹ The levels of menhaden meal, soybean meal, and synthetic amino acids listed in this table were added to a basal diet containing the following ingredients (percentage of total diet): corn gluten meal (60%), 7.7; distillers' dried grains with solubles, 10; wheat shorts, 10; vitamin and mineral premix (3), 0.65%. The calculated total protein level of each diet was 35% plus the substituted amino acids. Calcium, phosphorus and lipid levels were maintained at 1.5, 1.0, and 10% of diet, respectively, by the addition of calcium carbonate, dicalcium (monobasic) phosphate and tallow. Synthetic *L*-lysine was used to keep the total lysine level of each diet at 1.9% (5.4% of protein). Cellulose was used to bring the total ingredient level in each diet up to 100%. ² MM, menhaden meal (60% protein); SBOM, soybean meal (50% protein). ³ Solka-Floc, Brown Co., Berlin, N. H. 03570.

TABLE 2
Composition of diets in experiment 2¹

Diet	MM ²	SBOM ³	Cellulose ⁴	Total percentage of protein as			Supplemental	
				Met	Cys	Met + Cys	Met	Cys
	%	%	%	% of diet				
1	20	20	10.9	2.45	1.53	3.98	0	0
2	5	38	4.7	2.02	1.53	3.55	0	0
3	5	38	4.6	2.45	1.53	3.98	0.15	0
4	5	38	4.4	2.88	1.53	4.41	0.30	0
5	5	38	4.6	2.02	1.96	3.98	0	0.15

¹ The levels of menhaden meal, soybean meal and synthetic amino acids listed in this table were added to a basal diet containing the following ingredients (percentage of total diet): corn gluten meal (60%), 20.0; corn, 20.0; vitamin and mineral premix (3), 0.85. The total protein level of each diet was 34.7% + the supplemental amino acids. Calcium, phosphorus and lipid levels were maintained at 1.5, 1.0 and 10% by the addition of calcium carbonate, dicalcium (monobasic) phosphate and tallow. Synthetic L-lysine was used to keep the total lysine level of each diet at 1.9% (5.4% protein). Cellulose was used to bring the total ingredient level in each diet up to 100%. ² MM, menhaden meal (60% protein); SBOM, soybean meal (44% protein). ³ Solka-Floc, Brown Co., Berlin, N. H. 03570.

Total calcium, phosphorus, protein and lipid levels of all diets were kept constant by the addition of calcium phosphate dibasic, calcium carbonate, soybean meal and tallow in lieu of cellulose.

Experimental conditions

The diets described above were fed to triplicate replicates of 20 channel catfish fingerlings stocked in 0.9-m diameter fish culture tanks. The initial individual average weights of fish in experiments 1 to 4 were 14.1, 15.9, 4.2 and 4.3 g, respectively. The durations of the feeding trials in experiments 1 to 4 were 6, 8, 6 and 8 weeks, respectively. Feeding rates averaged 4% of biomass daily during the entire periods. Fish were fed twice daily (8:00 AM and 4:00 PM).

Water quality and temperature were controlled by the constant addition of 3.8 liters/minute of preheated (28°) well water. Water temperatures remained within one degree of the temperature of the incoming water. Dissolved oxygen levels were at least 7 ppm throughout the experimental periods.

At the end of each experiment, fish were weighed individually and growth was expressed in terms of average individual percentage gain. Growth data were analyzed for statistical significance by the method of Duncan (5).

RESULTS AND DISCUSSION

Experiments 1 and 2. In all cases, growth was significantly ($P < 0.05$) reduced when soybean meal was substituted on an iso-

TABLE 3
Composition of diets in experiments 3 and 4

Diet	Basal ¹	MM ²	SBOM ³	Cellulose	Supplement	%
	%	%	%	%		
1	40.76	20	20	10.61	None	0
2	40.76	5	39.5	2.69	None	0
3	40.76	0	46.0	0	None	0
4	40.76	0	46.0	0	MM ether extract ⁴	1.60
5	40.76	0	20.0	9.41	MM ether residue ⁵	19.6
6	40.76	0	20.0	9.41	MM CM residue ⁶	19.6
7	40.76	0	46.0	0	MM ash ⁷	3.67

¹ Provided a total dietary level of 20% corn gluten meal (60% protein) and 20% yellow corn and vitamin and mineral levels as used in a previous study (3). Tallow, calcium carbonate and calcium (monobasic) phosphate were added to each diet at a level that provided the total lipid, calcium and phosphorus content of each diet at 10.0, 1.5 and 1.0%, respectively. ² MM, menhaden meal (60% protein). ³ SBOM, soybean meal (50% protein). Added at a level that provided 34.5% protein in all diets. ⁴ Ether extract from a portion of menhaden meal that was equivalent to 20% of total diet. ⁵ Nonlipid residue from ether extract of a portion of menhaden meal that was equivalent to 20% of total diet. ⁶ Nonlipid residue from chloroform-methanol extract (2:1) of a portion of menhaden meal that was equivalent to 20% of total diet. ⁷ Ashed residue (500°) from a portion of menhaden meal that was equivalent to 20% of total diet.

TABLE 4
Effects of adding synthetic methionine and cystine to diets in which soybean meal had been substituted for menhaden meal (experiment 1)

Diet	Menhaden meal	Total percentage of protein as Met and Cys	Supplemental		Avg gain ¹	g feed/g gain
			Met	Cys		
	%			%		
1	30	3.80	0	0	188 ^a	1.1
2	15	3.44	0	0	168 ^b	1.3
3	15	3.80	0.36	0	170 ^b	1.3
4	0	3.08	0	0	130 ^c	1.4
5	0	3.44	0.36	0	128 ^c	1.5
6	0	3.80	0.72	0	134 ^c	1.5
7	0	4.16	1.08	0	127 ^c	1.6
8	0	4.52	1.44	0	132 ^c	1.5
9	0	3.44	0	0.36	125 ^c	1.6
10	0	3.80	0	0.72	129 ^c	1.5

¹ Average percentage gains from 3 replicate tanks containing 20 fish each. Values followed by the same letter are not statistically different ($P > 0.05$). The SEM from the analysis of variance of the average gains was 5.7.

nitrogenous basis for menhaden meal (tables 4 and 5). In experiment 1, the average gains of fish fed a diet with all the fish meal protein replaced by soybean meal (diet 4) were significantly less ($P < 0.05$) than those from fish fed diets in which only one-half of the fish meal had been replaced (diet 2).

In no case was growth significantly ($P < 0.05$) enhanced by the addition of synthetic methionine or cystine.

Food conversion ratios (g feed/g gain) reflected growth data.

These results suggest that either synthetic methionine or cystine were not efficiently utilized or that the methionine plus cystine requirement is 3.08% or less of protein. Studies with chinook salmon⁴ have demonstrated a growth response to synthetic methionine supplements and have also indicated that this species' methionine requirement in the presence of 1.0% dietary cystine is 0.5 to 0.6% of diet (1.2 to 1.5% of protein). This corresponds to a total methionine plus cystine level of 3.75 to 4.0% of crude protein, which was well above the minimum level of 3.08% used in the present study.

Although free amino acids are generally well utilized by animals, a recent report (6) has indicated that free amino acids may not be well utilized by carp. To the contrary, in reports on the qualitative amino acid requirements of catfish (1), a growth response was obtained when syn-

thetic amino acids (including methionine and cystine) were added to diets that were void of the given amino acid being studied. Likewise, in several studies on the amino acid requirements of salmonids⁴⁻⁷ (7-12) all of the essential amino acids have been fed in the free form with no apparent reduction in utilization efficiency.

If the methionine plus cystine requirement of catfish is 3.08% or less of protein (or the methionine requirement is 1.66% or less) then sulfur amino acids are not the growth-limiting factor as a result of this substitution. Because lysine was added to each diet in order to bring the total lysine level up to the level of the fish meal controls, lysine does not appear to be limiting. Also, the total lysine level (5.4% of protein) maintained in each diet in both experiments was well above the reported requirements for the salmon⁵ (5% of protein). Leucine and isoleucine are the only other essential amino acids whose levels are reduced as a result of isonitrogenous substitution of soybean meal for menhaden meal. In both cases the re-

⁴ Halver, J. E., DeLong, D. C. & Mertz, E. T. 1959. Methionine and cystine requirements of chinook salmon. *Federation Proc.* 18, 527. (Abstr.)

⁵ Halver, J. E., DeLong, D. C. & Mertz, E. T. 1959. Threonine and lysine requirements of chinook salmon. *Federation Proc.* 17, 1873. (Abstr.)

⁶ Halver, J. E. 1965. Tryptophan requirements of the chinook, sockeye and silver salmon. *Federation Proc.* 24, 229. (Abstr.)

⁷ Shanks, W. E., Gahlmer, G. D. & Halver, J. E. 1962. The indispensable amino acids for rainbow trout. *Bureau of Sport Fisheries and Wildlife. Progr. Fish Cult.*, April, 68-73.

TABLE 5

Effects of adding synthetic methionine and lysine to diets in which soybean meal had been substituted for menhaden meal (experiment 2)

Diet	Menhaden meal	Total percentage of protein as Met and Lys	Supplemental		Avg gain ¹	g feed/g gain
			Met	Lys		
	%			%		
1	20	3.98	0	0	480 ^a	1.50
2	5	3.55	0	0	438 ^b	1.60
3	5	3.98	0.15	0	450 ^b	1.60
4	5	4.41	0.30	0	444 ^b	1.61
5	5	3.98	0	0.15	441 ^b	1.59

¹ Average percentage gains from 3 replicate tanks containing 20 fish each. Values followed by the same letter are not statistically different ($P > 0.05$). The SEM from the analysis of variance of the average gains was 9.1.

duction is slight, with only a 0.1% of diet reduction when fish meal was totally replaced by soy protein. The lowest leucine and isoleucine level of any of the test diets was 8.7 and 5.0% of protein, respectively. These levels are twofold above the reported leucine (3.9% of protein) and isoleucine (2.2 to 2.7% of protein) requirements for salmon (7). Thus, it is highly unlikely that either of these amino acids was limiting in the present study.

After results from these two experiments indicated that methionine or lysine (the most likely possibilities) were not the unidentified factors that prohibited the substitution of soybean meal for fish meal in catfish diets, experiments 3 and 4 were designed in an attempt to determine what fractions of fish meal contained the growth factors.

Experiments 3 and 4. As was the case in experiments 1 and 2, growth was reduced

significantly ($P < 0.05$) by an isonitrogenous substitution of soybean meal for 15% of fish meal (table 6, diet 2) and was further reduced by replacing all of the fish meal (diet 3). Food conversion was also affected by these substitutions.

Addition of the ether extract (lipid) from a portion of menhaden meal, equivalent to 20% of total diet, to a diet in which soybean meal had been substituted for fish meal (diet 4) did not significantly ($P < 0.05$) enhance growth over that obtained from diet 3. Thus, the unidentified growth factors were not in the fat-soluble protein of the fish meal.

The isonitrogenous addition of the ether or chloroform-methanol (2:1) residue of fish meal (diets 5 and 6) to diet 3 resulted in similar gains and food efficiencies as obtained from the control diet with 20% whole menhaden meal (diet 1). This indicated that the growth factors were present

TABLE 6

Effects of dietary substitution of fish meal fractions on growth and feed conversion of catfish

Diet	Menhaden meal	Supplement ¹	Experiment 3		Experiment 4	
			Gain ²	g feed/g gain	Avg gain ²	g feed/g gain
	%		%		%	
1	20	None	237 ^a	1.51	465 ^a	1.50
2	5	None	215 ^b	1.75	412 ^b	1.72
3	0	None	195 ^c	2.01	360 ^c	1.90
4	0	MM ether extract	194 ^c	1.94	375 ^c	1.74
5	0	MM ether residue	242 ^a	1.48	469 ^a	1.45
6	0	MM CM residue			475 ^a	1.46
7	0	MM ash	163 ^d	2.05	358 ^c	1.87

¹ See footnotes in table 3 for a description of each supplement. ² Average percentage gain from 3 replicate tanks containing 20 fish each. Values followed by the same letter are not statistically different ($P > 0.05$). The SEM from the analysis of variance of the average gains from experiments 1 and 2 were 9.2 and 13.4, respectively.

in the nonlipid portion of fish meal. The fact that the growth response still occurred when the polar lipids were removed (diet 6, chloroform-methanol residue) demonstrated that the growth factors were not polar lipid components.

The addition of an ashed portion of fish meal (diet 7) did not result in an enhancement in growth. In experiment 3, growth was significantly reduced by this addition. Because the unidentified growth factors were not in the mineral elements (ash) in the nonlipid residue, they must be protein or other nonlipid components in the residue from the lipid extract.

The fact that adding methionine, cystine or lysine to the soybean meal-substituted diet did not enhance growth, raises the following possibilities: (1) the unidentified growth factors are not proteins or amino acids but are other nonlipid and nonmineral compounds; (2) free methionine or lysine are not efficiently utilized by catfish; (3) there may be differences between soy and fish-meal protein in availability of other amino acids, thus resulting in another amino acid being limiting; or (4) a very delicate amino acid balance is present in fish meal and the substitution of soybean meal results in a severe imbalance.

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