Effects of Lysine on Growth of Tilapia Fed Diets Rich in Corn Gluten Meal

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ABSTRACT

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Tilapia is a warmwater fish with mild flavor. Nearly 8.6 million kg are produced domestically, and ≈22.7 million kg are imported. Corn gluten meal (60% protein fraction) is a product obtained from wet-milling of corn. Diets (36% protein) containing 36–44% corn gluten meal with different levels of lysine and fish meal were formulated and fed to tilapia in aquaria for 12 weeks. Weight gain (WG) of tilapia fed diets containing the highest level of lysine (7.4% protein) with 4% fish meal was equal to that of fish fed a commercial control diet. Diets with lower lysine resulted in lower WG. The feed conversion ratio (FCR) and protein efficiency ratio (PER) of tilapia fed experimental diets containing adequate levels of essential amino acids and fish meal were the same as for fish fed the commercial control diet (also containing fish meal). Fish fed diets containing lower lysine levels had less favorable FCR and PER. This study shows that corn gluten meal is utilized at high levels in tilapia diets, particularly if essential amino acids are provided in adequate amounts.

The wet-milling process separates corn into starch, protein, oil, and fiber fractions. Corn gluten meal is the protein fraction and typically contains more than 60% protein. As more starch is converted to ethanol fuel and high-fructose corn syrup, more corn gluten meal is produced. As a result, it is desirable to find new uses for corn gluten meal in addition to the existing markets in cattle, swine, and poultry feeds.

For human and nonruminant animal diets, normal corn is deficient in lysine and tryptophan, but high-lysine corn contains increased levels of lysine and tryptophan (Mertz et al 1964). Use of high-lysine corn instead of normal corn can reduce the dietary protein supplement needed for swine feed. Because fish require a higher percentage of protein compared to swine, it would be especially beneficial to use high-lysine corn instead of normal corn to reduce the necessary dietary protein supplement. The use of high-lysine corn in fish feed has not been published previously.

Most commercial fish feeds contain fish meal as an ingredient. Fish meal is relatively expensive, frequently imported, and may not be available in the future. Replacement of fish meal with plant protein sources in feed has been an important objective of many investigations (Webster et al 1991, Wu et al 1995).

Seneriches and Chiu (1988) formulated five diets with similar proximate compositions and amino acid profiles based on corn gluten meal with or without the addition of fish meal for milkfish fry with an initial average weight of 8 mg. They found that growth was significantly better ($P \leq 0.01$) for milkfish fed a diet in which 30% of the protein originated from fish meal relative to milkfish fed diets with lower fish meal contents. Significantly ($P \leq 0.01$) poorer growth, survival, and feed efficiency were observed for milkfish fed diets containing corn gluten meal as the only protein source.

Webster et al (1991) evaluated distillers' grains with solubles for use as a partial replacement for soybean meal in diets for juvenile (10 g) channel catfish. They found that catfish fed 35% distillers' grains with solubles and 70% distillers' grains with solubles plus lysine diets (both diets met the lysine requirement of catfish) had larger weight gains ($P < 0.05$) than catfish fed a diet of 70% distillers' grains with solubles without supplemental lysine (this diet did not meet the lysine requirement of catfish).

Synthetic tryptophan and threonine are now available commercially. Although synthetic arginine, isoleucine, methionine (Lee and Bai 1997), and synthetic lysine (Viola et al 1994) are used in practical fish feed, little or no information has been published concerning the use of synthetic tryptophan or threonine. The purpose of this study was to investigate the effect of lysine on the growth response of tilapia fed diets containing high levels of corn gluten meal, high-lysine corn, and tryptophan, threonine, and lysine supplements, with and without fish meal.

MATERIALS AND METHODS

Corn gluten meal was a gift from Pekin Energy Company (Pekin, IL). High-lysine corn, SL48, was donated by Crow's Hybrid Corn Company (Milford, IL). Menhaden fish meal (Gulf) and menhaden fish oil, light cold pressed-feed grade (LCP-F), stabilized with 500 ppm of ethoxyquin, were gifts from Zapata Protein (Hammond, LA). Soy oil with 200 ppm of *tert*-butylhydroquinone, tryptosine 10:60 (10% L-tryptophan:60% L-lysine-HCl), L-threonine, and L-lysine-HCl were donated by Archer Daniels Midland Corp. (Decatur, IL). Vitamin premix for warmwater fish and L-ascorbyl-2-polyphosphate were gifts from Hoffman-LaRoche (Paramus, NJ). Mineral premix for catfish was supplied by Triple F Products (Des Moines, IA). The control diet was Purina 5144 Catfish Cage Chow (Purina, St. Louis, MO), a 36% protein pellet containing soybean meal, corn, meat, and bone meal, fish meal, brewers' dried yeast, animal fat, wheat middlings, dry whey, vitamins, and minerals.

The experimental tilapia diets were made with a twin-screw extruder (Leistritz Micro 18GL30D, Somerville, NJ). Table I lists the percent incorporation of ingredients for the experimental diets: increasing levels of lysine (L1, L2, and L3), additional vitamin C (L-ascorbyl-2-polyphosphate; C), and 4 or 8% fish meal (F4 and F8, respectively). Minimum levels of vitamin C are needed for optimum growth. Vitamin C also plays a role in wound healing (Jauncey et al 1985). Because most of the ascorbic acid will be destroyed by heat during extrusion, L-ascorbyl-2-polyphosphate, a more heat-stable form than ascorbic acid, was used. Additional L-ascorbyl-2-polyphosphate was added to some of the diets to determine whether they produce a better growth response.

Nitrogen, fat, ash, crude fiber, and moisture contents for diets were determined using Approved Methods (AACC 1995). Nitrogen (N) was analyzed by micro-Kjeldahl method, and protein was calculated at N × 6.25. Fat was measured after 4 hr of petroleum ether extraction. Ash content was determined from the weight remaining after heating samples for 2 hr at 600°C. Moisture was determined from weight loss after oven-drying at 135°C for 2 hr. Carbohydrates were determined by difference. Digestible energy was calcu-

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lated as 18.9, 16.8, and 37.7 kJ/g for protein, carbohydrate, and fat, respectively (Wang et al 1985). Samples for amino acid analyses were hydrolyzed at 145°C for 4 hr (Gehrke et al 1987), and methionine and cystine were oxidized with performic acid before hydrolysis (Moore 1963). Amino acids were determined in an amino acid analyzer (model 6300, Beckman Instruments, San Raman, CA) by cation-exchange chromatography. Tryptophan was measured by a colorimetric method after enzymatic hydrolysis by pronase (Spies and Chambers 1949, Holz 1972).

Tilapia niloticus with average initial weights of 8.3–8.9 g were used in feeding experiments. Groups of 10 fish each were fed in 76-L aquaria, in triplicate, for each diet. Fish were fed twice daily. The amount of feed offered per day was 8.5% of body weight at start and gradually decreased to 5.7% of body weight after two weeks, to 4.4% after six weeks, and to 2.2% at the end of 12 weeks. The feeding schedule was based on best available data from past performance of feeding tilapia. The fish in each aquarium were weighed as a group every two weeks, and the feed weight was adjusted after each weighing. The increase in fish weight was estimated between weighings, and the feed weight was changed daily.

TABLE I
Experimental Tilapia Diet Ingredients (%, as-is basis)

Ingredient	Experimental Diet[a]				
	L1	L2	L3C	L3F4	L3F8C
Corn gluten meal	43.78	43.01	42.43	39.09	35.76
High-lysine corn	44.62	44.96	45.03	44.78	44.30
Fish meal	0	0	0	4.0	8.0
Soy oil	2.0	2.0	2.0	2.0	2.0
Fish oil	2.0	2.0	2.0	2.0	2.0
Vitamin mix[b]	0.5	0.5	0.5	0.5	0.5
Mineral mix[b]	2.5	2.5	2.5	2.5	2.5
L-Ascorbyl-2-polyphosphate	0	0	0.1	0	0.1
Tryptosine 10/60[c]	2.34	2.42	2.44	2.32	2.21
L-Lysine-HCl	0	0.33	0.71	0.55	0.40
L-Threonine	0.31	0.33	0.34	0.31	0.28
Phosphate[d]	1.95	1.95	1.95	1.95	1.95

[a] L1, L2, and L3 indicate increasing levels of lysine; C indicates additional vitamin C (L-ascorbyl-2-polyphosphate); F4 and F8 indicate 4 and 8% fish meal added, respectively.

[b] Vitamin and mineral premixes supplied/kg of diet: 9,900 international units (IU) of vitamin A acetate; 2,200 IU of vitamin D_3; 82.5 IU of vitamin E; 0.014 mg of vitamin B_{12}; 18.2 mg of B_2; 10.7 mg of niacin from niacinamide; 37 mg of *d*-pantothenic acid from calcium *d*-pantothenate; 715 mg of choline from choline chloride; 6.1 mg of folic acid; 0.17 mg of *d*-biotin; 220 mg of ascorbic acid from L-ascorbyl-2-polyphosphate; 9 mg of K_3 from menadione sodium bisulfite complex; 16.2 mg of B_1 from thiamine mononitrate; 12 mg of B_6 from pyridoxine hydrochloride; 4.3 g of calcium from calcium carbonate and dicalcium phosphate; 2.6 g of phosphorus from dicalcium phosphate; 5.0 mg of copper from copper sulfate; 41 mg of iron from ferrous sulfate; 120 mg of manganese from manganous sulfate; 115 mg of zinc from zinc sulfate; 2.5 mg of iodine from ethylenediamine dihydriodide; 1.0 mg of cobalt from cobaltous carbonate; 153 mg of sulfur.

[c] 10% tryptophan/60% L-lysine-HCl by weight.

[d] KH_2PO_4:$NaH_2PO_4 \cdot H_2O$ (1:1).

Weight gain (WG) was calculated as final weight minus initial weight divided by initial weight and expressed as percent increase after 84 days. Feed conversion ratio (FCR) was calculated from dry feed offered divided by wet WG. Protein efficiency ratio (PER) was WG divided by amount of protein fed.

All aquaria were connected to the same biological filtration system. Nitrate, nitrite, and ammonia were measured by a spectrophotometer (Hach DREL 2000, Loveland, CO). Alkalinity and hardness were determined by a titration test kit (Lamotte, Chestertown, MD). Temperature and dissolved oxygen were assessed by a dissolved oxygen meter (model 58, Yellow Springs Instruments, Yellow Springs, OH). A pH meter (model 5669-20, Cole-Parmer, Vernon Hills, IL) was used to measure pH. Carbon dioxide was obtained from pH, temperature, and alkalinity values, using a chart. Water temperature, dissolved oxygen, and pH were measured daily; total ammonia-N, nitrate-N, and nitrite-N were measured weekly. Average (± standard deviation) water quality parameters were temperature at 27.8 ± 1.0°C; dissolved oxygen at 7.7 ± 0.5 mg/L; pH at 7.5 ± 0.2; ammonia-N at 1.14 ± 0.36 mg/L; nitrate-N at 18.1 ± 4.3 mg/L; and nitrite-N at 0.81 ± 0.29 mg/L.

Costs of ingredients for experimental diets were averaged from 14 values each for corn gluten meal, corn, and fish meal from January 1995 to April 1998, and averages for three values for soy oil and two values for fish oil were used. Tryptosine, lysine, and threonine prices were for April 1996. The processing cost for experimental diets was not available, although the cost will drop greatly if production is increased from 90.7 to 544 Mg (tonne) per year (Tudor et al 1996). The cost of control feed was $449/Mg in March 1996 and $467/Mg in May 1996, and a value of $454/Mg was used for the cost per WG calculation.

The data were analyzed by analysis of variance, and means were compared by *t*-tests of pairs of least square means, using statistical software (SAS Institute, Cary NC).

RESULTS AND DISCUSSION

Table II lists the proximate composition, digestible energy, and protein-to-energy ratio of the diets. Digestible energy values calculated for the diets ranged from 15.3 to 15.7 kJ/g, and protein:energy ratio ranged from 22.9 to 23.7 mg/kJ (Table II). Because both digestible energy and protein-to-energy ratio were very similar among diets, any differences observed in growth response of tilapia was due to other factors.

The essential amino acid levels, expressed as percentage of protein of diet, and the requirements for tilapia are listed in Table III (Santiago 1985). The arginine levels of diets L2 and L3C were borderline low. The diets met or exceeded requirements for the remaining essential amino acids.

Table IV shows the WG, FCR, and PER of tilapia fed the various diets. The largest WG was found in tilapia fed control diet P5144, but it was statistically the same as that for diet L3F4. Tilapia fed other diets had smaller WG than the control, although the WG of tilapia fed diet L3F8C was not significantly different ($P >$ 0.05) from the other four experimental diets.

TABLE II
Proximate Composition of Experimental and Control Tilapia Diets[a]

Diet[b]	Moisture (%)	Protein (%, N × 6.25)	Fat (%)	Ash (%)	Crude Fiber (%)	Digestible Energy[c] (kJ/g)	Protein/Energy (mg/kJ)
L1	9.0 (0.1)	35.7 (0.2)	3.3 (0.1)	5.4 (0.0)	1.4 (0.0)	15.5	23.0
L2	9.3 (0.1)	35.9 (0.2)	3.3 (0.1)	5.3 (0.0)	1.7 (0.1)	15.5	23.2
L3C	9.0 (0.2)	35.8 (0.3)	3.2 (0.0)	5.4 (0.0)	1.6 (0.0)	15.5	23.1
L3F4	8.3 (0.1)	35.8 (0.6)	3.7 (0.1)	6.3 (0.1)	1.4 (0.0)	15.6	22.9
L3F8C	7.7 (0.1)	36.9 (0.1)	4.3 (0.0)	7.0 (0.0)	1.5 (0.1)	15.7	23.5
Control	6.1 (0.1)	36.2 (0.2)	3.3 (0.1)	8.6 (0.1)	2.9 (0.2)	15.3	23.7

[a] Values are means (± standard deviations) of duplicate analyses.

[b] L1, L2, and L3 indicate increasing levels of lysine; C indicates additional vitamin C (L-ascorbyl-2-polyphosphate); F4 and F8 indicate 4 and 8% fish meal added, respectively.

[c] Calculated from 18.9, 16.8, and 37.7 kJ/g for protein, carbohydrate, and fat, respectively (Wang et al 1985).

The best FCR (1.81) was obtained from tilapia fed diet L3F4, although this value was not significantly different ($P > 0.05$) from tilapia fed L3F8C or control (Table IV). The FCR of tilapia fed diet L3F8C was not significantly different from that of tilapia fed any diet listed in Table IV.

The highest PER (1.42) was observed in tilapia fed diet L3F4, although it was not significantly different ($P > 0.05$) from tilapia fed the control diet (Table IV). Tilapia fed diet L3F8C had a PER similar to tilapia fed the control diet ($P > 0.05$).

Additional vitamin C supplied by extra L-ascorbyl-2-polyphosphate (Tables I and IV) did not provide any improvement in WG, FCR, and PER for tilapia fed diets L3C and L3F8C. Apparently, the amount of vitamin C supplied by the vitamin premix for experimental diets was adequate.

Fish fed diet L3F4 with 4% fish meal had higher PER than fish fed diet L3F8C with 8% fish meal. Although fish fed diet L3F4 also had higher WG and better FCR than fish fed diet L3F8C, the differences were not significant ($P > 0.05$). The higher PER of fish fed diet L3F4 compared to that of fish fed diet L3F8C may be due to the borderline higher lysine level of diet L3F4. Apparently, there was an advantage in using 4% fish meal in a tilapia diet rather than 8% fish meal, because the 4% fish meal diet produced better PER.

Costs of WG for tilapia fed various diets are listed in Table IV. The lowest cost per WG was from tilapia fed diet L3F4 with 4% fish meal, although it was not significantly different from tilapia fed diets L1 without fish meal and L3F8C with 8% fish meal ($P > 0.05$). Costs per WG for tilapia fed diets L2, L3C, and control were higher than that of fish fed diet L3F4 but not significantly higher than those of fish fed diets L1 and L3F8C. The amount of fish meal in the control diet is a trade secret. Diet L1 without fish meal may be used to achieve the lowest cost per WG ($P > 0.05$) compared to diets L3F4 and L3F8C with 4 and 8% fish meal, respectively.

The smaller WG of tilapia fed diets L2 and L3C may be explained by their borderline low arginine levels compared to required levels (Tables III and IV). The smaller WG of tilapia fed diet L1 compared to fish fed L3F4 may be explained by a lower level of lysine in diet L1 than in diet L3F4 (5.7 and 7.4 g/100 g of protein, respectively; Table III). Although the minimum requirement for lysine is 4.6 g/100 g of protein (Table III), it is possible that synthetic lysine is not utilized as efficiently as protein-bound lysine or that some synthetic lysine may be leached from the pellet if it is not consumed by the tilapia in a short time. Because 52–65% of the lysine from the experimental diets was derived from synthetic amino acids (Table III), availability of synthetic lysine is an important consideration. Tilapia fed a diet containing synthetic lysine and tryptophan exhibited a smaller WG than that of tilapia fed a diet without synthetic amino acids in 28% protein diets (Wu et al 1997). A higher level of essential amino acids than the requirement may be necessary for optimal growth of tilapia when synthetic amino acids are used.

CONCLUSIONS

An experimental diet containing 39% corn gluten meal and 45% high-lysine corn supplemented with synthetic lysine, threonine, and tryptophan resulted in equal WG, FCR, and PER when compared to a commercial control fed to tilapia. Diets with similar levels of corn gluten meal and high-lysine corn but with lower levels of lysine resulted in a less favorable growth response.

TABLE III
Essential Amino Acid Composition (% of protein) of Tilapia Diets

Amino Acid	Diet[a]						
	L1	L2	L3C	L3F4	L3F8C	Control	Requirement[b]
Arginine	3.3	3.1	3.0	3.4	3.5	6.7	3.5–4.4
Histidine	2.1	2.1	2.0	2.1	2.1	2.6	1.3–1.9
Isoleucine	3.6	3.4	3.1	3.5	3.5	4.0	3.1
Leucine	14.9	14.3	14.2	14.0	12.7	7.7	2.8–3.6
Lysine	5.7 (54)[c]	6.5 (60)	7.3 (65)	7.4 (58)	7.1 (52)	6.1	4.6–5.6
Methionine + cystine	4.0	3.9	3.8	3.8	3.7	3.4	3.2
Phenylalanine + tyrosine	10.5	10.2	10.1	10.1	9.3	7.9	5.0–6.1
Threonine	4.0 (22)	4.0 (23)	4.0 (24)	4.1 (21)	3.8 (20)	3.9	3.6
Tryptophan	1.5 (45)	1.5 (46)	1.5 (47)	1.5 (42)	1.5 (41)	1.2	0.7–1.3
Valine	4.3	4.1	3.8	4.2	4.2	4.6	2.3–3.0

[a] L1, L2, and L3 indicate increasing levels of lysine; C indicates additional vitamin C (L-ascorbyl-2-polyphosphate); F4 and F8 indicate 4 and 8% fish meal added, respectively.
[b] Santiago (1985).
[c] Values in parentheses indicate percent contribution from synthetic amino acid.

TABLE IV
Weight Gain (WG), Feed Conversion Ratio (FCR), and Protein Efficiency Ratio (PER) of Tilapia Fed Experimental and Control Diets[a]

Diet[b]	Initial Weight[c] (g)	Final Weight[c] (g)	WG[d]	FCR[e]	PER[f]	Cost/WG[g] ($/kg)
L1	8.3 (0.2)	46.2 (4.6)	458 (59)f	2.26 (0.20)d	1.14 (0.10)f	1.02d,e
L2	8.4 (0.6)	46.9 (3.9)	456 (22)f	2.24 (0.13)d	1.13 (0.07)f	1.03d
L3C	8.9 (0.2)	50.5 (9.7)	466 (100)f	2.34 (0.37)d	1.11 (0.19)f	1.12d
L3F4	8.8 (0.3)	66.4 (10.7)	652 (120)d,e	1.81 (0.15)e	1.42 (0.11)d	0.85e
L3F8C	8.4 (0.3)	57.6 (6.7)	586 (82)e,f	2.10 (0.04)d,e	1.19 (0.02)e,f	0.99d,e
Control	8.3 (0.5)	69.9 (11.6)	738 (84)d	1.90 (0.05)e	1.37 (0.03)d,e	1.12d

[a] Values are means (±standard deviations) of three replicates for each diet. Numbers followed by different letters in a column are significantly different ($P < 0.05$).
[b] L1, L2, and L3 indicate increasing levels of lysine; C indicates additional vitamin C (L-ascorbyl-2-polyphosphate); F4 and F8 indicate 4 and 8% fish meal added, respectively.
[c] Fish in each aquarium were weighed as a group, and the weights were divided by number of fish. Mortality was 0 for fish fed diet L2 and 3.3% for fish fed the other diets.
[d] Percent weight increase at the end of 84 days.
[e] Dry feed offered/wet WG.
[f] WG/protein fed.
[g] Costs of ingredients for experimental diets were averages of 14 values each for corn gluten meal, corn, and fish meal from January 1995 to April 1998 and averages of three values for soy oil and two values for fish oil.

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