Use of Corn-Derived Ethanol Coproducts and Synthetic Lysine and Tryptophan for Growth of Tilapia (*Oreochromis niloticus*) Fry

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Corn gluten meal, corn gluten feed, and corn distillers' grains with solubles, the coproducts from ethanol fermentation of corn, were incorporated in tilapia diets with 32 and 28% protein. The diets were balanced in amino acid composition by addition of soy flour and/or synthetic lysine and tryptophan. The diets were fed to tilapia fry of average initial weight of 0.5 g in aquaria for 8 weeks. Weight gain expressed as percentage increase after 56 days were best ($P < 0.05$) for a 28% protein diet with 67% corn gluten feed and 26% soy flour, a 32% protein diet with 54% corn gluten feed and 39% soy flour, and the control diet with 32% protein. Weight gain was positively correlated with protein content, lysine/protein, and protein/energy. Fish fed 32% protein diets exhibited the same feed conversion ratio and protein efficiency ratio ($P > 0.05$) as the control 32% protein diet. Fish fed 28% protein diet with 82% corn distillers' grains with solubles and synthetic lysine and tryptophan and 28% protein diet with 67% corn gluten feed and 26% soy flour also resulted in the same feed conversion ratio and protein efficiency ratio ($P > 0.05$) as the control diet. Fish fed the remaining two 28% protein diets exhibited higher feed conversion ratio and lower protein efficiency ratio ($P < 0.05$) than fish fed the control diet. It appears the 28% protein diet with 67% corn gluten feed and 26% soy flour is adequate for tilapia fry based on weight gain, feed conversion ratio, and protein efficiency ratio.

Keywords: *Tilapia; corn gluten meal; corn gluten feed; corn distillers' grains with solubles; synthetic amino acids; weight gain; feed conversion ratio; protein efficiency ratio*

INTRODUCTION

In the United States, corn is the major cereal grain for ethanol fermentation. Starch in corn is converted to glucose and then to ethanol, and protein, fat, and fiber are concentrated in the coproducts. The dry milling process uses ground corn to make ethanol, and the ethanol coproduct remaining is corn distillers' grains with solubles, which has 25−30% protein. The wet milling process separates corn into protein, starch, oil, and fiber fractions. Corn gluten meal is the protein fraction and contains >60% protein. Corn gluten feed contains about 20% protein and is composed of steep-water used to soak the corn kernel prior to wet milling and the fiber fraction. These protein-rich ethanol coproducts from corn are potential ingredients for fish feed.

Wu et al. (1994, 1995) reported that three diets containing corn distillers' grains with solubles and 32 or 36% protein as well as five diets containing corn gluten meal and 32 or 36% protein resulted in higher weight gain for tilapia (*Oreochromis niloticus*) compared with a commercial fish feed containing 36% protein and fish meal for tilapia with initial weight of 30 g. Since protein is relatively expensive in fish feed, it is of interest to find out if tilapia will grow satisfactorily on fish feed of lower protein contents. Also, it is important to find out the growth response of fish fed diet with high percentage of alcohol coproducts (54−92%) as well as fish fed diet supplemented with synthetic amino acids. This paper investigates the growth response of tilapia with initial weight of 0.5 g in aquaria for feeds containing high percentages of alcohol coproducts, synthetic lysine and tryptophan, and 28 and 32% protein contents.

MATERIALS AND METHODS

Soy flour (bakers' Nutri Soy) and soy oil were from Archer Daniels Midland Corp. (Decatur, IL). Corn gluten feed and corn gluten meal were supplied by Pekin Energy Co. (Pekin, IL). Menhaden fish oil was supplied by International Protein Corp. (St. Paul, MN). Corn distillers' grains with solubles came from Jack Daniels Distillery (Lynchburg, TN). Vitamin premix for warmwater fish was supplied by Hoffman-La Roche (Paramus, NJ). Mineral premix for catfish came from Triple F Products (Des Moines, IA). L-Lysine−hydrochloride was from Archer Daniels Midland (Fenton, MO). L-Tryptophan was supplied by Ajinomoto, USA (Raleigh, NC). The control diet was a 32% protein Silver cup tilapia pellet (Nelson & Sons, Inc., Murray, UT), containing soybean meal, wheat flour, wheat millrun, fish meal, blood meal, stabilized fish oil, lecithin, lignan sulfonate, vitamins, and minerals. The diet contained at least 32% protein and 6% fat as well as maximum of 5% crude fiber and 8% ash.

The ingredients for tilapia diets were mixed and fed into a Randcastle 12.7 mm diameter single-screw extruder (Cedar Grove, NJ). The extruded feed was air-dried overnight at room temperature to reduce the moisture content. Table 1 lists the percent ingredients of diets. Table 2 shows the proximate composition and trypsin inhibitor activity of diets and ingre-

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Table 1. Composition of Diets Fed to Tilapia (Percent As-Is Basis, Not Moisture-Free)

ingredient	diet[a]						
	28DM	28MF	28DS	28FS	32DS	32FS	32FSM
corn gluten meal	10	18.95	0	0	0	0	8
soy flour	0	0	15	26	30	39	28
corn gluten feed	0	73.27	0	67	0	54	57
corn distillers' grains with solubles	82.23	0	77.75	0	63	0	0
soy oil	2	2	2	2	2	2	2
fish oil	2	2	2	2	2	2	2
vitamin mix[b]	0.5	0.5	0.5	0.5	0.5	0.5	0.5
mineral mix[b]	2.5	2.5	2.5	2.5	2.5	2.5	2.5
L-lysine−HCl	0.75	0.72	0.25	0	0	0	0
L-tryptophan	0.02	0.06	0	0	0	0	0

[a] The first two digits of the experimental diets gave the approximate protein content on as-is basis. D is corn distillers' grains with solubles, M is corn gluten meal, F is corn gluten feed, and S is soy flour. [b] The vitamin and mineral premix supplied, per kilogram of diet: vitamin A, 9900 international units (IU) from vitamin A acetate; vitamin D_3, 2200 IU; vitamin E, 82.5 IU; vitamin B_{12}, 0.014 mg; riboflavin (B_2), 18.2 mg; niacin, 10.7 mg, from niacinamide; D-pantothenic acid, 37 mg, from calcium D-pantothenate; choline, 715 mg, from choline chloride; folic acid, 6.1 mg; D-biotin, 0.17 mg; ascorbic acid, 220 mg, from L-ascorbyl 2-polyphosphate; menadione (K_3), 9 mg, from menadione sodium bisulfite complex; thiamin (B_1), 16.2 mg, from thiamine mononitrate; pyridoxine (B_6), 12 mg, from pyridoxine hydrochloride; calcium, 4.3 g, from calcium carbonate and dicalcium phosphate; phosphorus, 2.6 g, from dicalcium phosphate; copper, 5.0 mg, from copper sulfate; iron, 41 mg, from ferrous sulfate; manganese, 120 mg, from manganous sulfate; zinc, 115 mg, from zinc sulfate; iodine, 2.5 mg, from ethylenediamine dihydroiodide; cobalt, 1.0 mg, from cobaltous carbonate; sulfur, 153 mg.

dients. The trypsin inhibitor activity of all diets in Table 2 was low and should have caused no complication in feeding tilapia.

Tilapia (*O. niloticus*) with an average initial weight of 0.50 g were used. Groups of 25 fish were stocked in 38-L aquaria in duplicate for each experimental diet and in four replicates for the control. The amount of feed introduced per day was based on previous feeding experience: 14% of body weight at the beginning of the experiment, 9.7% of body weight after 2 weeks, 7.2% after 4 weeks, 6.7% after 6 weeks, and 6.5% at 8 weeks. The fish were fed twice daily. Fish in each aquarium were weighed biweekly, and the feed weight was adjusted after each weighing. The increase in fish weight was estimated between weighings, and the feed weight was adjusted daily.

All aquaria were connected to the same biological filtration system. Nitrate, nitrite, and ammonia were measured by a Hach DREL 2000 spectrophotometer (Loveland, CO). Alkalinity and hardness were determined by a Lamotte titration test kit (Chestertown, MD). Temperature and dissolved oxygen were assessed by a YSI Model 58 dissolved oxygen meter (Yellow Springs Instruments, Inc., Yellow Springs, OH). A Cole-Parmer Model 5669-20 pH meter (Vernon Hills, IL) was used to measure pH. Carbon dioxide was obtained from pH, temperature, and alkalinity values by a chart. The number of measurements were as follows: temperature, 56; dissolved oxygen, 56; pH, 49; nitrite, 8; nitrate, 8; ammonia, 8; carbon dioxide, 7; alkalinity, 7; and hardness, 7. The average (± standard deviation) water quality parameters were as follows: temperature, 28.4 ± 1.1 °C; dissolved oxygen, 7.1 ± 0.7 mg/L; pH, 7.67 ± 0.13; nitrite-N, 0.79 ± 0.83 mg/L; nitrate-N, 66.5 ± 10.1 mg/L; ammonia-N, 1.08 ± 0.33 mg/L; carbon dioxide, 6.7 ± 0.9 mg/L; alkalinity, 166 ± 31 mg/L as calcium carbonate; and hardness, 304 ± 36 mg/L as calcium carbonate.

Duplicate nitrogen, ash, fat, crude fiber, and moisture contents and trypsin inhibitor activity were measured according to American Association of Cereal Chemists' Approved Methods 46-13, 08-03, 30-26, 32-10, 44-19, and 71-10, respectively (AACC, 1983). Nitrogen was determined according to micro-Kjeldahl, and protein was calculated by using the conversion factor of 6.25. Ash was from the weight remaining after the sample was heated to 600 °C for 2 h. Fat was from petroleum ether extraction, and moisture was from the weight loss after oven drying at 135 °C for 2 h. Crude fiber was loss on ignition of dried residue remaining after digestion of sample with 1.25% H_2SO_4 and 1.25% NaOH solutions under specific conditions. Cystine and methionine were oxidized by performic acid before hydrolysis (Moore, 1963). Samples for amino acid analyses were hydrolyzed by 6 N HCl for 4 h at 145 °C (Gehrke et al., 1987), and the amino acids were determined by cation exchange chromatography in a Beckman 6300 amino acid analyzer (Beckman Instruments, Inc., San Ramon, CA). Tryptophan was determined according to a colorimetric method after enzymatic hydrolysis by pronase (Spies and Chambers, 1949; Holz, 1972). One amino acid analysis was performed for each diet.

Weight gain was calculated as (final weight − initial weight)/initial weight and expressed as the percentage increase at the end of 56 days. Feed conversion ratio was calcualted as dry feed offered/wet weight gain. Protein efficiency ratio was calculated as wet weight gain/protein fed. The dietary digestible energy for tilapia was calculated by using values of 4.5, 4.0, and 9.0 kcal/g for protein, carbohydrate, and lipid, respectively (Wang et al., 1985). Crude fiber is not counted as carbohydrate, because cellulose is not digested by tilapia (Jauncey and Ross, 1982).

The data were analyzed by analysis of variance. Means were compared by *t*-tests of pairs of least-square means (SAS Institute Inc., 1987).

RESULTS AND DISCUSSION

The first two digits of the experimental diets in Table 1 give the approximate protein content on as-is basis (containing moisture after air-drying). D is corn distillers' grains with solubles, M is corn gluten meal, F is corn gluten feed, and S is soy flour. The diets were formulated to contain 28 and 32% proteins that met the amino acid requirement of tilapia (Santiago, 1985).

Table 3 gives the amino acid composition of tilapia and control diets, and the amino acid requirement for tilapia for a level of growth within the 95% confidence range of maximum growth (Santiago, 1985). The essential amino acid composition of experimental and control diets met the amino acid requirement of tilapia except diet 28MF was low in arginine (3.0 vs 3.5% required) and threonine (3.0 vs 3.6% required), diets 28DM, 28DS, 28FS, and 32FSM were borderline low in threonine (3.3 vs 3.6% required), and diet 28MF was borderline low in lysine (4.2 vs 4.6% required).

Table 4 lists weight gain, feed conversion ratio, protein efficiency ratio, and protein/energy of tilapia and control diets. Fish fed diets 28FS and 32FS and the control diet exhibited the highest weight gain, while fish fed diet 28MF had the lowest weight gain. There was no statistical difference ($P > 0.05$) among the feed conversion ratios for five of seven formulated and control diets. Fish fed diet 28FS had the highest protein efficiency ratio, while fish fed diets 28MF and 28DS had the lowest protein efficiency ratio. Protein/energy in general increased with increasing protein content.

The low weight gain of fish fed diet 28MF (Table 4) was probably due at least in part to deficient arginine, threonine, and, possibly, lysine levels (Table 3). The fact that fish fed diet 28FS had one of the best weight gains indicates a high level of corn gluten feed (67% in Table 1) may be used in diets fed to juvenile tilapia. Feeding diet 28DM with synthetic lysine and tryptophan (Table 1) resulted in lower weight gain than that of diet 28FS without synthetic amino acids, and this lower weight gain with synthetic lysine and tryptophan may indicate that the two synthetic amino acids in combination were less efficiently utilized by tilapia. Diet 28DS with lower

Table 2. Proximate Composition (Percent) and Trypsin Inhibitor Activity (Milligrams per Gram) of Tilapia Diets and Ingredients[a]

	protein	fat	ash	crude fiber	moisture	trypsin inhibitor
soy flour[b]	53.5 (0.2)	0.8 (0.0)	6.6 (0.1)	2.7 (0.0)	5.8 (0.2)	19.3 (0.5)
corn gluten meal	64.3 (0.1)	2.8 (0.0)	2.4 (0.1)	0.8 (0.0)	10.4 (0.2)	2.7 (0.0)
corn gluten feed	21.0 (0.1)	1.7 (0.0)	6.0 (0.0)	7.1 (0.0)	10.0 (0.1)	3.5 (0.1)
corn distillers' grains with solubles	25.6 (0.0)	9.2 (0.0)	3.9 (0.0)	9.9 (0.1)	11.8 (0.0)	2.2 (0.3)
28DM	29.2 (0.6)	13.2 (0.1)	5.7 (0.2)	7.2 (0.3)	7.2 (0.1)	1.8
28MF	29.0 (0.2)	5.7 (0.0)	6.8 (0.0)	5.4 (0.0)	9.1 (0.1)	2.6 (0.1)
28DS	30.2 (0.2)	12.2 (0.1)	6.1 (0.1)	7.2 (0.1)	6.6 (0.2)	2.0
28FS	28.9 (0.3)	4.9 (0.0)	7.6 (0.1)	5.5 (0.2)	8.3 (0.0)	2.5 (0.2)
32DS	34.0 (0.5)	10.5 (0.0)	6.5 (0.0)	6.4 (0.1)	6.6 (0.3)	2.2
32FS	33.0 (0.1)	4.9 (0.0)	7.7 (0.0)	4.8 (0.1)	7.8 (0.2)	3.1 (0.2)
32FSM	33.0 (0.2)	5.1 (0.0)	7.3 (0.0)	4.8 (0.1)	7.9 (0.1)	2.6
control	34.6 (0.1)	8.4 (0.0)	6.1 (0.0)	3.7 (0.1)	11.3 (0.1)	0.7 (0.0)

[a] All data on as-is basis. Means (standard deviation) of duplicate analysis. [b] Soy flour is used here for convenience, because it comes in a bag. Soy meal in bulk will be used in actual feed formulation because of lower cost and identical composition. [c] The experimental diets had 29−30 and 33−34% protein values instead of 28 (28.0−28.3) and 32 (32.1−32.2)% protein values implied in the abbreviations, because the experimental diets had lower moisture contents than the ingredients in general. Also experimental variation may contribute to this difference. The control diet had higher protein value, because the manufacturer wanted to meet the minimum value of 32% with safety.

Table 3. Essential Amino Acid Composition (Percent of Protein) of Experimental and Control Diets

amino acid	control	28DM	28MF	28DS	28FS	32DS	32FS	32FSM	requirement[a]
arginine	4.1	3.3	3.0	3.3	3.3	3.5	3.5	3.3	3.5
histidine	3.5	2.3	2.0	2.4	2.4	2.5	2.5	2.4	1.3
isoleucine	3.6	3.7	3.3	3.9	3.8	4.3	4.1	4.0	3.1
leucine	8.9	11.3	10.9	9.5	7.5	9.2	7.7	9.1	2.8
lysine	7.0	5.0	4.2	4.6	4.5	4.8	5.1	4.3	4.6
methionine + cystine	3.2	3.9	3.4	3.6	3.0	3.5	3.0	3.3	3.2
phenylalanine + tyrosine	8.4	8.4	7.6	8.0	6.9	8.3	7.5	7.8	5.0
threonine	4.1	3.3	3.0	3.3	3.3	3.5	3.5	3.3	3.6
tryptophan	1.2	0.9	0.8	0.9	0.9	1.0	1.1	0.9	0.7
valine	6.1	4.8	4.1	5.0	4.4	5.0	4.7	4.7	2.3

[a] Santiago (1985).

Table 4. Weight Gain (WG), Feed Conversion Ratio (FCR), Protein Efficiency Ratio (PER), and Protein/Energy (P/E) of Tilapia Fed Experimental and Control Diets[a]

diet	WG[b]	FCR[c]	PER[d]	P/E[e]	cost in $/kg[f]
28DM	1540 (580)c	1.76 (0.22)ab	1.82 (0.20)ab	73.1	0.257
28MF	767 (133)d	2.18 (0.17)b	1.44 (0.12)b	81.1	0.223
28DS	1920 (445)bc	2.25 (0.79)b	1.47 (0.53)b	76.0	0.243
28FS	2420 (197)ab	1.43 (0.05)a	2.21 (0.09)a	81.8	0.206
32DS	2180 (59)bc	1.62 (0.22)ab	1.71 (0.25)ab	86.8	0.261
32FS	2310 (269)abc	1.41 (0.02)a	1.98 (0.02)ab	91.6	0.237
32FSM	2210 (99)bc	1.63 (0.15)ab	1.72 (0.15)ab	91.2	0.234
control	2890 (415)a	1.25 (0.10)a	2.05 (0.16)a	92.3	

[a] Means (standard deviation) of duplicate aquaria for all formulated diets. Means of four replicate aquaria for control. Values within columns followed by different letters were significantly different ($P < 0.05$) as determined by analysis of variance. [b] Expressed as the percentage increase at the end of 56 days. [c] Dry feed/wet weight gain. [d] Wet weight gain/protein fed. [e] Protein/energy expressed as milligrams of protein per kilocalorie based on Wang et al. (1985). [f] Ingredient cost for experimental diets. Commercial diet composition is proprietary, therefore, ingredient cost cannot be calculated. Price of ingredients as of Jan 7, 1994, in $/kg were as follows: corn gluten feed, 0.067; corn gluten meal, 0.358; soy flour, 0.305; soy oil, 0.634; corn distillers' grains with solubles, 0.140; fish oil, 0.346; vitamin mix, 6.69; mineral mix, 1.14; lysine−HCl, 2.64; tryptophan, 27.13 (calculated from 4.30 for tryptosine with 10% tryptophan and 60% lysine−HCl).

amount of synthetic lysine and high levels of corn distillers' grains with solubles (Table 1) gave lower weight gain (although $P > 0.05$) than diet 28FS without synthetic lysine. Lipid levels in diet 28DM (13.2%) and in diet 28DS (12.2%) in Table 2 were higher than the 10% for 0.5−10 g of tilapia recommended by Jauncey and Ross (1982), and these higher levels of lipid were a possible cause for lower weight gain (Table 4). Comparison of diets 28FS and 32FS containing high levels of corn gluten feed (67 and 54% in Table 1) indicates that diet 28FS with 28% protein content equals the performance of diet 32FS with 32% protein content. Fish fed diets 32DS, 32FS, and 32FSM containing 32% protein and high levels of corn distillers' grains with solubles and corn gluten feed (Table 1) exhibited similar weight gains (Table 4).

Table 5. Correlation Coefficients of Protein/Energy Ratio (P/E), Protein Content, Fat Content, and Lysine/Protein of Diets with Weight Gain (WG), Feed Conversion Ratio (FCR), and Protein Efficiency Ratio (PER)

	WG[a]	FCR	PER
P/E	0.615**	−0.419	0.092
protein	0.672**	−0.332	−0.047
fat	−0.095	0.281	−0.252
lysine/protein	0.651**	−0.233	−0.004

[a] **, $P < 0.01$.

The feed conversion ratio and protein efficiency ratio of all 32% diets, diets 28DM and 28FS, and the control diet were the same ($P > 0.05$) despite the large difference in weight gain (Table 4).

Table 5 gives the correlation coefficients of protein/energy, protein content, fat content, and lysine/protein of diets with weight gain, feed conversion ratio, and protein efficiency ratio. Weight gain was positively correlated with protein/energy ($P < 0.01$), with protein content ($P < 0.01$), and with lysine/protein ($P < 0.01$).

CONCLUSION

Among the 28% protein diets, the best weight gains were from 28FS and 28DS with no or low synthetic amino acids; however, this disadvantage of synthetic amino acids was not observed for feed conversion ratio and protein efficiency ratio. Fish fed two diets, 28FS and 32FS, containing soy flour and a high percentage of corn gluten feed, performed equally well compared with fish fed the control diet containing fish meal in terms of weight gain, feed conversion ratio, and protein efficiency ratio. The 28% protein diet, 28FS, performed equally well compared with the 32% protein diet, 32FS. Since protein is relatively expensive compared with the other ingredients in fish feed, the 28% protein diet can be formulated at lower cost than the 32% protein diet (Table 4). On the basis of weight gain, feed conversion ratio, and protein efficiency ratio, there is a potential of all-plant diets for juvenile tilapia of initial weight of 0.5 g. Further study is needed for fish with initial weight of 100 g or more, because our results may not necessarily apply.

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