Utilization of Protein-Rich Ethanol Co-Products from Corn in Tilapia Feed[1]

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Corn distiller's grains with solubles (CDGS), which is the residue from ethanol fermentation of corn, were incorporated in tilapia (a warm-water fish) diets that contained either 36% protein without fish meal or 32% protein with and without fish meal. A 103-day feeding study indicated that the three diets containing CDGS resulted in higher weights of tilapia than fish fed a commercial fish feed containing 36% protein and fish meal. The difference in weight gains between 32 and 36% protein diets was not significant. Incorporating fish meal (6%) in diet had no advantage over a diet without fish meal.

KEY WORDS: **Corn, corn distiller's grains with solubles, corn protein, feed conversion ratio, fish meal, protein-rich ethanol co-products from corn, soy flour, tilapia, tilapia diet, weight gain.**

Corn is the major cereal grain to make ethanol *via* fermentation (1). Stillage, the residue remaining after ethanol is distilled, is screened or centrifuged to yield both solid and soluble fractions. The soluble fraction is usually concentrated and combined with wet solids and then dried to produce corn distiller's dried grains with solubles (CDGS). The solid fraction after drying gives corn distiller's grains (CDG).

Traditionally, CDG and CDGS have been used for animal feed. Satterlee *et al.* (2) prepared protein concentrates for potential human food uses from wheat and CDG. Tsen *et al.* (3,4) used CDG flour, mixed with wheat flour at 15 or 25% replacement level, for making cookies and studied the potential use of distiller's dried grain flour as an ingredient for preparing grain-type breads at 10 and 20% replacement levels for wheat flour. The potential use of CDG and CDGS in corn–soy–milk blended food products was also investigated (5,6).

O'Palka (7) incorporated CDGS in yeast breads and quick breads at 33 and 40% flour replacement, respectively. Rasco *et al.* (8) made chocolate-chip cookies, banana bread, white bread and whole-wheat bread with whole white wheat distiller's dried grains with solubles as a replacement for 30% of the all-purpose flour. Reddy *et al.* (9,10) evaluated dried distiller's grain flour as an ingredient in canned meat-based foods and in wheat muffins. Wu *et al.* (11) prepared spaghetti supplemented with 5, 10 and 15% CDG.

Tilapia is a widely cultured fish (12). The cost of feed is an important part of the total production cost. Commercial fish feeds contain fishmeal, which is expensive and may not always be available. CDGS is a protein-rich ethanol co-product from corn. An increasing amount of CDGS is produced as the demand for fuel ethanol increases. This study investigates the incorporation of CDGS in tilapia diets that contained 36% protein without fish meal or 32% protein with and without fishmeal.

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EXPERIMENTAL PROCEDURES

CDGS was supplied by Brown-Forman Corp. (Louisville, KY). Soy flour and soy oil were from Archer Daniels Midland Corp. (Decatur, IL). Menhaden fish meal and menhaden fish oil were from Zapata Haynie Corp. (Hammond, LA). Catfish trace mineral pre-mix was obtained from Triple F Products (Des Moines, IA). Vitamin pre-mix for warm-water fish was from Hoffmann-LaRoche (Paramus, NJ). L-Ascorbyl-2-polyphosphate instead of vitamin C was used in the vitamin mix for increased stability of vitamin C activity. Ground corn was purchased from a local commercial source.

CDGS was ground in an Alpine mill at 14,000 rpm. The experimental tilapia diets were made by an Insta Pro (Des Moines, IA) Model 600 Jr. extruder. The vitamin and mineral pre-mixes supplied the following per kilogram diet: vitamin A, 9900 international unit (IU); vitamin D, 2200 IU; vitamin E, 82.5 IU; vitamin B12, 0.014 mg; riboflavin (B2), 18.2 mg; niacin, 107 mg; pantothenic acid, 37 mg; choline, 715 mg; folic acid, 6.1 mg; biotin, 0.17 mg; ascorbic acid, 220 mg; menadione (K3), 9 mg; thiamine (B1), 16.2 mg; calcium, 4.3 g; phosphorus, 2.6 g; copper, 5.0 mg; iron, 41 mg; manganese, 120 mg; zinc, 115 mg; iodine, 2.5 mg; cobalt, 1.0 mg; and sulfur, 153 mg. The extruded pellets were air-dried overnight at room temperature to reduce the moisture content of the pellets to around 7–9%. The control diet was a Silver Cup catfish finisher feed with 36% protein which contained soybean meal, fish meal, wheat middlings, blood meal, feather meat, fish oil, vitamin and mineral mix (Nelson & Sons, Inc., Murray, UT).

Tilapia (*Oreochromus niloticus*, Syn, *Tilapia nilotica*) with an average initial weight of 30 g were used in the feeding experiment. Groups of 20 fish each were fed in cages in triplicate for each diet and in two triplicates for control. A solid plastic tray formed the bottom of the cage to prevent feed loss through the bottom into the tank. The top of each cage was equipped with a cover to prevent fish from leaving the cage. All cages were inside a tank that was part of a recirculating system.

Water quality, such as temperature, dissolved oxygen and pH, were measured daily; total ammonia and nitrite were monitored four times a week; and nitrate, alkalinity, hardness, phosphate, CO_2 and salinity were determined weekly. The average ($\pm$ standard error) water quality parameters during the study were: temperature, 27.1 $\pm$ 0.1°C; dissolved oxygen, 10.3 $\pm$ 0.2 mg/L; pH, 7.11 $\pm$ 0.01; total ammonia, 2.46 $\pm$ 0.13 mg/L; nitrite, 1.68 $\pm$ 0.17 mg/L; nitrate, 142.6 $\pm$ 15.1 mg/L; alkalinity, 172.9 $\pm$ 10.6 mg/L as calcium carbonate; hardness, 260.3 $\pm$ 7.9 mg/L as calcium carbonate; phosphate, 84.3 $\pm$ 18.3 mg/L; carbon dioxide, 21.4 $\pm$ 1.5 mg/L; and salinity, 1.07 $\pm$ 0.07 parts per thousand.

The fish were fed twice daily. The amount of feed introduced per day was 3.8% of the body weight at the beginning of the experiment and was gradually decreased to 2.8% of the body weight at the end of the experiment.

The fish in each cage were weighed biweekly, and the feed weight was adjusted after each fish weighing. Also, the increase in fish weight was estimated between weighings, and the feed weight was increased daily. The total feeding period was 103 d. Weight gain per fish per day was calculated as (final weight − initial weight)/initial weight and expressed as percentage increase for 103 d. Feed conversion ratio was calculated from as-is feed offered/wet weight gain.

Nitrogen, fat, ash, crude fiber, moisture contents and trypsin inhibitor activity were determined by the American Association of Cereal Chemists' Approved Methods (13). Nitrogen was determined by micro-Kjeldahl, and protein was estimated by using the conversion factor N × 6.25. Fat was from petroleum ether extraction, ash was from the weight remaining after heating the sample to 600°C for 2 h, and moisture was from the weight loss after even drying at 135°C for 2 h. Samples for amino acid analyses were hydrolyzed with 6N HCl for 4 h at 145°C (14). Cystine and methionine were oxidized with performic acid before hydrolysis (15). Amino acids were determined by cation exchange chromatography in a Beckman 6300 amino acid analyzer (Beckman Instruments, Inc., San Raman, CA). Tryptophan was quantitated by a colorimetric method after enzymatic hydrolysis by pronase (16,17).

RESULTS AND DISCUSSION

The tilapia diets were formulated to contain 32 and 36% proteins that meet the amino acid requirement of tilapia (18). Table 1 lists the composition of ingredients by weight for the diets. Vitamin and mineral mixes were added based on recommendations for warm-water fish. The proximate composition and trypsin inhibitor activity of tilapia diets and control are shown in Table 2. Diets 323 and 326 have similar proximate compositions and trypsin inhibitor activities, but only diet 326 contained fish meal. The control diet had the highest protein content (38.6%). All diets had low trypsin inhibitor activities and should have caused no complications in feeding tilapia. The amino acid compositions of the experimental and control diets are listed in Table 3, and all diets met the amino acid requirement of tilapia (18).

Weight gains and feed conversion ratios of tilapia fed experimental and control diets are shown in Table 4. P values from t-test comparisons of least square means for weight gains are listed in Table 5. Although all experimental diets showed higher weight gains than the control diet (Table 4), the difference between diet 323 and the control was not significant ($P = 0.48$ in Table 5). Weight gains for diets 326 and 363 were significantly higher than control ($P = 0.008$ and 0.026, respectively). The difference in weight gain between diets 323 and 326 was not significant ($P = 0.069$) and indicated that 6% fish meal in diet 326 had no advantage over diet 323 without fish meal. The difference in weight gains between diet 323 (32% protein) and 363 (36% protein) was not significant ($P = 0.16$) and indicated that 36% protein diet had no advantage over 32% protein diet.

Feed conversion ratios (Table 4) indicated that there were no significant differences among the three experimental and the control diets ($P > 0.05$). However, these feed conversion ratios were only useful for relative comparison between diets and control because some feed was lost through the sides of cages and not utilized by the fish. Parallel studies of feed conversion ratios in aquariums and in cages are planned.

Webster *et al.* (19) used 0, 35 and 70% CDGS in 36% protein diets for juvenile (10 g) channel catfish and found that growth in juvenile channel catfish fed a diet with 35% CDGS was equivalent to that in fish fed a commercially formulated diet with a high proportion of soybean meal. However, a diet containing 70% CDGS appeared to be lysine-deficient for juvenile channel catfish (19). Our diet 326 (32% protein) with 29% CDGS and diet 363 (36% protein) with 19% CDGS gave higher weight gains for tilapia than did the commercial control diet with 36% protein. Another diet with 22% CDGS (Table 1) also showed higher weight gain for tilapia than the commercial control diet,

TABLE 1

Percent Composition (as-is-weight basis) of Tilapia Diets

	Diet		
Ingredient	323	326	363
Ground corn	25	23	18
Soy flour	46	35	56
Corn distillers' grains with solubles	22	29	19
Fish meal	0	6	0
Soy oil	2	2	2
Fish oil	2	2	2
Vitamin mix	0.5	0.5	0.5
Mineral mix	2.5	2.5	2.5

TABLE 2

Proximate Composition and Trypsin Inhibitor Activity of Tilapia Diets and Control[a]

Diet	Moisture (%)	Protein (%) N × 6.25	Fat (%)	Ash (%)	Crude fiber (%)	Trypsin inhibitor (mg/g)
323	8.8	32.2	6.9	5.8	5.9	4.1
326	7.1	32.3	8.0	6.6	6.6	3.3
363	9.0	34.8	6.0	6.2	5.2	3.3
Control	7.7	38.6	6.4	8.3	4.3	0.7

[a]All data on as-is basis.

TABLE 3

Amino Acid Compositions (% as-is) of Tilapia and Control Diets

	Diet			
	323	326	363	Control
Hydroxyproline	0.00	0.11	0.00	0.21
Aspartic acid	3.30	3.14	3.75	3.57
Threonine	1.17	1.24	1.34	1.57
Serine	1.36	1.42	1.52	1.84
Glutamic acid	5.69	5.53	6.23	6.00
Proline	2.03	2.10	2.24	2.25
Lanthionine	0.00	0.00	0.00	0.08
Glycine	1.29	1.45	1.44	2.07
Alanine	1.60	1.79	1.74	2.25
Cystine	0.59	0.59	0.65	0.66
Valine	1.62	1.57	1.74	2.22
Methionine	0.54	0.60	0.55	0.64
Isoleucine	1.46	1.39	1.59	1.30
Leucine	2.87	2.93	3.06	3.34
Tyrosine	1.16	1.15	1.27	1.17
Phenylalanine	1.67	1.61	1.83	1.99
Hydroxylysine	0.00	0.02	0.02	0.00
Histidine	0.88	0.85	0.95	1.22
Ornithine	0.03	0.03	0.03	0.04
Lysine	1.79	1.77	2.03	2.52
Arginine	2.05	1.99	2.36	2.22
Tryptophan	0.43	0.38	0.50	0.49

TABLE 4

Weight Gains, Feed Conversions of Tilapia Diets[a]

Diet	Weight gain[b]	Feed[c] conversion ratio
323	344.7	3.02
326	408.2	2.84
363	392.8	2.84
Control	324.0	2.93

[a]Means of three replicate cages for diets 323, 326 and 363. Means of six replicate cages for control. Mortality percent was zero for all diets and control.
[b]Expressed as the percentage increase at the end of 103 d.
[c]As-is feed/wet weight gain. There was no significant difference between diets and control ($P < 0.05$).

TABLE 5

***P*-Value[a] from *t*-Test Comparisons of Least Square Means for Weight Gains**

	323	326	363
326	0.069		
363	0.16	0.65	
Control	0.48	0.008	0.026

[a]*P*-value is probability that means are equal.

although the difference is not significant. It appears that CDGS can be utilized in a balanced amino acid diet for tilapia without fish meal.

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