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Growth Response and Resistance to *Streptococcus iniae* of Nile Tilapia, *Oreochromis niloticus*, Fed Diets Containing Distiller's Dried Grains with Solubles

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Abstract

This study was conducted to evaluate the effect of dietary levels of distiller's dried grains with solubles (DDGS) on growth, body composition, hematology, and resistance of Nile tilapia, *Oreochromis niloticus*, to *Streptococcus iniae* challenge. Five isocaloric diets containing DDGS at levels of 0, 10, 20, and 40%, and 40% DDGS + lysine (Diets 1–5) as partial replacements of a combination of soybean meal (SBM) and corn meal (CM) on an equal protein basis were fed to juvenile Nile tilapia (9.41 ± 0.14 g) for 10 wk. Fish fed Diet 4 had the lowest weight gain (WG), feed efficiency ratio, protein efficiency ratio (PER), and whole-body protein. Supplementation of lysine to the 40% DDGS diet (Diet 5) improved WG and PER. Hematological and immunological parameters were not affected by dietary treatment. There were no significant differences among the average number of days to first mortality after *S. iniae* challenge and cumulative mortality 14 d postchallenge among fish in various treatments. DDGS can be incorporated in tilapia diet at a level of 20% as a substitute for a combination of SBM and CM without affecting their growth performance, body composition, hematological parameters, immune response, and resistance to *S. iniae* infection.

Feed is generally the single largest expenditure in semi-intensive and intensive culture operations, and protein is the most expensive component in feeds for aquatic species. Soybean meal (SBM), because of its low cost, consistent quality and availability, and high nutritional value, is the most commonly used plant ingredient in aquaculture feeds. Currently, SBM comprises over 40% of tilapia feeds used in intensive culture such as in cages, raceways, and tanks (Lim 1989). Replacement of SBM with less expensive protein sources would be beneficial in reducing feed costs. Distiller's dried grains with solubles (DDGS), a by-product of the ethanol distillery industry is less expensive than SBM on a per unit protein basis. According to Buchheit (2002), approximately 98% of the DDGS in North America is from plants that produced ethanol for fuel, while the remaining 1–2% is produced by the alcohol beverage industry. In 2001, the USA produced about 3.1 million tons of DDGS. As a result of the recent expansion and increase in ethanol production for fuels because of the shortage and rising cost of petroleum-based fuel, the DDGS production in the USA has been estimated to increase to approximately 8 million tons in 2006 (Shurson 2006).

DDGS has a relatively high protein content (~30% crude protein) without the presence of antinutritional factors commonly found in most plant protein sources. At present, DDGS is widely used as a protein supplement in terrestrial animal feeds, but its use in fish feed is limited because of its low content of essential amino acids, especially lysine (NRC 1993). However, according to Webster et al. (1995), research to evaluate the nutritional value of DDGS began as early as the 1940. More recent research has shown that DDGS is a promising feed ingredient for several fish species, such as rainbow trout (Cheng and Hardy 2004), channel catfish (Tidwell et al. 1990; Webster et al. 1991, 1992a, 1992b, 1993), and tilapia (Wu et al. 1996). Results of these studies, however, were based on growth performance and body composition. No studies have been conducted on the effect of dietary levels of DDGS on fish immune

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functions, although it is commonly known that modification of feed formulae or alteration of dietary nutrients could positively or negatively affect fish immunity and disease resistance. This study was conducted to evaluate the influence of the dietary levels of DDGS on growth performances, immune responses, and resistance of Nile tilapia, *Oreochromis niloticus*, to *Streptococcus iniae* challenge.

Materials and Methods

Five diets were formulated to contain approximately 32% crude protein and 2800 kcal of digestible energy per kg. The diets contain 0, 10, 20, and 40% DDGS (Diets 1–4) as partial replacements of a mixture of SBM and corn meal (CM) on an equal protein basis (Table 1). Diet 5 was identical to Diet 4 (40% DDGS), except that 0.4% lysine was added to obtain a lysine level equal to that of Diet 1. Because DDGS contains lower protein than SBM, a combination of SBM and CM was used. Diets were processed into 2-mm-diameter pellets, dried at room temperature to a moisture content of less than 10%, ground and sieved to appropriate size, and stored at −20 C until used (Peres et al. 2003). Proximate composition of experimental diets determined in triplicate is given in Table 1.

Nile tilapia juveniles spawned and raised at our laboratory (USDA-ARS, Aquatic Animal Health Research Unit) on commercial larval and fingerling diets were acclimated to the basal experimental diet for 2 wk before stocking. Fish with an average weight of 9.41 ± 0.14 g were randomly stocked into twenty 57-L glass aquaria at a density of 30 fish per aquarium. Aquaria were supplied with flow-through (0.6–1.0 L/min) dechlorinated tap water maintained

TABLE 1. *Percentage composition and analyzed nutrient content of experimental diets.*

	Experimental diets (%)[a]				
	1	2	3	4	5
Menhaden fish meal	8	8	8	8	8
Soybean meal	54	40	35	24.5	24.5
Corn meal	30	26.7	23.3	16.6	16.6
Wheat middlings	5	5	5	5	5
Distiller's dried grains with solubles	—	10	20	40	40
Corn oil	3.6	2.8	2	0.5	0.5
Carboxymethyl cellulose	3	3	3	3	3
Dicalcium phosphate	1	1	1	1	1
Vitamin premix[b]	0.5	0.5	0.5	0.5	0.5
Mineral premix[c]	0.5	0.5	0.5	0.5	0.5
Lysine HCl	—	—	—	—	0.4
Celufil	3.4	2.5	1.7	0.4	—
Analyzed β-glucan (g/kg diet)	<3.0	<3.0	<3.0	3.4	3.6
Proximate composition (%)					
Moisture	90.55	90.8	91.3	90.96	91.36
Protein	30.87	31.58	32.2	33.14	33.73
Lipid	5.46	5.56	5.45	5.48	5.35
Ash	6.84	5.87	6.85	6.8	6.94

[a] Diets 1, 2, 3, and 4 contained 0, 10, 20, and 40% DDGS, respectively, and Diet 5 contained 40% DDGS + 0.4% lysine hydrochloride.

[b] Vitamin premix, diluted in cellulose, provided by following vitamins (mg/kg diet): vitamin A (500,000 IU/g), 8; vitamin D_3 (1,000,000 IU/g), 2; vitamin K, 10; vitamin E, 200; thiamin, 10; riboflavin, 12; pyridoxine, 10; calcium pantothenate, 32; nicotinic acid, 80; folic acid, 2; vitamin B_{12}, 0.01; biotin, 0.2; choline chloride, 400; l-ascorbyl-2-polyphosphate (150 mg/g vitamin C activity), 400.

[c] Trace mineral premix provided by following minerals (mg/kg diet): zinc (as $ZnSO_4.7H_2O$), 150; iron (as $FeSO_4.7H_2O$), 40; manganese (as $MnSO_4.7H_2O$), 25; copper (as $CuCl_2$), 3; iodine (as KI), 5; cobalt (as $CoCl_2.6H_2O$), 0.05; selenium (as Na_2SeO_3), 0.09.

at 26–27 C. Water was continuously aerated and photoperiod maintained at 12:12 h light : dark schedule. Fish in quadruplicate aquaria were randomly assigned to each of the five experimental diets and were fed to apparent satiation twice daily (between 0730–0830 h and 1430–1530 h) for 10 wk. The amount of diet consumed was recorded daily. Fish in each aquarium were group weighed and counted biweekly to determine weight gain (WG) and survival.

At the beginning of the trial, 25 fish from the initial stock, and at the end of the trial, 4 fish from each aquarium, were randomly sampled, pooled, and stored at −20 C for determination of whole-body proximate composition. Each sample, in which the scales were removed, was analyzed in duplicate following the standard methods (AOAC 1990).

At the end of the 10-wk feeding trial, four fish per aquaria were randomly chosen and anesthetized with tricaine methanesulfate and blood samples were collected using heparinized tuberculin syringes for hematological (red and white blood cells counts, hemoglobin, and hematocrit) assays following the methods of Brown (1988). Serum was collected from another four fish from each tank and assayed in duplicate for serum total protein concentration using the modified Biuret method (Sigma Diagnostic Procedure No. 542, Sigma Diagnostics Inc., St. Louis, MO, USA) and lysozyme assay, which is based on lysis of lysozyme-sensitive gram-positive bacterium *Micrococcus lysodeikticus* (Yildirim et al. 2003).

Twenty remaining fish per aquaria were intraperitoneally (I.P.) challenged with 100 μL of 8.77×10^6 colony-forming units/mL of *S. iniae* (ARS-98-60). Fish mortality was recorded daily for 14 d. Postchallenge serum samples were collected at the end of the challenge trial for measurement of agglutinating antibody titers against *S. iniae*. Prechallenge samples were also included in the test to determine if they were negative for antibody to *S. iniae* using the method of Chen and Light (1994) as modified by Yildirim et al. (2003).

Data were analyzed by one-way ANOVA. Duncan's multiple range tests were used to determine differences between treatment means. Differences were considered significant at the 0.05 probability level. All analyses were performed using the SAS program (Statistic Analysis Systems, SAS Institute, Inc., Cary, NC, USA, 2001).

Results

Mean final WG, dry matter feed intake, feed efficiency ratio (FER) and protein efficiency ratio (PER), and survival after 10 wk of feeding with diets containing various DDGS levels are given in Table 2. Fish fed the 40% DDGS diet without lysine supplementation (Diet 4) had significantly ($P < 0.05$) lower WG and PER than fish fed lower levels of dietary DDGS or the control diet (Diet 1). WG and PER of fish fed the 40% DDGS + 0.4% lysine diet (Diet 5) were significantly lower than the groups fed Diets 2 (10% DDGS) and 1, respectively, but did not

TABLE 2. *Mean final weight gain, dry matter feed intake, feed efficiency ratio (FER), protein efficiency ratio (PER), and survival of Nile tilapia fed diets containing various levels of distiller's dried grains with solubles for 10 wk.*[1]

Diets	Weight gain (g)	Feed intake (dry matter basis, g)	FER[2]	PER[3]	Survival (%)
1	48.0^{ab}	73.23^{b}	0.66^{a}	1.93^{a}	97.5
2	51.1^{a}	81.40^{a}	0.63^{ab}	1.82^{ab}	93.3
3	48.9^{ab}	75.18^{ab}	0.65^{ab}	1.85^{ab}	93.2
4	41.1^{c}	71.50^{b}	0.58^{b}	1.59^{c}	98.2
5	44.7^{bc}	71.83^{b}	0.62^{ab}	1.70^{bc}	96.7
Pooled SEM	1.2	2.02	0.02	0.06	1.8

[1] Values are means of four replicates per treatment. Means in the same column with different superscripts are significantly different at $P < 0.05$.

[2] FER = weight gain (g)/dry feed fed (g).

[3] PER = wet weight gain (g)/crude protein fed (g).

differ from those fed other diets. FER of Diet 4 (40% DDGS without lysine) was also significantly lower than that of the control diet but did not differ from those of the other diets. FER of Diet 5 (40% DDGS + 0.4% lysine) did not differ from those of the other diets. Dry matter feed intake of Diet 2 (10% DDGS) was significantly higher than those of the other diets, except for Diet 3 (20% DDGS). Survival was not significantly affected by dietary levels of DDGS.

Percent whole-body protein of fish fed the 40% DDGS diet without added lysine was significantly lower than in fish fed the control diet but did not differ from other treatments (Table 3). No significant differences were observed among protein content of fish in other treatments. Body moisture, and fat and ash contents were not significantly influenced by dietary treatments.

Dietary treatments did not significantly affect hematological parameters (red and white blood cell counts, hemoglobin, and hematocrit) (Table 4) or immune responses such as serum protein, lysozyme activity, and antibody production against *S. iniae* at 15 d postchallenge (Table 5). Likewise, average numbers of days to first mortality after *S. iniae* challenge and cumulative mortality 14 d postchallenge did not differ among treatments (Table 6).

Discussion

Results of the present study suggest that 20% of DDGS can be included in the diet of Nile tilapia as a replacement of a combination of SBM and CM without affecting the overall growth performance. Wu et al. (1996), in a study to evaluate the growth response of Nile tilapia fry fed all-plant protein diets, reported that in diets containing 32, 36, and 40% crude protein, incorporation of 16–49% DDGS resulted in good WG, FER, and PER. A diet containing 15% DDGS has been reported to provide satisfactory growth of channel catfish (Hastings 1967). Lovell (1980) reported that, when used in combination with 10% fish meal, up to 30% DDGS can be used in channel catfish diets. Webster et al. (1993) also found that 30% DDGS can be used as a replacement of a mixture of SBM and CM in channel catfish diets containing 8% fish meal. Whether 30% DDGS can be included in Nile tilapia diet containing 8% fish meal without adversely affecting their performance is unknown because this level was not used in our study. It is suggested that this level of DDGS be include in future studies.

Tidwell et al. (1990) and Webster et al. (1991) found that 40 and 35% DDGS, respectively, can be used in catfish diets as substitutes for the combination of SBM and CM on an equal protein basis without requiring lysine supplementation. However, a diet containing 70% DDGS appeared to be deficient in lysine because supplementation of lysine at a level to meet lysine requirement improved the growth of catfish (Webster et al. 1991). In another study to evaluate a fixed percentage of DDGS (35%) and a variable percentage of SBM (35–49%) as a partial or total replacement of fish meal in channel catfish diets, Webster et al. (1992a) found that the WG of fish fed the diet with 0% fish meal, 35% DDGS, and 49% SBM was similar to that of the diet with 12% fish meal and 48% SBM. They observed, however, that there appeared to be a trend of decreasing growth in fish fed diets with 0 and 4% fish meal as compared to those fish fed diets with 12 and 8% fish meal. Improved WG was obtained when the 0% fish meal diet was supplemented with lysine. In our study, although all diets contained 8% fish meal, increasing dietary levels of DDGS to 40% without the addition of lysine significantly reduced WG and PER relative to those obtained

TABLE 3. *Whole-body proximate composition of Nile tilapia fed diets containing various levels of distiller's dried grains with solubles for 10 wk.*[1]

Diet	Moisture (%)	Percent wet weight basis (%)		
		Lipid	Ash	Protein
1	69.02	6.63	4.58	16.94[a]
2	69.87	6.70	4.47	16.35[ab]
3	68.91	7.40	5.58	16.28[ab]
4	71.57	6.74	4.68	15.60[b]
5	69.77	6.51	4.83	16.11[ab]
Pooled SEM	0.88	0.51	0.50	0.34

[1] Values are means of two determinations of pooled samples of four fish per tank and four tanks per treatment. Means in the same column with different superscripts are significantly different at $P < 0.05$.

TABLE 4. *Red blood cell count (RBC), white blood cell count (WBC), hemoglobin, and hematocrit of Nile tilapia fed diets containing various levels of distiller's dried grains with solubles for 10 wk.*[a]

Diets	RBC[b] ($\times 10^6/\mu L$)	WBC[b] ($\times 10^5/\mu L$)	Hemoglobin[c] (g/dL)	Hematocrit[b] (%)
1	2.04	3.39	9.47	28.51
2	2.03	3.54	9.11	25.57
3	1.90	3.56	9.10	28.53
4	1.90	3.74	9.34	27.44
5	1.91	3.50	8.29	27.00
Pooled SEM	1.24	0.27	0.30	1.82

[a] No significant differences were observed among treatment means at $P > 0.05$.
[b] Values are means of two determinations per fish, four fish per tank and four tanks per treatment.
[c] Values are means of four fish per tank and four tanks per treatment.

with diets containing lower DDGS levels (0, 10, and 20%). FER of this diet (40% DDGS) was also significantly lower than that of the control diet. The decreased performance of the 40% DDGS diet could be as a result of a deficiency of lysine because supplementation with 0.4% lysine hydrochloride improved WG and FER to levels comparable to those of the control diet.

Except for protein content in fish fed the 40% DDGS diet without lysine supplementation that was significantly lower than that in fish fed the control diet, whole-body proximate composition was not affected by dietary levels of DDGS. The lower protein content of fish fed the 40% DDGS diet without lysine may be related to smaller size fish that had less flesh. Imbalance of dietary essential amino acids such as deficiency of lysine may also contribute to reduced protein synthesis. Webster et al. (1992b) reported significantly lower protein content of dressed carcass of catfish fed a diet with 90% DDGS without added lysine than in fish fed the 55% DDGS diet. However, no significant differences were observed among carcass proximate composition of catfish fed diets containing 0, 10, 20, and 30% DDGS (Webster et al. 1993).

DDGS contains substantial amounts of yeast cells. Ingledew (1999) estimated that 3.9% of the total biomass of DDGS was yeast, with 5.3% of the protein content of this product being contributed by yeast protein. Yeasts are rich in protein, B-complex vitamins, and β-glucans. The concentrations of β-glucan in DDGS and experimental diets used in our study determined by a private laboratory (NP Analytical Laboratories, St. Louis, MO, USA), using the method of the AACC International, megazyme BBG5/03 AACC 32-23, were 5.7 g/kg of DDGS; <3.0 g/kg (below detection limit) for diets containing 0, 10, and 20% DDGS; and 3.4 and 3.6 g/kg for diets with 40% DDGS and 40% DDGS + lysine, respectively. β-Glucans, either in purified form, as a yeast by-product, or as live yeast, have been reported to stimulate immune responses in humans and animals including fish

TABLE 5. *Serum protein, lysozyme activity, and antibody titer against* Streptococcus iniae *at 15 d postchallenge of Nile tilapia fed diets containing various levels of distiller's dried grains with solubles for 10 wk.*[a]

Diets	Serum protein[b] (mg/mL)	Lysozyme activity[b] (μg/mL)	Antibody titer[c] ($\log_{10}$)
1	33.83	17.15	1.20
2	34.69	16.20	0.77
3	34.41	16.96	1.32
4	33.85	16.35	1.37
5	31.02	16.47	1.05
Pooled SEM	1.42	0.83	0.22

[a] No significant differences were observed among treatment means at $P > 0.05$.
[b] Values are means of two determinations per fish, four fish per tank and four tanks per treatment.
[c] Values are means of four fish per tank and four tanks per treatment.

TABLE 6. *Means number of days to first mortality and cumulative mortality of Nile tilapia 15 d postchallenge with* Streptococcus iniae.[a]

Diet	Days to first mortality	Cumulative mortality (%)
1	2.0	47.5
2	1.3	38.3
3	2.0	56.3
4	1.0	50.0
5	1.3	42.5
Pooled SEM	0.4	5.6

[a] Values are means of four replicates per treatment. No significant differences were observed among treatment means at $P > 0.05$.

(Chen and Ainsworth 1992; Robertson et al. 1994). However, no significant differences were observed in both hematological parameters and immune responses (serum protein, lysozyme activity, and antibody production against *S. iniae* at 15 d postchallenge) among tilapia fed diets containing various levels of DDGS. Similarly, dietary levels of DDGS had no effect on the average number of days to which the first mortality occurred and cumulative mortality 14 d postchallenge with *S. iniae*.

Results of most of the earlier studies reported the enhancement effect of β-glucan on nonspecific immune response. Enhanced macrophage, and neutrophil migration and phagocytosis were observed in channel catfish fed β-glucan-containing diets (Duncan and Klesius 1996). Yeast glucan has also been found to improve lysozyme activity in Atlantic salmon and rainbow trout by I.P. injection with 0.7–1.0 mL saline containing 10 mg microparticulate-glucan/mL (Engstad et al. 1992; Jorgensen et al. 1993). In contrast, results of a 14-wk feeding study by Wittington et al. (2005) showed that lysozyme activity of Nile tilapia 14 d postchallenge with *S. iniae* was not enhanced by dietary inclusion of β-glucan at levels up to 100 mg/kg, but this value significantly decreased in fish fed the diet containing 200 mg β-glucan/kg. Prolonged feeding and feeding high levels of β-glucan have been reported to increase the susceptibility of Atlantic salmon and gilthead seabream to bacterial infection (Robertsen et al. 1990; Couso et al. 2003). It has also been reported that the effective concentration of β-glucan varied with the source (Raa 1996). Thus, whether prolonged feeding duration, high feeding levels, and source and high levels of β-glucan used in our study may have contributed the nonsignificant differences among various immune parameters and resistance of tilapia to *S. iniae* could not be ascertained and require further investigations.

These data indicate that 20% DDGS can be included in the diet of juvenile Nile tilapia as a replacement of an SBM and a CM mixture. DDGS appears to be deficient in lysine for Nile tilapia because supplementation of this amino acid to the diet containing 40% DDGS restored WG and PER to levels comparable to those of the control diet (0% DDGS). Dietary DDGS, at levels of 0, 10, 20, and 40% in diets, had no effect on hematology, immune responses, and resistance of Nile tilapia to *S. iniae* infection. The stimulatory effect of dietary DDGS on immune parameters and resistance of fish to infectious pathogens needs further investigations.

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