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Growth Response and Resistance to *Edwardsiella ictaluri* of Channel Catfish, *Ictalurus punctatus*, Fed Diets Containing Distiller's Dried Grains with Solubles

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Abstract

A study was conducted to examine the effect of dietary levels of distiller's dried grains with solubles (DDGS) on growth, body composition, hematology, immune response, and resistance of channel catfish, *Ictalurus punctatus*, to *Edwardsiella ictaluri* challenge. Five diets containing 0, 10, 20, 30, and 40% DDGS with supplemental lysine (Diets 1–5) as partial replacements of a combination of soybean meal and cornmeal on an equal protein basis were fed to juvenile catfish (13.33 ± 0.25 g) for 12 wk. Growth performance and feed utilization efficiency were similar for fish in all treatments. Body lipid and moisture increased and decreased, respectively, in fish feed DDGS-containing diets relative to the control group. Dietary treatment had no effect on red and white blood cell counts. Hemoglobin and hematocrit were significantly higher in fish fed diets containing DDGS than in those fed the control diet. Fish fed 20–40% DDGS diets had increased serum total immunoglobulin, and those fed the 30% DDGS diet had significantly increased antibody titers 21 d following *E. ictaluri* challenge. Other immune variables evaluated were not affected by dietary treatments. Preliminary results on bacterial challenge showed an increased resistance against *E. ictaluri* in fish fed DDGS-containing diets (Diets 2–5).

Feed cost is the single largest expenditure in channel catfish culture operations, and protein is the most expensive component in catfish feeds. Efforts by nutritionists to reduce feed costs have resulted in increased use of alternative plant proteins in diet formulations as replacements of more expensive animal proteins. Soybean meal (SBM), because of its low cost, consistent quality and availability, and high nutritional value, is the most commonly used plant ingredient in fish feeds. Currently, SBM comprises 35 to more than 45% commercial grow-out feeds for catfish (NRC 1993; Robinson and Li 2005). Replacement of SBM with less expensive protein sources would be beneficial in reducing feed costs. Distiller's dried grains with solubles (DDGS), a corn coproduct obtained after fermentation of corn for ethanol production, is highly palatable to fish and less expensive than SBM and other protein sources 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 interest in increasing ethanol production for fuels to reduce the shortage and dependency on petroleum-based fuel, the DDGS production in the USA has been estimated to increase to approximately 8 million tons in 2006 (Shurson 2006). Thus, increased and new uses are needed to use these abundant DDGS supplies.

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 that is only about 40% of that found in SBM (NRC 1993). According to Webster et al. (1995), research to evaluate the nutritional value of DDGS in fish diets began as early as the 1940. More recent research has shown that DDGS is a promising

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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; Li and Robinson 2007), and tilapia (Wu et al. 1996; Lim et al. 2007). Except for the study of Lim et al. (2007) that investigated the effect of dietary levels of DDGS on immune response and resistance of Nile tilapia to *Streptococcus iniae* challenge, results of other studies were based mostly on growth performance and body composition. Therefore, this study was conducted to evaluate the influence of the dietary levels of DDGS on growth performance, immune response, and resistance of channel catfish, *Ictalurus punctatus*, to *Edwardsiella ictaluri* challenge.

Materials and Methods

Experimental Fish, Diets, and Feeding

Five diets were formulated to contain approximately 32% crude protein and 2800 kcal of digestible energy/kg. The diets contained 0, 10, 20, 30, and 40% DDGS (Diets 1–5) as partial replacements of a mixture of SBM and cornmeal (CM) on an equal protein basis (Table 1). Lysine hydrochloride 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 2.5-mm-diameter pellets, dried at room temperature using an electric fan to a moisture content of less than 10%, ground and sieved to an appropriate size, and stored at −20 C until used (Peres et al. 2003). Proximate composition of experimental diets (AOAC 1990) determined in triplicate is given in Table 1.

National Warmwater Aquaculture Center 103 strain channel catfish, *I. punctatus*, from a single spawn that have been reared at our laboratory (Aquatic Animal Health Research Unit, USDA-ARS) on commercial larval and fingerling diets from yolk sac fry to juvenile were acclimated to the basal experimental diet for 2 wk prior to stocking. Fish with an average weight of 13.33 ± 0.25 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 at 26 C. Water was continuously aerated, and photoperiod maintained at 12:12 h light : dark schedule. Water temperature and dissolved oxygen in four randomly chosen aquaria were measured once every other day in the morning using a YSI Model 58 Oxygen Meter (Yellow Springs Instrument, Yellow Springs, OH, USA). During the trial, water temperature averaged 25.5 ± 0.1 C and dissolved oxygen averaged 5.1 ± 0.1 mg/L.

Fish in four aquaria were randomly assigned to each of the five experimental diets and were fed to apparent satiation twice daily (between 0730–0830 and 1430–1530 h) for 12 wk. The amount of diet consumed was recorded daily by calculating the differences in weight of diets prior to the first and after the last feeding. Once a week, aquaria were scrubbed and accumulated wastes siphoned. On cleaning day, fish were fed only once, in the afternoon. Fish in each aquarium were group weighed and counted biweekly to determine weight gain (WG) and survival. Feed was not offered on sampling days.

Body Composition

Twenty fish at the beginning of the trial and 4 fish from each aquarium at the end of the trial were randomly sampled, pooled, and stored at −20 C for determination of whole-body proximate composition. Each sample was analyzed in duplicate following the standard methods (AOAC 1990). Moisture content was determined by drying fish samples in an oven at 105 C until constant weight was reached. Samples used for dry matter were digested with nitrite acid and incineration in a muffle furnace at 600 C for overnight for ash contents. Protein was measured by combustion method using an FP-2000 Nitrogen Analyzer (Leco Corporation, St. Joseph, MI, USA). Lipid content of samples was determined by petroleum ether extraction using a Soxtec System (2055 Soxtec Avanti; Foss Tecator, Höganäs, Sweden).

Hematology

At the end of the feeding period, four fish were randomly chosen from each tank and anesthetized with tricaine methanesulfonate (MS-222) at 150 mg/L. Blood samples were collected from

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

	Experimental diets (%)[1]				
	1	2	3	4	5
Ingredients					
Menhaden fish meal	8.0	8.0	8.0	8.0	8.0
Soybean meal	45.0	38.8	32.6	26.4	20.2
Cornmeal	25.0	21.5	18.1	14.7	11.2
Wheat middlings	5.0	5.0	5.0	5.0	5.0
DDGS	—	10.0	20.0	30.0	40.0
Corn oil	3.7	3.0	2.4	1.7	1.0
CMC	3.0	3.0	3.0	3.0	3.0
Dicalcium phosphate	1.0	1.0	1.0	1.0	1.0
Vitamin premix[2]	0.5	0.5	0.5	0.5	0.5
Mineral premix[3]	0.5	0.5	0.5	0.5	0.5
Lysine HCL	—	0.1	0.2	0.3	0.4
Corn starch	—	2.3	4.6	6.9	9.2
Celufil	8.3	6.3	4.1	2.0	—
Calculated β-glucan (g/kg diet)[4]	0	0.57	1.14	1.71	2.28
Proximate composition (%)					
Dry matter	91.91	94.66	94.63	94.31	94.8
Protein	30.12	30.26	30.43	30.45	30.66
Lipid	5.46	5.56	5.45	5.48	5.35
Ash	6.66	6.79	6.70	6.54	6.45

CMC = carboxymethyl cellulose; DDGS = distiller's dried grains with solubles.

[1] Diets 1, 2, 3, 4, and 5 contained 0, 10, 20, 30, and 40% DDGS, respectively.

[2] 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; and L-ascorbyl-2-polyphosphate (150 mg/g vitamin C activity), 400.

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

[4] Because of the sensitivity of the method used for glucan analysis (>3.0 g/kg), these values were calculated based on β-glucan content of DDGS (5.7 g/kg) determined by NP Analytical Laboratories.

the caudal vein with heparinized (100 IU/mL) tuberculin syringes for hematological assays. Red blood cell (RBC) and white blood cell (WBC) counts were performed in duplicate for each sample by diluting (1:10,000) whole blood in phosphate buffer saline (PBS) solution and enumerating in a Spencer Bright Line hemacytometer. Hemoglobin (Hb) was determined using a cyanomethemoglobin method (Sigma Chemical Co., St. Louis, MO, USA). Hb values were adjusted by cyanomethemoglobin correction factor for channel catfish as described by Larsen (1964). Hematocrit (Ht) of each fish was determined in duplicate using the microhematocrit method (Brown 1988).

Serum Protein and Total Immunoglobulin

An additional four fish per tank were bled using nonheparinized tuberculin syringes and allowed to clot at 4 C overnight. Serum samples were collected following centrifugation and stored at −80 C until used. Serum from each of the four fish per tank was assayed in duplicate for serum protein concentration using the modified biuret method. Total protein reagent (Sigma Chemical Co.) was added to each well of the microtiter plate at 250 μL/well. Then, 5 μL of serum was added to each well. After 30-min incubation at room temperature, the absorbance of the samples was read at 570 nm. Serum total protein concentrations were calculated using bovine serum albumin as an external standard.

Serum total immunoglobulin (Ig) was determined following the method of Siwicki and Anderson (1993). The assay was based on the measurement of total protein content in serum

prior and after precipitating the Ig molecules using 12% solution of polyethylene glycol.

Lysozyme Assay

Serum lysozyme activity was determined by the method of Litwack (1955) as modified by Sankaran and Gurnani (1972). The assay is based on lysis of lysozyme-sensitive gram-positive bacterium *Micrococcus lysodeikticus* (Sigma Chemical Co.) by the lysozyme present in the serum. Freeze-dried *M. lysodeikticus* suspension (0.25 mg/mL) was prepared immediately before use by dissolving in sodium phosphate buffer (0.04 M Na_2HPO_4, pH 6.0). Serum (15 μL/well in duplicate) from each of the four fish per tank was placed in a microtiter plate and 250 μL of bacterial cell suspension was added to each well. Hen egg white lysozyme was used as an external standard. The initial and final (0- and 20-min incubation at 35 C) absorbance of the samples was measured at 450 nm. The rate of reduction in absorbance of samples was converted to lysozyme concentration (μg/mL) using the standard curve.

Natural Hemolytic Complement Activity

Serum natural hemolytic complement activity (alternative pathway) was measured using an assay adapted from Sunyer and Tort (1995) and modified for use in microtiter plates. This assay is based on the hemolysis of sheep erythrocytes (Remel, Inc., Lenexa, KS, USA) by complement present in fish serum. Sheep erythrocytes were washed four times with potassium phosphate buffer containing $CaCl_2$, $MgCl_2$, and gelatin (PBS^{+++}) and standardized to 5×10^7 cells/mL in PBS^{+++} before use. A twofold serial dilution was made in 96-well round-bottom microtiter plates by adding 50 μL of serially diluted serum from four fish per tank into the wells plated with 50 μL of PBS^{+++}. The volume in each well was adjusted to 250 μL by adding 200 μL buffer to give final concentrations of 20, 10, 5, 2.5, 1.25, 0.625, 0.313, and 0.157%. Thereafter, 50 μL of sheep erythrocyte cell suspension was added to each well. Positive controls (100% lysis) of distilled water plus sheep erythrocytes (250 μL H_2O and 50 μL erythrocyte) and negative controls (spontaneous lysis) of buffers and sheep erythrocytes (250 μL PBS^{+++} and 50 μL erythrocyte) were also processed in each plate. Samples were incubated at room temperature (22 C) for 1 h with regular shaking. The reaction was stopped by placing plates on ice. The plates were centrifuged at 800 *g* for 10 min at 4 C to avoid unlyzed cells. Supernatants (250 μL) were transferred to a flat-bottom 96-well microtiter plate and the absorbance measured at 415 nm using an enzyme-linked immunosorbent assay plate reader.

Complement hemolytic activity was expressed as ACH_{50} value, which represents the volume of serum necessary to produce lysis of 50% of the target cells under standard conditions, and results presented as units/mL. The degree of hemolysis was estimated and the lysis curve for each sample was obtained by plotting $Y/(100 - Y)$ against the volume of serum added (mL) on a log–log scaled graph. The value Y (percentage of hemolytic activity at each dilution with respect to controls) was defined as:

$$Y = 100[\mathrm{Abs}(A) - \mathrm{Abs}(B)]/[\mathrm{Abs}(C) - \mathrm{Abs}(B)],$$

where A = supernatant of the test serum dilution, B = minimum hemolysis (negative control or spontaneous lysis), and C = maximum hemolysis (positive control or 100% lysis).

Isolation and Collection of Peritoneal Leucocytes

At the end of the 12-wk feeding period, four fish from each aquarium were intraperitoneally (IP) injected with 0.25 mL of squalene (Sigma Chemical Co.) as an attractant for macrophages. Fish feeding was continued with assigned diets. Six to 7 d later, fish were anesthetized with MS-222 and IP injected with 15 mL of ice-cold, sterile 0.85% PBS solution by using a 20-gauge needle attached to three-way valve. Then, PBS was removed along with the squalene-elicited exudate cells into a 50-mL centrifuge tube. The peritoneal fluid of fish from the same tank was combined and centrifuged at 1500 *g* for 10 min. The supernatant was discarded and the pellets were resuspended in 1 mL of calcium-, magnesium-, and sodium-free Hank's balanced

salt solution without phenol red (Gibco Laboratories, Grand Island, NY, USA). Number of macrophages of each sample was counted by staining with Yokayama's staining solution and enumerated in a Spencer Bright Line hemacytometer for use in macrophage chemotaxis ratio and superoxide anion production.

Macrophage Chemotaxis Assay

Macrophage chemotaxis was determined by a modification of the lower surface method (Boyden 1962) as described by Yildirim et al. (2003). Each sample was assayed in triplicate using chemotaxis chambers (Corning CoStar, Cambridge, MA, USA). To the bottom of the chambers was added either 200 μL (RPMI-1640) (Gibco Laboratories) containing 1% inactivated horse serum or 150 μL (RPMI-1640) + 50 μL of *E. ictaluri* exoantigen (2.80 mg protein/mL). The lower component of the chamber was separated from the upper compartment by an 8-μm-pore diameter polycarbonate membrane filter (Nucleopore, Pleasanton, CA, USA) presoaked for 5 min in RPMI-1640 containing 1% horse serum. Peritoneal macrophages (5×10^5 cells/chamber) were added to the upper compartment of the chamber. The chambers were incubated for 90 min at room temperature on a horizontal platform shaker (100 rpm). Following incubation, the filters were removed from the chambers, inverted, placed on a slide attached with clear fingernail polish, allowed to dry at room temperature, and stained with Leukostat. The numbers of macrophages on the surface of the filter were counted in five fields of triplicate filters at 100×.

Superoxide Anion Production

The respiratory burst of phagocytic cells, based on superoxide anion (O_2^-) production, was measured by nitroblue tetrazolium (NBT) reduction assay following the method of Secombes (1990) with some modifications. Previously isolated channel catfish peritoneal macrophage-rich leukocytes were adjusted in 0.85% PBS solution. Quadruplicate monolayers of 1×10^6 adherent cells were added to each well of a 96-well microtiter plate. After incubation for 2 h at room temperature in a humidified chamber, the wells were washed with 0.85% PBS solution to remove the nonadherent cells. NBT (0.2% w/v in saline) solution was incubated for 1 h with adherent leukocyte monolayers as an indicator of the respiratory burst activity. After removal of medium from wells, 100% methanol was added to stop the reaction and washed twice with 70% methanol and allowed to air-dry. The reduced formazan within adherent cells was solubilized by adding 120 μL 2 M potassium hydroxide (KOH) and 140 μL dimethyl sulphoxide (DMSO) to each well. After incubation on a horizontal platform shaker (100 rpm) for 10 min, the optical density (OD) of samples was measured at 630 nm using KOH/DMSO as a reference.

Bacterial Challenge

Edwardsiella ictaluri (AL-94-75), from a virulent outbreak of enteric septicemia of catfish, was used for infection challenge by bath immersion (Klesius and Sealey 1995). Stock culture of *E. ictaluri* frozen at −80 C (2 mL) was inoculated in 250 mL of brain–heart infusion broth and cultured for 24 h in a water bath shaker maintained at 26 C (Klesius 1992). The concentration of the culture was adjusted to an OD of 1.7 at 540 nm, using a spectrophotometer, to give an *E. ictaluri* concentration of approximately 2×10^{10} colony-forming units (CFU)/mL.

To determine the optimum bacterial cell concentration to use in experimental challenge, groups of 16 fish, which were held in separate aquaria and fed the control diet for 10 wk, were placed in 10-L perforated plastic buckets and immersed for 30 min in static, aerated 18.4-L buckets containing 10 L of *E. ictaluri* suspensions at concentrations of 0, 1×10^7, 2×10^7, 4×10^7, 8×10^7, and 1.6×10^8 cells/mL. Following challenge, each group of fish was transferred to a new, randomly assigned 57-L aquarium. Water flow and feeding were discontinued for the first 6 and 24 h after challenge, respectively. Mortality was recorded twice daily for 15 d. The LC_{50} (concentration lethal to 50% of exposed fish), which was calculated by the Karber's method (Plumb and Browser 1983), was 1×10^7 cells/mL and was the concentration used for the experimental challenge.

At the end of Week 12, the remaining 17 fish from each aquarium were challenged by bath immersion, as has been previously described, in water containing *E. ictaluri* at a concentration of 1×10^7 CFU/mL. Each group of fish was then returned to their respective aquarium where they continued to receive their assigned diets. Water flow and feeding were carried out as previously described. Mortality was recorded twice daily for 21 d.

Agglutination Antibody Titer

At the 21st day of the bacterial challenge, blood samples were collected from four surviving fish and serum was obtained following centrifugation. Agglutinating antibody titers against *E. ictaluri* in pre- and postchallenge serum samples were determined by modifying the method of Chen and Light (1994) as described in Yildirim et al. (2003). The agglutination end point was established as the last serum dilution where cell agglutination was visible after incubation. Agglutination titers were reported as $\log_{10}$ of the reciprocal of the highest serum dilution showing visible agglutination as compared to the positive control.

Statistical Analysis

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), apparent protein utilization (APU), and survival after 12 wk of feeding with diets containing various DDGS levels are given in Table 2. There were no significant differences among the values of these parameters of fish fed diets containing various levels of DDGS. However, there appeared to be a trend of improving WG, FER, PER, and APU with increasing levels of DDGS.

Percent whole-body moisture of fish fed Diets 3–5 was significantly lower than that of fish fed the control diet (Diet 1). The value for fish fed Diet 2 was not significantly different from those of other treatments (Table 3). No significant differences were observed among protein and ash contents of fish in various treatments. Body lipid contents of fish fed Diets 2–5 were similar and were significantly higher than those of fish fed Diet 1.

Dietary treatments had no effect on RBC and WBC counts (Table 4). Hb and Ht of fish fed the control diet (Diet 1) were significantly lower than those of the groups fed diets containing DDGS (Diets 2–5). The values of these variables did not differ among fish fed diets containing DDGS.

Serum total Ig of fish fed Diets 1 and 2 did not differ but was significantly lower than those of

TABLE 2. *Mean final weight gain, dry matter feed intake, FER, PER, APU, and survival of channel catfish fed diets containing various levels of distiller's dried grain with solubles for 12 wk.*[1]

Diet	Weight gain (g)	Feed intake (g)	FER[2]	PER[3]	APU[4]	Survival (%)
1	48.61	97.63	0.50	1.65	25.72	95.00
2	48.83	102.56	0.48	1.57	24.28	100.00
3	54.91	102.30	0.54	1.76	27.51	95.83
4	55.16	99.99	0.55	1.81	27.87	95.00
5	53.81	100.40	0.53	1.73	27.07	96.67
Pooled SEM	2.77	2.19	0.02	0.07	1.14	1.48

FER = feed efficiency ratio; PER = protein efficiency ratio; APU = apparent protein utilization.

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

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

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

[4] APU = 100 × [body protein gain (g)/crude protein fed (g)].

TABLE 3. *Whole-body proximate composition of channel catfish fed diets containing various levels of distiller's dried grain with solubles for 12 wk.*[1]

Diet	Moisture (%)	Percent wet weight basis		
		Protein	Lipid	Ash
1	71.99[a]	15.56	8.35[b]	3.85
2	71.21[ab]	15.43	9.35[a]	3.81
3	70.72[b]	15.56	9.64[a]	3.73
4	71.07[b]	15.38	9.20[a]	3.53
5	70.69[b]	15.50	9.21[a]	3.83
Pooled SEM	0.27	0.02	0.20	0.06

[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. *RBC, WBC, hemoglobin, and hematocrit of channel catfish fed diets containing various levels of distiller's dried grain with solubles for 12 wk.*[1]

Diet	RBC[2] ($\times 10^6/\mu L$)	WBC[2] ($\times 10^5/\mu L$)	Hemoglobin[3] (g/dL)	Hematocrit[2] (%)
1	2.28	2.85	8.88[b]	30.80[b]
2	2.46	2.73	10.81[a]	37.88[a]
3	2.71	3.05	11.16[a]	39.95[a]
4	2.77	2.79	10.91[a]	39.63[a]
5	2.68	3.25	10.24[a]	39.05[a]
Pooled SEM	0.03	0.07	0.35	1.56

RBC = red blood cell; WBC = white blood cell.

[1] Means in the same column with different superscripts are significantly different at $P < 0.05$.

[2] Values are means of two determinations per fish, four fish per tank, and four tanks per treatment.

[3] Values are means of one determination per fish, four fish per tank, and four tanks per treatment.

fish fed diets containing 20% or higher DDGS (Diets 3–5) (Table 5). There were no significant differences among serum protein, lysozyme activity, alternative complement, superoxide anion production, and macrophage chemotaxis ratio of fish in various treatments. Serum antibody titers against *E. ictaluri* at 21 d postchallenge of fish fed the diet containing 30% DDGS (Diet 4) were significantly higher than those of fish fed Diet 1 (Table 6). These values, however, were not significantly different from those of the groups fed other diets.

The average number of days to first mortality after *E. ictaluri* challenge was earliest for fish fed Diet 1 and was significantly lower than those of fish fed Diets 3–5 (Table 6). The value for fish fed Diet 2 did not differ from those of fish fed Diets 1 and 3 but was significantly lower than those of fish fed Diets 4 and 5. There were no significant differences among the numbers of days to first mortality in fish fed Diets 3–5. Cumulative mortality 21 d postchallenge was significantly highest in fish fed the control diet (Diet 1). No significant differences were observed among fish receiving other dietary treatments (Diets 2–5). Data on prechallenge antibody titer showed that fish were negative to *E. ictaluri*.

Discussion

Results of the present study suggest that, with lysine supplementation to a level equal to that of the control diet, at least 40% of DDGS can be included in the diet of juvenile channel catfish as a replacement of a combination of SBM and CM without affecting the growth performance, feed utilization efficiency, and survival. This is in agreement with the findings of Li and Robinson (2007) who showed that 40% DDGS and supplemental lysine can be included in the diet of catfish grown in ponds as a replacement of 36% SBM without affecting WG. In the same study, they obtained better WG of catfish fed the diet containing 30% DDGS plus supplemental lysine as a replacement of 30% SBM than fish fed the control diet or the diet in which all SBM was replaced by 19% DDGS and 44% cottonseed meal plus lysine. Tidwell et al. (1990) and Webster et al. (1991) found that 35–40% DDGS can be used in catfish diets containing 8–10% fish meal 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).

Lovell (1980) reported that, when used in combination with 10% fish meal, up to 30% DDGS can be used in channel catfish diets. Likewise, Webster et al. (1993) found that 30% DDGS can be used as a replacement of

TABLE 5. *Serum protein, total circulating immunoglobulin, lysozyme activity, natural hemolytic complement activity, superoxide anion production, and macrophage chemotaxis ratio channel catfish fed diets containing various levels of distiller's dried grain with solubles for 12 wk.*[1]

Diet	Serum protein[2] (mg/mL)	Total immunoglobulin[2] (mg/mL)	Lysozyme activity[2] (μg/mL)	Alternative complement activity[3] (units/mL)	Superoxide anion production[4] (OD_{630nm})	Macrophage chemotaxis ratio[5]
1	30.13	1.78[b]	3.31	45.46	0.207	0.77
2	28.49	2.28[b]	3.36	41.53	0.370	0.83
3	31.34	3.70[a]	3.34	37.33	0.432	0.80
4	30.49	3.79[a]	2.84	45.99	0.306	0.77
5	31.25	3.72[a]	3.54	40.87	0.407	0.71
Pooled SEM	0.77	0.36	0.32	6.22	0.09	0.09

OD = optical density.

[1] Means in the same column with different superscripts are significantly different at $P < 0.05$.

[2] Values are means of two determinations per fish, four fish per tank, and four tanks per treatment.

[3] Values are means of four fish per tank and four tanks per treatment.

[4] Values are means of eight determinations per tank and four tanks per treatment.

[5] Values are means of three determinations of pooled samples of four fish per tank and three tanks per treatment. Chemotaxis ratio represents the number of migrating cells in the presence of *Edwardsiella ictaluri* exoantigen divided by the sum of the number migrating cells without (control) and with *E. ictaluri* exoantigen.

a mixture of SBM and CM in channel catfish diets containing 8% fish meal. 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 of fish fed diets with 12 and 8% fish meal. Improved WG was obtained when the 0% fish meal diet was supplemented with lysine. Diets in our study also contained 8% menhaden fish meal. Whether supplemental lysine can be omitted from the 40% DDGS catfish diet containing 8% menhaden fish meal cannot be ascertained because diets without lysine supplementation were not included in our study. In Nile tilapia, Lim et al. (2007) showed that, even though all diets contained 8% fish meal, increasing dietary levels of DDGS to 40% without the addition of lysine significantly reduced WG and feed and protein efficiencies relative to those obtained with diets containing lower DDGS levels (0, 10, and 20%). The decreased performance of the 40% DDGS diet was attributed to a deficiency of lysine because supplementation with 0.4% lysine hydrochloride improved WG and feed efficiency to levels comparable to those of the control diet.

There were no differences among whole-body protein and ash of fish fed various experimental diets. In Nile tilapia, Lim et al. (2007) reported similar whole-body protein in fish fed diets containing 0, 10, 20% DDGS, and 40% DDGS supplemented with lysine. However, fish fed the 40% DDGS diet without added lysine had lower body protein content than those fed the SBM control diet. The decreased protein content in fish fed the 40% DDGS diet without

TABLE 6. *Mean number of days to first mortality and cumulative mortality of channel catfish 21 d postchallenge with* Edwardsiella ictaluri *and antibody production against the same bacterium.*[1]

Diet	Days to first mortality	Cumulative mortality (%)	Antibody titer (log_{10})
1	7.75[c]	32.4[a]	1.41[b]
2	11.00[bc]	11.8[b]	1.71[ab]
3	16.00[ab]	10.3[b]	1.75[ab]
4	20.00[a]	2.9[b]	1.96[a]
5	19.75[a]	4.4[b]	1.69[ab]
Pooled SEM	2.24	4.27	0.11

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

lysine supplementation was attributed to smaller size fish, which had less flesh and/or the imbalance of dietary essential amino acids, such as deficiency of lysine, which may contribute to reduced protein synthesis. Webster et al. (1992b) obtained 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.

Fish fed diets containing DDGS, however, had significantly lower body moisture (except that of fish fed Diet 2) and higher fat content than those fed the control diet. Because diets used in our study were isonitrogenous, were isocaloric, and contained approximately the same lipid level, the higher whole-body fat content in fish fed diets containing DDGS may be because of improved diet digestibility leading to higher available energy. Lim et al. (2007) obtained similar body fat content of Nile tilapia fed isocaloric and isolipidic diets containing up to 40% of DDGS. Li and Robinson (2007) reported that fillet fat was higher or tended to be higher in catfish fed diets containing SBM and DDGS plus supplemental lysine as compared to the group fed the SBM control diet, due possibly to higher fat content of the DDGS-containing diets. Webster et al. (1991) reported significantly higher body fat in catfish fed DDGS diets (fat content ranging from 8.15 to 11.52%) than the group fed the control diet (5.80% fat). In another study, no significant differences were found among carcass proximate composition of catfish fed diets containing 0, 10, 20, and 30% DDGS with dietary lipid contents of 5.00, 5.70, 6.36, and 7.06, respectively (Webster et al. 1993).

In the current study, RBC and WBC counts were not affected by inclusion levels of DDGS. However, fish fed diets containing DDGS (10–40%) had significantly higher Hb and Ht than the group fed the control diet. Welker et al. (2007) also observed no significant differences in RBC and WBC in catfish fed diets supplemented with yeast, *Saccharomyces cerevisiae*, or yeast subcomponents (β-glucans: MacroGard and Betagard A, and mannan: Bio-Mos) but significantly higher Ht in the MacroGard group than in the control group after 4 wk of feeding. Lim et al. (2007) did not observe significant differences among hematological parameters (RBC, WBC, Hb, and Ht) of Nile tilapia fed diet containing 0, 10, 20, 40% DDGS, and 40% DDGS + lysine. It is unknown whether the increase in Hb and/or Ht values observed in our study and by Welker et al. (2007) was related to the effect of β-glucan or the presence of additional nutrients, especially vitamins present in DDGS-containing diets because yeasts present in DDGS are a rich source of B-complex vitamins, but further investigations are warranted.

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 concentration of β-glucan in DDGS used in our study determined by a private laboratory (NP Analytical Laboratories, St. Louis, MO, USA), using megazyme BBG5/03 according to the method of the American Association of Cereal Chemists (AACC 32-23), was 5.7 g/kg of DDGS. β-glucans, in purified form, yeast by-product, or live yeast, have been shown to stimulate immune responses and increase the host resistance to various microbial pathogens in mammals and other animals including fish (Chen and Ainsworth 1992; Ainsworth et al. 1994; Robertsen et al. 1994; Jorgensen and Robertsen 1995; Duncan and Klesius 1996; Raa 1996; Sakai 1999; Gannam and Schrock 2001).

Evidence on the effect of β-glucans on improving immune function and the resistance of fish to infections is not consistent. We observed that catfish fed diets containing 20–40% DDGS (1.14–2.28 g glucan/kg diet) had significant increase in serum total Ig, but inclusion levels of DDGS had no effect on serum protein, lysozyme activity, alternative complement, superoxide anion production, and macrophage chemotaxis ratio of fish in various treatments. However, preliminary data on *E. ictaluri* challenge showed a delayed mortality that may indicate reduced accumulative mortality 21 d postinfection challenge in fish fed DDGS-containing diets (Diets 2–5) compared to the control group. Serum agglutinating

antibody titers against *E. ictaluri* at 21 d post-challenge of fish fed the diet containing 30% DDGS (Diet 4) were significantly higher than those of fish fed Diet 1 (Table 6). The increase in serum Ig may be responsible for the delay of onset of mortality and decreased mortality of fish fed DDGS-containing diets following *E. ictaluri* challenge. Shoemaker et al. (2007) reported that fish Ig is capable of specifically binding epitopes on the surface of bacteria. They also suggested that Ig is a potential activator of complement and a very effective opsonin and agglutinin that facilitate clearance of pathogen. Lim et al. (2007) reported no differences among serum total protein and lysozyme activity and mortality and antibody titer 15 d post-challenge with *St. iniae* of Nile tilapia fed diets containing 0, 10, 20, 40% DDGS, and 40% DDGS + lysine. Welker et al. (2007) obtained no significant differences in immune function (serum alternative complement, lysozyme activity, superoxide anion production, and macrophage bactericidal activity) and resistance to *E. ictaluri* challenge in catfish fed diets supplemented with *S. cerevisiae* or yeast subcomponents (glucan and mannan). Duncan and Klesius (1996) reported that channel catfish fed the β-glucan-containing (0.2%) diet had enhanced macrophage and neutrophil migration and phagocytosis, whereas fish fed the *S. cerevisiae* diet (2.7%) had enhanced phagocytic activity of peritoneal exudate cells. This, however, had no effect on the resistance of fish to *E. ictaluri* infection. Chen and Ainsworth (1992) obtained increased antibody titers and reduced mortality in catfish challenged with *E. ictaluri* by intraperitoneal injection with β-glucan from baker's yeast 4 and 7 d prior to the challenge. Ainsworth et al. (1994) reported no improved resistance to *E. ictaluri* challenge in catfish fed 0.1% β-glucan but obtained increased antibody titers to *E. ictaluri*. Jorgensen and Robertsen (1995) observed a marked increase in respiratory burst activity of head kidney macrophages of Atlantic salmon 4–7 d after treatments with 0.1–1 μg/mL of glucan. Despite the stimulatory effect of glucan on respiratory burst activity, they reported that these macrophages did not show enhanced bactericidal activity against the avirulent and virulent strain of *Aeromonas salmonicida*. Differences in species, fish size, source and concentration of glucans, mode of administration, feeding duration and levels, challenge method and concentration, and virulence of the pathogens may have contributed to variations among various research results.

Data of the current study suggest that, with lysine supplementation to a level equal to that of the control diet, at least 40% DDGS can be included in the diet of juvenile channel catfish as a replacement of an SBM and CM mixture without affecting their growth performance and feed utilization efficiency. However, fish fed diets containing DDGS (Diets 2–5) had increased body fat. Dietary inclusion of DDGS also improved Ht, Hb, serum total Ig, and antibody titers against *E. ictaluri*, although the optimum levels varied among parameters evaluated. Preliminary data on bacterial challenge showed a delayed mortality and increased resistance against *E. ictaluri* infection in fish fed DDGS-containing diets. However, because of the termination of the study at 21 d, further work extending the challenge period is suggested. More research is also needed to identify compounds in DDGS that have immunostimulatory effect and improve the resistance of fish to infectious pathogens.

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