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Abstract
A feeding trial investigated the use of a diet comprised of yeast and distillers' dried grain with solubles (DDGS) to improve growth, feed utilization, and stress resistance of tilapia. Six diets were formulated (as-fed basis) with 8% menhaden fishmeal and 20%, 25%, or 30% DDGS with or without 0.125% yeast probiotic. Aquaria (110 L) were stocked with eight Nile tilapia (mean weight = 43.6 ± 1.3 g) with four replicate aquaria per diet. Weight gains, food conversion ratios (FCR), protein efficiency ratios (PER), blood cortisol levels, and survival rates did not significantly differ ($P \geq 0.1$) among or between fish fed differing diets. Overall, results indicated that the inclusion of low-level yeast probiotics did not increase the utilization of DDGS nor did it improve the growth or acute stress resistance (i.e., short-term stressor) of juvenile Nile tilapia.

Keywords
Nile tilapia, Oreochromis niloticus, DDGS, yeast, probiotic, stress resistance

Disciplines
Agriculture | Aquaculture and Fisheries | Bioresource and Agricultural Engineering

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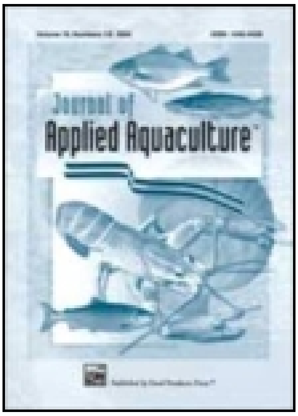

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Growth and Stress Resistance of Advanced Sized Nile Tilapia Fed Diets Containing Fuel-Based DDGS and Yeast

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A feeding trial investigated the use of a diet comprised of yeast and distillers' dried grain with solubles (DDGS) to improve growth, feed utilization, and stress resistance of tilapia. Six diets were formulated (as-fed basis) with 8% menhaden fishmeal and 20%, 25%, or 30% DDGS with or without 0.125% yeast probiotic. Aquaria (110 L) were stocked with eight Nile tilapia (mean weight = 43.6 ± 1.3 g) with four replicate aquaria per diet. Weight gains, food conversion ratios (FCR), protein efficiency ratios (PER),

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blood cortisol levels, and survival rates did not significantly differ ($P \geq 0.1$) among or between fish fed differing diets. Overall, results indicated that the inclusion of low-level yeast probiotics did not increase the utilization of DDGS nor did it improve the growth or acute stress resistance (i.e., short-term stressor) of juvenile Nile tilapia.

KEYWORDS Nile tilapia, Oreochromis niloticus*, DDGS, yeast, probiotic, stress resistance*

INTRODUCTION

Less than 10 years ago, distillers' dried grains with solubles (known as DDGS) were primarily produced in alcoholic beverage plants. Nowadays, the majority of DDGS is a co-product of dry grind fuel ethanol manufacturing (Rosentrater & Muthukumarappan 2006). Due to its relative availability, low cost, and nutrient composition, DDGS may provide an economical, partial replacement for fish meal in aquafeeds (Chevanan et al. 2005). Several studies have indicated positive performance of Nile tilapia fed diets containing beverage-based DDGS (Wu et al. 1996, 1997). However, other studies have indicated that Nile tilapia do not perform as well when fed diets exceeding 20% inclusion of fuel-based DDGS compared to fishmeal-based diets (Lim et al. 2006; Schaeffer et al. 2010). The effectiveness of fuel-based DDGS utilization in fish diets may depend upon the use of supplementary ingredients.

Yeast such as *Saccharomyces cerevisiae* may serve as a probiotic and aid in the use of DDGS and other plant-based aquaculture diets (Verschuere et al. 2000; Olivia-Teles & Goncalves 2001; Li & Gatlin 2004; Li et al. 2005). Probiotics contain several immunostimulating compounds, including β-glucans, nucleic acids, and manna oligosaccharides (Li & Gatlin 2004), and have been observed to enhance immune (Siwicki et al. 1994; Ortuño et al. 2002) and growth responses (Lara-Flores et al. 2003) of various fish species. Probiotic yeast is generally produced as a co-product of the brewing industry (Li & Gatlin 2004), but can also be commercially cultured and sold as a feed additive. Inclusion of yeast as a probiotic diet additive could provide feed manufacturers and culturists with cost-effective means to increase the overall health and growth of cultured fishes when replacing fishmeal with plant-based alternatives.

To our knowledge, no research has been performed to test a combination of DDGS and yeast probiotics in fish feeds. A few studies have discussed the processing of DDGS-based aquafeeds (Chevanan et al. 2007, 2007a, 2007b, 2009, 2010), but no studies have simultaneously examined the relationships between the characteristics of DDGS-based aquaculture diets and growth performance. The objectives of this study were: (1) to determine if the addition of low levels of yeast would increase utilization of DDGS by Nile tilapia; (2) to determine if the addition of low levels of yeast improve stress resistance of Nile tilapia; and (3) to examine the relationships between feed properties and the performance of Nile tilapia fed these diets.

METHODS

A two-by-three factorial design was used to produce six experimental diets that were formulated to contain 20%, 25%, and 30% DDGS by weight, with or without 0.125% of a commercial yeast culture product (DVAQUA, Diamond V Mills, Cedar Rapids, IA, USA). Diets were formulated to obtain similar crude protein [28.03 ± 0.03% (mean ± SE)] and estimated digestible energy levels (2.32 ± 0.09 kcal/g; Table 1). Fuel-based DDGS was obtained from Dakota Ethanol, LLC (Wentworth, SD, USA) and analyzed for proximate

TABLE 1 Diet Formulation (As-Fed Basis) with Estimated Proximate Composition and Digestible Energy (DE) Values of Experimental Diets Containing Distillers' Dried Grains with Solubles (DDGS) and a Commercial Yeast Probiotic

	Diets					
Ingredient	1	2	3	4	5	6
DDGS[a]	20	25	30	20	25	30
Yeast probiotic[b]	0.125	0.125	0.125	0	0	0
Corn flour[c]	25.075	22.525	20.075	25	22.5	20
Menhaden fishmeal[d]	8	8	8	8	8	8
Soybean meal[e]	44	41.55	39	44.2	41.7	39.2
Soybean oil[f]	2	2	2	2	2	2
Vitamin mix[g]	0.6	0.6	0.6	0.6	0.6	0.6
Ascorbic acid[h]	0.2	0.2	0.2	0.2	0.2	0.2
Proximate composition (%)						
Crude protein	31.44	31.56	31.64	31.51	31.61	31.71
Crude lipid	5.41	5.82	6.23	5.41	5.82	6.23
Crude fiber	5.24	5.50	5.76	5.23	5.49	5.75
Ash	5.68	5.84	5.99	5.68	5.84	5.99
DE (kcal/g)	3.74	3.67	3.60	3.74	3.67	3.60

[a]Dakota Ethanol, Wentworth, SD, USA.
[b]DVAQUA, Diamond V, Cedar Rapids, IA, USA.
[c]#505 Yellow Corn Flour, Cargill Dry Corn Ingredients, Minneapolis, MN, USA.
[d]IPC 740, International Proteins Corp., Minneapolis, MN, USA.
[e]Solvent Extracted, Dakota Land Feeds, Huron, SD, USA.
[f]OF1870E, Consumers Supply Distributing, Sioux City, IA, USA.
[g]Vitamin premix #30 (Rangen Inc., Buhl, ID, USA): pantothenic acid, 26,500 mg/kg; pyridoxine, 7700 mg/kg; riboflavin, 13,200 mg/kg; niacin, 55,100 mg/kg; folic acid, 2200 mg/kg; thiamine, 8800 mg/kg; biotin, 88.2 mg/kg; vitamin B_{12}, 5.5 mg/kg; menadione, 2.76 mg/kg; vitamin E, 88,200 IU/kg; vitamin D, 110,250 IU/kg; vitamin A, 1,653,750 IU/kg.
[h]Rovimix Stay-C at 35% active; Rangen Inc., Buhl, ID, USA.

TABLE 2 Composition (%, Dry Matter Basis) of Fuel-Based Distillers' Dried Grain with Solubles (DDGS) Used in this Study. Dry Matter of DDGS Used in this Study was 95.3%

Property	Values (%)
Crude protein	27.9
Crude lipid	11.5
Crude fiber	6.6
Ash	4.0

composition by Servi-Tech Laboratories (Hastings, NE, USA; Table 2). Other ingredients were obtained locally.

As described in Chevanan et al. (2007, 2007b, 2009, 2010), a Plasti-Corder PL 2000, ¾-in (19.05 mm) barrel diameter single screw extruder (Brabender, South Hackensack, NJ, USA), with a barrel length:diameter ratio of 20:1, screw compression ratio of 3:1, and a 2.4 mm diameter circular die, was used to process diets. A constant barrel temperature profile of 70°C −80°C −90°C and a constant screw speed of 150 rpm were used during processing. After exiting the extruder die, the pellets were cooled and dried at room temperature, crumbled to 2-mm lengths, sieved to remove fines, and stored at −20°C.

Proximate compositions of the diets were estimated using inclusion levels and composition values of each ingredient from the National Research Council (NRC; 1993). Digestible energy was estimated using composition values of each ingredient multiplied by the physiological fuel values of 5.64, 9.44, and 4.11 kcal for proteins, lipids, and carbohydrates, respectively (NRC 1993).

Juvenile Nile tilapia (initial mean weight = 43.6 ± 1.3 g) from MinAqua Fisheries (Renville, MN, USA) were acclimated for two-weeks and then randomly stocked into 24, 110-L glass aquaria with eight fish per aquarium and four replicate aquaria per diet. Fish were fed to apparent satiation twice daily for 62 days. Total fish biomass per tank was measured weekly.

A common biological and mechanical filter system was used to recirculate water and maintain similar water quality among treatments. Each aquarium was supplied with recirculated water at an approximate rate of 3.8 L/min and cleaned with a siphon when needed. A blower and air stones provided continuous aeration. Water temperatures in aquaria were maintained at 25.3 ± 0.1°C. Foil-backed bubble insulation was used to surround each aquarium to reduce temperature fluctuations and minimize disturbances. A photoperiod of 14:10 h light:dark was maintained. Weekly measurements of nitrate (Hach 2008, method 8039), nitrite (Hach 2008, method 8153), and nitrogen ammonia (Hach 2008, method 8038) were obtained using a Hach DREL 2000 spectrophotometer (Hach Company, Loveland, CO, USA). Weekly dissolved oxygen (DO) and temperature measurements were obtained using a YSI Model 55 DO meter (Yellow Springs Instrument Corp., Yellow Springs, OH, USA).

Performance was determined by: weight gain (WG, %) = 100 × ([final weight − initial weight]/initial weight), food conversion ratio (FCR) = (weight of diet fed/total wet weight gain), and protein efficiency ratio (PER) = (weight gain/crude protein fed). Food conversion ratios and PER were estimated by subtracting the weight of uneaten feed from the total feed fed. One hundred pellets per diet were randomly selected and weighed to determine the mean mass per pellet. Counts of uneaten pellets were performed 30 min post feeding in each tank to allow satiation but prior to pellet disintegration. The number of uneaten pellets was multiplied by the mean mass per pellet for each diet then subtracted from the total food mass fed to each tank. Estimated consumption was then used to calculate FCR and PER.

Following the 62-day feeding trial, stress trials were applied to treatment fish. Initially, fish were subjected to an acute stressor by suspending individuals in a dip net out of water for three minutes; this stressor was applied to all fish four times at three-day intervals. Fish were fed *ad libitum* rations of treatment diets between acute stress events. Survival (%) was determined immediately after the final acute stress period for all treatments. After obtaining survival estimates, an additional stress period was induced on fish fed 20% DDGS, with or without yeast probiotic, and plasma cortisol levels were collected. Only fish fed 20% DDGS, with or without yeast probiotics, were sacrificed to ensure enough fish remained for the chronic stress trial. For both treatments, two fish each from three tanks were removed one hour after application of the acute stressor to obtain peak plasma cortisol levels as indicated by Barcellos et al. (1999). Fish were anaesthetized in unbuffered MS222 (200 mg/L). Blood was collected into heparinized test tubes by severing the caudal vein with an ethanol-rinsed scalpel. Plasma was separated by centrifugation and stored at −80°C, pending analysis.

In the second stress trial, fish were starved for a 20-d period to impose chronic stress. For the chronic stress trial, only fish fed 25% and 30%, with and without 0.125% yeast probiotic, were used in analyses. Survival was calculated as percent survival (%) = 100 × (number of surviving fish/number of stocked fish).

All comparisons among experimental diets were analyzed using a two-way general linear model. If significant treatment effects existed, post-hoc least significant difference tests were applied to determine where significant differences ($P < 0.1$) occurred among treatment means. Comparisons of growth metrics, plasma cortisol levels, and survival between diet treatments containing similar levels (20%, 25%, or 30%) of DDGS with or without yeast probiotics were analyzed using *t*-tests and considered significant at $P < 0.1$. Linear correlation (Pearson product moment) analyses were then used to determine if relationships existed between feed physical properties

(Kannadhason et al. 2010) and observed WG, FCR, and PER values. Systat (version 11; SPSS Inc., Chicago, IL, USA) and Microsoft Excel (Microsoft Office 2003 Edition; Microsoft Corporation, Redmond, WA, USA) software were used to perform all analyses.

RESULTS

Mean WG of Nile tilapia ranged from 96%–120%; no significant differences occurred among treatments (Table 3). Mean FCR of Nile tilapia ranged from 1.23–1.66; no significant differences occurred between treatments (Table 3). Mean PER ranged from 1.99–2.90; no significant differences occurred among treatments (Table 3).

For the acute stress trial, no significant differences ($P = 0.81$; df = 4; $t = 0.26$) occurred in the mean plasma cortisol levels (20% DDGS with or without probiotic; Table 4). Similarly, no significant differences were seen in mean survival between fish fed 25% DDGS with or without probiotic, ($P = 0.23$; df = 4; $t = 1.32$) and 30% DDGS with or without probiotic, ($P = 0.60$; df = 4; $t = 0.55$) in the chronic stress trial (Table 4).

DISCUSSION

Results from this study indicated that Nile tilapia can effectively utilize up to 30% DDGS; however, inclusion of yeast did not result in increased growth at any DDGS inclusion level. Our growth and feed utilization results are similar to those found by He et al. (2009), where juvenile hybrid tilapia (*Oreochromis niloticus* × *O. aureus*) were fed six isonitrogenous and isoenergetic diets containing increasing levels of DVAQUA (0%, 0.125%,

TABLE 3 Mean (± Standard Error) Weight Gain (WG), Food Conversion Ratio (FCR), and Protein Efficiency Ratio (PER) for Nile Tilapia Fed Experimental Diets Containing Three Levels of Distillers' Dried Grains with Solubles (DDGS) with or without a Commercial Yeast Probiotic. Statistical Analyses Revealed no Significant Differences Among or Between Treatments ($P \geq 0.1$)

DDGS (%)[a]	Probiotic (%)[b]	WG	FCR	PER
20	0	96 (10)	1.23 (0.19)	2.90 (0.05)
	0.125	120 (10)	1.57 (0.17)	2.12 (0.24)
25	0	108 (8)	1.63 (0.05)	2.02 (0.10)
	0.125	101 (8)	1.48 (0.11)	2.27 (0.23)
30	0	100 (9)	1.59 (0.23)	2.29 (0.18)
	0.125	104 (7)	1.66 (0.15)	1.99 (0.19)

[a]Dakota Ethanol, Wentworth, SD, USA.
[b]DVAQUA, Diamond V, Cedar Rapids, IA, USA.

TABLE 4 Mean Plasma Cortisol Levels (± Standard Error) for Fish Fed Diets Containing 20% Distillers' Dried Grains with Solubles (DDGS) with or without a Commercial Yeast Probiotic. Mean Survival (± Standard Error) for Fish Fed Diets Containing 25% and 30% DDGS with or without Yeast Probiotic. No Significant Differences were Detected Between any DDGS Treatment Pairings ($P \geq 0.1$)

DDGS (%)[a]	Probiotic (%)[b]	Plasma (μg/dL)	Survival (%)
20	0	21.3 (3.9)	
	0.125	23.6 (7.9)	
25	0		84.4 (11.8)
	0.125		100.0 (0)
30	0		84.4 (6.9)
	0.125		88.7 (3.8)

[a]Dakota Ethanol, Wentworth, SD, USA.
[b]DVAQUA, Diamond V, Cedar Rapids, IA, USA.

0.25%, 0.5%, 1.0%, and 2.0%). He et al. (2009) indicated that no significant differences in WG, specific growth rate, FCR, daily feed intake, or survival occurred.

However, our results directly contrast those of Lara-Flores et al. (2003), which indicated that Nile tilapia fed diets supplemented with a commercial yeast product (*S. cerevisiae*, BioSaf, SafAgri, Minneapolis, MN, USA) resulted in higher growth compared to a control diet or fish fed diets supplemented with a commercial mixture of bacteria probiotics (*Streptococcus faecium* and *Lactobacillus acidophilus*, ALL-LAC, AllTech, Nicholasville, KY, USA). Likewise, Barnes et al. (2006) investigated the effects of DVAQUA using 0.125% and 0.25% inclusion levels on the growth and survival of rainbow trout (*Oncorhynchus mykiss*; initial mean weight = 0.5 mg) during initial hatchery rearing; results indicated that mortality was significantly reduced during the initial four weeks in fish fed diets containing any DVAQUA, while growth was statistically greater for fish receiving diets supplemented with 0.25% DVAQUA (Barnes et al. 2006). Further, after four to eight weeks post-initial feeding, only fish in tanks supplemented with 0.25% DVAQUA had statistically lower mortality. In another study, Barnes and Durben (2009) found that rainbow trout fed 0.125 g/kg DVAQUA from initial feeding to 408 d exhibited lower mortality, were significantly larger, and had improved FCR. Our study indicated that FCR did not differ with the inclusion of DVAQUA yeast, but Lara-Flores et al. (2003) reported improved FCR values. Another study reported improved intestinal microbial balance in hybrid striped bass (*Morone chysops* × *M. saxatilis*), which resulted in increased growth and feed utilization (Li & Gatlin 2004).

Several differences between these studies may explain some of the contradictory findings. Diet compositions, primarily inclusion of fishmeal, varied greatly. Lara-Flores et al. (2003) included 36.60%–54.23% anchovy fishmeal and reported improved diet and protein digestibility with the addition of probiotics. The current study utilized 8% menhaden fishmeal in each diet. Differences in fish meal inclusion and subsequent digestibility suggest that additional growth from yeast probiotics only occurs in diets containing higher amounts of proteins compared to those containing high amounts of carbohydrates.

Size of fish and colonization of yeast probiotics within the digestive system may affect the overall utilization of probiotics. Colonization of yeast probiotics within the gastrointestinal tracts must occur prior to the establishment of competitive microbiota; high doses of yeast probiotic temporarily produce its dominance and benefits following this initial installation (Balcázar et al 2006). Fish used in the current study were of a size (initial mean weight = 43.6 ± 1.3 g) used to initiate the growout phase of commercial production, while Lara-Flores et al. (2003) and Barnes et al. (2006) utilized fry (initial mean weight = 0.153 g and 0.5 mg, respectively.). These differences suggest that smaller sized fish (i.e., fry) may gain additional benefits from yeast probiotics at a greater capacity than larger fish, since colonization and permanence of yeast probiotic microbiota within the gastrointestinal tract can be initiated at a larval life stage. Conversely, larger fish may require a longer time period and greater amounts of yeast probiotics to adjust microbial balances within the intestine, with only temporary benefits occurring (i.e., until yeast probiotic supplementation in the diet ceases). However, He et al. (2009) indicated that supplementation of DVAQUA affected the autochthonous gut bacteria of larger-sized hybrid tilapia (initial mean weight = 50.89 ± 0.27 g) by selectively stimulating beneficial bacteria while conversely depressing harmful species.

Elevated circulating cortisol levels result in suppressed immune response and disease resistance in fish, and have been associated with natural or artificial stressors that occur in normal aquaculture production (Lewis & Thomas 1988). No increase in cortisol levels between fish fed diets with or without yeast probiotics in this study indicated that the inclusion of yeast probiotic did not alter immune response or disease resistance to a common acute stressor. This observation was similar to findings by Li et al. (2005), who reported that yeast probiotics had no apparent effect on the cortisol response of red drum subjected to handling stress (i.e., confinement stress test). However, supplementation of dietary nucleotides has been shown to reduce cortisol release and other stress responses in rainbow trout (Leonardi et al. 2003). These differing results, along with limited research on the topic, suggest that no evident patterns exist between the supplementation of yeast probiotic and cortisol concentrations; therefore, the connection remains poorly resolved and requires further investigation.

Several studies have shown increased survival of fish when fed supplemental probiotics. Lara-Flores et al. (2003) indicated the highest survival of Nile tilapia fry occurred with yeast probiotic treatments; along similar lines, survival of hybrid striped bass exposed to *Streptococcus iniae* was significantly higher in fish supplemented with yeast probiotics (Li & Gatlin 2004). Results from this study indicated that survival did not increase with the supplementation of yeast probiotics when combined with either 25% or 30% DDGS. These results concur with Li et al. (2005), who stated that survival of red drum did not significantly increase with the inclusion of probiotics. He et al. (2009) indicated that the optimum dose level of DVAQUA for non-specific immune parameters of hybrid tilapia was from 0.226–0.5 g/kg. Therefore, larger-sized Nile tilapia may require higher amounts (>0.125% inclusion rate) to shift the microbiota of the gastrointestinal tract and influence survival.

In conclusion, the addition of yeast probiotics as a dietary supplement to DDGS-based diets did not appear to improve feed utilization, enhance growth, or improve stress resistance in juvenile Nile tilapia. These results differ from those of other studies where enhanced growth and increased stress resistance occurred in Nile tilapia fry (Lara-Flores et al. 2003), hybrid striped bass (Li & Gatlin 2004), and rainbow trout (Barnes et al. 2006; Barnes & Durben 2009). Such differences may be attributed to the size and species of fish used, the dose and exposure time of yeast probiotics used in the diets, the potential changes to the microflora in fish digestive tracts, and the degree of stress put on the fish. Further research is required to determine specific relationships between fish size and yeast probiotic inclusion rates, as well as commensurate effects of yeast probiotics on the gastrointestinal tracts of various species. Also, more research is needed to determine if inclusion of yeast probiotics can increase utilization of plant-based protein sources (such as DDGS or soybean meal) in aquaculture diets.

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