Utilization of diets containing graded levels of ethanol production co-products by Nile tilapia

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Utilization of diets containing graded levels of ethanol production co-products by Nile tilapia

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Introduction

Aquaculture diet costs often account for over 50% of production for aquaculture species with protein composing the most expensive dietary constituent (Coyle et al., 2004). Fishmeal provides an important source of protein in aquaculture diets, containing well-balanced profiles of amino acids, fatty acids, digestible energy, vitamins, and minerals (Abdelghany, 2003). However, because of the rising cost and uncertain availability of fishmeal from over-fished marine stocks, researchers have begun to investigate alternative protein sources (Jauncey and Ross, 1982; Fontaínhas-Fernandes et al., 1999; Coyle et al., 2004). One plant nutrient source available for aquaculture feeds is distillers dried grains with solubles (DDGS) (Webster et al., 1992a,b, 1993; Wu et al., 1996, 1997; Coyle et al., 2004; Lim et al., 2006). Unlike beverage-based DDGS, where various kinds of grains are fermented and distilled to obtain beverage alcohol (Hertrampf and Piedad-Pascual, 2000), fuel-based DDGS are a co-product of dry mill processing, where primarily corn is used to manufacture fuel ethanol (Rosentrater and Muthukumarappan, 2006). In 2008, 174 operating ethanol plants in the United States produced a total of 41.0 billion liters, with an estimated industry expansion of 9.1 billion liters (RFA, 2009). With this exponential growth in ethanol production, significant quantities of distillers grains are being produced. Distillers dried grains with solubles do not contain anti-nutritional factors (e.g. trypsin inhibitors and gossypol) that are present in fishmeal.

Keywords
distillers dried grain with solubles, ethanol co-product, Oreochromis niloticus, feeding value

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Summary

A feeding trial was performed to investigate inclusion levels of distillers dried grains with solubles (DDGS) as a fishmeal replacement for juvenile Nile tilapia (Oreochromis niloticus). On a dry matter basis, five isocaloric [19.3 ± 0.4 kJ/g (mean ± SE)], isonitrogenous (39.1 ± 0.5% crude protein) diets were formulated to contain 17.5%, 20%, 22.5%, 25%, and 27.5% DDGS and compared against a 0% DDGS, reference diet (gross energy = 14.5 kJ/g; crude protein = 39.8%). The reference diet resulted in significantly higher body weight gain (BWG), food conversion ratio (FCR), and protein efficiency ratio (PER) than experimental diets except that 17.5% DDGS provided similar FCR and PER. The diet containing 27.5% DDGS had significantly lower FCR and PER values than all other diets even though apparent digestibility did not significantly differ among experimental diets. Although DDGS can be incorporated at higher levels, 20% DDGS provided the highest apparent BWG among experimental diets, while 17.5% promoted the best FCR and PER. Fishmeal may be replaced with low levels of fuel-based DDGS to reduce feeding cost; however, additional supplements should be considered to enhance fish performance.
in soybean meal (Wilson and Poe, 1985; Shiau et al., 1987) and cottonseed meal (Jauncey and Ross, 1982; Robinson, 1991). However, DDGS usually contain lower amounts of lysine and methionine, the most limiting amino acids, when compared to fishmeal (Cheng and Hardy, 2004).

Due to the relative availability, low cost, and nutrient composition of DDGS, this ethanol co-product may be used as an inexpensive protein supplement to provide lower-cost diet formulations. Several studies have indicated positive performance of Nile tilapia (Oreochromis niloticus) fed diets containing varying levels of DDGS (Wu et al., 1996, 1997; Coyle et al., 2004; Lim et al., 2006). However, earlier studies used a broad range of beverage-based DDGS (usually 10% increments). Further, these studies determined approximate amounts of DDGS that could be incorporated but did not specify a level of DDGS that could support maximum growth or examine currently produced fuel-based DDGS. Thus, we conducted a feeding trial to determine an optimal level of fuel-based DDGS to incorporate in the diets of Nile tilapia.

**Materials and methods**

**Experimental diets and fish**

Five experimental diets were formulated to contain 17.5%, 20%, 22.5%, 25%, and 27.5% DDGS, each with 5% fishmeal; a reference diet contained 0% DDGS and 15% fishmeal. Each experimental diet was balanced with a combination of soybean meal and yellow corn meal to obtain, on a dry matter basis, similar crude protein (39.1 ± 0.5% (mean ± SE)) and gross energy levels (19.3 ± 0.4 kJ/g; Table 1). Fuel-based DDGS were obtained from the Dakota Ethanol Plant (Wentworth, SD, USA), and were analyzed for proximate composition prior to use in experimental diets by Servi-Tech Laboratories (Hastings, NE, USA; Table 2). Additional ingredients were obtained locally (Ag First Farmer's Cooperative, Brookings, SD, USA). Vitamin premix #30 and Rovimix Stay-C were obtained from Rangen (Buhl, ID, USA). Following the procedures used by Chevanan et al. (2007), a pilot-scale Wegner TX-52 twin screw extruder (Wenger, Kansas City, MO, USA) was used to process and pellet all diets. Diets were processed into 2-mm diameter pellets, dried at room temperature, crumbled and sieved to obtain homogenous pellet sizes, and stored at −20 °C. Additional details about the extrusion processing and the physical properties of the pellets can be found elsewhere (Kannadhason et al., 2008). Dry matter was calculated from the moisture content of each diet in accordance with method 935.29 (AOAC, 2009). Diets were analysed for crude protein (AOAC, 2009, method 2001.11), crude lipid (AOAC, 2009, method 2003.05; modified by substituting petroleum ether for diethyl ether), crude fibre (AOAC, 2003, method 978.10), and ash content (AACC, 2000, method 08–03) (Table 1) and amino acid profiles by ion exchange chromatography with post-column ninhydrin derivatization (AOAC, 2009, methods 994.12 and 988.15) (Table 3). Tryptophan was estimated using ingredient values from the National Research Council (NRC, 1993). Bomb calorimetry was used to obtain total gross energy of each diet (Table 1).
Juvenile Nile tilapia (initial mean weight = 34.9 ± 1.4 g), obtained from MinAqua Fisheries (Renville, MN, USA), were fed the reference diet for a 2-week conditioning period. After acclimation, individual fish were randomly selected and stocked (n = 7) into 24, 110 l glass aquaria to provide four replicate aquaria per diet. Fish were fed to apparent satiation three times per day for 55 days, and total tank weights were measured every 10 days. Three fish per aquarium were euthanized at the end of the trial to obtain whole body, liver, visceral, and fillet weights for calculating organosomatic indices and muscle ratios (MR).

After day 50, fish were fed diets containing chromium (III)-oxide (Cr₂O₃) for 5 days to determine apparent digestibility of diets. Diets remained similar to those in Table 1 except 0.5% Cr₂O₃ and soybean meal were added and subtracted respectively. After the fifth feeding day, three fish per aquarium were euthanized and the lower 50% of intestines were removed and stripped to obtain fecal samples. The amounts of Cr₂O₃ in the diets and fecal samples were determined using the graphite furnace atomic absorption method (APHA, 2009, method 3113B). This study was performed in compliance with the South Dakota State University Institutional Animal Care and Use Committee (Study #07-E016).

### Performance metrics

Growth performance was determined by the following parameters: body weight gain (BWG, %) = 100 × [(final weight – initial weight)/initial weight] (Wu et al., 1996), food conversion ratio (FCR) = weight of diet fed/total wet weight gain (Wu et al., 1996), and protein efficiency ratio (PER) = weight gain/crude protein fed (Wu et al., 1996). 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. Condition was determined using muscle ratio (MR, %) = 100 ×...
[fillet weight (skin off)/weight of whole fish] (Lovell, 1975) and hepatosomatic index (HSI, %) = 100 × (liver weight/weight of whole fish) (Strange, 1996). Apparent digestibility was measured using digestion coefficients of protein (DCP, %) = 100 – 100 × [(percent Cr2O3 in feed × percent crude protein in feces)/(percent Cr2O3 in feces × percent crude protein in feed)] (NRC, 1993). Survival was determined using survival (%) = 100 × (number of surviving fish/number of stocked fish).

Statistical analyses

All performance metrics were analyzed using a one-way analysis of variance (ANOVA). If significant treatment effects existed, least significant difference (LSD) tests were applied to determine significant differences (p < 0.05) occurring between treatment means. SYSTAT (version 11) software (SPSS, Chicago, IL, USA) was used to perform all statistical analyses.

Results

All experimental diets met known amino acid requirements of Nile tilapia (Table 3); however, lysine did decrease with increased amounts of DDGS. Mean BWG of Nile tilapia ranged from 74.4% to 128.2%; mean BWG for Nile tilapia fed the reference diet were significantly higher than all other diets followed by fish fed 20% DDGS (Table 4). Mean FCR of Nile tilapia ranged from 2.39 to 4.99; the reference diet FCR was statistically lower than all treatments except FCR of fish fed 17.5% DDGS, while fish fed 27.5% DDGS had a significantly higher FCR than all other treatments (Table 4). Mean PER ranged from 0.53 to 1.09; the reference diet PER was statistically higher than all diets except PER of fish fed 17.5% DDGS, while fish fed 27.5% DDGS had significantly lower PER than all other treatments (Table 4). Mean MR ranged from 31.7% to 35.6%; no significant differences occurred among treatments (Table 4). Mean HSI ranged from 1.8% to 2.3%; no significant differences occurred among treatments (Table 4). Mean treatment DCP ranged from 70.3% to 79.1%, however, no significant differences occurred among treatments (Table 4). Mean treatment survival for Nile tilapia ranged from 72.4% to 100%, no significant differences occurred among treatments (Table 4).

Discussion

Although not statistically significant, results indicated 20% DDGS may produce the highest BWG of Nile tilapia in relation to the fish fed other experimental diets; however, it was significantly lower from the reference diet in BWG, FCR, and PER. These BWG results are comparable to a study conducted by Lim et al. (2006); however, in the current study Nile tilapias with a larger initial weight (34.9 ± 1.4 g) were used in relation to smaller fish (9.4 ± 0.1 g) by Lim et al. (2006). It could be argued that 17.5% DDGS may provide an optimal dietary level because FCR and PER values did not significantly differ from the reference diet; whereas, these values were statistically lower for the 20% DDGS diet in relation to the reference diet. However, BWG of fish fed 17.5% DDGS was still significantly lower than those fed the reference diet. Further research may be needed to determine the optimal inclusion level of DDGS and

Table 4 Mean body weight gain (BWG), estimated food conversion ratio (FCR), estimated protein efficiency ratio (PER), muscle ratio (MR), hepatosomatic indices (HSI), digestion coefficients of protein (DCP), and survival of Nile tilapia fed diets containing various levels of distillers dried grains with solubles (DDGS) and the reference diet (0% DDGS) for 50 days

<table>
<thead>
<tr>
<th>DDGS (%)</th>
<th>BWG (%)</th>
<th>FCR</th>
<th>PER</th>
<th>MR (%)</th>
<th>HSI (%)</th>
<th>DCP (%)</th>
<th>Survival (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>17.5</td>
<td>80.8 (5.8)</td>
<td>2.68 (0.11)</td>
<td>0.93 (0.04)</td>
<td>89.2 (0.7)</td>
<td>1.9 (0.2)</td>
<td>70.3 (1.6)</td>
<td>90.0 (10.0)</td>
</tr>
<tr>
<td>20</td>
<td>91.2 (12.5)</td>
<td>3.18 (&lt;0.01)</td>
<td>0.79 (&lt;0.01)</td>
<td>89.6 (0.4)</td>
<td>2.0 (0.1)</td>
<td>76.8 (1.2)</td>
<td>85.7 (8.2)</td>
</tr>
<tr>
<td>22.5</td>
<td>74.4 (5.0)</td>
<td>3.21 (0.13)</td>
<td>0.78 (0.03)</td>
<td>89.3 (0.5)</td>
<td>2.0 (0.1)</td>
<td>79.1 (3.6)</td>
<td>87.3 (4.6)</td>
</tr>
<tr>
<td>25</td>
<td>75.4 (12.5)</td>
<td>2.99 (0.16)</td>
<td>0.89 (0.05)</td>
<td>87.9 (0.6)</td>
<td>2.1 (0.1)</td>
<td>76.0 (5.4)</td>
<td>72.4 (18.4)</td>
</tr>
<tr>
<td>27.5</td>
<td>82.2 (2.9)</td>
<td>4.99 (0.28)</td>
<td>0.53 (0.03)</td>
<td>90.0 (0.5)</td>
<td>1.8 (0.1)</td>
<td>78.5 (1.2)</td>
<td>78.6 (13.7)</td>
</tr>
<tr>
<td>Reference</td>
<td>128.2 (6.6)</td>
<td>2.39 (0.27)</td>
<td>1.09 (0.10)</td>
<td>89.1 (0.6)</td>
<td>2.3 (0.2)</td>
<td>77.4 (1.5)</td>
<td>100.0 (0.0)</td>
</tr>
</tbody>
</table>

Values are treatment means (±SE) for experimental and reference diets. Values not significantly different (p > 0.05) have the same letter within a given column.

1FCR = weight of diet fed/total wet weight gain; diet fed estimated by subtracting uneaten pellets from total food fed.
2PER = weight gain/crude protein fed; protein fed was estimated by subtracting uneaten pellets from total food fed then multiplying by crude protein (%).
3MR (%) = 100 × (percent Cr2O3 in feed × percent crude protein in feces)/(percent Cr2O3 in feces × percent crude protein in feed).
supplements that may increase the effectiveness of DDGS use by juvenile Nile tilapia.

Due to their aqueous environment where carbohydrates are scarce, digestive and metabolic systems of fish generally utilize proteins and lipids for energy better than carbohydrates (Lovell, 1989). Significantly poorer BWG, FCR, and PER of fish fed the DDGS based diets in relation to the reference diet, except for 17.5% DDGS, seems to suggest that juvenile Nile tilapia use diets containing higher amounts of fishmeal better than those with similar proximate compositions. However, carbohydrates have several beneficial functions in aquaculture diets, including: pellet binders, precursors to dispensable amino acids and nucleic acids needed for growth, and a sparing effect on dietary protein utilization as an inexpensive source of energy (NRC, 1993; Lim and Webster, 2006). *Tilapia* species have been found to utilize carbohydrates more efficiently than salmonids, seabass *Seriola* spp., and yellowtail *Seriola dorsalis lalandi* (NRC, 1993; Lim and Webster, 2006). Anderson et al. (1984) stated that Nile tilapia (initial weight ≈2 g) exhibited superior growth when fed diets containing glucose, sucrose, dextrin and starch in relation to carbohydrate-free diets; weight gain increased when dietary carbohydrates were raised from 0% to 40%. Likewise, other carbohydrate ingredients (i.e., soybean and corn meal) along with the DDGS may have affected the overall performance of Nile tilapia within the study; however, because similar inclusion levels were used across diets, comparisons made within this study remain valid.

Muscle ratios and HSI values were similar across treatments. Webster et al. (1993) reported similar results in channel catfish (*Ictalurus punctatus*) fed diets containing 10%, 20%, and 30% DDGS. However, Webster et al. (1992a) found that channel catfish fed a 90% DDGS diet had a significantly lower MR than catfish fed diets containing 0.35% and 54.75% DDGS, but when 0.60% lysine was added to the 90% DDGS diet, MR was similar across treatments. Muscle ratios from this study studies along with DCP values indicated that Nile tilapias are capable of incorporating DDGS into edible muscle mass at rates similar to a fishmeal-based diet and maintain adequate condition. Even though fish fed experimental diets were able to produce MR values comparable to the fishmeal-based reference diet, they were unable to provide similar BWG; therefore, a tradeoff may occur with the use of DDGS in tilapia diets requiring a longer grow-out period to reach a harvestable size.

Digestion coefficients of protein indicated that DDGS were utilized by Nile tilapia at rates similar to the fishmeal-based reference diet. Cheng and Hardy (2004) found differing results when incorporating DDGS into the diets of rainbow trout (*Oncorhynchus mykiss*). Apparent retention of crude protein (ARCP) was statistically similar between the reference (0% DDGS), 15%, and 22.5% DDGS diets, while the 7.5% DDGS diet had a significantly lower value than the reference (Cheng and Hardy, 2004). In another study, ARCP in rainbow trout varied with differing levels of soybean meal combined with 18.5% DDGS (Cheng et al., 2003). Due to the conflicting results among studies, it becomes apparent that information on species-specific digestion of DDGS is needed. Our study indicated that Nile tilapia appear to digest DDGS at rates independent of the inclusion level, allowing DDGS inclusion levels to vary. Conversely, rainbow trout better utilize certain amounts of DDGS within diets, potentially limiting the use of DDGS for that species.

Even though survival was not statistically different among treatments, Nile tilapia fed the reference diet experienced 100% survival, while fish fed experimental DDGS diets experienced some mortality. Lim et al. (2006) reported that Nile tilapia fed higher amounts of DDGS experienced lower survival rates; however, differences in survival rates were not statistically significant. Likewise, Lim et al. (2006) found no significant difference among the average number of days to first mortality after Nile tilapia were challenged with a pathogen (*Streptococcus iniae*). Mbahinzirek et al. (2001) indicated that tilapia cannot be raised successfully when fed diets based on cottonseed meal as the primary protein source. It is possible that diets containing mainly DDGS and other plant-based proteins could reduce the immune response of fish when stressors exist (e.g. periodic handling, poor water quality, etc.) which may result in increased mortality in relation to a primarily fishmeal-based diet.

Processing conditions can also affect feed quality. Extrusion data for these feeds indicated that temperatures at the fourth head of the twin screw extruder exceeded 100 °C (Kannadhason et al., 2008), which may have led to denaturation of protein structures or alteration of proteins themselves. Mango et al. (2007) indicated that denaturation occurred in whey protein concentrates above 90 °C. However, Kitabatake et al. (1989) indicated that the denaturation temperature of soy protein increased from 102.3 to 170.7 °C as moisture content decreased from 94% (w/w) to 29% (w/w). Although unlikely, dena-
uration of protein structures may have occurred and led to decreased utilization of diets by Nile tilapia resulting in reduced growth performance. However, because all diets were extruded under the same conditions (Kannadhason et al., 2008), comparisons made within this study remain viable.

Additional factors can influence the overall composition and use of DDGS as a dietary constituent. Nutritional differences in DDGS can occur between ethanol plants, to some extent from the composition and quality of the raw corn grain used; however, differences are primarily due to processing equipment, parameters, additives, and techniques used (i.e., beverage vs. fuel production) (Chevanan et al., 2005). Numerous studies have been performed on DDGS to determine its chemical and nutritional values (Chevanan et al., 2005; Rosentrater and Muthukumarappan, 2006). However, as new or modified fermentation and production processes are deployed, resulting ethanol co-products will change both physically and chemically (Rosentrater and Muthukumarappan, 2006). Moreover, many feeding studies do not provide DDGS proximate composition or fail to mention whether the co-product was fuel- or beverage-based DDGS. Thus, we urge researchers to report the basic values of ethanol co-products used in dietary studies to advance the understanding of DDGS utilization in aquaculture feeds.

**Conclusion**

In conclusion, diet supplementation of DDGS as a primary or secondary protein source may provide aquaculture growers with a cost effective substitute for some fishmeal replacement. However, the use of DDGS and other plant-based proteins may result in decreased performance when compared to a fishmeal-based diet, until plant protein diets can be optimized. Future research should likely focus on additional supplements to DDGS-based feeds to increase the overall uptake, growth performance, and survival.

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