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Performance Characteristics of Nile Tilapia (*Oreochromis niloticus*) Fed Diets Containing Graded Levels of Fuel-Based Distillers Dried Grains with Solubles

[1]T.W. Schaeffer, [1]M.L. Brown and [2]K.A. Rosentrater
[1]Department of Wildlife and Fisheries Sciences,
South Dakota State University, Brookings, South Dakota, USA
[2]United States Department of Agriculture,
Agricultural Research Service, Brookings, South Dakota, USA

Abstract: A feeding trial was performed to investigate levels of corn-based Distillers Dried Grains with solubles (DDGS), a co-product of fuel ethanol manufacturing, used as a nutrient source for juvenile Nile tilapia (*Oreochromis niloticus*). Three isocaloric (15.7±0.7 MJ kg^{-1} dry matter), isonitrogenous (29.6±1.0% crude protein) experimental diets were formulated to contain 20, 30 and 40% DDGS; a commercial diet containing fishmeal was used as a reference diet. Glass aquaria (110 L) were stocked with Nile tilapia (mean weight = 6.7 g) with four replicates per diet. Weight gains, conversion ratios and hepatosomatic indices did not significantly differ between fish fed 20% DDGS and reference diets. Dressing percentages were similar among fish fed 20 and 30% DDGS and reference diets, while no difference occurred in protein efficiency ratios among diets. These results indicate that aquaculture diets incorporating 20% DDGS may produce similar performance results to commercial diets of similar energy content.

Key words: DDGS, fuel ethanol, *Oreochromis niloticus*, glass aquaria, Nile tilapia, USA

INTRODUCTION

Within aquaculture operations, diet costs can account for over 50% of production with protein compromising the most expensive dietary constituent (Coyle *et al*., 2004). Due to its well-balanced profiles of amino acids, fatty acids, digestible energy, vitamins and minerals, fishmeal provides an important source of protein in aquaculture diets and is commonly used in most diets (Abdelghany, 2003).

However, researchers have examined numerous alternative protein sources, due to the rising cost and uncertain availability of fishmeal sources from over-fished marine stocks (Coyle *et al*., 2004; Jauncey, 2000; Fontainhas-Fernandes *et al*., 1999). One alternative protein source available for aquaculture feeds is Distillers Dried Grains with Solubles (DDGS) (Webster *et al*., 1992, 1993a, b; Wu *et al*., 1996, 1997; Coyle *et al*., 2004; Lim *et al*., 2006).

Less than 10 years ago, DDGS were primarily produced in beverage alcohol plants. However, a majority of currently produced DDGS occurs as a coproduct of dry mill fuel ethanol manufacturing (Rosentrater and Muthukumarappan, 2006). The nutritional composition of DDGS has changed over the years as ethanol production has grown and as technology and processing techniques have advanced. Cromwell *et al*. (1993) reported a crude protein level of 26.9% for DDGS while Lodge *et al*. (1997) found crude protein and lipid levels for DDGS at 29.2 and 11.4%, respectively. Belyea *et al*. (2004) analyzed DDGS samples from 1997-2001 and indicated an increase in crude protein and lipid levels from 28.3 and 10.9% to 33.3 and 12.6%, respectively. Similarly, Spiehs *et al*. (2002) analyzed DDGS samples from 1997-1999 and compared these results to a sample from an older mid-western ethanol plant. Results indicated higher crude protein and lipid concentrations of 30.2 and 10.9% when compared against 28.1% crude protein and 8.2% crude lipid levels of the older samples. All of these studies indicate an increasing trend in protein and lipid concentrations in DDGS as changes occur in fuel ethanol production.

Due to the relative availability, low cost and nutrient composition, DDGS may provide an inexpensive protein supplement to produce lower-cost feeds, particularly for omnivorous species. Several studies have explored the use of DDGS as a protein source in tilapia diets and indicated positive performance characteristics with the potential for including DDGS to replace fishmeal used in tilapia (*Oreochromis* sp.) feeds (Wu *et al*., 1996, 1997; Coyle *et al*., 2004; Lim *et al*., 2006). Most of the previous

Corresponding Author: Kurt A. Rosentrater, United States Department of Agriculture, Agricultural Research Service, North Central Agricultural Research Laboratory, 2923 Medary Ave., Brookings, South Dakota 57006, USA

studies however, used DDGS processed at beverage alcohol plants, not fuel ethanol plants. Because of these earlier findings with beverage DDGS, there is growing interest in feed manufacturing vis-a-vis using fuel-based DDGS in tilapia feeds as well.

For example, Chevanan *et al.* (2008) produced feeds containing up to 40% DDGS using a laboratory-scale extruder and found that as the inclusion of DDGS increased, resulting feed quality decreased.

Chevanan *et al.* (2009) found that including whey at 5% of the diet did help improve pellet quality, especially durability. Additionally, Chevanan *et al.* (2007) investigated the effects of die dimensions while Chevanan *et al.* (2010) examined the effects of moisture content, screw speed and barrel temperature on the processing performance and pellet quality of tilapia diets containing up to 40% DDGS. Also, advancements in ethanol technology and processing have led to a change in the nutrient concentrations of current DDGS; therefore, studies which incorporated DDGS processed with older methods may no longer give an adequate description of DDGS inclusion in aquaculture diets. Thus, the researchers conducted a feeding trial to determine an appropriate level of DDGS, processed with current technology while attempting to maintain adequate growth and body composition of Nile tilapia.

MATERIALS AND METHODS

Preparation of feeds and fish: Three experimental diets were formulated to contain 20, 30 and 40% DDGS with a combination of soybean meal and corn flour to obtain similar crude protein (29.6±1.0%) and estimated gross energy levels (15.7±0.7 MJ kg^{-1} dry matter; Table 1). Distillers dried grains with solubles were obtained from the Dakota Ethanol Plant (Wentworth, South Dakota, USA). Other ingredients (Table 1) were obtained locally (Ag First Farmer's Cooperative, Brookings, South Dakota). As described by Chevanan *et al.* (2009), a Plasti-Corder PL 2000 single screw extruder (Brabender, South Hackensack, New Jersey) with a barrel length:diameter ratio of 20:1 and a screw compression ratio of 3:1 was used to process diets which were formed into 2 mm diameter pellets, dried at room temperature, crumbled and sieved to appropriate size and stored at -20°C. Diets were analyzed for crude protein (AOAC, 2000) and crude fat (Table 1). Gross energy content of each feed was estimated using proximate composition multiplied by the physiological fuel values of 5.64, 9.44 and 4.11 kcal for proteins, lipids and carbohydrates, respectively (NRC, 1993).

Table 1: Feed ingredients and proximate compositions (percent dry matter) with estimated Gross Energy (GE) values of experimental diets containing Distillers Dried Grains with Solubles (DDGS) and a reference diet

	Diets			
Ingredients	1	2	3	Reference[a]
DDGS	20.00	30.00	40.00	0
Soybean meal	33.00	29.00	25.00	-
Corn flour[b]	35.00	29.00	23.00	-
Menhaden fishmeal	5.00	5.00	5.00	-
Cod liver oil	2.00	2.00	2.00	-
Soybean oil	2.00	2.00	2.00	-
Vitamin mix[c]	1.00	1.00	1.00	-
Mineral mix[d]	2.00	2.00	2.00	-
Proximate composition (%)				
Crude protein	28.50	30.00	30.40	36.70
Crude lipid	8.12	8.79	10.50	5.90
Crude fiber	3.72	3.65	4.45	3.21
Ash	7.59	7.28	7.30	8.52
DE (MJ kg^{-1} dry matter)	16.50	15.70	15.00	N/A

[a]Rangen Inc. Tilapia EXTR 350 Feed; [b] Cargill 505 Yellow Corn Flour; [c] Land O' Lakes, Cattle Vita Pak: vitamin A, 2,204,586 IU kg^{-1}; vitamin D, 440,917 IU kg^{-1}; vitamin E, 8818 IU kg^{-1}. [d]Land O' Lakes, Lean Gain Premix w/Lysine: lysine, 3.95%; calcium, 19.1%; phosphorus, 8.40; salt, 11.6; selenium, 8.6 mg kg^{-1}; zinc, 3000 mg kg^{-1}; iodine,14.6 mg kg^{-1}; vitamin A, 227,072 IU kg^{-1}; vitamin B12, 0.79 mg kg^{-1}; choline, 2.60 mg kg^{-1}

Juvenile Nile tilapia (initial mean weight = 6.7 g), obtained from MinAqua Fisheries (Renville, Minnesota) were acclimated to the control diet for a 2 weeks conditioning period and then randomly (n = 7) stocked in 16, 110-L glass aquaria providing four replicate aquaria per diet. Fish were fed to apparent satiation twice daily for 42 days. Total tank weights were measured weekly and rations adjusted according to tank weight and amount of feed consumed. Three fish per aquarium were euthanized at the end of the trial to obtain Dressing Percentages (DP) and organosomatic indices for comparisons. This study was performed in compliance with the South Dakota State University Institutional Animal Care and Use Committee (#07-E016).

Culture system: 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 2 L min^{-1} and cleaned with a siphon when needed. A blower and airstones provided continuous aeration. Water temperatures in aquaria were maintained at 24°C. Foil-backed bubble insulation was used to surround each aquarium to reduce temperature fluctuations and minimize disturbances. A photo-period of 14:10 h light:dark was maintained throughout the trial. Weekly measurements of nitrate-nitrogen, nitrite-nitrogen and total ammonia were obtained using a Hach DREL 2000 spectrophotometer (Hach Company, Loveland, Colorado). Weekly measurements of dissolved oxygen and temperature were obtained using a YSI Model 55 dissolved oxygen meter (Yellow Springs Instrument Corp., Yellow Springs, Ohio).

Performance metrics and statistical analyses: Growth performance and feed utilization were assessed by the following indices: Weight Gain (WG) = 100 x ((final weight - initial weight)/initial weight), Food Conversion Ratio (FCR) = weight of diet fed/wet weight gain and Protein Efficiency Ratio (PER) = wet weight gain/protein fed (Wu *et al*., 1996). FCR and PER were estimated by determining the weight of uneaten feed (estimated from pellet counts). One hundred pellets per diet were randomly selected and weighed to determine the mean mass per pellet. Pellet counts were done 30 min post feeding in each tank to allow satiation feeding, 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 fed to each tank. Estimated consumption was then used to calculate FCR and PER. Condition was determined using Dressing Percentage (DP) = 100 x (weight of dressed fish/weight of whole fish) (Lovell, 1975) and the Hepatosomatic Index (HSI) = 100 x (liver weight/whole body weight) (Strange, 1996). Comparisons between individual experimental diets and the reference diet were independently analyzed using t-tests and considered significant at $p<0.1$. Systat (version 11; SPSS Inc., Chicago, Illinois) and Microsoft Excel (Office, 2003 Edition; Microsoft Corporation, Redmond, Washington) software was used to perform all analyses.

RESULTS

Mean WG (%) of Nile tilapia fed experimental diets ranged from 48.6-66.5% (Table 2). The highest mean WG (66.5%) resulted from fish fed diet 1 and was not statistically different from the reference diet while mean WG of fish fed diets 2 and 3 were statistically lower. Mean FCR of Nile tilapia fed experimental diets ranged from 2.88-3.83 (Table 2). The best mean FCR (2.88) occurred for fish fed diet 1 and was not statistically different from the reference diet while diets 2 and 3 were statistically lower. Mean PER ranged from 0.99-1.31 (Table 2). The highest mean PER (1.31) was observed for fish fed diet 1 with the lowest mean ratio occurring in fish fed diet 2 (0.99); all experimental diets were statistically similar to the reference. Mean DP (%) of Nile tilapia fed experimental diets ranged from 31.9-32.6% (Table 2). The highest mean DP occurred from fish fed diet 1 (32.5%) and diet 3 (32.6%); neither was significantly different from the reference diet.

Mean HSI (%) ranged from 1.9-2.7 (Table 2). Both fish fed the reference diet and diet 1 exhibited the highest mean HSI values (2.7%) and were statistically similar, while fish fed diets 2 and 3 had statistically lower mean HSI values than the reference diet.

DISCUSSION

Results from the feeding trial demonstrated that 20% corn-based DDGS can be used as a suitable protein source for use in Nile tilapia diets without greatly affecting performance. Compared to a fishmeal-based reference diet, 20% DDGS provided statistically similar WG and FCR (Table 2). These results concur with Lim *et al*. (2006) that 20% fuel-based DDGS can be included in Nile tilapia diets without significantly affecting overall growth performance. However, Lim *et al*. (2006) stated that fish fed diets containing 30% DDGS had similar WG, PER and Feed Efficiency Ratio (FER) as those fed a control diet, while fish fed 40% DDGS had significantly lower WG, PER and FER than those on the control diet. Results from the study indicate the same trend except for fish fed 30% DDGS performed poorer than those on the reference diet. Likewise, HSI values indicated that the use of 20% DDGS in diets resulted in no negative effects to Nile tilapia condition, while the use of higher levels of DDGS may begin to negatively affect condition and growth (Table 2). To the knowledge, this is the first study to use HSI values to indicate Nile tilapia condition in DDGS feeding trials; therefore, the researchers could not compare the results to any other studies.

Several other studies have found varying results when using DDGS in Nile tilapia feeds. Wu *et al*. (1996) indicated 35% beverage-based DDGS diets did not have statistically different WG and FCR from a reference

Table 2: Mean Weight Gain (WG), Food Conversion Ratio (FCR), Protein Efficiency Ratio (PER), Dressing PERCENTAGE (DP) and Hepatosomatic Indices (HSI) of Nile tilapia fed diets containing one of three levels (1 = 20%, 2 = 30% and 3 = 40%) of distillers dried grains with solubles or a reference diet for 42 days. Values are treatment means (±SD) for experimental and reference diets

Diets	WG (%)	FCR[a]	PER[b]	DP (%)	HSI (%)
1	66.5 (20.5)	2.88 (0.79)	1.31 (0.32)	32.5 (2.9)	2.7 (1.3)
2	48.6 (15.8)*	3.83 (1.08)*	0.99 (0.27)	31.9 (2.6)*	2.3 (0.6)*
3	49.7 (10.2)*	3.58 (0.87)*	1.04 (0.23)	32.6 (2.3)	1.9 (0.6)*
Reference[c]	73.8 (16.3)	2.53 (0.50)	1.16 (0.21)	34.1 (2.3)	2.7 (0.7)

[a]FCR = weight of diet fed/total wet weight gain; diet fed estimated by subtracting uneaten pellets from total food fed; [b]PER = weight gain/protein fed; protein fed estimated by subtracting uneaten pellets from total food fed then multiplying by crude protein; [c]Rangen Inc. Tilapia EXTR 350 feed. Asterisks (*) indicate treatment responses that significantly differ ($p<0.1$) from the reference diet

diet; however, the study shows contrasting results with WG and FCR both being significantly different from a commercial, reference diet. In a followup study by Wu *et al.* (1997), WG for a diet containing 63% beverage-based DDGS was significantly lower than the control diet while FCR and PER values did not differ. Similar to Wu, the study showed that higher amounts of DDGS (30-40%) resulted in decreased WG while FCR and PER were higher and lower, respectively. However compared with the results of Wu *et al.* (1996, 1997), the study indicated poorer performance. Coyle *et al.* (2004) found that Nile tilapia fed a combination of 30% DDGS (+ 8% fishmeal) did not significantly differ in growth performance from a reference diet while fish fed a diet containing 30% DDGS (+46% soybean meal) had significantly lower WG and PER and significantly higher FCR.

The feeding trial used a diet containing 30% DDGS (+5% fishmeal and 29% soybean meal) and indicated growth performance was statistically different from the reference diet with growth performance being lower than observed results by Coyle *et al.* (2004). These discrepancies in performance between studies likely resulted from the use of differing DDGS sources, smaller Nile tilapia fry (initial mean weight = 0.4, 0.5 and 2.7 g), higher protein concentrations and different combinations of ingredients by Wu *et al.* (1996, 1997) and Coyle *et al.* (2004), resulting in higher relative growth performance compared to Nile tilapia (initial mean weight = 6.7 g) used in the study. More research on the type and combination of constituents is needed to determine those ingredients that compliment DDGS by increasing growth performance.

Dressing percentages indicated similar condition of Nile tilapia fed 20 and 40% DDGS to the reference diet. Webster *et al.* (1993a, b) reported similar results in channel catfish (*Ictalurus punctatus*) fed diets containing 10, 20 and 30% DDGS. However, Webster *et al.* (1992) found that channel catfish fed a 90% DDGS diet had a significantly lower DP in catfish fed diets containing 0, 35 and 54.75 % DDGS but when 0.60% lysine was added to the 90% DDGS diet, DP became similar across treatments. Future studies incorporating DDGS in the diets of Nile tilapia should determine DP to provide another metric which can then be compared across studies.

It appears that current processing techniques of fuel-based DDGS do not yet provide any significant benefits over older beverage-based DDGS. Even though studies have shown the increasing trends in protein and lipid levels of DDGS (Spiehs *et al.*, 2002; Belyea *et al.*, 2004) which should be beneficial to aquaculture diets, the current study indicated that fish fed lower levels of DDGS (20%) had similar growth responses as those fed a fishmeal-based commercial diet, while studies using older

Table 3: Average percent composition and amino acid profiles of fuel-based Distillers Dried Grain with Solubles (DDGS) and DDGS used in this study

Property	Reported values (%)[a]	Reported values (%)[b]
Dry matter	86.2-93.0	95.30
Crude protein	26.8-33.7	27.90
Arginine	0.9-2.200	-
Cystine	0.4-0.800	-
Histidine	0.6-1.000	-
Isoleucine	0.9-1.500	-
Leucine	2.4-4.000	-
Lysine	0.5-1.100	-
Methionine	0.5-0.800	-
Phenylalanine	1.3-1.700	-
Threonine	0.8-1.300	-
Tryptophan	0.2-0.300	-
Tyrosine	0.8-1.000	-
Valine	1.3-1.800	-
Alanine	1.8	-
Aspartic acid	1.8	-
Glutamic acid	4.6	-
Glycine	1.0	-
Hydroxyproline	0.2	-
Proline	2.6	-
Serine	1.4	-
Crude lipid	3.5-12.80	11.50
Ash	2.0-9.800	3.96
Carbohydrate	39.2-61.9	-
Crude fiber	5.4-10.6	6.60
Total dietary fiber	24.2-39.8	-
Starch	4.7-5.900	-
Nitrogen free extract	33.8-54.0	50.00
Neutral detergent fiber	25.0-51.3	-
Acid detergent fiber	8.0-21.00	-

[a]Rosentrater and Muthukumarappan (2006); [b]Fuel-based DDGS (Dakota Ethanol Plant, Wentworth, South Dakota, USA) used in study

DDGS had similar results using higher amounts of DDGS (>35%; Wu *et al.*, 1996, 1997). Some potential explanations for these contradictions could be that nutritional differences between older and current DDGS may be small enough that performances differences become lost in the experimental error and variation that occurs during fish feeding trials.

The shift from beverage-based to fuel-based DDGS, along with advancements in technology and processing has led to increasing shift in the overall nutrient composition of DDGS. Nutrient content and quality of DDGS can differ between ethanol plants; the quality of the raw corn grain and processing conditions contribute greatly to these differences (Chevanan *et al.*, 2005). Compared to corn, nutrient concentrations in DDG and DDGS approximately tripled due to the fermentation process but the amino acid profile of DDGS remained similar to whole corn and is retained through the fermentation process (Chevanan *et al.*, 2005; Table 3).

However, overheating in the production process may lead to a reduction in available lysine and partial destruction in cystine; therefore, other ingredients rich in amino acids must be added to balance the amino acid requirements of fish (Chevanan *et al.*, 2005). Fuel-based

DDGS do provide an adequate source of vitamins and minerals (Hertrampf and Piedad-Pascual, 2000). Compared to raw grain, DDGS has a minimum three-fold increase in riboflavin, niacin, pantothenic acid, folic acid and choline. Similarly, DDGS can provide minerals within a diet; however, there is wide variation in the mineral content of DDGS. Generally, DDGS provides an adequate source of phosphorus while calcium is low (Hertrampf and Piedad-Pascual, 2000).

CONCLUSION

In this study, the data indicated that 40% fuel-based DDGS can be incorporated into the diets of juvenile Nile tilapia; however, 20% DDGS should provide the highest growth response more closely related to commercial diets. Supplementation of DDGS as a primary protein source may provide aquaculture operations with a cost effective substitute for fishmeal.

Future research should focus on supplementation of other ingredients in addition to DDGS to increase consumption and growth performance.

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