DDGS Produced from Illinois as an Ingredient in Nile Tilapia Feeds

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Introduction

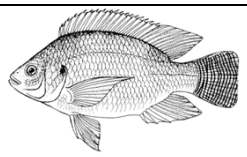
Aquaculture is the fastest food producing sector and it comprises almost half of the total fish supply. Hence, increasing the global aquaculture production helps to keep pace with human population growth and relieve world hunger. Aquaculture greatly contributes to the world food security, and yet, aquaculture faces many challenges. The global fisheries production equals 170 million tons annually, of which 88% is used for human consumption and the rest is utilized for non-food products, such as fishmeal and fish oil production (1). However, the steady decline in catches of wild fish and the increased demands for livestock and aquaculture feeds, have resulted in a rapid decrease in the availability of these raw materials and in their concurrent price increase. The cost of aquaculture feeds represents 40–70% of the cost of the fish produced and hence, it is expected that the future of aquaculture will depend on human capacity to reduce the reliance on raw materials of animal origin with plant-based products and other ingredients from rendered animals and animal waste (2). In 2007, the National Oceanic and Atmospheric Administration (NOAA) and the U.S. Department of Agriculture (USDA) launched an Alternative Feeds Initiative, which included developing a road map for identifying the research needed for using plant-based diets (3). Specifically, distiller's dried grains with soluble (DDGS), the co-products from corn to ethanol processing, was considered a viable ingredient for fishmeal. There are numerous benefits to utilize DDGS in aquafeed: competitive price, high protein and low fiber content from modified processing, enriched vitamins and phytochemicals from yeast fermentation, no concern of antinutritional factors known to induce intestinal inflammation, and cohesive, stable, floating pellets (4, 5). Since the profit margins to make ethanol has kept low for the corn to ethanol industry, DDGS becomes an important product for maintaining ethanol plant profitability and continued operation. Finding a diverse and strong market for DDGS is vital to the U.S. farmers and rural America.

The National DDGS Library established at the National Corn to Ethanol Research Center (NCERC) since 2007, has provided a representative sample base to understand the nutritional and risk factors in DDGS produced from the corn to ethanol industry in the U.S. (6). To expedite the usage of DDGS as a common aquafeed ingredient, NCERC has collaborated with the Center for Fisheries, Aquaculture, and Aquatic Sciences (CFAAS) of Southern Illinois University-Carbondale, and conducted a feeding trial on tilapia using DDGS from one DDGS library contributor, an ethanol plant in Illinois (IL 196, according to reference 6). The DDGS was produced under first generation corn to ethanol processing conditions with de-oil in the back end.

Red tilapia (*Oreochromis sp.*) is native of Africa and is one of the most cultured fish species worldwide due to its fast growth, good market acceptance, and high performance in intensive culture systems (7). Also, tilapia breeds throughout the year and is highly resistant to disease, high temperatures, high water ammonia levels, high stocking densities and low oxygen levels (8). The aim of the study was to evaluate the effect of dietary inclusion of DDGS on growth performance and protein digestibility in Red Tilapia *Oreochromis sp*.

Methodology

I. Experimental setup

Species	Red tilapia *Oreochromis sp.*	
Origin	Commercial vendor, Tilapia Depot (St. Augustine, Florida, EU)	

a. Acclimatization and feed

Fish were acclimatized and fed during two weeks upon arrival with commercial feed (Zeigler, Gardners, PA), before starting the experiment until fish reached 4.3 g in average weight.

b. Preparation of the recirculation aquaculture system

Before starting the experiment, each tank was cleaned up and disinfected to avoid and prevent any disease outbreak. The recirculated aquaculture system (RAS) has been set up with a sand filter, biological filter, and heater for running the experiment, and a total of 30 black cylinder tanks (280 L), in which each tank had water inlet and outlet, and its respective aeration inlet as well.

c. Experimental design

Four triplicate diets were set up as follows, and each tank, considered as an individual observation unit, was labeled and connected to RAS. All treatments were randomly assigned to the experimental tanks. A total of 30 fish was distributed to each tank (1 fish / 9.3L) and fed by hand 3 times per day with a restriction rate of 6% of biomass. The temperature was regulated to 29 ± 1 °C. Mortality was measured every day.

Tank	Diet
1	D4
2	Control
3	D2
4	D3
5	D1

Tank	Diet
6	D2
7	Control
8	D1
9	D4
10	D3

Tank	Diet
11	D2
12	Control
13	D4
14	D3
15	D1

II. Experimental diet

Four different inclusion levels of DDGS were tested. Treatments included: Control = diet resembling commercial formulation for tilapia, soybean meal-based, D1 = diet in which 10% of soybean meal was replaced by DDGS-IL196, D2 = 20% DDGS-IL196, D3 = 30% DDGS-IL196 and D4 = 40% DDGS-IL196. All diets were prepared according to the formulation presented in Table 1. All ingredients were finely ground, thoroughly mixed, and dry pelleted using a pellet mill through a 2 mm die. Diets were dried in a dehydrator for 24 h at 40 °C and then stored at −20 °C until used.

Table 1. Diets Formulation (%, dry weight basis)

Diets	Control (0% DDGS)	D1 (10% DDGS)	D2 (20% DDGS)	D3 (30% DDGS)	D4 (40% DDGS)
Ingredients (%)					
DDGS (IL-196)	0	10.8	21.6	32.4	43.2
Soybean Meal	45.24	37.64	30.06	22.5	14.87
Fish meal	10	10	10	10	10
Corn gluten	10	10	10	10	10
Starch	20.06	17.76	15.47	13.15	10.87
CMC	2	2	2	2	2
Vegetable oil	5.83	4.94	4.04	3.14	2.24
Mineral mix	2.5	2.5	2.5	2.5	2.5
Vitamin mix	2.5	2.5	2.5	2.5	2.5
Dicalcium Phosphate	1.5	1.5	1.5	1.5	1.5
Vitamin C	0.05	0.05	0.05	0.05	0.05
Choline chloride	0.1	0.1	0.1	0.1	0.1
Histidine	0.14	0.14	0.14	0.14	0.14
Methionine	0.08	0.06	0.03	0.01	0.00

III. Data collection

The following growth and feeding performance parameters were assessed: mortality, specific growth rate, feed conversion rate, weight gain (g and %), and protein efficiency ratio. All performance values were calculated after 98 days of experimental period. All biomass in every tank was measured biweekly. Fish were starved for 12 h prior any handling.

The assessed growth parameters were calculated as follows:

Average Weight gain

$$\text{AWG (\%)} = \frac{W_f - W_i}{W_i}\ x\ 100$$

W_f = final weight

W_i = initial weight

Specific growth rate

$$\text{SGR (\%)} = \frac{\ln W_f - \ln W_i}{t}\ x\ 100$$

Where:

W_f = final weight

W_i = initial weight

t = time in days

Factor Convertion Ratio

FCR = weight of diet fed (kg)/total wet weight gain (kg)

Survival rate

$$\text{SR (\%)} = \frac{FF}{IF}\ x\ 100$$

FF = final number of fish

IF = initial number of fish

IV. Proximate analysis

a. Proximate analysis on fish

One fish from each experimental tank at the end of the trial was sampled and frozen at −20 °C for whole-body composition analysis. All fish samples collected were thawed, homogenized (Powergen 1000, Fisher Scientific, USA), and finally freeze dried (Freezone 6, Labconco, USA) before analysis of proximate composition. Lyophilized samples were pulverized before determination of moisture, lipid, protein and ash. Proximate analysis of the whole fish body was made by the following procedures: moisture, by oven drying (Precision 25EG, Thermo Scientific, USA) the samples at 105 °C for 24h and was calculated by mass difference; lipid was determined following a chloroform–methanol extraction method modified from Folch et al. (9); protein contents were measured by combustion method using an FP-528 Nitrogen/Protein Analyzer (LECO Corporation, St. Joseph, MI, USA) according to AOAC International (10); ash was calculated by mass difference, the samples were incinerated in a muffle furnace (Lindberg/Blue M Moldatherm Box Furnace, Thermo Scientific, USA) at 450 °C for 18 h according to AOAC International (11).

b. Proximate analysis on feed

Triplicate samples of control and 30% DDGS diet were pulverized before determination for crude protein, lipid, moisture and ash composition. The samples were analyzed following the same process for proximate composition on fish. Table 2 shows the results of analysis of proximate composition of the experimental diet for digestibility trial.

Table 2. Percent Moisture, Protein, Lipid, and Ash (%, dry weight basis) of Diets for Digestibility Trial in Red Tilapia *Oreochromis sp.*

Diet	Moisture (%)	Protein (%)	Lipids (%)	Ash (%)
Control	90.64±0.1	29.2±0.05[a]	7.17±0.37	9.05±0.09
30% DDGS	91±0.2	28.5±0.06[b]	8.08±0.87	9.03±0.06
P value	*0.052*	*0.003*	*0.168*	*0.836*

Analysis of variance was used to determine significant differences ($P<0.05$), different letters in same column means significantly different.

V. Digestibility analysis

Fish remaining after the final sampling of the growth trial were used for digestibility trial. A new batch of each experimental diet was prepared adding to each diet 1% of yttrium oxide as external marker (only control and 30% DDGS diets). The fish were fed by hand twice a day, to apparent visual satiety. Feces were collected removing them from water column of tanks during 15 consecutive days, three hours after the morning meal and after the noon meal. Feces were immediately stored at −20 °C. The samples were lyophilized (Freezone 6, Labconco, USA) until analysis.

The analysis of samples was performed by Experiment Station Chemical Laboratories of University of Missouri Agricultural using a combustion analysis to protein analysis (LECO) (10)

and yttrium content (12). Apparent digestibility coefficients (ADC) of protein of the experimental diets were calculated as follows:

$$ADC\ (\%) = 100\ x\ \left[1 - \left(\frac{Yttrium_{feed}}{Yttrium_{feces}}\ x\ \frac{Protein_{feces}}{Protein_{feed}}\right)\right]$$

VI. Statistical analysis

Data are presented as mean ± standard deviation. The growth performance, proximate analysis, and digestibility were analyzed using one-way ANOVA, followed Tukey's test by R-Software version 3.6.3. A value of $P < 0.05$ was considered significantly different.

Results

Throughout the study, water quality parameters were within suitable ranges for red tilapia (temperature 26~29 °C, and pH 6~8). All growth performance data were analyzed biweekly and summarized in Table 3.

After 98 days of feeding, significant differences occurred between treatments in some measured responses. The final weight of fish was different between treatments (79 - 98 g) as shown in Figure 1. The average fish weight (AFW) did not present differences in initial weight. Fish fed diets D1 and D2 (soybean meal replaced by DDGS at 10 and 20%, respectively) showed only numerically higher average final fish weight, compared to D3 and D4, but no statistical differences were detected. The diets D1 and D2 had the highest AWG compared to D3 and D4, but not different compared with control treatment. The AWG range was 1711 to 2157%, which was an expected outcome considering the fish size and water conditions used. The SGR showed a similar trend, it was both significantly higher in D1 and D2 treatments compared to D4, but not different compared to D3 or control. The FCR range was 1.8 to 2. Numerically the lowest FCR was found in D1 and D2 compared to D3, D4, and control groups, but there were no significant differences detected between treatments.

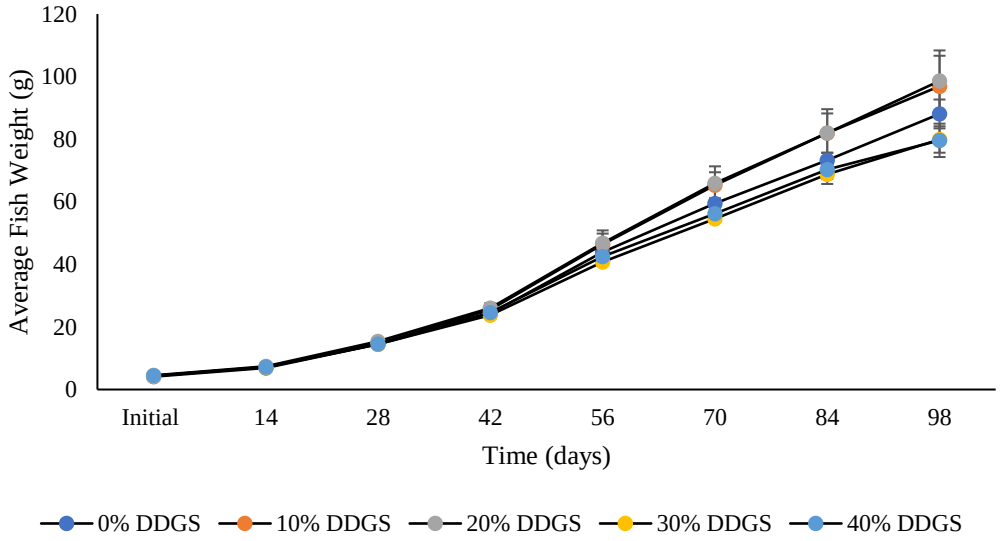


Figure 1. Average fish weight of Red Tilapia *Oreochromis sp.* after 98 days of experimental treatment with different levels of inclusion of DDGS.

The data demonstrated that lower level of DDGS (below 30%) had better effect on growth performance and FCR of tilapia. According to Abo-State *et al.* (13), DDGS replacing soybean meal by 25-50% can significantly improve growth performance of Nile Tilapia with an initial weight of 2 g. Chatvijitkul *et al*., (14) demonstrated that moderate levels of DDGS can be incorporated into practical diets for tilapia (<30% DDGS), but when high levels of DDGS are used (>40% DDGS), it will likely require supplementation of essential amino acid lysine and lipids. All our tested diets were formulated to have the same lipid and essential amino acid levels. Similarly as in our study, Li *et al.* (15) have shown that DDGS can be incorporated in tilapia diet at a level of 20%, as a substitute for a combination of soybean meal and corn meal, without affecting their growth performance and body composition.

Table 3. Effect of different levels of DDGS on growth performance of Red Tilapia *Oreochromis sp.*

Diet	Control	D1	D2	D3	D4	*P value*
AFW (g)	88.1±4.62	96.9±9.74	98.6±9.74	79.9±4.23	79.4±5.33	*0.143*
AWG (%)	2042±109^{AB}	2157±150^{A}	2087±234^{A}	1745±125^{B}	1711±140^{B}	*0.006*
SGR (%/day)	3.12±0.05^{A}	3.17±0.07^{A}	3.14±0.11^{A}	2.97±0.07^{AB}	2.95±0.08^{B}	*0.014*
FCR	1.899±0.08	1.838±0.05	1.854±0.07	2.057±0.12	2.072±0.17	*0.059*
SR (%)	99±1.92	99±1.92	99±1.92	96±1.89	99±1.92	*0.249*

Analysis of variance was used to determine significant differences (P<0.05), different letters in same row means significantly different.

No significant differences were found in survival among all treatments. Survival rate was high and ranged form 96% to 99% for fish fed all diets. The individual mortalities were not caused by any of the dietary treatments.

The whole-body composition was not affected by dietary replacement of soybean meal by DDGS (Table 4). The D2 and D4 diets showed numerically higher percent of moisture (32.11±2.1 and 32.79±0.8 % respectively) and lipids (28.89±2.1 and 31.55±3.8 % respectively) compared to the other groups; and diet D3 had numerically the highest percent of ash (16.1±1.6 %) among all the groups. For other species it has been shown that high dietary DDGS inclusion levels reduced whole-body lipids (16) which was not the case in the present study. The results were similar to Diógenesis *et al*. (17) on gilthead seabream (*Sparus aurata*) for dry matter and lipids (32.9-33.7 and 12-13.4 % respectively) with 15 and 35% of DDGS inclusions as soybean meal replacement. The control and D3 diets showed numerically higher percent of protein composition (46.3±2.64 and 45.6±3.42 % respectively), and no significant differences were detected between diets.

Table 4. Whole-body Composition of Red Tilapia *Oreochromis sp.* Fed with Experimental Diets

Diet	Control	D1	D2	D3	D4	*P value*

Dry Matter (%)	30.93±1.6	32.05±0.7	32.11±2.1	29.84±2.9	32.79±0.8	*0.376*
Protein (%)	46.3±2.64	43.3±0.85	43.8±3.87	45.6±3.42	40.1±2.31	*0.138*
Lipids (%)	25.83±3.3	28.89±2.1	27.04±4.3	22.81±4.3	31.55±3.8	*0.121*
Ash (%)	14.27±1.6	13.36±0.7	14.37±2	16.1±1.6	14.73±1.4	*0.363*

Analysis of variance was used to determine significant differences ($p<0.05$), different letters in same row means significantly different.

Digestion trials are difficult with fish because fecal and other metabolic excretions are usually suspended or dissolved in large quantities in the water (18). Reported apparent digestion coefficients obtained by other feces extraction methods have been higher than those obtained by the present study, likely because of possible contamination of the fecal matter with uneated feed particles. There is no data available on protein ADC of diets based on DDGS in tilapia. The present study showed significant difference between control and 30% DDGS diets (*P=0.011*) (Table 5). Smith et al. (18) and Cheng and Hardy (19) conducted experiments with corn-based DDGS on apparent digestibility coefficients (ADCs) in rainbow trout (*Oncorhynchus mykiss*), and found that ADCs of crude protein in DDGS supplemented groups of were 71.9 and 80 % respectively in diets with 25 and 30 % of DDGS, significantly higher compared with results obtained from the current study. However, the tested diets were supplemented with microbial phytase, which was effective to increase ADC values in trout.

Table 5. Apparent digestibility coefficients (ADC %, dry weight) of the experimental diets in Red tilapia.

Diet	ADC (%)	Yttrium (%)		Protein (%)	
		Feed	Feces	Feed	Feces
Control	52.69±6.19[a]	0.35±0.007	0.50±0.01	34.34±0.11	23.42±2.38
30% DDGS	30.31±6.05[b]	0.36±0.003	0.20±0.02	33.84±0.03	13.07±0.72

Analysis of variance was used to determine significant differences in ADC results ($P<0.05$), different letters in same column means significantly different.

Conclutions

The results of the present study demonstrate that DDGS can be easily included in tilapia diets at levels up to 20% as a practical replacement for soybean meal without jeopardizing tilapia growth performance, feed utilization, and proximate composition, while slightly improving feed cost per kg of fish produced. The apparent digestibility coefficient of protein in feeds containing DDGS was lower compared to what was reported in carnivorus species. More studies are needed to improve DDGS utilization and allow for higher inclusion levels in commercial feed formulations for tilapia and other omnivorous fish species..

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