Lipid Extracted Distillers Dried Grains with Solubles (LE-DDGS) as a Partial Replacement for Soybean Meal in Hybrid Tilapia (*Oreochromis niloticus* × *Oreochromis aureus*)

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Abstract

Feed costs are primarily driven by the cost of protein sources in the feed. Substitution of expensive protein sources with lower cost ingredients would potentially reduce feed cost. Lipid-extracted distillers dried grains with solubles (LE-DDGS) is a relatively new product of distillers dried grains with solubles (DDGS) which could be used as alternative protein source in tilapia feed formulations. Two growth trials were conducted to evaluate the substitution of soybean meal with LE-DDGS in practical diets for the hybrid tilapia (*O. niloticus* × *O. aureus*). The first trial evaluated five levels of LE-DDGS (0, 20, 30, 40 and 50 g/kg) in a practical diet containing 360 g kg^{-1} protein and 60 g kg^{-1} lipid. Increasing percentages of LE-DDGS generally resulted in a reduced growth of hybrid tilapia with a significant depression in performance when incorporated in the diet at 40 and 50 g/kg. The second study evaluated high levels of inclusion (0, 20, 40 and 50%) with lysine supplements and a fifth diet with and additional 20 g/kg lipids (2.7 g kg^{-1} lysine and 80 g kg^{-1} lipid) with all diets promoting good growth in hybrid tilapia. Overall, results from these studies concluded that LE-DDGS could be a promising protein source in combination with soybean meal in formulated diets containing 360 g kg^{-1} protein for hybrid tilapia.

KEY WORDS: alternative protein sources, nutrient retention, distillers dried grains, ethanol industry

Introduction

Reducing feed cost in aquaculture is important for the long term sustainability of this industry. One way to reduce costs is to systematically reduce or replace the more expensive components of the feed. This must be done in such a way as to reduce overall production costs while ensuring that such substitution will not compromise the growth performance of fish. Towards this goal, numerous studies have been conducted with the purpose of reducing fish meal-based protein with plant protein sources in feed formulations (Brinker and Reiter, 2011). The use of plant-based proteins in aquatic feeds has increased as they are cost effective protein sources with consistent quality and worldwide availability (Watanabe, 2002). Distillers dried grains with solubles (DDGS) is a co-product from ethanol industry. As the ethanol industries continue to mature, DDGS has been found to be a cost effective protein source in feed formulations. The properties of DDGS offer several potential advantages to animals including moderate protein and lipid contents, as well as phosphorus, vitamins, and trace minerals. Another benefit of DDGS is that it does not contain anti-nutritional factors found in other plant protein sources such as trypsin inhibitor and phytate which are presented in soybean meal (Wilson and Poe, 1985; Shiau et al., 1987) and gossypol which is contained in cottonseed meal (Jauncey and Ross, 1982; Robinson, 1991). That can cause negative impacts to aquatic digestive system and may influence its palatability.

Although DDGS contains moderate proportion of protein, it usually contains lower levels of lysine, the most limiting amino acid, compared to fishmeal (Cheng and Hardy, 2004). Therefore, it might be necessary to supplement lysine when using high levels of DDGS as a protein source in fish diets. Numerous research projects have shown that DDGS supplemented with lysine is a suitable protein source in fish diets (Webster et al., 1991; Wu et al., 1996, 1997; Belyea et al., 2004; Lim et al., 2007). The nutrient compositions of the resulting DDGS vary considerably depending on the sources of grains and the production process used in ethanol industry. Corn is the most widely used feedstock for ethanol production in the United States. Other grains such as sorghum (Corredor et al., 2006), wheat (Ojowi et al., 1997; Nyachoti et al., 2005), and barley (Mustafa et al., 2000), are also used in ethanol industry. Due to new and emerging technologies in fuel ethanol production, some plants are modifying their processing to increase the value of ethanol co-products. Extracting the lipids from DDGS (lipid-extracted DDGS, LE-DDGS) allows for a higher protein and amino acid contents. LE-DDGS contains about 440 g kg^{-1} crude protein and 300 g kg^{-1} crude fat, while DDGS contains 270 g kg^{-1} crude protein and 100 g kg^{-1} crude fat.

Information on LE-DDGS as an alternative protein source in aquatic feeds has been limited. Therefore, in this study, two growth trials were conducted to identify the response of hybrid tilapia using corn LE-DDGS with and without lysine supplementation as a partial replacement for soybean meal.

Materials and methods

Experimental diets

In the first trial, six isonitrogenous diets (360 g kg^{-1} crude protein) were designed to replace soybean meal with corn LE-DDGS at increasing levels of inclusion 0, 200, 300, 400, and 500 g kg^{-1} of diet (D0-1, D200-1, D300-1, D400-1, D500-1) on an isonitrogenous basis (Table 1). Lysine was supplemented to diets containing the highest levels of LE-DDGS (D500L-1). Amino acid composition of the experimental diets was reported in Table 2. Corn oil was adjusted to maintain similar lipid level at 60 g kg^{-1} among dietary treatments. In the second trial, five diets were designed to contain 360 g kg^{-1} protein and formulated to contain LE-DDGS at increasing levels of 0, 200, 400, and 500 g kg^{-1} (D0-2, D200-2, D400-2, D500-2) as substitutes for soybean meal (Table 3). Also, lysine was supplemented in diets contained LE-DDGS 400 and 500 g kg^{-1} (D400L-2, D500L-2). Amino acid composition of the experimental diets is reported in Table 4. Corn oil was adjusted to maintain a similar lipid level across all but one diet. In the second trial one diet was formulated to contain 500 g kg^{-1} LE-DDGS with lysine supplement and an additional 20 g kg^{-1} lipid. (D500LF-2). All diets were manufactured at the Aquatic Nutrition Laboratory at Auburn University (Auburn, AL, USA) by using standard procedures for the laboratory production of fish diet. Feed ingredients and oil were placed in a food mixer (Hobart Corporation, Troy, OH, USA) for 15 minutes. Hot water was then added to the mixture in order to attain an appropriate consistency for pelleting. Diets went through a 4-mm-diameter meat grinder, dried at 70° C to a moisture content of less than 10%, and stored in the freezer at -20° C until used. Feed samples were collected to determine proximate and amino acids composition following AOAC (1995) procedures.

Experimental fish, feeding, and sampling

Hybrid tilapia (*O. niloticus* × *O. aureus*) were obtained from a commercial fingerling producer in Florida (Aquasafra, Bradenton, FL, USA). Juveniles were reared in nursery tanks before they were acclimated to the control diet for 2 weeks. In the first trial, the experiment consisted of six treatments with four replicates. Juvenile tilapia with average weight 6.00 ± 0.11 g (mean ± S.E.M., n=50) were randomly placed into 24 aquaria (50 L) at a stocking density of 25 fish per aquarium. Aquaria were supplied with flow-through (0.6–1.0 L min^{-1}), heated, and dechlorinated tap water maintained at a temperature of 27° C. Water was continuously aerated, and photoperiod maintained at 12:12 h light:dark schedule. Water temperature and dissolved oxygen were randomly measured in three random aquaria every other day using an YSI Model 58 Oxygen Meter (Yellow Springs Instrument Model 58, Yellow Springs, OH, USA). Fish were fed to apparent satiation twice daily (0800h and 1500h) for an 8-wk period. The amount of feed consumed was recorded daily, calculated by the differences in diet weights prior the first and last feeding. Fish from each aquarium were group weighed and counted to determine weight gain and survival every other week. At the end of the experiment, four fish from each aquarium were randomly sacrificed to determine the whole body composition.

In the second trial, juvenile tilapia with average weight 2.23 ± 0.10 g (mean ± S.E.M., n=50) were placed into 20 tanks (150 L) at a stocking density of 20 fish per tank in a recirculating system. Water temperature and dissolved oxygen were randomly measured twice daily in the morning and afternoon (800h and 1600h) using a YSI Model 58 Oxygen Meter (Yellow Springs Instrument Model 58, Yellow Springs, OH, USA). Fish in four tanks were randomly assigned to each of the five experimental diets. Feed input was calculated between 50 to 70 g kg^{-1} of average fish weight every other week. Test diets were applied twice daily at 0800 and 1600 h for a 12-wk experimental period. Fish in each tank were group weighed and counted biweekly to determine weight gain (WG) and survival as well as readjust the daily feed input. At the conclusion of the 12-wk growth trial, fish were counted and group weighed to obtain final weight (FW). Weight gain, feed conversion ratio (FCR) and survival were determined using equation 1.1, 1.2 and 1.3, respectively. Four fish from each aquarium were randomly sampled to determine the whole body composition.

$$\textit{Weight gain (g)} = \textit{Average final weight (g)} - \textit{Average initial weight (g)} \qquad 1.1$$

$$Feed\ conversion\ ratio = Dry\ feed\ fed\ (g)\ /\ Weight\ gain\ (g) \quad 1.2$$

$$Survival\ (\%) = (Initial\ fish\ number - Final\ fish\ number) \times 100 \quad 1.3$$

Analytical Method

Moisture content of both fish and feed samples were determined using equation 2.1, by recording their original weight (wet), placing them in ceramic crucibles, drying them in isothermal oven at 105°C for 8 hours, placing them in desiccators until they were at room temperature and recording their final weight (dry).

$$Moisture\ content\ (\%) = \frac{Ww - Wd}{Ww} \times 100 \quad 2.1$$

where: Ww = wet weight of the sample

Wd = weight of sample after drying

Protein content in the first trial was measured by combustion method using an FP-2000 Nitrogen Analyzer (Leco Corporation, St. Joseph, MI, USA). Protein content in the second experiment was analyzed using The Kjeldahl method (Ma and Zauzago, 1942). Energy contents of all samples were determined using a bomb calorimeter (1425 Semimicro Calorimeter, Illinois, USA). Apparent net protein retention (ANPR) and apparent net energy retention (ANER) were calculated by using equation 2.2 and 2.3, respectively.

$$\text{ANPR}\ (\%) = \frac{(\text{P}f \text{ in fish } \times \text{ FW } \times \text{ DM}f \text{ of fish}) - (\text{P}i \text{ in fish } \times \text{ FW } \times \text{ DM}i \text{ of fish})}{\text{P in feed } \times \text{amount of feed consumed}} \quad 2.2$$

Where: $\text{P}f$ = Protein in final fish

$\text{P}i$ = Protein in initial fish

$\text{DM}f$ = Dry matter of final fish (%)

DMi = Dry matter of initial fish (%)

$$\text{ANER (\%)} = \frac{(\text{E}f \text{ in fish } \times \text{ FW } \times \text{ DM}f \text{ of fish}) - (\text{E}i \text{ in fish } \times \text{ FW } \times \text{ DM}i \text{ of fish})}{\text{E in feed } \times \text{amount of feed consumed}} \qquad 2.3$$

Where: Ef = Energy in final fish
Ei = Energy in initial fish
DMf = Dry matter of final fish (%)
DMi = Dry matter of initial fish (%)

Statistical Analysis

All data were statistically analyzed using one-way analysis of variance to determine significant differences ($P<0.05$) among treatments, which was followed by the Tukey multiple comparison test to determine significant differences among treatment means. All statistical analyses were carried out using SAS (V9.2 SAS Institute, Cary, NC, USA).

Results

The laboratory trials were conducted without any noticeable problems with water quality. Water quality parameters were within suitable ranges for the culture of this specie (Table 5). For the first trial, the growth performance (FW, WG, FCR, survival, ANPR and ANER) were summarized in Table 6. Fish fed D0-1 had the highest FW and WG, and was significantly higher than the other treatments ($P<0.05$), except the D200-1 and D300-1 treatment. Survival ranged from 98 to 100%. No significant differences were found in survival among all treatments. Feed conversion ratio ranged from 0.91 to 1.02. The lowest FCR was found in the D0-1 treatment, and the highest FCR in the D500-1 treatment. In regards to ANPR and ANER, control diet (D0-1) had the highest ANPR and ANER and was significantly higher than other treatments, except the D300-1 treatment for ANPR and the D200-1 treatment for ANER. ANPR and ANER ranged from 39.90 to 46.35 % and 34.81 to 43.02 %, respectively.

Growth performance of hybrid tilapia in Trial II was summarized in Table 7. The response of juvenile tilapia fed at increasing levels of LE-DDGS (D0-2, D200-2, D400L-2, D500L-2, and D500LF-2) were not significantly different with regards to FW, WG, FCR, survival, ANPR, and ANER ($P>0.05$). The D500LF-2 treatment had the highest FW, WG, and ANER, though these were not significantly different among treatments. The D500L-2 treatment had the lowest FW, WG, and FCR. The D200-2 treatment had the lowest ANPR and ANER. At the conclusion of the experimental period, FW ranged from 55.82 to 65.55 g. WG ranged from 2,469.03 to 2,808.73 %. FCR ranged from 1.04 to 1.14. Survival ranged from 77.10 to 89.75%. ANPR and ANER ranged from 35.60 to 41.93% and 32.06 to 34.25%, respectively.

Discussion

A number of fish feeding studies evaluating the efficacy of DDGS from ethanol industry have been conducted. Several studies have evaluated composition of DDGS in order to estimate nutritional value as a feedstuff (Chevanan et al., 2005; Rosentrater and Muthukumarappan, 2006). Belyea et al. (2004) analyzed DDGS samples from 1997 to 2001, and found that protein and lipid levels ranged from 283 to 333 g kg^{-1} and 109 to 126 g kg^{-1}, respectively. Likewise, Spiehs et al. (2002) evaluated DDGS samples from 1997 to 1999 and concluded an average protein and lipid concentration of 302 g kg^{-1} and 109 g kg^{-1}, which were higher than those reported in the past at the level of 281 g kg^{-1} protein and 82 g kg^{-1} lipid. There is a trend of increasing DDGS protein and lipid resulted from new technology in ethanol production. Due to new developments and technology advancements, some ethanol plants are modifying their processing and extracting lipid from DDGS resulting in an increased protein and reduced lipid contents. Although DDGS has relatively high fiber content, tilapia can utilize carbohydrates more efficiently than cultured piscivorous fishes (NRC, 1983; Lim and Webster, 2006).

Distillers dried grains with solubles has been utilized in aquatic feeds since 1940s; however, inclusion levels in diets were fairly low (Phillips, 1949; Phillips et al., 1964). Result from the first trial indicated that diet inclusion rates of 200, 300, 400, and 500 g kg^{-1} LE-DDGS caused a reduced performance on juvenile tilapia growth. This might be due to the fact that LE-DDGS contains low level of lipid which can provide less energy to the diets than DDGS. Results from several studies demonstrated that the inclusion of DDGS in aquatic feed can be utilized up to 300 g kg^{-1} of diet. Wu et al. (1994) observed that tilapia fry fed a commercial diet (360 g kg^{-1} crude protein, fish-based protein) had a lower weight gain than fish fed diet containing 290 g kg^{-1} DDGS formulated with 360 g kg^{-1} crude protein. Coyle et al. (2004) concluded that diet (300 g protein kg^{-1} of diet) containing DDGS at a level of 300 g $kg^{-1}$of diet in combination with meat and bone meal and soybean meal provided an effective performance to hybrid tilapia (*O. niloticus* × *O. aureus*). Similarly, Zhou et al. (2010) evaluated fuel-based DDGS to replace soybean meal and corn meal in juvenile hybrid catfish diet (320 g protein kg^{-1} of diet). They suggested that diet containing 300 g kg^{-1} DDGS also provided good growth, protein retention, and feed conversion in catfish. Result from the present study also demonstrated that LE-DDGS can be used in hybrid tilapia diet at the inclusion level of 300 g kg^{-1} without causing any negative effects on fish growth.

Results from the first experiment revealed that juvenile tilapia offered a diet with 500 g kg^{-1} LE-DDGS had significantly lower FW and WG ($P<0.05$) as compared to fish fed the control diet (D0-1). The supplementation of lysine (D500L-1) did not appear improve final weights of the fish. This indicates that either crystalline lysine was not utilized or there was another more limiting nutrient, possibly energy. Results of the second experiment indicated an effective utilization of all the diets and hence the lysine supplement by tilapia fed high level of LE-DDGS. There were no significant differences between performance of fish fed control diet (D0-2) and diets containing 400 and 500 g kg^{-1} LE-DDGS with lysine supplementation (D400L-2 and D500L-2). Although not significant there is a general trend of reduced weight gain as LE-DDGS is increased as was seen in the first experiment.

Robinson and Li (2008) evaluated a combination of DDGS and cotton seed meal as a replacement of soybean meal, and demonstrated that diet (290 g kg^{-1} crude protein) containing 300-400 g kg^{-1} DDGS with lysine supplement could be used to replace soybean meal without an adverse effect on performance of catfish. Webster et al. (1991) fed channel catfish diets with increasing levels of DDGS (0, 350, and 700 g kg^{-1} of diet), and they found that channel catfish fed a diet with 700 g kg^{-1} DDGS grew significantly less than other dietary treatments. However, fish fed diet containing 700 g kg^{-1} DDGS with 4 g kg^{-1} lysine had a similar growth compared to fish fed diets containing 0 and 350 g kg^{-1} DDGS. Shelby et al. (2008) reported the growth performance of tilapia fed diets (325 g kg^{-1} crude protein) with increasing levels of DDGS (0, 300, and 600 g kg^{-1}) as a replacement of a combination of soy and corn meals. This study showed that diet containing 600 g kg^{-1} DDGS with no lysine supplementation led to a negative growth performance of tilapia. The addition of lysine to the 600 g kg^{-1} DDGS diet improved fish weight gain to a level that was not significantly different from control fish.

Distillers grains are moderate protein and energy sources, however, the information on energy availability for aquatic animals is often limited. Smith et al. (1980) reported digestible energy of distillers dried soluble in rainbow trout was 2,436 kcal kg^{-1} diet. There is no data on the energy and protein digestibility of DDGS in tilapia; therefore, in these studies, the digestible energy and digestible protein of DDGS was calculated based on the numbers of rainbow trout as reference. Cheng and Hardy (2004) conducted an experiment on the effect of microbial phytase in corn-based DDGS on apparent digestibility coefficients (ADCs) in rainbow trout and found that ADCs of crude fat, crude protein, gross energy, minerals, and amino acids in DDGS supplemented with different dosages of phytase were 78.9-88.9, 80.0-91.9, 50.5-66.6, 7.3-99.7, 73.9-96.8%, respectively. Smith et al. (1980) conducted experiment in rainbow trout (*Salmo gairdneri*), and reported that their digestibility of corn DGS was 71.9% for protein and 58.6% for carbohydrate (Lovell, 1977). In the first trial, the addition of crystalline lysine to diet containing 500 g kg^{-1} LE-DDGS (D500L-1) did not improve fish growth. Fish fed diet containing 500 g kg^{-1} LE-DDGS with lysine supplement (D500L-1) obtain similar growth performance with fish fed diet containing 500 g kg^{-1} LE-DDGS without lysine supplement (D500-1), and obtained FW, WG ANPR, and ANER lower than fish fed the basal diet (D0-1). This suggested a limitation in other nutrients which retard growth of fish. DDGS contains relatively high crude fiber, which could result in a limited use as an energy source. An earlier study reported that Nile tilapia fry exhibited the best growth with a protein to energy ration of 110 mg kcal^{-1} (El-Sayed and Teshima 1992). Furthermore, Kubaryk (1980) pointed out that the protein to energy ratios between 108 to 120 mg kcal^{-1} led to the best growth in Nile tilapia fry. Estimating GE and DE indicated that, as inclusion levels of LE-DDGS in the diets increased from 200 to 500 g kg^{-1} of diets, the ratio of digestible energy to protein generally decreased approximately from 10.77 to 8.42 kcal g^{-1} digestible protein or 111.1 to 133.3 mg digestible protein kcal^{-1} (Table 8). In the second experiment, the ratio of digestible energy to protein was increased by adding 20 g kg^{-1} lipid to the diet containing 500 g kg^{-1} LE-DDGS with lysine from 7.94 to 8.27 kcal g^{-1} digestible protein (Table 9). Thus, the poor growth of fish fed diets containing increasing level of LE-DDGS observed in this study is likely due to a deficiency in dietary digestible energy. As lipid content in diet contained 500 g kg^{-1} LE-DDGS with lysine supplement increased from 60 (D500L-2) to 80 g kg^{-1} (D500LF-2), weight gain of juvenile tilapia improved more than 300% compared to fish fed diet contained 500 g kg^{-1} LE-DDGS with lysine (D500L-2). Stickney and Wurts (1986) stated that the performance of *O. aureus* could be improved as fish oil was provided at 75-100 g kg^{-1} of the diet; however, best growth was achieved with menhaden oil at 100 g kg^{-1} of the diet. Chou and Shiau (1996) observed that 5 g kg^{-1} of dietary lipid was sufficient to meet the minimal requirement of juvenile hybrid tilapia (*O. aureus* × *O. niloticus*), but a level of 12 g kg^{-1} was needed for maximal growth. Jauncey and Ross (1982) reported that the diet containing lipid in excess of 12 g kg^{-1} caused a depressed growth of hybrid tilapia (*O. aureus* × *O. niloticus*).

Results of the present study, demonstrate that LE-DDGS can be incorporated into practical diets for hybrid tilapia at 300 g kg^{-1}. Furthermore, results indicate that if 500 g kg^{-1} LE-DDGS are utilized, energy may be limiting and that increasing the digestible energy content of the diet may improve performance.

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Table 1 Ingredient compositions (g 100 g^{-1} as is) of seven experimental diets formulated to contain 360 g kg^{-1} protein and 60 g kg^{-1} lipid used in trial I. Diets contained increasing levels of LE-DDGS (0, 200, 300, 400, and 500 g kg^{-1}) as well as lysine supplement in diets with high levels of LE-DDGS (500 g kg^{-1} of diet) as a substitute for soybean meal. Proximate (% as is), were analyzed at Midwest Laboratories, Inc. (Omaha, NE, USA).

Ingredient	D0-1	D200-1	D300-1	D400-1	D500-1	D500L-1
Menhaden fishmeal[1]	5.00	5.00	5.00	5.00	5.00	5.00
Soybean meal[2]	49.80	39.80	34.80	29.80	24.80	24.20
DDGS-lipid extracted[3]	0.00	20.00	30.00	40.00	50.00	50.00
Menhaden fish oil[1]	0.82	0.82	0.82	0.82	0.80	0.82
Corn oil	2.92	1.74	1.15	0.56	0.00	0.00
Whole wheat[4]	33.06	24.74	20.58	16.42	12.25	12.58
Trace mineral premix[5]	0.50	0.50	0.50	0.50	0.50	0.50
Vitamin premix[6]	0.80	0.80	0.80	0.80	0.80	0.80
Choline chloride[4]	0.20	0.20	0.20	0.20	0.20	0.20
Stay C 25%[7]	0.10	0.10	0.10	0.10	0.10	0.10
Dicalcium phosphate[8]	2.30	1.80	1.55	1.30	1.05	1.05
Soy lecithin[9]	0.50	0.50	0.50	0.50	0.50	0.50
Corn gluten meal[10]	4.00	4.00	4.00	4.00	4.00	4.00
L-Lysine HCl[11]	0.00	0.00	0.00	0.00	0.00	0.25
Crude protein	35.70	37.80	36.70	37.40	36.60	36.80
Crude fat	6.61	6.86	6.33	6.19	6.57	6.37

Crude fiber	2.69	4.03	3.52	3.79	4.62	2.79
Moisture	8.56	6.37	7.62	5.42	6.70	6.81
Ash	6.69	6.72	6.25	6.40	6.66	6.17

[1] Omega Protein Inc., Reedville, VA, USA.
[2] Faithway Feed Co., Guntersville, AL, USA.
[3] Poet Dakote Gold Inc., Sioux Falls, SD, USA.
[4] Gold Medal, General Mills Inc., Minneapolis, MN, USA.
[5]Trace mineral (g/100g Premix): Cobalt chloride 0.004, Cupric sulfate pentahydrate 0.25, Furrous sulfate 4.0, Magnesium sulfate anhydrous 13.862, Manganous sulfate monohydrate 0.65, Potassium iodide 0.067, Sodium selenite 0.01, Zinc sulfate hepahydrate 13.193, cellulose 67.964.
[6]Vitamin (g/kg Premix): Thiamin HCl 0.44, Riboflavin 0.63, Pyridoxine HCl 0.91, D pantothenic acid 1.72, Nicotinic acid 4.58, Biotin 0.21, Folic acid 0.55, Inositol 21.05, Menadione sodium bisulfite 0.89, Vitamin A acetate (500,000 IU g^{-1}) 0.68, Vitamin D_3 (400,000 IU g^{-1}) 0.12, DL-alpha-tocoperol acetate (250 IU g^{-1}) 12.63, cellulose 955.59.
[7] Stay-C® (L-ascorbyl-2-polyphosphate), DSM, Pendergrass GA USA
[8] MP Biochemicals Inc., Solon, OH, USA.
[9] Solae Company, St. Louis, MO, USA.
[10] Grain Processing Corporation, Muscatine, IA, USA.
[11] Aldrich-Sigma, St. Louis, MO, USA.

Table 2 Amino acid composition (g 100 g^{-1} as is) of the experimental diets containing increasing levels of LE-DDGS (0, 200, 300, 400 and 500 g kg^{-1}) as well as lysine supplement in diets with high levels of LE-DDGS (500 g kg^{-1} of diet).

Component	D0-1	D200-1	D300-1	D400-1	D500-1	D500L-1
Alanine	1.52	1.72	1.88	2.08	2.22	2.07
Arginine	2.39	2.19	2.29	2.29	2.06	2.20
Aspartic Acid	3.40	3.08	2.83	2.93	2.71	2.85
Cystine	0.54	0.79	0.83	0.65	0.65	0.69
Glutamic Acid	6.56	6.53	6.25	6.31	6.15	6.23
Glycine	1.45	1.48	1.49	1.54	1.55	1.50
Histidine	0.90	1.06	0.92	1.11	1.17	1.06
Isoleucine	1.41	1.49	1.45	1.41	1.36	1.37
Leucine	2.69	2.97	3.07	3.23	3.18	3.31
Lysine	1.83	1.82	1.73	1.72	1.61	1.81
Methionine	0.53	0.63	0.66	0.66	0.66	0.66
Phenylalanine	1.61	1.68	1.82	1.72	1.51	1.63
Proline	2.28	2.52	2.57	2.74	2.66	2.73
Serine	1.83	1.75	1.53	1.78	1.59	1.78
Threonine	1.46	1.38	1.28	1.39	1.29	1.37
Tyrosine	1.33	1.35	1.47	1.48	1.31	1.42
Tryptophan	0.31	0.34	0.31	0.27	0.30	0.30

Diets were analyzed by Midwest Laboratories, Inc., Omaha, NE, USA.

Table 3 Ingredient compositions (g 100 g^{-1} as is) of five experimental diets formulated to contain 360 g kg^{-1} protein and 60 g kg^{-1} lipid used in trial II containing increasing levels of LE-DDGS (0, 200, 400 and 500 g kg^{-1}) with lysine supplement in diets with high levels of LE-DDGS (400, and 500 g kg^{-1} of diet) as well as lipid supplement in diets containing 500 g kg^{-1} LE-DDGS with lysine supplement as a substitute for soybean meal. Proximate (% as is), were analyzed at Midwest Laboratories, Inc. (Omaha, NE, USA).

Ingredient	D0-2	D200-2	D400L-2	D500L-2	D500LF-2
Menhaden fishmeal[1]	5.00	5.00	5.00	5.00	5.00
Soybean meal[2]	48.90	38.98	28.72	23.60	24.50
DDGS-lipid extracted[3]	0.00	20.00	40.00	50.00	50.00
Menhaden Fish Oil[1]	0.82	0.82	0.82	0.80	0.80
Corn Oil	3.13	1.92	0.70	0.12	2.15
Whole wheat[4]	33.55	25.18	17.07	12.61	9.73
Trace Mineral premix[5]	0.50	0.50	0.50	0.50	0.50
Vitamin premix[6]	0.80	0.80	0.80	0.80	0.80
Choline chloride[4]	0.20	0.20	0.20	0.20	0.20
Stay C 25%[7]	0.10	0.10	0.10	0.10	0.10
Dicalcium phosphate[8]	2.50	2.00	1.45	1.50	1.45
Soy lecithin[9]	0.50	0.50	0.50	0.50	0.50
Corn Gluten meal[10]	4.00	4.00	4.00	4.00	4.00
L-Lysine HCl[11]	0.00	0.00	0.14	0.27	0.27
Crude protein	35.30	37.20	34.50	33.60	34.00

Crude fat	6.90	7.13	7.10	6.79	8.95
Crude fiber	5.42	4.74	5.17	6.99	6.20
Moisture	9.66	9.95	10.14	11.30	11.68
Ash	7.18	7.15	6.71	6.89	6.62

[1] Omega Protein Inc., Reedville, VA, USA.
[2] Faithway Feed Co., Guntersville, AL, USA.
[3] Poet Dakote Gold Inc., Sioux Falls, SD, USA.
[4] Gold Medal, General Mills Inc., Minneapolis, MN, USA.
[5]Trace mineral (g/100g Premix): Cobalt chloride 0.004, Cupric sulfate pentahydrate 0.25, Furrous sulfate 4.0, Magnesium sulfate anhydrous 13.862, Manganous sulfate monohydrate 0.65, Potassium iodide 0.067, Sodium selenite 0.01, Zinc sulfate hepahydrate 13.193, cellulose 67.964.
[6]Vitamin (g/kg Premix): Thiamin HCl 0.44, Riboflavin 0.63, Pyridoxine HCl 0.91, D pantothenic acid 1.72, Nicotinic acid 4.58, Biotin 0.21, Folic acid 0.55, Inositol 21.05, Menadione sodium bisulfite 0.89, Vitamin A acetate (500,000 IU g^{-1}) 0.68, Vitamin D_3 (400,000 IU g^{-1}) 0.12, DL-alpha-tocoperol acetate (250 IU g^{-1}) 12.63, cellulose 955.59.
[7] Stay-C® (L-ascorbyl-2-polyphosphate), Roche Vitamins Inc., Parsippany, NJ, USA.
[8] MP Biochemicals Inc., Solon, OH, USA.
[9] Solae Company, St. Louis, MO, USA.
[10] Grain Processing Corporation, Muscatine, IA, USA.
[11]Aldrich-Sigma, St. Louis, MO, USA.

Table 4 Nutrient composition (g 100 g^{-1} as is) of the experimental diets containing increasing levels of LE-DDGS (0, 200, 400 and 500 g kg^{-1}) with lysine supplement in diets with high levels of LE-DDGS (400, and 500 g kg^{-1} of diet) as well as lipid supplement in diets containing 500 g kg^{-1} LE-DDGS with lysine supplement.

Component	D0-2	D200-2	D400L-2	D500L-2	D500LF-2
Alanine	1.72	1.90	2.03	2.08	2.06
Arginine	2.27	2.18	1.99	1.87	1.87
Aspartic Acid	3.32	3.27	2.91	2.79	2.71
Cystine	0.51	0.54	0.55	0.55	0.52
Glutamic Acid	6.69	6.39	5.98	5.55	5.38
Glycine	1.61	1.66	1.57	1.52	1.53
Histidine	0.89	0.92	0.92	0.91	0.90
Isoleucine	1.49	1.49	1.44	1.40	1.38
Leucine	2.94	3.15	3.36	3.46	3.39
Lysine	1.93	1.85	1.82	1.83	1.83
Methionine	0.57	0.63	0.62	0.63	0.60
Phenylalanine	1.79	1.82	1.81	1.81	1.79
Proline	2.20	2.33	2.42	2.40	2.36
Serine	1.52	1.54	1.52	1.51	1.49
Threonine	1.31	1.36	1.32	1.34	1.31
Tyrosine	1.19	1.24	1.25	1.26	1.23
Tryptophan	0.44	0.42	0.37	0.31	0.36

Diets were analyzed by Midwest Laboratories, Inc., Omaha, NE, USA.

Table 5 Summary of water quality variables for growth trials with O. *niloticus* reared under flow-through (trial I) and recirculating system (trial II). Values represent the average, standard deviation, minimum, and maximum readings.

Parameter	Average	Standard deviation	Minimum	Maximum
First trial (flow-through system)				
DO (mg L^{-1})[a]	6.06	0.46	4.50	6.80
Temperature (˚C)	27.68	0.73	25.00	29.20
Second trial (recirculating system)				
DO (mg L^{-1})[a]	5.95	0.56	4.05	6.89
Temperature (˚C)	28.21	1.78	23.50	30.80
Salinity (ppt)	0.30	0.45	0.10	2.00
TAN (mg L^{-1})[b]	0.26	0.31	0.06	1.19
Nitrite (mg L^{-1})	0.41	0.40	0.03	1.35

[a]Dissolved oxygen.

[b]Total ammonia-nitrogen

Table 6 Response over a 8-wk growth period for hybrid tilapia (6.0 $\pm$ 0.11 g, initial weight), fed diets containing levels of LE-DDGS (0, 200, 300, 400, and 500 g kg^{-1} of diet) as a substitute for soybean meal, reared under a flow-through system in indoor tanks (Trial I).

Diet	FW (g)	WG (%)	Survival (%)	FCR[a]	ANPR[b]	ANER[b]
D0-1	81.38[a]	1238.15[a]	100.00	0.91[a]	46.35[a]	43.02[a]
D200-1	76.47[ab]	1157.08[ab]	100.00	0.93[a]	41.79[b]	39.54[ab]
D300-1	75.58[ab]	1156.27[ab]	100.00	0.94[a]	42.61[ab]	37.61[b]
D400-1	73.50[b]	1118.30[b]	98.00	0.97[ab]	41.32[b]	36.32[b]
D500-1	72.30[b]	1094.46[b]	98.00	1.02[b]	39.90[b]	34.81[b]
D500L-1	72.02[b]	1125.62[b]	99.00	0.97[ab]	40.58[b]	36.12[b]
P-value[c]	0.0081	0.0058	0.6691	0.0008	0.0015	0.0014
PSE[d]	1.66	22.86	1.22	0.01	0.91	1.17

[a]Feed conversion ratio = Total feed offered / biomass increase.
[b]Apparent net protein retention and apparent net energy retention
[c]Analysis of variance was used to determine significant differences ($P<0.05$) among treatment means (n=4).
[d]Pooled standard error of treatment means

Table 7 Response over a 12-wk growth period for hybrid tilapia (2.23 $\pm$ 0.11 g, initial weight), fed diets containing levels of LE-DDGS (0, 200, 400, and 500 g kg^{-1} of diet) as a substitute for soybean meal, reared under a closed recirculating system in indoor tanks (Trial II).

Diet	FW (g)	WG (%)	Survival (%)	FCR[a]	ANPR[b] (%)	ANER[b] (%)
D0-2	63.15	2701.26	77.10	1.08	38.03	32.66
D200-2	61.03	2672.92	87.42	1.14	35.60	32.06
D400L-2	61.09	2645.15	89.75	1.08	38.51	33.89
D500L-2	55.82	2469.03	85.00	1.04	41.93	32.73
D500LF-2	65.55	2808.73	78.30	1.05	40.45	34.25
P-value[c]	0.47	0.78	0.40	0.53	0.32	0.66
PSE[d]	1.67	84.69	2.41	0.02	0.96	0.53

[a]Feed conversion ratio = Total feed offered / biomass increase.
[b]Apparent net protein retention and apparent net energy retention
[c]Analysis of variance was used to determine significant differences (P<0.05) among treatment means (n=4).
[d]Pooled standard error of treatment means

Table 8 Estimated energy and protein contents, as well as digestible energy, digestible protein, and ratio of digestible energy to digestible protein in diets containing increasing levels of LE-DDGS (0, 200, 300, 400 and 500 g kg^{-1}).

Diet	GE[a] (kcal/g)	CP[b] (%)	DP[c] (%)	DE[d] (kcal/g)	DE:CP (kcal/g)	DE:DP (kcal/g)
D0-1	4.31	35.70	29.98	3.23	9.05	10.77
D200-1	4.49	37.80	31.05	3.03	8.04	9.78
D300-1	4.67	36.70	31.59	2.94	8.01	9.31
D400-1	4.61	37.40	32.12	2.84	7.61	8.86
D500-1	4.67	36.60	32.66	2.75	7.51	8.42
D500L-1	4.54	36.80	32.43	2.75	7.48	8.49

[a]GE = Gross energy
[b]CP = Crude protein
[c]DP = Digestible protein
[d]DE = Digestible energy

Table 9 Estimated energy and protein contents, as well as digestible energy, digestible protein, and ratio of digestible energy to digestible protein in diets containing increasing levels of LE-DDGS (0, 200, 400 and 500).

Diet	GE[a] (kcal/g)	CP[b] (%)	DP[c] (%)	DE[d] (kcal/g)	DE:CP (kcal/g)	DE:DP (kcal/g)
D0-2	4.43	35.70	29.64	3.04	8.52	10.26
D200-2	4.45	37.80	30.74	2.85	7.54	9.26
D400L-2	4.68	36.70	31.72	2.66	7.24	8.38
D500L-2	4.56	37.40	32.18	2.55	6.83	7.94
D500LF-2	4.71	36.60	32.28	2.67	7.30	8.27

[a]GE = Gross energy
[b]CP = Crude protein
[c]DP = Digestible protein
[d]DE = Digestible energy