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

by

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Abstract

Feed costs are primarily driven by the cost of protein sources used to produce the feed. Substitution of expensive protein sources with lower cost ingredients would potentially reduce the cost of the feed. Lipid-extracted distillers dried grains with solubles (LE-DDGS) is a relatively new product of distillers dried grains (DDG) which could be used as alternative feedstuffs in tilapia feed formulations. Compared to other plant-based protein sources, DDGS has moderate protein and higher fiber contents. Tilapia require a relatively low level of protein and can utilize high inclusion level of plant-based proteins. Given the relatively low price, LE-DDGS may be a suitable alternative protein source in hybrid tilapia diet. Hence, 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*). Results from these studies indicated that formulated diets containing increasing percentages of LE-DDGS without lysine supplementation resulted in a reduced growth of hybrid tilapia. However, positive performances including growth, feed conversion ratio, survival, protein retention, and energy retention were demonstrated in fish fed diet using 40 % LE-DDGS of diet with lysine supplementation. Moreover, the inclusion of LE-DDGS at 50% of diet with lysine and an additional 2% lipid promoted good growth in hybrid tilapia. Overall, results from these studies revealed that LE-DDGS with lysine supplement is a promising protein source in combination with soybean meal in formulated diets containing approximately 36% protein for hybrid tilapia.

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CHAPTER 1

INTRODUCTION

According to the Food and Agriculture Organization, aquaculture is expanding more rapidly than all other animal food industries. Tilapia is one of the most important cultured fish species which has steadily increased over the last decade. The tilapia production grew from 1.0 million tonnes in 2001 to 2.5 million tonnes in 2010 (FAO, 2012). The expansion of the tilapia industry has been associated with high feed costs which usually represents 60% of expenses in commercial farms (Tan and Dominy, 1997). Therefore, it is a considerable concern to reduce the cost of feed in order to improvee profitability. The first step in reducing feed costs is identifying the most expensive ingredient in feed formulation which is generally protein (Li *et al*., 2012). Fish-based protein is one of the most common protein sources utilized in commercial feed due to its nutritional property, high protein content, balanced amino acid profile, and suitable digestibility as well as palatability (Abdelghany, 2003). Fish meal used in aquatic feed has shown a strong growth from 0.96 million tonnes in 1992 to 3.06 million tonnes in 2006 (Tacon, *et al*., 2006, 2007). Aquaculture is estimated to have used 63.0 percent of world fish meal production in 2009, which is the largest user of animal husbandry subsectors (International Fishmeal and Fish Oil Organization, 2011). Due to rising prices, fixed production, and environmental pollution (primary phosphorus), considerable research has been conducted regarding the replacement of fish meal with alternative protein ingredients in commercial diets (Jauncey and Ross, 1982; Fontainhas- Fernandes *et al*., 1999; Coyle *et al*., 2004; Brinker and Reiter, 2011). Thus, terrestrial animal by-products such as poultry meal, blood meal, hydrolyzed feather meal, and meat and bone meal, have been introduced into aquatic feeds (Tacon, 1993). These feedstuffs may be deficient in some of the essential amino acids such as lysine and methionine in poultry by-product meal, isoleucine in blood meal, and methionine in hydrolyzed feather meal, that if not corrected can impair fish performance (NRC, 1983; Tacon and Jackson, 1985). Nevertheless, the imbalance of amino acids could be overcome by proper ratio of various protein sources or supplementing synthetic forms of the amino acid to the feed. The use of renewable plant-based proteins has become the focus of protein substitution studies in aquatic feeds. This is due to their inexpensive cost, acceptable protein level, suitable amino acid content, and consistent quality (Watanabe, 2002). Soybean meal is the most widely-used protein source in commercial feed formulations for aquatic species because of its moderate protein content, high digestibility, acceptable-balanced amino acid profile, and reasonable price (Amaya *et al*., 2007a, b; Davis and Arnold, 2000; Hertrampf and Piedad-Pascual, 2000). However, soybean meal has low levels of methionine; therefore, a total replacement of fish meal with soybean meal generally results in a reduced growth performance of animals if methionine levels of the feed are not adjusted (Jackson *et al*., 1982). This study is in agreement with Dabrowski *et al*. (1989) who demonstrated that soybean meal inclusion above 50% of diet caused a decline in growth and amino acid absorption in rainbow trout. They determined that the activity of anti-nutritional factors such as trypsin inhibitor might have affected fish performance (Dabrowski and Kozak, 1979; Wilson and Poe, 1985). Distillers grains are co-products of the ethanol industry which are historically less expensive plant protein sources and can also be used as ingredients in aquatic feeds. Since 1989, DDGS price has been in the $80-90 per ton of dry matter, excluding the cost of transportation over the last several years; however, its cost varies with energy and corn prices.

In 2012, DDGS price is about $200 per ton which is lower than the cost of fish meal and soybean meal (Liu and Rosentrater, 2012). Advances in processing techniques have produced several types of co-products such as wet distillers grains (DWG), distillers dried grains (DDG), distillers dried solubles (DDS), and distillers dried grains with solubles (DDGS) (Bonnardeaux, 2007). These co-products have different nutrient compositions. For instance, DDS has a lower crude fiber content but a higher ash content as compared to DDG (Lim and Yildirim-Aksoy, 2008). With regards to vitamin and mineral content of corn-based distillers grains, DDS contains the highest concentrations of most vitamins and minerals, while DDG has the lowest, except for vitamin A, sodium, and sulfur, which are higher in DDG (Liu and Rosentrater, 2012).

In 2001, the United States produced about 3.1 million tons of DDGS. The production of DDGS has increased rapidly from 16.4 million tonnes in 2006 to 35.3 million tonnes in 2010 (Renewable Fuels Association, 2010). Therefore, DDGS has become a promising protein ingredient due to its low cost and abundant supply. Distillers dried grains with solubles is produced through a process of fermentation in which yeast converts starch to ethanol and carbon dioxide (Mustafa *et al*., 2000). Whole corn kernel that is fermented through this process, results in two primary products, ethanol and DDGS. Each bushel of corn (56 lb) is converted into approximately 18 and 17 lb of ethanol and DDGS, respectively (Jacques *et al*., 2003). After the fermentation process, nutrients except starch found in DDGS are available at a higher concentration compared to whole corn (Chevanan *et al*., 2007). Thus, DDGS can provide nutritional values to livestock animals and can be utilized as a promising ingredient in the commercial feed industry.

Cromwell *et al*. (1993) evaluated nutrient composition of DDGS and found that DDGS contains crude protein, fat, ash, acid detergent fiber, and neutral detergent fiber ranged from

26.0-31.7%, 9.1-14.1%, 3.7-8.1%, 11.4-20.8%, and 33.1-43.9%, on dry matter basis, respectively. Nutrient concentrations of DDGS except starch are higher than original grains about threefold (Han and Liu, 2010). However, some nutrients, Na, S, Ca, and P, have an increase higher than threefold due to the action of yeast during fermentation process on the grains (Liu and Han, 2011). Although protein content in DDGS is increased over that in original grains, the quality of protein in terms of amino acid composition is not improved over that of the original grains. Protein quality of DDGS is incomplete due to the low level of some essential amino acid contents particularly lysine (Liu and Rosentrater, 2012). Spiehs *et al*. (2002) analyzed DDGS contained about 30% crude protein 119 samples for 10 essential amino acids and reported that the average values of lysine, methionine, tryptophan, threonine, arginine, histidine, phenylalanine, isoleucine, leucine, and valine were 0.85, 0.55, 0.25, 1.13, 1.20, 0.76, 1.47, 1.12, 3.55, and 1.50%, respectively. The lipid profile of corn distillers grains is similar to that of corn. However, distillers grains contain higher amounts of free fatty acids than the original feedstocks (6-8% in DDGS and 1-2% in corn, based on extracted oil weight) (Winkler-Moser and Vaughn, 2009; Majoni and Wang, 2010; Moreau *et al*., 2010). A number of studies have been conducted to determine mineral composition in DDGS. Major minerals in DDGS are Ca, P, K, Mg, S, and Na, while minor minerals include Zn, Mn, Cu, Fe, Al, and Se. However, variation in mineral contents in DDGS is much wider than the composition of other nutrients due to an exogenous addition of some minerals mainly sulfur during processing. For example, ethanol plants may use sulfuric acid to adjust pH for optimum enzyme activity during liquefaction or meeting yeast requirements during fermentation (Spiephs *et al*., 2002; Batal and Dale, 2003; Belyea *et al*., 2006; Liu and Han, 2011).

Two production processes have been used in the ethanol industry; wet milling and dry grind process (Rausch and Belyea, 2006). Over 80% of the U.S. ethanol plants use the dry grain process because of its low investment and operational requirements as well as advances in fermentation technology (Renewable Fuels Association, 2010). In the dry grind processing, the whole grain is dry milled, and then mixed with water. The starch is converted into sugar by enzymes and fermented into ethanol by yeast (Bothast and Schlicher, 2005). In the wet milling process, kernel is fractionated into individual components of starch, protein, fiber, germ, and soluble solids using an aqueous medium. Wet mill separates the grain kernel into various fractions, which allows for the production of multiple products including ethanol, corn oil, and corn gluten meal (Blanchard, 1992). The production cost of DDGS obtained from the wet milling industry is higher than those from dry grind process; therefore, most of the U.S. ethanol plants currently use the dry grind process (Ramirez *et al*., 2009). Moreover, new technologies have been developed for extraction of oil in ethanol operation which produce lipid extracted-distillers dried grains with solubles (LE-DDGS). Removing of oil affects the nutritional profile of DDGS primary by reducing fat and energy content, and increasing protein concentration. The oil in DDGS has potential value as a third co-product from ethanol industry. It can be used in food and feed products, and as feedstock for biodiesel production (Liu and Rosentrater, 2012).

Processing conditions play an important role on DDGS quality. All steps of operation, including cooking, fermentation, distillation, centrifugation, evaporation, blending, and drying have effects on the resulting DDGS. However, some processing steps are more influential than others. For instance, in the drying process, an additional reduction of essential amino acid results from overheating relying upon temperatures and processing times used (Chevanan *et al*., 2005). The types of dryers may also affect the quality of DDGS. In the case of older generation plants using rotary dryers, DDGS is typically dark color, large particle size, and wide particle size distribution (Liu and Rosentrater, 2012). Size and shape of DDGS influence aquatic feed because they have effects on physical properties of diet including bulk density, compressibility, and flowability (Ganesan *et al*., 2008). Also, the ratio of condensed distillers solubles to wet distillers grains has impacts on both physical properties and chemical compositions of DDGS. As the levels of condensed distillers solubles increase, crude protein and non-digestible fiber values generally decrease. Conversely, crude fat and ash are increased with increasing levels of condensed distillers solubles (Liu and Rosentrater, 2012).

Sources and qualities of grain contribute to a wide variation of nutrient content in DDGS (Li *et al*., 2011). 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. Corn DDGS has been evaluated as an ingredient in feed for several fish species including rainbow trout (Cheng and Hardy, 2004), channel catfish (Tidwell *et al*., 1990; Webster *et al*., 1991, 1992, 1993; Robinson and Li, 2008; Lim *et al*., 2009; Li *et al*., 2010, 2011), sunshine bass (Thompson *et al*., 2008), and tilapia (Wu *et al*., 1994, 1996, 1997; Coyle *et al*., 2004; Lim *et al*., 2007; Shelby *et al*., 2008; Schaeffer *et al*., 2009). The amount of DDGS from sorghum produced by the U.S. ethanol industry is relatively small compared to corn DDGS. Sorghum DDGS is slightly higher in crude protein, acid detergent fiber, and ash, but lower in crude fat and lysine compared to corn DDGS. Currently, decortication which is an abrasive dehulling process has been used to remove non-fermentable fiber from sorghum grains. This process increases the quality of sorghum DDGS resulting in a higher in protein and lower in fiber (Corredor *et al*., 2006). Wheat is an important feedstock for fuel ethanol production in Canada, Europe, and Australia. Compared to corn, wheat DDGS has a higher protein level (38.0%) and ash (5.3%) and a lower in crude fat (4.6%) (Lim and Yildirim-Aksoy, 2008). The lower fat level leads to a reduced energy content compared to corn DDGS. Wheat DDGS has been primarily used in ruminant diets (Greter *et al*., 2008; Penner *et al*., 2009). In regards to barley, it has been only used sparingly for ethanol production due to several disadvantages. First, barley has silica in the hull that causes damage to grain handling and processing equipment. To solve this problem, hull needs to be removed by abrasive techniques such as pearling and scarification in order to improve the quality of barley DDGS (Wang *et al*., 1997; Flores *et al*., 2007). The second drawback is that barley has a low level of starch resulting in a lower ethanol production. However, breeding programs have developed to produce a higher starch and lower neutral detergent fiber barley (Griffey *et al*., 2010). The third limitation is a presence of β-glucan which causes high viscosity in aqueous solution. This has led to difficulties in mixing, pumping, saccharification, and fermentation; nevertheless, β-glucanase enzyme can be added in order to reduce viscosity.

Both corn and sorghum contain low concentrations of lysine compared to wheat and barley. However, distillers dried grains with solubles produced from these grains have a higher level of lysine than original grains due to the contribution of yeast protein (Liu and Rosentrater, 2012). Yeast is an important component of DDGS. Belyea *et al*. (2004) determined that yeast can contribute up to 50% of the protein in DDGS. Regarding essential amino acid profile, DDGS is also influenced by the source of grains. Amino acid composition of DDGS, compared to fish meal and soybean meal, varies; for instance, leucine content of DDGS from corn and sorghum are higher than soybean meal and fish meal. DDGS from corn, wheat, and sorghum have higher methionine content than soybean meal, but are lower than fish meal. In contrast, barley DDGS contains a lower level of methionine than soybean meal (Lim and Yildirim-Aksoy, 2008).

Although DDGS has been successfully utilized in aquatic feeds as an alternative plant protein source, the use of DDGS is still limited because of its low starch and high fiber content (Chin *et al*., 1989). The lack of starch has a detrimental effect on feed floatation. As starch content decreases, the expansion of diet during the extrusion process is reduced leading to poor floatation of feed. In regards to fiber content, corn DDGS has 10% crude fiber compared to fiber from fish meal (0.7%). As fiber is an indigestible ingredient, diets have a high level of fiber resulting in a low nutritional value and increased carbon loading of the culture system. Another disadvantage of increasing fiber is on the physical strength of feed, which will decrease as fiber content increases. Binders can be used to overcome issues with cohesiveness in sinking pellet (Chevanan *et al*., 2009) and/or fiber levels can be restricted. Fiber in DDGS can be separated by using air classification process. This method was able to remove pericarp from germ fraction.

Another disadvantage of using DDGS in feed formulation is due to the limitation of some essential amino acids, especially lysine and methionine. The amino acid level in fish meal, soybean meal, and DDGS are: lysine 50.0, 29.0, 6.5 g kg^{-1}, methionine 15.0, 5.5, 6.5 g kg^{-1}, respectively (Cheng and Hardy, 2003). Lysine in DDGS is highly susceptible to heat damage; therefore, lysine content and digestibility are primary concerns when using DDGS as a feed ingredient. Cromwell *et al*. (1993) indicated that lysine concentration was low in the dark-colored DDGS and high in the light-colored DDGS. Similarly, the digestibility of lysine in the darker DDGS was lower than that in the lighter DDGS. This resulted from the Maillard reaction, which destroyed a significant amount of lysine during excessive heating. Thus, color analysis can be used as an indicator estimating amino acid content in DDGS, primarily lysine (Batal and Dale, 2006). Comparing tilapia amino acid requirement with the average values of DDGS, it is demonstrated that lysine is the most limiting amino acid (Liu and Rosentrater, 2012). Therefore, supplementing lysine to improve the nutritional values of diets containing high levels of DDGS is necessary. Cheng and Hardy (2004) also reported that juvenile rainbow trout fed diet containing 15.0% DDGS produced a positive growth performance, while a concentration of 22.5% were acceptable with lysine and methionine supplementation at 0.41 and 0.14% of diet, respectively. Currently, nutritionists have been attempting to create a high protein and lysine DDGS by using an alkaline protein extraction along with the hydrolysis of the remaining fiber converted to sugar, which are then fermented to ethanol. They noted that alkaline extraction improved lysine concentration in DDGS (Bals *et al*., 2009).

A number of studies evaluating the addition of DDGS to aquatic diets have been reported since 1940s. Inclusion levels of DDGS in commercial diets vary with fish species, nutrients provided by other feed ingredients, and supplementation of deficient nutrients, particularly lysine (Li *et al*., 2011). DDGS is a promising protein source for several fish species such as rainbow trout (Cheng and Hardy, 2004), channel catfish (Tidwell *et al*. 1990; Webster *et al*. 1991, 1992, 1992b, 1993; Li *et al*. 2010), and tilapia (Wu *et al*. 1996; Lim *et al*. 2007). Compared to other aquaculture species, tilapia require a relatively low level of dietary protein. Furthermore, they can utilize high inclusion levels of plant-based proteins (Twibell and Brown, 1998). The use of DDGS in the diet of tilapia has received considerable attention with acceptable results. However, there is limited information available on the utilization in LE-DDGS which has different nutrient composition from DDGS, primary in terms of lipid and protein contents, in hybrid tilapia feeds. The overall objective of this research was to demonstrate the feasibility of using lipid extracted distillers dried grains with solubles (LE-DDGS) in practical feeds for the hybrid tilapia (*O. niloticus* × *O. aureus*). Two growth trials are conducted to identify the response of hybrid tilapia to increasing levels of LE-DDGS. Three objectives are included to identify final weight, weight gain, survival, feed conversion ratio, apparent net protein retention, and apparent net energy retention of hybrid tilapia to dietary treatments:

1. To evaluate final weight, weight gain, survival, feed conversion ratio, apparent net protein retention, and apparent net energy retention of hybrid tilapia to increasing levels of dietary LE-DDGS.

2. To evaluate final weight, weight gain, survival, feed conversion ratio, apparent net protein retention, and apparent net energy retention of hybrid tilapia to the use of high levels of LE-DDGS supplemented with lysine in practical diets.

3. To evaluate final weight, weight gain, survival, feed conversion ratio, apparent net protein retention, and apparent net energy retention of hybrid tilapia to the use of a high level of LE-DDGS in combination with lysine and lipid supplements in practical diets.

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CHAPTER II

LIPID EXTRACTED DISTILLERS DRIED GRAINS WITH SOLUBLES AS A PARTIAL REPLACEMENT FOR SOYBEAN MEAL IN HYBRID TILAPIA (*Oreochromis niloticus* × *Oreochromis aureus*)

Abstract

Two feeding trials were conducted using juvenile hybrid tilapia (*O. niloticus* × *O. aureus*) to evaluate the acceptability of lipid-extracted distillers dried grains with solubles (LE-DDGS) as a partial replacement for soybean meal. In the first trial, LE-DDGS was included at 0, 20, 30, 40, and 50 % as substitutes for soybean meal on an isonitrogenous basis (36% protein, 6% lipid). Additional two diets with 40 and 50% LE-DDGS were supplemented with lysine. A total of 700 fish (initial body weight of 6.08 ± 0.11 grams) were randomly stocked into 28 aquariums with 25 fish per aquaria. Four replicate aquariums were randomly assigned to each of the 7 dietary treatments. Fish were fed to apparent satiation twice per day for an 8-wk period. At the conclusion of the experiment mean final weight, percent weight gain, survival, and feed conversion ratio were evaluated. The results indicated that increasing percentages of LE-DDGS without lysine supplementation led to a reduced performance of tilapia. Fish offered the diet containing 40% LE-DDGS with lysine demonstrated an improved performance with no significant differences ($P \geq 0.05$) from fish fed the basal diet (0% LE-DDGS). Supplementing lysine to the diet containing 50% LE-DDGS did not result in an improved performance of the

fish. Consequently, a second growth trial was conducted with 500 fish (initial body weight 2.23 ± 0.10 grams) fed diets containing 0, 20, 40, and 50% LE-DDGS. Lysine was supplemented in the diet contained 40 and 50% LE-DDGS. An additional diet was formulated to contain 50% LE-DDGS and 8% lipid. Fish were fed based on percent body weight twice a day for a 12-wk period. Fish were randomly stocked into 20 tanks with 20 fish per tank. Four replicate tanks were randomly assigned to each of the 5 dietary treatments. The result demonstrated that there were no significant differences or notable trends in fish performance variables among test diets. Although not statistically significant, fish fed the diet with 50% LE-DDGS with lysine but without a lipid supplement performed the poorest. Supplementing this diet with 2% lipid favorably influenced the response of the fish.

Introduction

Over the last three decades, global aquaculture has grown substantially and production has increased by almost 12 times, at an average annual rate of 8.8%. World aquaculture production attained 60 million metric tons with an estimated total value of $119 billion in 2010 (FAO, 2012). 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 expand, 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, as well as phosphorus, vitamins, and trace minerals. Distillers dried grains with solubles also contains yeast and glucan which potentially improve immune system and resistance to some diseases in aquatic animals (Webster *et al*., 1993). Lim *et al*. (2009) demonstrated that diet containing 40% DDGS provided resistance to *Edwardsiella ictaluri* due to increased hemoglobin and hematocrit, increased total serum immunoglobulin, and increased antibody titers 21 day post-challenge in channel catfish. Many nutritionists have presumed that the factors contributing to these positive responses are biologically active compounds derived from yeast.

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. Moreover, concerns about environmental sustainability from aquaculture activities have increased tremendously during the last decade. Excessive waste of nutrients, particularly nitrogen and phosphorus, is the primary cause of pollution. The effective method to minimize phosphorus excretion is to decrease the levels of animal protein such as fish meal by using plant-based DDGS. Distillers dried grains with solubles is relatively moderate protein ingredient and contains much less phosphorus than fish meal; 6.6 and 17.0 phosphorus per kilogram in DDGS and fish meal, respectively (NRC 1993). Unlike soybean meal there is also no phytate, which reduces the bioavailability of phosphorus. Thus, distillers dried grains with soluble does not only minimize fish diet costs, but also reduce environmental pollution due to lower discharges of phosphorus and other minerals from fish industry.

Although DDGS contains moderate proportion of protein (270 g crude protein per kg), it usually contains lower levels of lysine, the most limiting amino acid, compared to fish meal (Cheng and Hardy, 2004). Therefore, it is 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, 1992; Wu *et al*. 1996, 1997; Lim *et al*. 2007; Belyea *et al*. 2004). In general, fish need a well-balanced mixture of essential amino acids. Tilapia require the same 10 indispensable amino acids: arginine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, and valine, as other fish. The requirement of lysine in tilapia is 5.12 % of dietary protein (Santiago and Lovell, 1988).

Nutrient compositions of DDGS varies considerably among the different methods used by each ethanol plant. As the ethanol industry has grown, a number of challenges associated with the utility of distillers grains have increased. New developments and technology advancements include new strains of more effective enzymes, cold cook technologies, improved drying systems, fractionation systems, and decreased pollutant emissions, have been introduced to improve the quality of the resulting DDGS and develop more value streams of the ethanol co-products. Several plants have been combining capacities to concentrate nutrient streams including oil, protein, and fiber into specific fractions, which can be used for targeted markets. For instance, food-grade corn oil can be produced by removing germ from corn kernel prior to ethanol process. However, if lipids are instead removed from the DDGS, they can be used to produce biodiesel (Rosentrater and Muthukumarappan, 2006). In 2010, the U.S. produced 13 billion gallons of ethanol, generating over 30 million metric tons of DDGS and 3 metric tons of oil. About 95% of commercial corn oil produced in the U.S., is extracted from corn germ from ethanol industry (USDA, 2009).

Currently, some ethanol plants are modifying their processing and extracting the lipids from DDGS resulting in a reduced lipid product. In addition, removing the oil increases the protein concentration of DDGS which makes it a more valuable feed component (Rausch and Belyea, 2006). A modified DDGS with lower fat and higher protein is desirable for aquaculture diet. As this is a relatively new process, there is limited data on these products. Thus, there is an interest in using lipid-extracted distillers dried grains with solubles (LE-DDGS) in tilapia feed formulations. The objective of this study is to evaluate the response of hybrid tilapia to increasing levels of LE-DDGS as replacement of soybean meal in practical diets for hybrid tilapia, and to determine if lysine supplement could improve their growth response.

Materials and Methods

First Experiment

Experimental diets

Seven isonitrogenous experimental diets (36% crude protein) were designed to replace soybean meal with LE-DDGS at increasing levels of inclusion (0, 20, 30, 40, and 50% of diet) on an equal protein basis (Table 1). Lysine was supplemented to diets containing the two highest levels of LE-DDGS (40, and 50% of diet) to determine if lysine was the limiting amino acid. All experimental diets contained a low level of fish meal (5%) and fish oil to help with palatability across the graded levels of LE-DDGS. Corn oil was adjusted to maintain similar lipid level (6%) among dietary treatments.

Test diets were manufactured at Auburn North Station, Auburn, AL under laboratory conditions. Feed ingredients and oil were placed in a food mixer (Hobart Corporation, Troy, OH, USA) for 15 min. 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 (Table 2 and 3).

Culture method

Hybrid tilapia (*O. niloticus* × *O. aureus*) juveniles were obtained from a commercial fingerling producer in Florida. Juveniles were reared in nursery tank before they were acclimated to the control diet for 2 weeks. The experiment consisted of seven treatments with four replicates. On stocking day, samples of 50 fish were individually weighed to determine average initial weight. Juvenile tilapia with 6.0 $\pm$ 0.11 g initial weight were placed into 50 L aquariums at a stocking density of 25 fish per aquarium in a flow through system. The first three days after stocking, fish mortalities were observed and re-stocked with reserve fish of similar weight. Aquariums were supplied with flow-through (0.6–1.0 L/min) heated dechlorinated municipal 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 aquariums every other day in the morning (0800h) using a YSI Model 58 Oxygen Meter (Yellow Springs Instrument Model 58, Yellow Springs, OH, USA).

Feeding

Fish were fed to apparent satiation twice daily (0800h and 1500h) for an 8-wk period. The quantity of feed consumed was recorded daily, calculating by the differences in diet weights prior the first and last feeding. Aquariums were scrubbed and solid wastes were siphoned out once weekly at which time fish were fed once in the afternoon. Every other week, fish from each aquarium were group weighed and counted to determine weight gain and survival. Feed was not offered to fish on sampling days.

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 Student–Neuman–Keuls multiple comparison test to evaluate significant differences among treatment means. All statistical analysis were carried out using SAS (V9.2 SAS Institute, Cary, NC, USA).

Second experiment

Experimental diets

The basal diet was designed to contain 36% protein and 6% lipid using primarily plant-based protein sources. The basal and experimental diets were formulated to meet the nutritional requirements of tilapia. The basal diet was modified to five diets with similar levels of protein, but with increasing levels of the LE-DDGS as well as diets with lysine supplements (Table 4). Experimental diets were formulated to contain increasing levels of LE-DDGS (0, 20, 40, and 50%) as substitutes for soybean meal on an equal protein basis. Lysine was supplemented in diets contained 40 and 50% LE-DDGS. Additionally, corn oil was added to the 50% LE-DDGS with lysine to increase the total lipid content to 8%. Corn oil was adjusted to maintain a similar lipid level across the diets, except diet 5. The test diets were prepared in the feed laboratory of Auburn University, Auburn, AL, USA using standard practices. Pre-ground dry ingredients and oil were mixed in a food mixer (Hobart Corporation, Troy, OH, USA) for 15 min. Hot water was blended into the mixture to attain an appropriate consistency for pelleting. Each diet was pelleted using a meat grinder and a 4-mm die. After pelleting, diets were dried to a moisture content of 8-10% and stored at -20° C until used. A sample of each feed was collected and analyzed for proximate and amino acid compositions following AOAC (1995) procedures by the New Jersey Feed Laboratory, Inc. (Table 5 and 6).

Culture method

Hybrid tilapia (*O. niloticus* × *O. aureus*) juveniles were obtained from a commercial fingerling producer in Florida. Juveniles were reared in nursery tank before they were stocked into experimental tanks. Commercial diets were applied twice per day at 0800h and 1500h. On stocking day, samples of 50 fish were individually weighed to determine average initial weight. Juvenile tilapia were placed into 150-L tanks at a stocking density of 20 fish per tank in a recirculating system. The first three days after stocking, fish mortalities were observed and re-stocked with reserve fish at the same size. Tanks were supplied with flow-through tap water maintained at a temperature of 28° C. Water was continuously aerated, and photoperiod maintained at 12:12 h light : dark schedule. Water temperature and dissolved oxygen were randomly be 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).

Feeding

Fish in four tanks were randomly assigned to each of the five experimental diets. Feed input was calculated between 5 to 7 % 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 and survival as well as readjust the daily feed input. On sampling day, fish were fed only once in the afternoon. At the conclusion of the 12-wk growth trial fish were counted and group weighed. Mean final weight, weight gain, feed conversation ratio, and survival were determined.

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 Student–Neuman–Keuls 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

First Experiment

Water quality parameters throughout the 8-wk period were within suitable ranges for the culture of this species (Table 7). The growth performance (final weigh, FW; weight gain, WG; feed conversion ratio, FCR; survival; apparent net protein retention, ANPR; and apparent net energy retention, ANER) were summarized in Table 8. The 0 % LE-DDGS treatment had the highest FW and was significantly higher than the other treatments, except the treatment 40% LE-DDGS with lysine supplement. The highest WG was obtained with 0% LE-DDGS treatment and had no significantly differences with 20%, 30 %, and 40% plus lysine supplement. 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 0% LE-DDGS treatment, and the highest FCR in the 50% LE-DDGS treatment. In regards to ANPR and ANER, there were no significant differences in any of the dietary treatments. ANPR and ANER ranged from 39.9 to 46.3 % and 34.8 to 43.0 %, respectively.

Second Experiment

Water quality conditions were within an acceptable range for adequate growth and survival of juvenile tilapia (Table 7). Growth performance of hybrid tilapia reared under circulating system was summarized in Table 9. The response of juvenile tilapia fed at increasing levels of LE-DDGS (0, 20, 40 + lysine, 50 + lysine, and 50% + lysine + 2% lipid) were not significantly different with regards to mean final weight (FW), weight gain (WG), feed conversion ratio (FCR), survival, apparent net protein retention (ANPR), and apparent net energy retention (ANER). At the conclusion of the production period, FW ranged from 55.82 to 65.55 g. WG ranged from 2,496.03 to 2,808.73 %. FCR ranged from 1.03 to 1.14. Survival ranged from 71.1 to 89.7%. ANPR and ANER ranged from 35.60 to 41.93 % and 32.06 to 33.89 %, respectively. The 50% LE-DDGS with lysine and lipid supplement treatment had the highest FW, WG, ANPR, and ANER, though these were not significantly different among treatments. The 20% LE-DDGS treatment had the lowest ANPR and ANER, and the 50% LE-DDGS with lysine supplement treatment had the lowest FW and WG.

Discussion

A number of fish feeding studies evaluating the efficacy of DDGS have been conducted. Distillers dried grains with solubles are produced from fuel ethanol and beverage alcohol cereal grain dry–grind processes. Nutrient composition of DDGS from beverage alcohol plants differs from DDGS produced at fuel ethanol plants. Corn DDGS-based fuel has a protein level up to 30% which is almost equal to 50% of the crude protein content found in menhaden fish meal (65% crude protein). The crude lipid content in DDGS is typically 11.0% which is similar to the level of lipid in menhaden fish meal (NRC, 1993). Proximate composition of DDGS has differences among varieties of original grains and production technology. Several studies have evaluated composition of DDGS in order to estimate nutritional value as a feedstuff (Chevanan, 2005; Rosentrater and Muthukumarappan, 2006). Cromwell *et al*. (1993) reported that DDGS had a level of crude protein at 26.9%, whereas Lodge *et al*. (1997) demonstrated that protein and lipid content in DDGS were 29.2% and 11.4% on dry weight basis, respectively. Belyea *et al*. (2004) analyzed DDGS samples from 1997 to 2001, and found that protein and lipid levels ranged from 28.3 – 33.3 % and 10.9 to 12.6 %, respectively. Likewise, Spiehs *et al*. (2002) evaluated DDGS samples from 1997 to 1999 and concluded an average protein and lipid concentration of 30.2% and 10.9%, which were higher than those reported in the past at the level of 28.1% protein and 8.2% 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 the lipids from DDGS resulting in an increased protein and reduced lipid content. According to this experiment, diet containing LE-DDGS resulted in a positive performance in hybrid tilapia. Although DDGS has relatively high fiber content, tilapia can utilize carbohydrates more efficiently than cultured piscivorous fishes (NRC, 1993; Lim and Webster, 2006). Anderson *et al*. (1984) proved that tilapia fed a diet containing glucose, sucrose, dextrin, and starch achieved higher weight gain compared to fish fed carbohydrate-free diet. Furthermore, El-Sayed and Garling (1988) also found that *Tilapia zillii* could utilize dextrin at the same level as Nile tilapia, but more efficiently than channel catfish. Although, there is no specific requirement for carbohydrate, high levels have been recommended for tilapia (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. Deyoe and Tiemeier (1969) stated that channel catfish could be fed diet containing 10% DDGS. Result from the first experiment indicated that diet inclusion rates of 20, 30, 40, and 50% LE-DDGS caused a reduced performance on juvenile tilapia growth. Phillips *et al*. (1964) concluded that trout fed a diet with 24% fish meal, 5% cottonseed meal, 10% brewer's yeast, and 21% DDGS had similar growth to trout fed a diet formulated from meat-meal mixtures. Moreover, broodstock female trout fed diet with DDGS had 86% total hatch and 88% survival to the eyed stage. However, the results from several studies demonstrated that the inclusion of DDGS in aquatic feed can be utilized up to 30% of diet. Wu *et al*. (1994) observed that tilapia fry fed a commercial diet (36% crude protein, fish-based protein) had a lower weight gain than fish fed diet containing 29% DDGS formulated with 36% crude protein. Coyle *et al*. (2004) concluded that DDGS at a level of 30% 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. They suggested that diet containing 30% DDGS also provided good growth, protein retention, and feed conversion in catfish. Likewise, Tidwell *et al*. (1990) demonstrated that channel catfish fed a 32% protein diet containing 30% DDGS, 8% menhaden fish meal, 30% soybean meal, and 8% corn had a similar performance in terms of final weight, feed conversion ratio, and total length as fish fed a diet with 8% menhaden fish meal, 50% soybean meal, and 38% corn. This result was confirmed by Webster *et al*. (1992) stated that diet containing a combination of plant-protein sources including 49.9% soybean meal, and 35% DDGS provided the same growth as a diet with 12% menhaden fish meal, and 48% soybean meal of diet.

Results from both experiments demonstrated that juvenile tilapia could effectively utilize crystalline lysine supplementation in diets containing high percentages of LE-DDGS. Based on the first feeding trial, fish fed diet containing 40% LE-DDGS without the addition of lysine had significantly lower final weights as compared to fish fed the control diet. However, the value of this variable did not differ when lysine was added to the 50 % LE-DDGS treatment. Regarding the second experiment, there were no significant differences between performance of fish fed control diet and diets containing 40% and 50% LE-DDGS with lysine supplementation. Similar to channel catfish, Robinson and Li (2008) evaluated a combination of DDGS and cotton seed meal as a replacement of soybean meal, and demonstrated that diet (29% crude protein) containing 30-40% DDGS with lysine supplement could be used to replace soybean meal without an adverse effect on performance of fingering catfish. Webster *et al*. (1991) fed channel catfish diets with increasing levels of DDGS (0, 35, and 70% of diet). They found that channel catfish fed a diet with 70% DDGS grew significantly less than other dietary treatments. However, fish fed diet containing 70% DDGS with 0.4% lysine had a similar growth compared to fish fed diets containing 0% and 35% DDGS. Shelby *et al*. (2008) reported the growth performance and immune function of tilapia fed diets (32.5% crude protein) with increasing levels of DDGS (0, 30, and 60%) as a replacement of a combination of soy and corn meals. This study showed that diet containing 60% DDGS with no lysine supplementation led to a negative growth performance of tilapia. The addition of lysine to the 60% DDGS diet improved fish weight gain to a level that was not significantly different from control fish. In regards to rainbow trout, Cheng and Hardy (2004) demonstrated that DDGS could be utilized as a partial replacement of fish meal up to 50% of diet. Nevertheless, if lysine was supplemented to diet, DDGS could be replaced as high as 75% of the fish meal in rainbow trout diet.

Distillers grains are moderate protein and energy sources, however, the information on energy value 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 diet, respectively. There is no data on the energy and protein digestibility of DDGS in tilapia; therefore, in these studies, the digestible energy of DDGS was calculated by using the numbers of rainbow trout as references. Cheng *et al*., 2004 conducted the 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 trouts (*Salmo gairdneri*) and reported that their digestibility of corn DGS was 71.9% for protein and 58.6% for carbohydrate. In channel catfish (*Ictalulus punctatus*), the apparent protein digestibility was 67.0% (Lovell, 1977). In the first growth trial, the addition of crystalline lysine to diet containing 50% LE-DDGS did not improve fish growth. Fish fed diet containing 50% LE-DDGS with lysine supplement obtained FE, ANPR, and ANER lower than fish fed the basal diet, indicating a limitation of other nutrient. DDGS contains relatively high crude fiber resulting in a limited use as an energy source. In general, protein and lipid are the primary sources of energy. If insufficient non-protein energy intake exists, protein will be utilized for energy and leads to a reduced growth. An earlier study reported that Nile tilapia fry exhibited the best growth with a protein to energy ration of 110 mg/kcal (El-Sayed and Teshima, 1992). Furthermore, Kubaryk (1980) pointed out that the protein to energy ratios between 108 to 120 mg/kcal 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, the ratio of digestible energy to protein generally decreased approximately from 9.0 to 7.5 kcal/g crude protein or 111.1 to 133.3 mg crude protein/ kcal (Table 10). In the second experiment, the ratio of digestible energy to protein was increased by adding 2% lipid to the diet containing 50% LE-DDGS with lysine from 7.94 to 8.27 kcal/g digestible protein (Table 11). Thus, the poor growth of fish fed diets containing increasing level of LE-DDGS observed in this study is likely due to deficient in dietary digestible energy. As lipid content in diet increased from 6 to 8%, weight gain of juvenile tilapia improved more than 300% compared to fish fed diet contained 50% LE-DDGS with lysine. Stickney and Wurts (1986) stated that the performance of *O. aureus* could be improved as fish oil was provided at 7.5-10.0% of the diet; however, best growth was achieved with menhaden oil at 10% of the diet. Chou and Shiau (1996) observed that 5% dietary lipid was sufficient to meet the minimal requirement of juvenile hybrid tilapia (*O. aureus* × *O. niloticus*), but a level of 12% was needed for maximal growth. Jauncey and Ross (1982) reported that the diet containing lipid in excess of 12% 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 40 % provided that lysine was supplemented. Furthermore, results indicate that if 50% LE-DDGS are utilized energy may be limiting and that increasing the digestible energy content of the diet may improve performance. These results confirm that high levels of LE-DDGS can be utilized in practical feed formulation and that there use in practical diets should be encouraged.

Table 1. Ingredient composition (g 100 g^{-1} as is) of seven experimental diets used in trial I containing increasing percentages of LE-DDGS (0, 20, 30, 40, and 50) as well as lysine supplement in diets with high levels of LE-DDGS (40 and 50% of diet) as a substitute for soybean meal

Ingredient	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5	Diet 6	Diet 7
Menhaden fishmeal[1]	5.00	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	29.50	24.20
DDGS-lipid extracted[3]	0.00	20.00	30.00	40.00	50.00	40.00	50.00
Menhaden fish oil[1]	0.82	0.82	0.82	0.82	0.80	0.82	0.82
Corn oil	2.92	1.74	1.15	0.56	0.00	0.57	0.00
Whole wheat[4]	33.06	24.74	20.58	16.42	12.25	16.58	12.58
Trace mineral premix[5]	0.50	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	0.80
Choline chloride[4]	0.20	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	0.10
Dicalcium phosphate[8]	2.30	1.80	1.55	1.30	1.05	1.30	1.05
Soy lecithin[9]	0.50	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	4.00
L-Lysine HCl[11]	0.00	0.00	0.00	0.00	0.00	0.13	0.25

[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: Cobalt chloride, Cupric sulfate pentahydrate, Furrous sulfate, Magnesium sulfate anhydrous, Manganous sulfate monohydrate, Potassium iodide, Sodium selenite, Zinc sulfate hepahydrate, cellulose
[6] Vitamin: Thiamin HCl, Riboflavin, Pyridoxine HCl, DL pantothenic acid, Nicotinic acid, Biotin, Folic acid, Vitamin B_{12}, Inositol, Vitamin A acetate, Vitamin D_3, Vitamin E, cellulose
[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 2. Nutrient composition (g 100 g^{-1} as is) of the experimental diets containing increasing percentages of LE-DDGS (0, 20, 30, 40, and 50) as well as lysine supplement in diets with high levels of LE-DDGS (40 and 50% of diet)

Component	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5	Diet 6	Diet 7
Moisture	8.56	6.37	7.62	5.42	6.70	6.04	6.81
Crude Protein	35.70	37.80	36.70	37.40	36.60	37.10	36.80
Crude Fat	6.61	6.86	6.33	6.19	6.57	6.94	6.37
Crude Fiber	2.69	4.03	3.52	3.79	4.62	4.30	2.79
Ash	6.69	6.72	6.25	6.40	6.66	6.52	6.17
Sulfur	0.37	0.50	0.57	0.64	0.71	0.66	0.72
Phosphorus	1.32	1.27	1.28	1.28	1.27	1.29	1.27
Potassium	1.29	1.42	1.46	1.47	1.50	1.48	1.48
Magnesium	0.22	0.26	0.29	0.31	0.33	0.31	0.33
Calcium	1.33	1.04	0.93	0.88	0.77	0.87	0.76
Sodium	0.07	0.12	0.15	0.18	0.20	0.18	0.21

Diets were formulated to contain 36% protein and 6% lipid.

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

Table 3. Amino acid composition (g 100 g^{-1} as is) of the experimental diets containing increasing percentages of LE-DDGS (0, 20, 30, 40, and 50) as well as lysine supplement in diets with high levels of LE-DDGS (40 and 50% of diet)

Component	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5	Diet 6	Diet 7
Alanine	1.52	1.72	1.88	2.08	2.22	1.87	2.07
Arginine	2.39	2.19	2.29	2.29	2.06	1.99	2.20
Aspartic Acid	3.40	3.08	2.83	2.93	2.71	2.75	2.85
Cystine	0.54	0.79	0.83	0.65	0.65	0.65	0.69
Glutamic Acid	6.56	6.53	6.25	6.31	6.15	6.00	6.23
Glycine	1.45	1.48	1.49	1.54	1.55	1.39	1.50
Histidine	0.90	1.06	0.92	1.11	1.17	1.14	1.06
Isoleucin	1.41	1.49	1.45	1.41	1.36	1.35	1.37
Leucine	2.69	2.97	3.07	3.23	3.18	3.12	3.31
Lysine	1.83	1.82	1.73	1.72	1.61	1.69	1.81
Methionine	0.53	0.63	0.66	0.66	0.66	0.64	0.66
Phenylalanine	1.61	1.68	1.82	1.72	1.51	1.59	1.63
Proline	2.28	2.52	2.57	2.74	2.66	2.53	2.73
Serine	1.83	1.75	1.53	1.78	1.59	1.65	1.78
Threonine	1.46	1.38	1.28	1.39	1.29	1.26	1.37
Tyrosine	1.33	1.35	1.47	1.48	1.31	1.37	1.42
Tryptophan	0.31	0.34	0.31	0.27	0.30	0.25	0.30

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

Table 4. Ingredient compositions (g 100 g^{-1} as is) of five experimental diets used in trial II containing increasing percentages of LE-DDGS (0, 20, 40, and 50) with lysine supplement in diets with high levels of LE-DDGS (40 and 50% of diet) as well as higher lipid supplement in diet containing 50% LE-DDGS with lysine supplement

Ingredient	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5
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
Whole wheat[4]	33.55	25.18	17.07	12.61	9.73
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
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

[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: Cobalt chloride, Cupric sulfate pentahydrate, Furrous sulfate, Magnesium sulfate anhydrous, Manganous sulfate monohydrate, Potassium iodide, Sodium selenite, Zinc sulfate hepahydrate, cellulose
[6] Vitamin: Thiamin HCl, Riboflavin, Pyridoxine HCl, DL pantothenic acid, Nicotinic acid, Biotin, Folic acid, Vitamin B_{12}, Inositol, Vitamin A acetate, Vitamin D_3, Vitamin E, cellulose
[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 5. Nutrient composition (g 100 g^{-1} as is) of the experimental diets containing increasing percentages of LE-DDGS (0, 20, 40, and 50) with lysine supplement in diets with high levels of LE-DDGS (40 and 50% of diet) as well as lipid supplement in diets containing 50% LE-DDGS with lysine supplement

Component	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5
Moisture	9.66	9.95	10.14	11.30	11.68
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
Ash	7.18	7.15	6.71	6.89	6.62
Sulfur	0.37	0.50	0.57	0.64	0.71
Phosphorus	1.33	1.32	1.26	1.31	1.20
Potassium	1.21	1.24	1.24	1.25	1.15
Magnesium	0.22	0.25	0.28	0.31	0.28
Calcium	1.29	1.13	0.89	0.89	0.82
Sodium	0.07	0.10	0.14	0.17	0.15

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

Table 6. Nutrient composition (g 100 g^{-1} as is) of the experimental diets containing increasing percentages of LE-DDGS (0, 20, 40, and 50) with lysine supplement in diets with high levels of LE-DDGS (40 and 50% of diet) as well as lipid supplement in diets containing 50% LE-DDGS with lysine supplement

Component	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5
Alanine	1.51	2.05	2.16	2.37	2.07
Arginine	2.28	2.30	2.02	1.74	1.95
Aspartic Acid	2.87	3.39	2.76	2.58	2.38
Cystine	0.49	0.54	0.54	0.57	0.53
Glutamic Acid	6.01	6.57	6.23	6.20	5.63
Glycine	1.30	1.53	1.38	1.49	1.42
Histidine	0.99	0.97	0.90	0.82	0.75
Isoleucin	1.34	1.50	1.35	1.41	1.19
Leucine	2.59	2.98	3.08	3.23	3.04
Lysine	1.78	1.88	1.68	1.83	1.77
Methionine	0.56	0.63	0.61	0.64	0.61
Phenylalanine	1.67	1.90	1.71	1.71	1.64
Proline	2.24	2.42	2.49	2.55	2.31
Serine	1.81	2.19	1.67	1.57	1.62
Threonine	1.23	1.44	1.36	1.33	1.36
Tyrosine	1.32	1.41	1.49	1.52	1.33
Tryptophan	0.29	0.29	0.25	0.28	0.21

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

Table 7. Summary of water quality variables for growth trials with hybrid tilapia 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)				
DO (mg L^{-1})[a]	6.06	0.46	4.50	6.80
Temperature (˚C)	27.68	0.73	25.0	29.2
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-N (mg L^{-1})	0.41	0.40	0.03	1.35

[a]Dissolved oxygen.

[b]Total ammonia-nitrogen

Table 8. Response over a 8-wk growth period for hybrid tilapia (6.0 ± 0.11 g, initial weight), fed diets containing of LE-DDGS (0, 20, 30, 40, and 50% of diet) as a substitute for soybean meal, reared under a flow-through system in indoor tanks

Diet	FW (g)	WG (g)	WG (%)	Survival (%)	FCR[a]	ANPR[b] (%)	ANER[b] (%)
0% LE-DDGS	81.38[a]	75.30[a]	1238.15[a]	100.0	0.91[a]	46.3[a]	43.0[a]
20% LE-DDGS	76.47[b]	70.38[ab]	1157.08[ab]	100.0	0.93[a]	41.8[b]	39.5[b]
30% LE-DDGS	75.58[b]	69.56[ab]	1156.27[ab]	100.0	0.94[a]	42.6[b]	37.6[b]
40% LE-DDGS	73.50[b]*	67.46[b]*	1118.30[b]*	98.0	0.97[ab]*	41.3[b]	36.3[b]
50% LE-DDGS	72.30[b]*	66.24[b]*	1094.46[b]*	98.0	1.02[b]*	39.9[b]	34.8[b]
40% LE-DDGS + Lys	77.01[ab]	70.98[ab]	1176.30[ab]	100.0	0.93[a]	43.4[b]	37.4[b]
50% LE- DDGS + Lys	72.02[b]*	66.15[b]	1125.62[b]*	99.0	0.97[ab]*	40.6[b]	36.1[b]
P-value[c]	0.0047	0.0044	0.0032	0.6500	0.0004	0.0007	0.0009
PSE[d]	0.59	1.52	8.10	0.43	0.01	0.33	0.42

[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 9. Response over a 12-wk growth period for hybrid tilapia (2.23 ± 0.11 g, initial weight), fed diets containing of LE-DDGS (0, 20, 40, and 50% of diet) as a substitute for soybean meal, reared under a closed recirculating system in indoor tanks

Diet	FW (g)	WG (g)	WG (%)	Survival (%)	FCR[a]	ANPR[b] (%)	ANER[b] (%)
0%LE-DDGS	63.15	60.89	2701.26	77.1	1.08	38.03	32.66
20%LE-DDGS	61.03	58.82	2672.92	87.42	1.14	35.60	32.06
40%LE-DDGS+ Lys	61.09	58.86	2645.15	89.75	1.08	38.51	33.89
50%LE-DDGS+ Lys	55.82	53.64	2469.03	85.00	1.04	40.03	32.73
50%LE-DDGS + Lys+2% Lipid	65.55	63.28	2808.73	78.3	1.05	40.45	34.25
P-value[c]	0.47	0.90	0.78	0.40	0.53	0.32	0.66
PSE[d]	1.67	1.03	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 10. Estimated energy and protein contain, as well as digestible energy, digestible protein, and ratio of digestible energy to digestible protein in diets containing increasing percentages of LE-DDGS (0, 20, 30, 40, and 50)

Diet	GE[a] (kcal/g)	CP[b] (%)	DP[c] (%)	DE[d] (kcal/g)	DE:CP (kcal/g)	DE:DP (kcal/g)
0% LE-DDGS	4.31	35.70	29.98	3.23	9.05	10.77
20% LE-DDGS	4.49	37.80	31.05	3.03	8.04	9.78
30% LE-DDGS	4.67	36.70	31.59	2.94	8.01	9.31
40% LE-DDGS	4.61	37.40	32.12	2.84	7.61	8.86
50% LE-DDGS	4.67	36.60	32.66	2.75	7.51	8.42
40% LE-DDGS+Lys	4.53	37.10	32.01	2.85	7.68	8.90
50% LE-DDGS+Lys	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 11. Estimated energy and protein contain, as well as digestible energy, digestible protein, and ratio of digestible energy to digestible protein in diets containing increasing percentages of LE-DDGS (0, 20, 40 and 50)

Diet	GE[a] (kcal/g)	CP[b] (%)	DP[c] (%)	DE[d] (kcal/g)	DE:CP (kcal/g)	DE:DP (kcal/g)
0% LE-DDGS	4.43	35.70	29.64	3.04	8.52	10.26
20% LE-DDGS	4.45	37.80	30.74	2.85	7.54	9.26
40% LE-DDGS+Lys	4.68	36.70	31.72	2.66	7.24	8.38
50% LE-DDGS+Lys	4.56	37.40	32.18	2.55	6.83	7.94
50%LE-DDGS+Lys+2%Lip	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

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CHAPTER III

SUMMARY AND CONCLUSIONS

High feed ingredient prices around the world have encouraged nutritionists to search for lower cost alternative feed ingredients to minimize cost of production. Distillers dried grains with solubles is a low cost alternative protein source used in animal feeds. In general, distillers dried grains with solubles is used as a partial replacement for some of the more expensive ingredients which could contribute to reduce feed prices as compared to fish meal or soybean meal. Its high energy, moderate protein, high digestible phosphorus content, and low cost of DDGS make it very attractive in aquatic feed industries. Furthermore, distillers dried grains with solubles does not contain anti-nutritional factors found in other protein sources such as soybean meal and cottonseed meal. Although DDGS has a moderately high protein content, supplemental lysine is needed at high dietary inclusion rates in order to meet animal requirements because of the relatively low levels of this amino acid in DDGS. Since DDGS is added to properly formulated feeds, it attributes to a positive fish performance. The variability in nutrient composition and digestibility among DDGS sources can be a challenge for nutritionists in evaluating economic and feeding value for aquatic animals. Different sources of feedstuffs such as corn, wheat, barley, or sorghum are used to produce ethanol and DDGS. The nutrient compositions of the resulting DDGS also vary considerably depending on the sources of grains. These shifts must be considered when using DDGS produced from different grain sources. Currently, there are no grading methods that define the quality standard for DDGS.

However, the color of DDGS has been used as an indicator to determine the quality of DDGS. A dark colored DDGS may indicate reduced essential amino acid contents due to heat damage during the process.

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 has been introduced to produce an increased protein and reduced lipid product. The use of 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 lipid extracted distillers dried grains with solubles as a partial replacement for soybean meal in hybrid tilapia. The first study determined tilapia performance fed increasing levels of LE-DDGS (0, 20, 30, 40, and 50% of diets), as well as with lysine supplementation at the levels of 40 and 50% LE-DDGS over an 8-wk period. In the second study, experimental diets were formulated to contain increasing levels of LE-DDGS (0, 20, 40, and 50% of diets). Lysine was supplemented in the diets containing 40 and 50% LE-DDGS. Additionally, a diet was formulated with 50% LE-DDGS and 8% lipid. Fish performance was evaluated for a 12-wk period. Results from these studies confirmed that LE-DDGS was found to be an acceptable protein ingredient in hybrid tilapia feed. Fish fed diet using high levels of LE-DDGS, up to 40% of, diet with lysine supplementation obtained a positive performance without affecting growth, feed conversion ratio, survival, apparent net protein retention, and apparent net energy retention. Moreover, the inclusion of LE-DDGS at 50% of the total diet with lysine and energy supplements also provided good growth in hybrid tilapia. In conclusion, LE-DDGS with lysine supplement can be used as a promising protein source in combination with soybean meal in hybrid tilapia diets when feeds were formulated to contain well balanced of nutrients.