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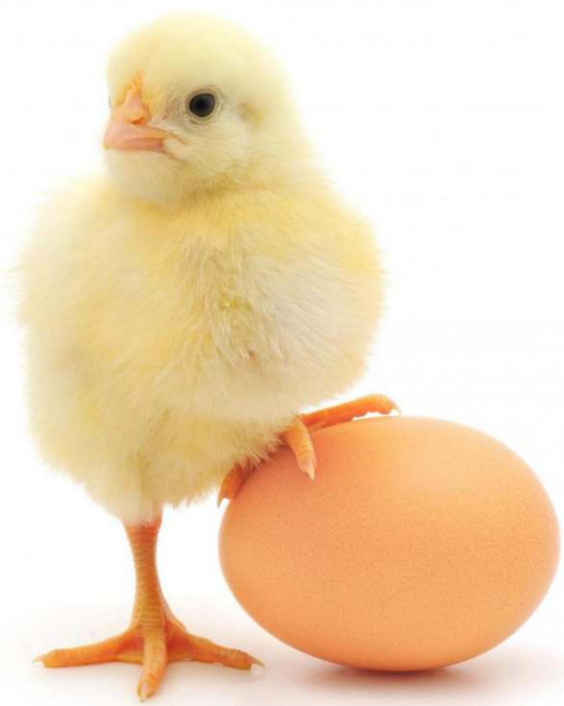

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Use of Distillers Dried Grains with Solubles (DDGS) as Replacement for Soybean Meal in Laying Hen Diets

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Abstract: An experiment was conduced to evaluate the inclusion of Distillers Dried Grains with Solubles (DDGS) in commercial layer diets with Avizyme 1500® supplementation. Two hundred eighty-eight 40-week-old Bovans brown layers were distributed in a completely randomized experimental design in a 2 x 4 factorial arrangement, with the variables being (DDGS) substitution for soybean meal at four levels (0 or 25% or 50% or 75%) and Avizyme 1500 at two levels (0 or 0.075%). Layer performance, egg quality, nutrients digestibility and blood parameters were evaluated. Results showed that average egg production, egg weight, egg mass, feed conversion ratio and the change in body weight of laying hens were significantly ($p<0.05$) decreased as dietary inclusion of DDGS increased. Avizyme supplementation relatively improved ($p<0.05$) egg production, egg mass and feed conversion ratio for DDGS inclusion levels at 25 and 50% when compared to those fed diets without Avizyme supplementation. No significant effect of DDGS, Avizyme, or their interaction on feed intake was observed. Yolk color was significantly increased, when DDGS was included in the diet. No significant differences in digestion coefficient values of Dry Matter (DM), Organic Matter (OM), Ether Extract (EE) and Nitrogen Free Extract (NFE) while, there were significant differences ($p<0.05$) for Crude Protein (CP) and Crude Fiber (CF) digestibility. Dietary inclusion of DDGS without Avizyme linearly reduced cost of feed. Although Avizyme supplementation increased the cost per kilogram of formulated diet, but improves economical efficiency value. The results suggest that diets containing DDGS at less than 15.45% level (50% of SBM) with Avizyme 1500® supplementation could improve the nutritive value of DDGS for layers.

Key words: Distillers dried grains with solubles, avizyme supplementation, laying hen performance

INTRODUCTION

Feed is the main cost component of poultry production and often accounts for 60-65% of total costs of commercial egg production. Soybean meal is the conventional protein source in layer diets and it is influenced by external fluctuations. Increasing ingredient prices remain the greatest single item that determines profit margins in poultry production. The best strategy to reduce costs is the development of diet formulation using alternative, locally available, ingredients, thereby decreasing feed costs. Corn DDGS is a well-established and relatively inexpensive protein source for poultry diets (Harpster, 2007).

Distillers Dried Grains with Solubles (DDGS) is a by-product of ethanol production. Because of the high content of readily fermentable starch, corn is the main grain used in the fuel ethanol industry. During fermentation, approximately equal portions of ethanol, DDGS and CO_2 are formed (Lumpkins *et al.*, 2005). Because corn starch is converted to ethanol and CO_2, the concentration of the remaining nutrients in DDGS increases 2-3 folds. Use of DDGS in poultry diets was primarily as a source of "unidentified growth factors" at low inclusion levels (Noll *et al.*, 2001). In previous studies on laying hens, it was shown that DDGS could be used at 5-20% inclusion levels in diets, even as a source of one-third of the protein supply, without affecting performance (Matterson *et al.*, 1966; Harms *et al.*, 1969; Jensen *et al.*, 1974) and had a positive effect on Haugh unit values (Jensen *et al.*, 1978).

Distillers dried grains with solubles are higher in Nonstarch Polysaccharides (NSP), crude protein, crude fat and minerals than the parent grain. However, mono-gastric do not digest feedstuffs high in NSP efficiently. As a result, the ME of DDGS is lower than in corn (2,820 vs. 3,420 kcal/kg, based on moisture content adjustment) (Wang *et al.*, 2007; NRC, 1994). However, supplementing monogastric diets with exogenous enzymes may improve the available energy of DDGS by degrading the fiber content and increasing the digestibility of other components. Amylase improves starch digestion, xylanase reduces gut viscosity and breaks down cereal cell walls and protease affects soybean meal anti-nutritional factors and storage proteins (Graham and Aman, 1991; Muramatsu *et al.*, 1991).

Several studies have shown beneficial effects of supplemental enzymes on feed intake, egg production, egg weight, egg mass and egg-specific gravity when added to laying hen diets (Francesch *et al.*, 1995; Pan *et*

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al., 1998; Jaroni *et al*., 1999). Recent studies have suggested that the addition of Avizyme 1500, an enzyme product containing a mixture of xylanase, protease and amylase, improved nutrient utilization, bird performance and reduced feed costs in broilers and layers (Zanella *et al*., 1999; Douglas *et al*., 2000; Sohail *et al*., 2003; Scheideler *et al*., 2005). The objective of the current experiment was to study the effect of partial replacement of graded levels of corn Distillers Dried Grains with Solubles (DDGS) for soybean meal on egg production, egg quality, digestibility and blood parameters in laying hens. The possibility of improving the nutritional value of DDGS containing diets by using Avizyme supplementation was also evaluated.

MATERIALS AND METHODS

The experimental work was carried out at Poultry Nutrition Research Station, Animal Production Department, Faculty of Agriculture, Cairo University.

Birds, diets and experimental design: Two hundred eighty-eight Bovans brown laying hens were used in this experiment. A completely randomized design was used, with three replications of 12 birds each; three birds were housed per (45 x 45 x 45 cm) wire cage, given feed and water for *ad libitum* intake and subjected to a photoperiod of 16 h light/day. Hens were equally distributed in the upper and lower cage levels to minimize cage-level effect. Each experimental diet was formulated to meet nutrients recommendation of Bovians brown management guide which met or exceeded the NRC (1994) recommendations. Experimental diets were isocaloric (2900 kcal ME/kg) and isonitrogenous (18% CP). The hens were fed experimental diets between 40-56 weeks of age. The diets were arranged in a 2 x 4 factorial design, with the variables being Dried Distillers Grains with Solubles (DDGS) substituted for soybean meal at four levels (0 or 25% or 50% or 75%) and Avizyme 1500® supplementation at two levels (0 or 0.075%). The diets were fed as a mash. Diets formulation and their analysis are shown in Table 1. Avizyme 1500® the exogenous cellulolytic enzyme was purchased from the nutritional additive company in Egypt (multivita Co.) and added at 0.075% being supplied with 150U glucanase, 300U xylanase, 400U amylase, 25U polygalacturonase and 4000U protease/kg diet.

Measurements: Measurements during the experiment included body weights which were recorded at the beginning and the end of the study to determine body weight changes. The number of eggs laid, egg weights and feed consumption (g/hen) were recorded daily and accordingly, the egg production (%), egg mass (g/hen/d) and feed conversion (g feed/g egg mass) were calculated. To measure egg quality, fifteen eggs per treatment were sampled monthly; first the egg was weighed, next the egg length (long axis) and width (short axis) were measured with an electronic caliper. The width to length ratio was shown in percentage points and constituted the egg shape index. After the removal of the egg content, the eggshell was dried. Subsequently, the eggshell was weighed to the nearest 0.01g. Eggshell thickness without the inner membranes was measured (mm) with the micrometer. Haugh unit was measured using the formula of Eisen *et al*. (1962):

$$\text{Haugh units} = 100 \text{ Log } \frac{[H - \sqrt{G}(30W^{0.37} - 100) + 1.9]}{100}$$

Where H = Thick albumen height (mm), W = Egg weight, G= Gravitional constant (32.2).

Yolk index was calculated as yolk diameter/yolk height and albumen index as albumen height/average of short and long diameter of albumen. Yolk colour score was measured with Roche Yolk Colour Fan (Vuilleumier, 1969).

Digestion trial: At the end of the experiment, five hens from each treatment were placed into individual balance cages. After one-week adaptation period, a total collection of excreta was carried out over 5 days and feed consumption for each hen was recorded. The excreta were stored in plastic bags at -20°C for two weeks and after thawing, were dried in an oven at 60°C to a constant weight, then weighed and finely ground and placed in a screw-top glass jars until analysis. The procedure described by Jakobson *et al*. (1960) was used for separating fecal nitrogen from urine nitrogen in excreta samples. The chemical analysis of CP, EE and CF content of the excrement as well as that of the feed were determined according to AOAC (1990) and expressed on a dry matter basis. The apparent digestibility of Crude Protein (CP), fat (EE), fiber (CF) and OM was calculated by dividing the daily amount retained (g/d) by the amount of intake (g/d). The daily amount retained is equal to the amount of intake (% nutrient in feed x amount of feed consumed) minus that voided in the excreta (% nutrient in excreta, except for nitrogen of which the fecal nitrogen was used x amount of dry matter excreta voided).

Blood analyses: At the end of the experiment, blood samples were collected from 5 birds per treatment for haematological and serum biochemical analysis. Haematological samples were collected into sample tubes containing Ethylene Diaminetetraacetic Acid (EDTA) as an anticoagulant while samples for serology were collected into anticoagulant free tubes. A portion of fresh blood was used for hematocrit (PCV) determination using capillary tubes and a microhematocrit centrifuge. The hematocrit figures were

Table 1: Composition and analysis of experimental diets

	Replacement level of corn DDGS for soybean meal (%)							
	No Avizyme supplementation mg/kg				Avizyme supplementation 750 mg/kg			
Ingredients%	0	25	50	75	0	25	50	75
Yellow corn	52.53	51.50	49.30	46.50	52.53	51.50	49.30	46.50
Soybean meal (44%)	30.90	23.17	15.45	7.72	30.90	23.17	15.45	7.72
Corn DDGS*	0.00	7.72	15.45	23.17	0.00	7.72	15.45	23.17
Gluten meal (60%)	0.00	2.20	4.65	7.00	0.00	2.20	4.65	7.00
Soybean oil	5.65	4.85	4.45	4.30	5.65	4.85	4.45	4.30
Limestone	9.37	9.20	9.41	10.10	9.37	9.20	9.41	10.13
Di-calcium phosphate	0.80	0.55	0.40	0.16	0.80	0.55	0.40	0.18
NaCl	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30
Vit.-min. premix**	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30
DL-methionine	0.15	0.11	0.10	0.10	0.15	0.11	0.10	0.10
L-lysine HCL	0.00	0.10	0.19	0.35	0.00	0.10	0.19	0.30
Total	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
Calculated analysis[1]								
Metabolizable energy (kcal/kg)	2900.00	2900.00	2901.00	2902.00	2900.00	2900.00	2901.00	2902.00
Crude protein (%)	18.06	18.04	18.05	18.00	18.06	18.04	18.05	18.00
Crude fiber (%)	3.32	3.49	3.63	3.76	3.32	3.49	3.63	3.76
Available phosphorus (%)	0.39	0.39	0.40	0.40	0.39	0.39	0.40	0.40
Calcium (%)	3.83	3.72	3.79	4.00	3.83	3.72	3.79	4.00
Methionine (%)	0.44	0.43	0.45	0.47	0.44	0.43	0.45	0.47
Lysine (%)	0.96	0.93	0.89	0.92	0.96	0.93	0.89	0.87
Determined analysis[2] (%)								
Dry matter	89.81	89.56	89.79	89.68	89.81	89.56	89.79	89.68
Crude protein	17.83	17.92	17.23	18.22	17.83	17.92	17.23	18.22
Crude fat	4.68	5.11	5.73	6.31	4.68	5.11	5.73	6.31
Crude fiber	3.51	3.79	4.18	5.22	3.51	3.79	4.18	5.22

*Chemical composition of corn DDGS used in the experiment analyzed according to AOAC (1990), was: %: dry matter 91.2; crude protein 27.79; crude fat 10.32; crude fiber 8.34; crude ash 4.36; calcium 0.1; phosphorus 0.73.

**Vitamin and trace minerals provided the following per kilogram: vitamin A (retinyl acetate, 6,600 IU); vitamin E (DL-α-tocopheryl acetate, 10 IU); vitamin K3 (menadione dimethpyrimidinol, 2.0 mg); riboflavin (4.4 mg); pantothenic acid (6.6 mg); niacin (24.2 mg); choline (110 mg^{-1}); vitamin B7 (biotin, 8.8 mg^{-1}) and ethoxyquin (1.1 mg/kg). Mn (MnO, 88 mg); Cu ($CuSO_4H_2O$, 6.6 mg); Fe ($FeSO_4H_2O$, 8.5 mg); Zn (ZnO, 88 mg) and Se (Na_2SeO_3, 0.30 mg).

[1]Calculated values (NRC, 1994)

[2]Determined values (AOAC, 1990)

measured after spinning the microhematocrit for 12 min. Blood Haemoglobin (Hb) and serum total protein were determined according to Henry *et al.* (1974), serum albumin was determined according to Doumas *et al.* (1977). Serum globulin was calculated by subtracting albumin from total protein. Plasma total lipids was determined according to Eisemann *et al.* (1986), IgG and IgM were determined according to Fahey and Mackelvey (1965) and Cho and Kramer (1970).

Economical efficiency: The experimental treatments were economically evaluated based upon the local market prices at year 2009, in terms of the feed cost consumed during experiment and the total egg number produced.

Statistical analysis: Data were analysed using the general linear model procedure in SAS (2004). A 4 x 2 factorial design was used to analyze data of performance as a response to four levels of DDGS and two levels of Avizyme 1500. Differences among treatment means were detected using two way Analysis of Variance (ANOVA). Duncan's Multiple Range Test was applied to separate means (Duncan, 1955). Statements of statistical significance were based on a probability of ($p<0.05$). The model used was

$$Y_{ij} = \mu + D_i + A_j + (DA)_{ij} + e_{ij}$$

Where, Y_{ij} = Observed value of a given dependent variable, μ = Overall mean; D_i = Fixed effect of DDGS levels, i = 1, 2, 3, 4; A_j = Fixed effect of the Avizyme levels; j = 1, 2, $(DA)_{ij}$ = Fixed effect of interaction between DDGS levels and Avizyme levels and e_{ij} = Random error associated to each observation.

RESULTS AND DISCUSSION

The chemical composition of corn DDGS used in the experiment is shown in Table 1. The results of proximate analysis for DDGS yielded values of 91.2% for dry matter, 27.79% for Crude Protein (CP), 10.32% for Ether Extract (EE), 8.34 % for Crude Fiber (CF), 4.36% for ash.

Table 2: Effect of different dietary corn DDGS levels and Avizyme supplementation on productive performance of laying hens

Dietary treatments		Egg production (%)	Egg weight (g)	Egg mass (g/h/day)	Feed intake (g/h/day)	Feed Conversion (g feed: g egg)	Body weight change (g)
DDGS%	0	83.74[a]	60.28[a]	50.48[a]	114.25	2.25[d]	+125.79[a]
	25	82.08[b]	59.55[a]	48.88[b]	114.00	2.33[c]	+118.87[a]
	50	79.45[c]	58.27[b]	46.31[c]	114.66	2.48[b]	+119.41[a]
	75	75.71[d]	56.97[c]	43.15[d]	115.45	2.68[a]	+82.87[b]
	SEM	0.346	0.295	0.231	0.597	0.014	0.295
Avizyme	without	79.29[b]	58.60	46.51[b]	114.41	2.47[a]	+111.97
	With	81.20[a]	58.93	47.89[a]	114.77	2.40[b]	+111.5
	SEM	0.244	0.208	0.164	0.422	0.01	0.208
Interaction between DDGS% and Avizyme supplementation							
DDGS% without Avizyme	0	83.68[a]	60.36[a]	50.51[a]	113.91	2.25[d]	+130.16[a]
	25	80.80[b]	59.35[ab]	47.96[b]	113.66	2.37[c]	+120.25[a]
	50	76.98[c]	57.63[c]	44.36[c]	115.33	2.50[b]	+121.08[a]
	75	75.71[c]	57.06[c]	43.23[d]	116.35	2.65[a]	+76.41[b]
DDGS% with Avizyme	0	83.80[a]	60.20[a]	50.45[a]	114.58	2.26[d]	+121.41[a]
	25	83.36[a]	59.74[ab]	49.80[a]	114.33	2.29[d]	+117.50[a]
	50	81.92[b]	58.92[b]	48.26[b]	114.00	2.36[c]	+117.75[a]
	75	75.73[c]	56.86[c]	43.05[d]	116.16	2.70[a]	+89.33[b]
SEM		0.28	0.186	0.295	0.296	0.017	0.186
Probabilities							
DDGS%		*	*	*	NS	*	*
Avizyme		*	NS	*	NS	*	NS
DDGS% x Avizyme		***	*	**	NS	*	*

[a-d]Means within a column and under each main effect with no common superscripts differ significantly at *($p<0.05$) or **($p<0.01$) or ***($p<0.001$), SEM = Standard Error of Means, NS = Not Significant

It is clear that its moisture content was less than 10%, indicating the possibility of storing DDGS for a long time without deleterious effect. Crude protein value of DDGS was higher than that recorded by Choi *et al.* (2008) 26.53% and lower than that recorded 30% by Spiehs *et al.* (2002). Ether Extract (EE) value of DDGS obtained in this study 10.32% was approximately the same as those recorded by Spiehs *et al.* (2002) 10.50% and by Waldroup *et al.* (2007) being10.08%. Belyea *et al.* (2004) indicated that the high variation in composition of different samples of DDGS is not related to corn composition but rather to variations in processing techniques. Great variability in chemical composition of corn DDGS was also found by Cromwell *et al.* (1993).

In general, reliable values for the nutrient content of feed constituents are essential to create more precise diet formulations. However, several factors affect the nutritional and physical characteristics of DDGS causing variability. This includes the variability of nutrient levels in the corn sources, proportion of distillers soluble added to DDG before drying (Martimez-Amezuca and Parsons, 2007), efficiency of converting starch to ethanol and temperature and duration of drying (Olentine, 1986). Although the nutrient content of DDGS is relatively consistent within the same processing source (Noll *et al.*, 2007a), the main problem in the use of DDGS as a feed is the high variability of nutrient concentration and quality among different DDGS sources. Thus, the complete chemical analysis of each source of DDGS in accordance with a standardized methods (AFIA, 2007) should be performed before formulating diet for poultry.

Laying hen productive performance: Effects of different dietary corn DDGS levels and Avizyme supplementation on productive performance are shown in Table 2. In general, there was a negative relationship ($p<0.05$) between the corn DDGS level and the indices of productive performance such as egg production, egg weight, egg mass, feed conversion ratio and the change in body weight of laying hens when compared to those of the control treatment. Moreover, the decrease in egg production significantly reached an extent of 1.9, 5.1 and 9.5% for 25, 50 and 75% corn DDGS substitution of soybean meal, respectively. Feed intake was not significantly affected by either DDGS level, Avizyme supplementation or their interactions (Table 2). Avizyme supplementation significantly improved egg production by 2.4%, egg mass by 2.9% and feed conversion ratio by 2.8%. While, Avizyme supplementation had no significant affect on egg weight and change in body weight. It can be stated that enzymes mixture supplementation to layer feeds have been reported to improve layers performance including feed conversion ratio (Benabdeljelil and Arbaoui, 1994; Vukic Vranjes and Wenk, 1995), energy utilization (Wyatt and Goodman, 1993; Vukic Vranjes and Wenk, 1995). This improvement appears to be a result of microbial fermentation of solubilized NSPs (Vukic Vranjes and Wenk, 1995) and the subsequently higher absorption of volatile fatty acids (Choct *et al.*, 1995) produced.

Significant interaction between corn DDGS levels and Avizyme supplementation were observed for egg production, egg weight, egg mass and feed conversion

Table 3: Effect of different dietary corn DDGS levels and Avizyme supplementation on egg quality of laying hens

Dietary treatments		Egg shell weight (g)	Egg shell thickness (mm)	Egg shape index (%)	Haugh unit	Egg albumen index (%)	Egg yolk index (%)	Egg yolk color
DDGS%	0	5.91^{a}	0.393	75.56	82.98	9.92	40.36	4.54^{d}
	25	5.83^{ab}	0.394	75.05	82.54	9.94	40.48	5.15^{c}
	50	5.80^{b}	0.393	74.92	83.01	10.02	40.45	5.34^{b}
	75	5.82^{ab}	0.391	75.46	83.09	10.07	39.82	6.46^{a}
	SEM	0.034	0.003	0.228	0.189	0.50	0.215	0.051
Avizyme	Without	5.84	0.392	74.99	82.99	9.99	40.42	5.35
	With	5.83	0.393	75.51	82.81	9.97	40.15	5.40
	SEM	0.024	0.002	0.161	0.133	0.036	0.152	0.036
Interaction between DDGS% and Avizyme supplementation								
DDGS% without Avizyme	0	5.92	0.396	75.42^{ab}	83.00	9.89	40.59^{ab}	4.55^{c}
	25	5.90	0.393	74.76^{ab}	81.71	9.95	4049^{ab}	5.13^{b}
	50	5.80	0.391	74.57^{b}	83.25	10.02	40.92^{a}	5.33^{b}
	75	5.85	0.385	75.19^{ab}	83.05	10.08	39.67^{b}	6.38^{a}
DDGS% with Avizyme	0	5.77	0.387	75.70^{a}	82.95	9.94	40.13^{ab}	4.53^{c}
	25	5.82	0.392	75.33^{ab}	82.37	9.93	40.46^{ab}	5.16^{b}
	50	5.84	0.393	75.26^{ab}	82.81	10.03	40.03^{ab}	5.35^{b}
	75	5.79	0.393	75.73^{a}	83.12	10.06	39.97^{ab}	6.55^{a}
SEM		0.017	0.001	0.116	0.094	0.025	0.11	0.068
Probabilities								
DDGS%		**	NS	NS	NS	NS	NS	***
Avizyme		NS	NS	NS	NS	NS	NS	NS
DDGS% x Avizyme		NS	NS	**	NS	NS	**	***

$^{a-d}$Means within a column and under each main effect with no common superscripts differ significantly at *(p<0.05) or **(p<0.01) or ***(p<0.001), SEM = Standard Error of Means, NS = Not Significant

ratio. It is clear that Avizyme addition relatively improved (p<0.05) egg production, egg mass and feed conversion ratio for DDGS inclusion levels at 25 and 50% when compared to those fed diets without Avizyme supplementation. In this regard, the literature stated that DDGS is being recommended at a maximum inclusion level of 15% for Poultry. However, at levels higher than 15%, there was an overall 3% reduction in egg production, with a consistent decrease in egg production after wk 32 of production in poultry (Roberson, 2003; Lumpkins *et al.*, 2005; Roberson *et al.*, 2005). The decrease in egg production when DDGS was included at 75% level substituted for SBM was due to the inclusion of this product at higher level (23.17% of dietary matter) compared to the maximum guideline of 15%. According to Noll *et al.* (2007a,b), a level of 15% DDGS can be included in layer diets without negative effect and higher levels may be added with careful adjustment of amino acids and energy levels.

Egg quality: Effects of dietary levels of DDGS and Avizyme supplementation on egg quality of laying hens are shown in Table 3. Both main effects of DDGS levels replacing for soybean meal and Avizyme supplementation insignificantly (p<0.05) affected egg shell thickness, egg shape index, Haugh unit, egg albumen index and egg yolk index. Similar results were obtained by Lumpkins *et al.* (2005) and Roberson *et al.* (2005). Previous work done by Jensen *et al.* (1978) showed no effect of a 10% DDGS diet on shell thickness and shell breaking strength. However, DDGS positively affected interior egg quality measured as Haugh units. The level of DDGS had a negative effect on egg shell weight (p<0.05). As the DDGS inclusion level increased, egg shell weight decreased. It is due to the consumption of sulfur from sulfur-rich DDGS which may interfere with absorption of dietary calcium from the small intestines (Leeson and Summers, 2001; 2005), thereby reducing eggshell quality, although, poultry are differ than other species as they can tolerate higher levels of dietary sulfur.

The opposite was observed for egg yolk color. Eggs of hens fed the 75% DDGS diet substituted for SBM had a significantly higher yolk color score (Table 3).Yolk color, however, is affected by the inclusion of the xanthophyll-rich DDGS, which increased quickly with a 10% DDGS diet and by two months with 5% DDGS diet (Roberson *et al.*, 2005; Roberts *et al.*, 2007; Pineda *et al.*, 2008). In contrast, Lumpkins *et al.* (2005) found no effects of an experimental diet with 15% corn DDGS on this parameter.

Nutrients digestibility: The impact of different dietary treatments on nutrients digestibility of experimental diets is shown in Table 4. No significant differences were observed among DDGS levels for DM, OM, EE and NFE digestibilities, while there were significant differences (p<0.05) for CP and CF digestibility. The highest DDGS inclusion level (75% substitution for soybean meal) had the lowest CP and CF digestibility, being 65.58 and 22.56%, respectively (p<0.05). It was expected that increasing the DDGS level should decrease total

Table 4: Effect of different dietary corn DDGS levels and Avizyme supplementation on nutrients diestibility of experimental diets

Dietary treatments		DM (%)	OM (%)	CP (%)	EE (%)	CF (%)	NFE (%)
DDGS%	0	73.76	79.23	67.68[a]	74.56	24.70[a]	78.65
	25	74.10	78.66	67.63[a]	74.94	24.45[a]	79.13
	50	74.03	78.98	67.39[a]	73.46	22.91[b]	78.26
	75	73.90	78.76	65.58[b]	74.28	22.56[b]	77.30
	SEM	0.216	0.332	0.247	0.722	0.308	0.576
Avizyme	Without	73.91	78.88	66.90	74.62	23.60	78.09
	With	73.99	78.94	67.24	74.00	23.71	78.58
	SEM	0.153	0.235	0.174	0.510	0.218	0.407
Interaction between DDGS% and Avizyme supplementation							
DDGS% without Avizyme	0	73.70	79.06	67.47[a]	74.46	24.72[a]	78.63
	25	74.16	78.8	67.59[a]	75.50	24.50[a]	78.73
	50	73.83	78.86	67.27[a]	73.56	22.76[b]	77.56
	75	73.93	78.83	65.29[b]	74.96	22.43[b]	77.43
DDGS% with Avizyme	0	73.85	79.4	67.90[a]	74.67	24.71[a]	78.66
	25	74.04	78.53	67.18[a]	74.38	24.40[a]	79.53
	50	74.23	79.1	68.00[a]	73.36	23.06[b]	78.96
	75	73.86	78.73	65.87[b]	73.60	22.70[b]	77.16
SEM		0.096	0.147	0.216	0.334	0.233	0.291
Probabilities							
DDGS%		NS	NS	**	NS	*	NS
Avizyme		NS	NS	NS	NS	NS	NS
DDGS% x Avizyme		NS	NS	**	NS	*	NS

[a-d]Means within a column and under each main effect with no common superscripts differ significantly at *(p<0.05) or **(p<0.01) or ***(p<0.001), SEM = Standard Error of Means, NS = Not Significant

Table 5: Effect of different dietary corn DDGS levels and Avizyme supplementation on hematological indices of laying hens

Dietary treatments		Hb (%)	PCV (%)	RBCs ($x10^3$/ mm^3)	WBCs ($x10^3$/ mm^3)	Total protein (g/dl)	Total albumin (g/dl)	Total globulin (g/dl)	Total lipids (g/dl)
DDGS%	0	21.07[b]	34.38	2.37	23.54	4.85[ab]	2.58	2.24	915.83[ab]
	25	22.29[ab]	34.57	2.50	22.99	6.03[a]	2.55	3.37	919.53[a]
	50	22.44[ab]	35.27	2.47	23.78	5.20[ab]	2.14	3.05	903.16[ab]
	75	24.71[a]	34.98	2.36	23.34	4.28[b]	2.13	2.12	861.71[b]
	SEM	0.775	0.713	0.052	0.717	0.435	0.178	0.465	17.73
Avizyme	Without	22.28	34.91	2.44	23.44	5.29	2.30	2.94	893.89
	With	22.97	34.69	2.41	23.38	4.88	2.41	2.45	906.22
	SEM	0.548	0.504	0.037	0.507	0.308	0.126	0.329	12.54
Interaction between DDGS% and Avizyme supplementation									
DDGS% without Avizyme	0	21.02[b]	34.59	2.41	23.45	5.16	2.55	2.57	901.34
	25	21.83[ab]	34.23	2.57	21.82	6.11	2.63	3.30	929.1
	50	22.19[ab]	35	2.43	24.55	5.38	2.22	3.16	878.49
	75	24.10[ab]	35.82	2.37	23.96	4.53	1.78	2.75	866.67
DDGS% with Avizyme	0	21.13[b]	34.17	2.34	23.63	4.54	2.62	1.92	930.34
	25	22.76[ab]	34.92	2.44	24.16	5.95	2.47	3.44	909.96
	50	22.68[ab]	35.55	2.51	23.02	5.02	2.06	2.95	927.83
	75	25.31[a]	34.13	2.35	22.72	4.03	2.48	1.49	856.76
SEM		0.431	0.322	0.026	0.345	0.229	0.095	0.235	9.368
Probabilities									
DDGS%		*	NS	NS	NS	*	NS	NS	*
Avizyme		NS	NS	NS	NS	NS	NS	NS	NS
DDGS% x Avizyme		**	NS	NS	NS	NS	NS	NS	NS

[a-d]Means within a column and under each main effect with no common superscripts differ significantly at *(p<0.05) or **(p<0.01) or ***(p<0.001), SEM = Standard Error of Means, NS = Not Significant

digestibility of nutrients due to the unbalanced dietary amino acids and the high fiber content of DDGS. Avizyme supplementation had no significant effect on all nutrient digestibility. The results showed that digestibility values were not significantly affected by the interaction between corn DDGS and Avizyme supplementation, except for CP and CF.

Hematological and immunization parameters: Effect of DDGS levels and Avizyme supplementation on some hematological parameters of laying hens is shown in Table 5. The present results showed that there was no significant difference among treated groups for all hematological parameters, except of Hb%, total protein and total lipids. Main effect of DDGS level at 75%

Table 6: Effect of different dietary corn DDGS levels and Avizyme supplementation on immunization of laying hens

		WBCs count (x10^3/mm^3)						
Dietary treatments		Lymph.	Mono.	Basophilus	Eosinophils	Neutrophils	IgM (mg/dl)	IgG (mg/dl)
DDGS%	0	48.81	2.18	10.06	10.43	28.89	75.08^{b}	464.00
	25	48.83	2.32	9.88	9.82	28.23	74.35^{b}	455.00
	50	48.58	2.35	9.73	10.2	27.51	91.06^{b}	696.00
	75	49.3	2.07	10.07	10.57	28.45	140.66^{a}	554.00
	SEM	0.531	0.095	0.252	0.356	0.548	11.63	136.42
Avizyme	Without	48.82	2.2	10.04	10.19	28.1	80.69^{b}	722.40
	with	48.94	2.26	9.82	10.31	28.44	109.88^{a}	362.75
	SEM	0.376	0.067	0.178	0.252	0.388	8.22	96.46
Interaction between DDGS% and Avizyme supplementation								
DDGS% without Avizyme	0	48.96	2.14^{ab}	10.13	10.38	28.1	85.68^{bc}	411.93^{ab}
	25	48.3	2.40^{a}	9.79	10.52	27.12	51.50^{c}	703.66^{ab}
	50	48.66	2.21^{ab}	9.99	10.48	29.69	58.33^{c}	1047.46^{a}
	75	48.86	2.30^{ab}	9.66	9.88	27.9	127.26^{ab}	726.56^{ab}
DDGS% with Avizyme	0	49.02	2.33^{ab}	9.93	9.48	29.01	64.48^{c}	517.06^{ab}
	25	48.97	1.93^{b}	10.33	10.4	28.18	97.20^{c}	206.40^{b}
	50	48.61	2.31^{ab}	9.83	10.16	27.45	123.80^{ab}	346.03^{b}
	75	48.87	2.23^{ab}	9.81	10.73	28.71	154.06^{a}	381.53^{b}
SEM		0.234	0.048	0.113	0.168	0.28	8.707	77.51
Probabilities								
DDGS%		NS	NS	NS	NS	NS	*	NS
Avizyme		NS	NS	NS	NS	NS	**	NS
DDGS% x Avizyme		NS	**	NS	NS	NS	**	*

$^{a-d}$Means within a column and under each main effect with no common superscripts differ significantly at *(p<0.05) or **(p<0.01) or ***(p<0.001), SEM = Standard Error of Means, NS = Not Significant

Table 7: Effect of DDGS at various levels with or without Avizyme supplementation on the economical efficiency of egg production

		Replacement level of DDGS for soybean meal (%)							
		Without Avizyme				With Avizyme			
Items		0	25	50	75	0	25	50	75
Price/k feed (L.E.)	A	2.52	2.43	2.36	2.30	2.55	2.46	2.39	2.33
Total feed intake (kg)	B	12.75	12.72	12.92	13.03	12.83	12.80	12.76	13.00
Total feed cost (L.E.)	A x B = C	32.14	30.93	30.48	29.97	32.72	31.50	30.51	30.31
Total number of eggs	D	94.00	91.00	87.00	85.00	94.00	94.00	92.00	85.00
Price/egg (L.E.)	E	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Total price of eggs (L.E.)	D x E = F	47.00	45.50	43.50	42.50	47.00	47.00	46.00	42.50
Net revenue (L.E.)	F - C = G	14.85	14.56	13.01	12.52	14.27	15.49	15.48	12.18
Economical Efficiency (E.Ef.)	G/C = H	0.46	0.47	0.42	0.41	0.43	0.49	0.50	0.40
Relative E.Ef.	R	100.00	101.95	92.43	90.49	100.00	112.79	116.32	92.15

A….based on average price of diets during the experimental time. E…according to the local market price at the experimental time.
G/C…net revenue per unit feed cost. R…assuming that economical efficiency of the control groups equals 100

substitution for SBM significantly increased Hb%, decreased total protein and total lipids compared to the control.

White blood cells differentiation counts for laying hens were insignificant for both main effects of DDGS levels and Avizyme supplementation (Table 6). Moreover, the interaction between DDGS levels and Avizyme supplementation had no significant effect on all white blood cell counts except for monocyts cells. All these findings confirmed that DDGS could be used safely in laying hen diet without negative effects, up to 50% of SBM.

Economical efficiency: The oscillating costs of conventional feedstuffs for poultry has been a prime stimulant for the continuing search for alternative feedstuffs to reduce cost of poultry production (Joseph *et al.*, 2000; Emenalom, 2004; Esonu *et al.*, 2004). The Economical Efficiency (E.Ef.) and the Relative Economical Efficiency (REE) values are shown in Table 7. Hens fed diet containing 50% DDGS substitution for soybean meal with Avizyme supplementation were economically the best treatment which had economical and relative efficiency values of 0.50 and 116.32%, respectively, followed by hens fed diet containing 25% DDGS substituted for SBM with Avizyme supplementation which had values of 0.49 and 112.79%, respectively. Hens fed diet containing 75% DDGS substituted for SBM without Avizyme had the worst corresponding values, being 0.41 and 90.49%, respectively. Compared to the control, the change in relative efficiency varied between -9.51 to +16.32%.

Conclusion: The results of the current study indicated that corn DDGS should be included in layers diet at less than 15.45% of total dietary level, supplemented with Avizyme 1500® in order to improve egg productive performance. Higher levels of corn DDGS negatively affected egg quality and productive performance.

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