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Evaluation of Distillers Dried Grains with Solubles as Feed Ingredient in Laying Hen Diets

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Abstract: This experiment was conducted to evaluate the effect of different dietary levels (0, 5, 10, 15 or 20%) of Distillers Dried Grains with Solubles (DDGS). Each level was fed without or with two enzyme supplementation, (Kemzyme plus dry® supplemented at 250 gm/ton feed or Polytec Binder plus® supplemented at 500 gm/ton feed). A total number of 450 Inshas hens (30 wks old) and 45 cocks were distributed into 15 treatments of 30 hens, each in three replicates (10 hens and one cock, each). Diets were formulated to contain 16% CP and 2700 Kcal ME/Kg at laying period. Results show that no significant differences in digestibility coefficient values of Crude Protein (CP), Ether Extract (EE), Crude Fiber (CF), Nitrogen Free Extract (NFE), Body Weight Gain (BWG), feed intake and egg quality were observed by inclusion DDGS in laying hen diets. Also, results indicated that no significant effect on semen quality, fertility, hatchability and body weight of chicks in hatch by inclusion DDGS, enzyme supplementation or interaction between DDGS levels and enzyme supplementation in laying hen diets. Inclusion of 5% DDGS in laying diets significantly increased egg production %, egg number and egg mass, compared with the other levels. However, increasing DDGS to 15 or 20% in laying hen diets significantly increased yolk color and shell thickness and significantly decreased egg production %, egg number, egg weight and egg mass and gave the worst feed conversion compared with the other levels (0, 5 and 10%). Supplementation of Kemzyme plus dry® (enzyme 1) to diets containing DDGS had significantly higher percentage of digestibility coefficient value of ether extract and increased egg production %, egg number and egg mass than those fed the other treatments. In conclusion, the present results show that DDGS can be successfully fed at levels up to 10% in laying hen diet without adverse effect on laying performance. Also, enzyme supplementation could improve the utilization of DDGS to levels up to 20% of the diet.

Key words: Dietary levels, laying hen, poultry diet

INTRODUCTION

Distiller's Dried Grains with Soluble (DDGS) is a co-product of dry mill ethanol plants and is a source of protein/amino acids, energy and available phosphorus for poultry Creswell (2006). Corn contains about 62% starch, 3.8% oil, 8.0% protein, 11.2% fiber and 15% moisture. Because most of the starch is converted to ethanol during fermentation, the resulting nutrient fractions (protein, oil and fiber) are 2 to 3 times more concentrated in DDGS compared to corn. The DDGS from modern ethanol plants may be an attractive alternative ingredient for layer diets. Lumpkins *et al.* (2005) suggested a maximal inclusion rate of 10-12% DDGS in diets for laying hens. In addition, Roberson *et al.* (2005) reported that 15% DDGS did not adversely affect performance of laying hens but suggested that lower levels of DDGS is preferred when introducing it into the diet. Also, Swiatkiwicz and Koreleski (2006) reported that up to 15% DDGS could be used in layer feeds while, inclusion of 20% negatively affected laying rate and egg weight.

Recently, Roberts *et al.* (2007) found that using 10% DDGS in laying hens diets had no negative effects on egg production or egg quality parameters. Parson *et al.* (1992) suggested that excessive heat applied during the drying process may cause mallard reactions between the lysine residues and carbohydrate moieties, subsequently a reduction in lysine availability.

Enzyme using is well documented across different types of poultry diets, amylase, Jiang *et al.* (2008), protease, Wang *et al.* (2008), xylanase, Cowieson *et al.* (2005), beta-glucanase, Mathlouth *et al.* (2002) , mixes of two or more of the aforementioned activities Cowieson and Ravindran (2008) are among the many that can be found in the scientific literature. Ward *et al.* (2008) noted that arabinoxylans and cellulase were the predominant NSP (Non-starch polysaccharide) in DDGS from modern ethanol plants. Addition of NSPases in the 44- to 68-week feed phase helped offset the drop in laying rate and daily egg mass noted by Swiatkiwicz and Koreleski (2006) in diets with 20 % DDGS versus the non-supplemented diet. Also Shalash *et al.* (2009a,b) found

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that addition of radish root extract to 12% DDGS diet increased body weight at 28 and 42 days in broiler compared to birds fed DDGS alone.

In Egypt, there is a lack of information on DDGS as feed ingredient in laying diets. Therefore, the present study was design to evaluate using different levels (0, 5, 10, 15 or 20%) DDGS without or with two enzyme supplementation in Inshas laying diets in a 5 x 3 factorial design.

MATERIALS AND METHODS

The experiment was carried out at poultry experimental unite Sakha, Animal Production Research Institute, Egypt.

A total number of 450 Inshas hens (30 wks old) and 45 cocks were distributed into 15 treatments of 30 hens and 3 cocks each in three replicates (10 hens and one cock each). The experimental treatments were arranged as 5 x 3 factorial design with five levels (0, 5, 10, 15 and 20%) of DDGS without or with two enzyme supplementation. The tested two commercial enzyme preparations were Kemzyme plus dry® (supplemented at 250 gm/ton feed) is a mixture of α-amylase, beta-glucanase, cellulase, protease and xylanase) and Polytec Binder plus® (supplemented at 500 gm/ton feed) is a mixture of xylanase, α-amylase, beta-glucanase and protease). Diets were formulated (Table 1) to contain 16% CP and 2700 Kcal ME/Kg at laying period.

Hens were housed in floor pens (280 cm long x 220 cm wide) / replicate and kept under similar conditions of management and exposed to 16 L:8 D daily lighting schedule during the experimental period which lasted up to 42 weeks of age.

The tested raw material was analyzed for moisture, CP, EE, CF and ash by the methods outlined by Official Methods of Analysis AOAC (1990). Amino acid concentrations in DDGS were analyzed with Biochrom 20 amino acid Analyzer based on the described method of Spackman *et al.* (1958). Methionine and cystine were determined in samples oxidized with performic acid.

Digestibility coefficients of nutrients were determined for experimental diets using 3 cockerels each treatment (42 wks old). Samples of experimental diets and dried excreta were assigned for proximate chemical composition according to Official Methods of Analysis AOAC (1990). Faecal nitrogen was determined according to method outlined by Ekman *et al.* (1949), while the urinary organic matter fraction was calculated according to Abou-Raya and Galal (1971).

Body Weight (BW) was measured at 30 wks of age and at the end of experimental period (40 wks of age). Feed Intake (FI), Egg Production (EP), Egg Weight (EW) were recorded, while, feed conversion (feed consumption/egg mass) and egg mass (by multiplying egg number by average egg weight) were calculated every week intervals throughout the entire experimental period.

Egg quality measured was preformed on eggs produced at the end of the experimental period using fifteen eggs/ treatment. Shape and yolk index were measured according to Romanoff and Romanoff (1949). Egg shell

Table 1: Composition and calculated analysis of experimental diets

Ingredients	Control diet	5% DDGS	10% DDGS	15% DDGS	20% DDGS
Yellow corn	64.00	62.16	60.69	59.00	57.00
Soybean meal 44%	23.70	21.20	18.30	15.68	12.76
Wheat bran	1.85	1.30	0.70	-	-
DDGS	-	5.00	10.00	15.00	20.00
Limestone	8.00	7.95	8.00	8.07	8.06
Di-calcium phosphate	1.63	1.60	1.53	1.47	1.41
NaCl	0.45	0.40	0.33	0.28	0.21
Vitamin and Mineral premix	0.30	0.30	0.30	0.30	0.30
DL-methionine (99%)	0.07	0.06	0.06	0.05	0.05
L-Lysine HCl (98%)	-	0.03	0.09	0.15	0.21
Total	100.00	100.00	100.00	100.00	100.00
Calculated					
CP (%)	16.04	16.09	16.01	16.02	16.01
ME (kcal/kg)	2700.00	2700.00	2706.00	2709.00	2704.00
CF (%)	4.64	4.78	4.93	5.07	5.25
Methionine	0.36	0.35	0.36	0.36	0.37
Methionine + Cystine	0.62	0.62	0.62	0.62	0.62
Lysine	0.79	0.79	0.79	0.79	0.79
Ca (%)	3.46	3.44	3.44	3.45	3.42
Av. P (%)	0.43	0.43	0.43	0.42	0.42
Na (%)	0.19	0.19	0.19	0.19	0.19

Supplied per kg of diet: Vit. A, 12000 IU; Vit. D3, 2200 ICU; Vit. E, 10 mg; Vit K3, 2 mg; Vit. B1, 1 mg; Vit. B2 5 mg; B6 1.5 mg; B12 10 mcg; Nicotinic acid 30 mg; Folic acid 1 mg, Pantothenic acid 10 mg; Biotein 1.5 mcg; Choline 250 mg; Copper 10 mg; Iron 30 mg; Manganse 60 mg; Zinc 50 mg; Iodine 1 mg; Selenium 0.1 mg; Cobalt 0.1 mg thickness was measured using micrometer to the nearest 0.01 mm at the equator. Shell weight per unit of surface area was calculated according to Paganelli *et al.* (1974). Egg yolk color score was measured by matching the yolk with one of the 15 bands of a 1961 Roche Improved Yolk Color Fan.

Cockerels were massaged and semen was collected at the end of the experiment to determine some semen physical properties (ejaculated volume (ml), advanced motility %, alive %, dead % and abnormality % sperm %).

Eggs were collected and numbered daily and then placed in incubators. On day 18th of incubation, the eggs were translated in order to test fertility percentage and to be moved to the hatcher, for hatchability percentage estimation. Hatching chicks were weight to the nearest grams.

Data were subjected to a (5 x 3) factorial design, statistical analysis using General Linear Model of SAS Institute (2004). Means were separated by Duncan Multiple Range Test Duncan (1955).

RESULTS AND DISCUSSION

Chemical composition of the experimental ingredients: The chemical and some amino acids composition of the distillers dried grains with solubles, yellow corn and soybean meal are summarized in Table 2. The moisture, crude protein, ether extract, crude fiber and ash values of DDGS, yellow corn and soybean meal are within the normal range of NRC (1994). However, the protein content of DDGS has been reported to vary between 23 and 32% with average 27.5% Batal and Dale (2006). This wide range is likely because of differences in the protein content of the corn grain used to produce DDGS and because of differences in residual starch content (diluting the concentrations of protein and other nutrients) caused by differences in fermentation efficiency. Recently, Shalash *et al.* (2009a,b) reported that the protein content of DDGS was 27.65%. Amino acids profile of soybean meal is rich in lysine, but deficient in methionine. However, DDGS was lower in methionine and lysine compared with soybean meal as a source of protein (Shalash *et al.*, 2009a,b). A reduced concentration of lysine is a characteristic of cereal grains, explaining the reduced concentration of this amino acid in corn co-products when compared with soybean (oilseed) co-products such as soybean meal, in addition to the heating process which reduce the lysine availability in DDGS Parson *et al.* (1992). Differences in processing procedures may lead to large variations in the nutritional value of DDGS Cromwell *et al.* (1993). In addition, the nutritional value of DDGS is related to its lysine content and is more closely related to acid detergent fiber and acid detergent insoluble nitrogen than to neutral detergent fiber content.

Nutrient of digestibility coefficients: Digestibility coefficient values of Crude Protein (CP), Crude Fiber (CF), Ether Extract (EE) and Nitrogen Free Extract (NFE) were not significantly affected by dietary DDGS levels or enzyme supplementation (Table 3). However, chicks fed diets containing DDGS supplemented with Kemzyme plus dry (enzyme 1) had significantly higher percentage of digestibility coefficient value of ether extract than those fed the other treatments. These results generally agree with those reported by Abou-Raya *et al.* (1971) who mention that the digestibility of EE by poultry was usually high digestion coefficient not less than 70% which lies in the range of values obtained in the present study. Recently, Shalash *et al.* (2009a,b) found that feeding cockers 100% DDGS reduced EE to 69.3% compared to 82.37 for those fed 50% DDGS. Insignificant interactions between dietary DDGS levels and enzyme supplementations were observed for CP, CF, EE and NFE. However, the highest value of CP digestibility was observed for chicks fed diet containing 5% DDGS supplemented with kemzyme plus dry (enzyme 1).

Laying hen performance: Results in Table 4 indicated that increasing DDGS level from 0.0-20% had no significant effect on Body Weight Gain (BWG) and feed intake. However increasing DDGS to 15 or 20% in laying hen diets significantly ($p\leq0.01$) decreased egg production %, egg number, egg weight and egg mass and gave the worst feed conversion compared with the other levels (0, 5 and 10%). No significant differences

Table 2: Chemical composition of the experimental feed ingredients

	DDGS	Corn	Soybean meal
Moisture (%)	10.95	9.20	9.50
Crude protein (%)	27.15	8.10	44.20
Ether extract (%)	10.03	2.50	1.50
Crude fiber (%)	9.30	4.50	6.50
Ash (%)	4.30	1.82	6.50
Lysine (%)	0.84	0.26*	2.69*
Methionine (%)	0.54	0.18*	0.62*
Cystine (%)	0.60	0.18*	0.66*
Methionine + Cystine (%)	1.14	0.36*	1.28*

*Amino acids of corn and soybean meal According to NRC (1994)

Table 3: Effect of different levels of DDGS and enzyme supplementation on digestibility coefficients of nutrients

Items	CP (%)	CF (%)	EE (%)	NFE (%)
Dietary DDGS levels				
Control diet (without DDGS)	78.99	21.41	70.91	73.10
5%	80.19	22.17	71.71	73.20
10%	79.56	23.08	71.40	73.56
15%	79.32	22.18	72.02	72.67
20%	78.84	22.39	71.73	72.04
SEM	1.559	2.465	1.377	3.427
P-value	0.916	0.934	0.507	0.999
Enzyme supplementation				
Without- enzyme	79.24	21.93	70.87[b]	73.23
Enzyme (1)	79.19	22.55	72.50[a]	72.91
Enzyme (2)	79.71	22.25	71.30[b]	72.60
SEM	0.363	0.586	0.383	0.756
P-value	0.600	0.794	0.008	0.883
Interaction				
Cont	79.20	21.83	70.67	73.53
Cont x Enz (1)	78.73	21.10	70.63	73.53
Cont x Enz (2)	79.77	21.30	71.43	72.23
5% DDGS x Cont	80.00	21.73	70.43	73.97
5% DDGS x Enz (1)	80.80	23.57	72.33	72.83
5% DDGS x Enz (2)	79.23	21.20	72.37	72.80
10% DDGS x Cont	79.67	23.50	72.00	73.87
10% DDGS x Enz (1)	79.77	21.93	71.87	72.87
10% DDGS x Enz (2)	79.37	23.80	70.33	73.93
15% DDGS x Cont	78.53	21.50	71.00	73.17
15% DDGS x Enz (1)	80.07	22.70	74.17	73.17
15% DDGS x Enz(2)	78.80	22.33	70.90	72.67
20% DDGS x Cont	78.53	21.10	70.23	72.60
20% DDGS x Enz (1)	79.20	23.43	73.50	72.17
20% DDGS x Enz (2)	79.03	22.63	71.47	71.37
SEM	0.213	0.335	0.240	0.436
P-value	0.985	0.851	0.092	0.949

Enz1 = Kemzyme plus dry; Enz2 = Polytec Binder plus; Cont = Control diet without supplementation.

[a,b]Means in the same column with different letters differ significantly

can be detected in egg weight, egg production %, egg number, egg mass and feed conversion between groups of hens fed different dietary levels of DDGS (0 and 10%) during experimental period. However, inclusion of 5% DDGS in laying diets significantly increased egg production % egg number and egg mass, compared with the other treatments. The present results show that DDGS can be successfully fed at levels up to 10% in laying hen diet which agrees with previous research (Lumpkins *et al*., 2005). Moreover, Roberts *et al*. (2007) found that using 10% DDGS in laying hens diets had no effects on egg production. Inclusion of 15% DDGS did not adversely affect performance of laying hens but suggested that lower levels of DDGS can be used when introducing it into the diet (Roberson *et al*., 2005). While, inclusion of 20% DDGS in laying hen diets negatively affected egg production, weight, number, mass and feed conversion Swiatkiwicz and Koreleski (2006). These results disagree with that reported by Scheideler *et al*. (2008), who found that increasing graded levels of DDGS from 0-25% for White Leghorn-type hens (24 wks) had no negative effect on egg production, feed intake and body weight gain. The decreased egg weight, egg production %, egg number, and egg mass, with high level of DDGS (15 and 20%) may be due to the high percentage of crude fiber, unpalatable and the sulfur content of the DDGS (between 0.3-1.9%) Pineda *et al*. (2008) and lysine deficiency (Sherr *et al*., 1989) and Hansen and Millington (1979) where they reported that, The lysine deficiency may be due to the mallard reaction which reduces the digestibility of lysine by competing with absorption of lysine or inhibition of carboxy peptidases. While, the soybean protein is known to have more favorable amino acid pattern for birds performance than DDGS. Furthermore, the diet with the highest content (20%) of DDGS contained low level of starch because most of the starch is converted to ethanol during fermentation Creswell (2006), meaning that the hens relied solely on converting part of dietary amino acids to glucose through the gluconeogenesis pathway to maintain normal glucose concentrations in the blood

Table 4: Effect of different levels of DDGS and enzyme supplementation on performance

Items	BWG (g)	Feed intake (g/day)	Egg weight (g)	Egg production (%)	Feed conversion	Egg number/ hen/period	Egg mass (kg/hen/ period)
Dietary DDGS levels							
Control diet (without DDGS)	223.89	101.10	49.51[a]	64.46[b]	3.17[c]	45.13[b]	2.23[b]
5%	221.11	102.03	49.66[a]	65.58[a]	3.13[c]	45.90[a]	2.28[a]
10%	215.00	101.50	49.56[a]	64.64[b]	3.17[c]	45.25[b]	2.24[b]
15%	210.56	101.31	49.27[b]	63.51[c]	3.24[b]	44.46[c]	2.20[c]
20%	195.78	101.39	49.05[c]	62.70[c]	3.30[a]	43.89[c]	2.15[d]
SEM	31.859	1.485	0.167	0.948	0.055	0.664	0.031
P-value	0.0725	0.286	0.0001	0.0004	0.0001	0.0004	0.0001
Enzyme supplementation							
Without-enzyme	207.67	101.37[ab]	49.44[ab]	63.73[b]	3.22	44.61[b]	2.21[b]
Enzyme (1)	212.33	102.33[a]	49.49[a]	64.65[a]	3.20	45.26[a]	2.24[a]
Enzyme (2)	219.80	100.70[b]	49.32[b]	64.15[ab]	3.18	44.91[ab]	2.21[b]
SEM	7.627	0.363	0.0786	0.326	0.189	0.246	0.0146
P-value	0.905	0.019	0.0266	0.0326	0.205	0.039	0.015
Interaction							
Cont x Cont	220.00	100.67	49.63	64.40	3.15	45.08	2.24
Cont x Enz (1)	211.67	101.97	49.55	65.06	3.16	45.54	2.26
Cont x Enz (2)	240.00	100.67	49.36	63.93	3.19	44.75	2.21
5% DDGS x Cont	211.67	101.27	49.79	65.30	3.12	45.71	2.28
5% DDGS x Enz (1)	225.00	103.20	49.72	66.07	3.14	46.25	2.30
5% DDGS x Enz (2)	226.67	101.63	49.49	65.36	3.14	45.75	2.26
10% DDGS x Cont	215.00	100.87	49.50	63.93	3.19	44.75	2.21
10% DDGS x Enz (1)	213.33	102.63	49.66	65.00	3.18	45.50	2.26
10% DDGS x Enz (2)	216.67	101.00	49.54	65.00	3.14	45.50	2.25
15% DDGS x Cont	203.33	101.27	49.29	63.15	3.25	44.21	2.18
15% DDGS x Enz (1)	211.67	102.10	49.36	63.87	3.24	44.71	2.21
15% DDGS x Enz (2)	216.67	100.57	49.17	63.51	3.22	44.46	2.19
20% DDGS x Cont.	188.33	100.80	49.98	61.85	3.40	43.29	2.12
20% DDGS x Enz (1)	200.00	101.73	48.15	63.27	3.27	44.29	2.18
20% DDGS x Enz (2)	199.00	99.63	49.03	62.48	3.23	44.08	2.16
SEM	4.750	0.231	0.0417	0.2015	0.0124	0.141	0.008
P-value	0.825	0.587	0.599	0.898	0.1051	0.898	0.629

Enz1 = Kemzyme plus dry; Enz2 = Polytec Binder plus; Cont = control diet without supplementation.

[a-d]Means in the same column with different letters differ significantly

and relying increasingly on fatty acid oxidation to supply energy. The DDGS diets contained relatively high amounts of fat, protein and fiber, which differ widely in their energy losses as heat increment.

Supplementation of enzyme preparation to diets containing DDGS (Table 4) increased egg production and egg number compared with the control diets (without enzyme supplementation). These results agreed with those reported by Swiatkiwicz and Koreleski (2006), who found that addition of NSPases in the 44- to 68-week feed phase helped offset the drop in laying rate and daily egg mass in diets with 20% DDGS versus the non-supplemented diet. In addition, Yakout *et al.* (2003) reported that egg mass was significantly improved by enzyme addition. Moreover, Nelson (1989) noted that a multi-enzyme preparation containing a variety of enzymes, which degrade cell walls and liberate nutrients improved laying performance. This was not reported by El-Deek *et al.* (2003) as egg production% was not significantly affected by enzyme addition or type of diet. Supplementation of Kemzyme plus dry to laying diets recorded the highest egg weight, egg production %, egg number and egg mass compared with Polytec Binder plus or control diet. The beneficial effect of Kemzyme plus dry preparation including mixture of beta-glucanase, protease, amylase and the high level of xylanase may work synergistically to improve the nutritive value of DDGS diets for laying hens. In addition to its content of cellulase which improve fiber digestibility. Concerning the interaction between dietary levels of DDGS and enzyme supplementation, there were insignificantly increase in egg production, egg number and egg mass for treatments fed (5, 10, 15 and 20 %) DDGS with the enzyme compared to the same levels without enzyme specially, with the kemzyme plus dry. Also, feed conversion was slightly improved for chicken fed diets containing the levels (10, 15 and 20%) DDGS supplemented with the two different enzyme compared to the same level without enzyme supplementation. These insignificant increment may be due to the synergistic effect of enzymes which improve the nutritive value of the DDGS levels. These results agree with (Nelson, 1989) who stated that laying performance was improved by adding multi enzyme preparations containing variety of enzyme. Also (Shalash *et al*., 2009a,b) reported that there was an increase in broiler body weight at 28 and 42 day when they fed diet containing 12% DDGS supplemented with radish root extract enzyme. In addition (Ali *et al*., 2006) found with quail in both growing and laying period that addition of radish root extract enzyme and Polytec Binder plus each alone or in combination significantly improve feed conversion of diet containing 30% wheat bran, putting in mind that the problem in wheat bran was the high content of crude fiber and phenolic compounds while in DDGS the problem was the high content of crude fiber and mallard reactions. Moreover, Abaza *et al*. (2004) found that diets containing 35% wheat bran and supplemented with enzyme Polytec Binder plus alone or with radish root extract enzyme improve feed conversion in locals hens. The present results indicate that the supplementation of enzymes could improve the utilization of the DDGS with the high levels (15, 20%).

Egg quality: Results in Table 5 indicated that no significant effect on egg weight, albumin weight %, yolk weight %, shell weight %, egg shape % and yolk index % by inclusion dietary levels of DDGS in laying hens diets. These results are in agreement with those reported by Lumpkins *et al*. (2005) and Roberts *et al*. (2007) who mention that egg quality parameters were not affect by feeding White Leghorn-type laying hens (23 to 58 wks of age) diets containing 10% DDGS. Moreover, Pineda *et al*. (2008) reported that egg quality was not affected by the DDGS inclusion. Increasing DDGS to 15 or 20% in laying hen diets (Table 5) significantly ($p \leq 0.01$) increased yolk color compared with the other levels. These results agree with those reported by Pineda *et al*.

Table 5: Effect of different levels of DDGS and enzyme supplementation on egg quality

Items	Egg weight (g)	Albumin weight (%)	Yolk weight (%)	Shell weight (%)	Egg shape (%)	Yolk color	Yolk index (%)	Shell thickness
Dietary DDGS levels								
Control diet (without DGS)	49.76	56.90	32.39	10.71	77.15	6.20^b	48.54	0.352^b
5%	49.64	56.86	32.33	10.82	76.77	6.33^b	47.52	0.362^{ab}
10%	49.69	56.66	32.51	10.82	76.91	6.50^b	47.43	0.360^{ab}
15%	49.53	56.76	32.27	10.98	76.98	6.87^a	48.17	0.367^{ab}
20%	49.76	56.65	32.49	10.87	77.03	7.20^a	47.83	0.375^a
SEM	0.807	0.740	0.763	0.537	1.643	0.666	3.471	0.028
P-value	0.824	0.266	0.973	0.136	0.971	0.0001	0.533	0.038
Enzyme supplementation								
Without-enzyme	49.56	56.75^{ab}	32.25	10.90	76.78	6.40^b	47.80	0.383^a
Enzyme (1)	49.75	56.94^a	32.35	10.71	77.08	6.72^a	48.02	0.348^c
Enzyme (2)	49.72	56.60^b	32.49	10.91	77.05	6.74^a	47.85	0.359^b
SEM	0.112	0.104	0.104	0.078	0.212	0.102	0.501	0.028
P-value	0.453	0.079	0.586	0.116	0.613	0.018	0.911	0.0001
Interaction								
Cont	49.94	56.59	32.46	10.95	76.86	5.90	48.51	0.370
Cont x Enz (1)	49.51	57.44	32.28	10.28	77.21	6.50	48.72	0.338
Cont x Enz (2)	49.84	56.66	32.43	10.91	77.38	6.20	48.51	0.349
5% DDGS x Cont	49.50	56.94	32.28	10.79	76.81	5.90	47.94	0.380
5% DDGS x Enz (1)	49.67	56.90	32.27	10.83	76.96	6.70	46.38	0.343
5% DDGS x Enz (2)	49.74	56.74	32.43	10.83	76.55	6.40	48.24	0.363
10% DDGS x Cont	49.28	56.60	32.36	11.04	76.26	6.10	45.59	0.377
10% DDGS x Enz (1)	49.99	56.85	32.52	10.62	77.48	6.60	48.34	0.341
10% DDGS x Enz (2)	49.79	56.54	32.65	10.80	76.99	6.80	48.25	0.362
15% DDGS x Cont	49.43	57.11	32.08	10.80	76.96	6.70	48.49	0.387
15% DDGS x Enz (1)	49.69	56.67	32.20	11.13	76.60	6.80	49.59	0.351
15% DDGS x Enz (2)	49.47	56.48	32.52	11.00	77.37	7.10	46.43	0.363
20% DDGS x Cont	49.65	56.52	32.56	10.92	77.01	7.40	48.95	0.399
20% DDGS x Enz (1)	49.89	56.87	32.49	10.68	77.14	7.00	47.36	0.366
20% DDGS x Enz (2)	49.74	56.60	32.40	11.00	76.94	7.20	47.69	0.359
SEM	0.064	0.0610	0.0605	0.044	0.1304	0.063	0.282	0.082
P-value	0.682	0.336	0.9718	0.1538	0.8701	0.129	0.244	0.366

Enz1 = Kemzyme plus dry; Enz2 = Polytec Binder plus; Cont = control diet without supplementation.

[a-c]Means in the same column with different letters differ significantly

(2008). This observation was expected, because corn contains relatively high contents of xanthophylls, which are a primary contributor of yolk pigmentation NRC (1994). In addition, Roberson *et al.* (2005) show that egg yolk is visually changed within one month when 10% or higher of a lightly colored DDGS is fed and by two months with 5% DDGS. At the present study shell thickness was significantly increased by the DDGS inclusion in laying hen diets. These results disagree with those reported by Pineda *et al.* (2008) who indicated that reducing egg shell quality by the DDGS inclusion in laying hen diets which contain sulfur may interfere with absorption of dietary calcium from the small intestines. The difference between the two results may be due to the type of hens (commercial vs local strain) used in experiment, where the commercial line with high production expected to be more affected than the local one(commercial needs more available Ca to meet their high productions of eggs).

Supplementation of enzyme preparations to diets containing DDGS had no significant effect on egg weight, yolk weight %, shell weight %, yolk index % and egg shape %. However, supplementation of enzyme preparations had significant effect on albumin weight %, yolk color and shell thickness. These results agreed with those reported by El-Deek *et al.* (2003), who observed that yolk color increased significantly due to enzyme addition. This may be due to dietary pigmentation released from cell wall contents Graham (1991). Insignificant interactions between dietary DDGS levels and enzyme supplementations were observed for egg quality in general.

Semen quality, fertility and hatchability: Results in Table (6 and 7) indicate that no significant effect on volume of semen, motility %, life sperm %, dead sperm % and abnormality sperm %, fertility %, total egg set %, fertile egg % and body weight of chicks at hatch by inclusion DDGS and enzyme supplementation or interaction between DDGS levels and enzyme supplementation in laying hen diets. These results agree partially with those reported by Attia *et al.* (1997)

Table 6: Effect of different levels of DDGS and enzyme supplementation on semen quality of cocks

Items	Volume of semen (ml)	Motility (%)	Life sperm (%)	Dead sperm (%)	Abnormality sperm (%)
Dietary DDGS levels					
Control diet (without DDGS)	0.443	74.44	86.11	11.56	2.33
5%	0.440	76.11	86.33	11.44	2.22
10%	0.434	74.44	86.33	11.22	2.44
15%	0.411	73.33	85.22	12.33	2.44
20%	0.419	73.33	85.33	12.11	2.55
SEM	0.529	5.725	2.338	2.440	1.033
P-value	0.639	0.837	0.742	0.854	0.968
Enzyme supplementation					
Without-enzyme	0.430	74.33	86.40	11.13	2.47
Enzyme (1)	0.432	74.67	85.40	12.00	2.60
Enzyme (2)	0.427	74.00	85.80	12.07	2.13
SEM	0.012	1.307	0.546	0.566	0.234
P-value	0.961	0.951	0.507	0.513	0.453
Interaction					
Cont	0.440	76.66	87.00	11.00	2.00
Cont x Enz (1)	0.457	73.33	85.33	12.00	2.66
Cont x Enz (2)	0.433	73.33	86.00	11.66	2.33
5% DDGS x Con	0.460	75.00	86.33	11.00	2.66
5% DDGS x Enz (1)	0.433	76.66	85.33	12.66	2.00
5% DDGS x Enz (2)	0.427	76.66	87.33	10.66	2.00
10% DDGS x Cont	0.457	73.33	87.33	10.33	2.33
10% DDGS x Enz (1)	0.423	76.66	85.33	12.00	2.66
10% DDGS x Enz (2)	0.423	73.33	86.33	11.33	2.33
15% DDGS x Cont	0.393	73.33	85.66	11.66	2.66
15% DDGS x Enz (1)	0.420	73.33	85.66	11.66	2.66
15% DDGS x Enz (2)	0.420	73.32	84.33	13.66	2.00
20% DDGS x Cont	0.400	73.33	85.66	11.66	2.66
20% DDGS x Enz (1)	0.427	73.33	85.33	11.66	3.00
20% DDGS x Enz (2)	0.430	73.33	85.00	13.00	2.00
SEM	0.070	0.738	0.0314	0.326	0.136
P-value	0.936	0.9923	0.963	0.944	0.963

Enz1 = Kemzyme plus dry; Enz2 = Polytec Binder plus; Cont = control diet without supplementation

Table 7: Effect of different levels of DDGS and enzyme supplementation on fertility, total egg set%, hatching egg% and body weight of chicks at hatch (g)

Items	Fertility (%)	Total egg set (%)	Hatching egg (%)	Body weight of chicks at hatch (g)
Dietary DDGS levels				
Control diet (without DDGS)	90.93	81.85	90.03	35.17
5%	91.29	82.22	90.07	35.22
10%	90.55	81.85	90.39	35.05
15%	90.18	81.11	89.99	35.01
20%	90.18	80.74	89.53	35.17
SEM	1.247	2.018	2.207	0.358
P-value	0.746	0.524	0.950	0.671
Enzyme supplementation				
Without-enzyme	90.55	81.22	89.69	35.12
Enzyme (1)	90.66	81.55	89.98	35.17
Enzyme (2)	90.66	81.88	90.24	35.09
SEM	0.479	0.464	0.497	0.083
P-value	0.985	0.668	0.727	0.813
Interaction				
Cont	91.11	82.22	90.24	35.30
Cont x Enz (1)	91.11	80.11	89.05	35.13
Cont x Enz (2)	90.55	82.22	90.80	35.08
5% DDGS x Con	91.11	81.66	89.64	35.22
5% DDGS x Enz (1)	91.11	82.22	90.25	35.32
5% DDGS x Enz (2)	91.66	82.77	90.31	35.13
10% DDGS x Cont	90.55	81.66	90.20	35.03
10% DDGS x Enz (1)	90.00	81.66	90.72	35.05
10% DDGS x Enz (2)	91.11	82.22	90.26	35.07
15% DDGS x Cont	90.00	80.00	88.84	34.88
15% DDGS x Enz (1)	90.00	81.66	90.84	35.15
15% DDGS x Enz (2)	90.55	81.66	90.26	34.98
20% DDGS x Cont	90.00	80.55	89.51	38.15
20% DDGS x Enz (1)	91.11	81.11	89.02	35.20
20% DDGS x Enz (2)	89.44	80.55	90.08	35.17
SEM	0.271	0.272	0.287	0.047
P-value	0.983	0.980	0.970	0.991

Enz1 = Kemzyme plus dry; Enz2 = Polytec Binder plus; Cont = control diet without supplementation

and El-Deek *et al.* (2003) who found that enzyme addition exhibited no deteriorating effect on fertility and hatchability of total eggs set.

Conclusion: DDGS could be incorporation in laying hen diets up to 10% without adverse effect on productive performance or reproductive of laying hens in both sexes. While, enzyme addition to DDGS diets give a hand in improving the utilization of DDGS levels even with the high levels 15 or 20%.

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