

Effect of Corn Distillers Dried Grains With Soluble With or Without Xylanase Supplementation in Laying Hen Diets on Performance, Egg Quality and Intestinal Viscosity

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Article Code: KVFD-2017-18832 Received: 10.10.2017 Accepted: 17.01.2018 Published Online: 18.01.2018

How to Cite This Article

Yildiz T, Ceylan N, Atik Z, Karademir E, Ertekin B: Effect of corn distillers dried grains with soluble with or without xylanase supplementation in laying hen diets on performance, egg quality and intestinal viscosity. *Kafkas Univ Vet Fak Derg*, 24 (2): 273-280, 2018. DOI: 10.9775/kvfd.2017.18832

Abstract

A 14-week experiment was conducted to evaluate the effect of Corn Distillers Dried Grains with soluble (DDGS) with or without xylanase on layers' performance and egg quality traits as well as intestinal viscosity. Four hundred and eighty Atak-S Brown laying hens (32-week-old) were randomly distributed among the eight dietary treatments with six replicates of ten birds each. There was no significant ($P>0.05$) interaction between DDGS levels and enzyme supplementation for any of the studied parameters ($P>0.05$). The addition of either 0.0, 10.0, 20.0 or 30.0% DDGS to the diet had no significant ($P>0.05$) effect on egg production, egg weight, feed intake, feed conversion ratio, body weight gain, livability, eggshell breaking strength, eggshell thickness, eggshell ratio, egg albumen height and haugh unit. The addition of any level of DDGS positively affected egg yolk color. On the other hand, 30% of DDGS in the diet caused a significant ($P<0.05$) decrease in egg mass. The supplementation of enzyme to diets significantly ($P<0.05$) increased the percentage of egg production. In addition, enzyme supplementation resulted an overall reduction in intestinal viscosity. So, it can be concluded that up to 20% DDGS can be used in laying hen nutrition without adversely affecting any of performance and egg quality parameters, besides xylanase based enzyme supplementation could improve egg production and decrease intestinal viscosity regardless of the inclusion rate of DDGS.

Keywords: Laying hens, DDGS, Xylanase, Egg production, Egg quality, Viscosity

Kurutulmuş Damıtma Çözünürü Taneleri İçeren Yumurta Tavuğu Yemlerine Ksilanaz Enzim İlavesinin Performans Yumurta Kalitesi ve Bağırsak Viskozitesi Üzerine Etkisi

Öz

Ondört hafta yürütülen çalışmada farklı oranlarda mısır damıtma çözünürleri kuru (DDGS) içeren yumurtacı tavuk yemlerine ksilanaz enzimi ilavesinin performans, yumurta kalitesi ve bağırsak viskozitesi üzerine etkisi değerlendirilmiştir. Çalışma 32 haftalık yaşta 480 adet ATAK-S kahverengi yumurtacı tavuk kullanılarak 6 tekerrürlü ve her bir alt grupta 10 adet tavuk olmak üzere 8 grupta tesadüf parselleri deneme düzeninde yürütülmüştür. Araştırma sonucunda yumurta tavuğu yemlerinde DDGS kullanım seviyeleri ile ksilanaz enzimi ilavesi arasında araştırmada incelenen performans yumurta kalitesi ve incebağırsak viskozitesi parametrelerinin hiçbirinde önemli bir interaksyon tespit edilmemiştir ($P>0.05$). Ayrıca yemlerde farklı oranlarda DDGS bulunmasının yumurta verimi, yem tüketimi, yem değerlendirme sayısı, yumurta ağırlığı, yaşama gücü, canlı ağırlık yanında yumurta kabuk kırılma direnci, kabuk kalınlığı, kabuk oranı, albumen yüksekliği ve haugh birimi gibi yumurta kalite kriterleri üzerine de önemli bir olumsuz etkisi bulunmamışken ($P>0.05$), DDGS varlığı yumurta sarı rengini önemli oranda iyileştirmiştir ($P<0.05$). Ancak %30 DDGS seviyesi yumurta kütlelerinin önemli düzeyde azalmasına sebep olmuştur ($P<0.05$). Ksilanaz enzimi uygulaması yumurta verimini önemli oranda artırırken ($P<0.05$), ince bağırsak viskozitesini de düşmüştür ($P<0.05$). Araştırmada yumurta tavuğu yemlerinde %20 seviyesine kadardır DDGS kullanımının yumurta tavuklarının beslenmesinde verim ve yumurta kalitesini olumsuz etkilemeksizin kullanılabileceği ve ksilanaz esaslı enzimin DDGS düzeyinden bağımsız olarak yumurta verimini önemli düzeyde arttırabileceği, bağırsak viskozitesini ise düşüreceği sonucuna varılmıştır.

Anahtar sözcükler: Yumurta tavuğu, DDGS, Ksilanaz, Yumurta verimi, Yumurta kalitesi, Viskozite



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INTRODUCTION

Nowadays renewable energy has gained great importance as the population of the world and the energy demand increase today. Kyoto Protocols and the most recent Paris Protocols are calling for the use of clean, green and renewable transportation fuels to replace gasoline, diesel and jet fuel [1]. The production of biofuels is important as an alternative energy source to fossil fuels and having much concern because of low and competitive production costs and, being harmless to environment. Biofuels can be produced from dry distillers grains with solubles (DDGS), that are leftover from corn consumed in ethanol production, using a chemical process.

One of the main purpose of nutritional researches is to minimize the cost of poultry production and increase profit by enhancing the utilization of nutrients in feeds. The rising trends in grain and soybean meal prices are putting pressure on poultry producers, thus the feed industry is looking for ways to reduce feed costs using new feed ingredients [2]. With growing demand for food, feed and short resources, the animal livestock industry does not have the luxury of letting anything go to waste. Maize-based dried distillers' grain with solubles (DDGS) is a byproduct obtained during the dry-milling process of maize to produce ethanol after the fermentation of maize-starch by selected yeasts [3]. As well as being potentially cost effective [4,5], maize DDGS are also a good source of energy, protein, vitamins and minerals [6,7]. There have been a number of recent studies done on the use of high quality maize DDGS in layer diets supporting that it is an excellent partial substitution for maize and soybean meal and supports high layer performance and egg quality [8]. On the other hand, there are certain anti-nutritional factors including high level of phytate and insoluble fibers such as arabinoxylans in DDGS which limit its use in poultry nutrition [9,10]. Insoluble fibres hold water building more bulk in the animal's gut, which can reduce feed intake and subsequent production [11]. As a result of the high amount of non starch polysaccharides content, mainly arabinoxylans, the optimal inclusion level of DDGS in poultry diets for maximum production is controversial [12]. The supplementation of exogenous enzymes to animal feeds in order to improve nutrient digestion is not a new idea [13,14]. Most of the commercial enzyme products have been targeted for poultry [15,16] and are typically added to diets containing barley, wheat, oats, rye, or peas [11]. Nevertheless there are not many studies evaluating xylanase based enzymes at the high level of DDGS inclusion in laying hen diets [17]. Therefore, the objective of this study was to examine if increasing the level of DDGS in diet with and without the addition of an enzyme complex would affect production parameters, egg quality and intestinal viscosity.

MATERIAL and METHODS

The animal care protocol used in this study was reviewed and approved by the Ethics Committee of the Poultry Research Institute Ankara, Turkey (21.01.09-2009.05)

The experiment was carried out at Ankara Poultry Research Institute. Four hundred and eighty ATAK-S laying birds of thirty two weeks of age were used. The experiment lasted for 14 weeks. ATAK-S is a commercial brown egg heavy hybrid bred in the Turkish Republic. The birds were placed in individually cages in the experimental house of the capacity of 480 cages. The house's temperature control system was set to maintain a daily minimum of 21°C at the middle tier level of the cages by controlling the ventilation rate. The house was windowless with artificial light (16 h light and 8 h dark; from 05:00 to 21:00 h light) by 40 Watt tungsten bulbs. The experimental cages were 25 cm wide, 47 cm high and 55 cm deep. Prior to the experiment, wheat, DDGS, corn, sunflower seed meal, soybean meal were analyzed for crude protein, ether extract and crude fiber using standard AOAC procedures [18], and diets were formulated based upon the obtained values. The results of the analysis showed that DDGS was composed of 92.4% dry matter, 22.80% crude protein, 11.5% ether extract, 12.3% starch, 1.7% sugar and 4.9% ash. The ingredients used and the calculated nutrient content of the diet formulations used in this study are shown in *Table 1*. The normal daily feed intake would be approximately 120 g/day for ATAK-S [19]. Based on the expected average feed intake, four diets were formulated. The diets were formulated as isocaloric (2750.00, 2751.00, 2750.00 and 2750.50 kcal/kg of ME) and isonitrogenous (16.42, 16.42, 16.43 and 16.41 CP). For all diets, nutrient specifications were set to meet or exceed National Research Council (NRC) nutrient requirements [9].

Before starting the experiment, a two-week period was allowed for the birds to adapt to the control diet (without enzyme supplementation). After that, egg production and egg weight were measured. Birds of similar body weight and egg production were then equally distributed to each replicate and treatment.

All diets contained 5% sunflower seed meal and 5% wheat. The experiment was designed according to the 4 × 2 factorial design, four inclusion levels (0, 10, 20 and 30%) of DDGS, without or with enzyme supplementation (0 and 400 g/tonne). Each of the eight treatments (60 birds per group) were randomly assigned to six replicates (10 birds per replicate). According to the manufacturer, the enzyme complex (Hostazym X250, Huvepharma, location) contained xylanase activity of 6500-7500 endo-pentosanase units/g with side-activities of cellulase, hemicellulase, amylase and protease. Diets, in mash form, were offered *ad-libitum* and water was freely available.

Table 1. Composition of the experimental diets

| Ingredients Composition | Diets (g/kg) | | | |
|---|--------------|----------|----------|----------|
| | Control | 10% DDGS | 20% DDGS | 30% DDGS |
| Wheat | 50.00 | 50.00 | 50.00 | 50.00 |
| Sunflower seed meal, 28% CP | 50.00 | 50.00 | 50.00 | 50.00 |
| DDGS | 0.00 | 100.00 | 200.00 | 300.00 |
| Yellow corn | 511.85 | 458.50 | 404.25 | 350.25 |
| Soybean meal, 48% CP | 243.50 | 199.50 | 155.50 | 111.00 |
| DCP ¹ | 14.00 | 11.00 | 8.00 | 5.00 |
| Limestone | 95.00 | 96.35 | 98.00 | 100.00 |
| Salt (NaCl) | 4.00 | 4.00 | 4.00 | 4.00 |
| Vegetable oil | 27.00 | 25.50 | 24.50 | 23.50 |
| Lysine HCL | 0.20 | 1.00 | 1.90 | 2.70 |
| DL-methionine | 1.25 | 0.95 | 0.65 | 0.35 |
| Vitamin/Mineral premix ² | 1.70 | 1.70 | 1.70 | 1.70 |
| Salmonella inhibitor | 1.50 | 1.50 | 1.50 | 1.50 |
| Nutrient Composition | | | | |
| Metabolizable energy (Kcal/kg) ³ | 2750.00 | 2751.00 | 2750.00 | 2750.50 |
| Crude protein, % ⁴ | 16.42 | 16.42 | 16.43 | 16.41 |
| Ether extract, % ⁴ | 5.46 | 6.04 | 6.67 | 7.31 |
| Crude fiber, % ⁴ | 4.03 | 4.43 | 4.83 | 5.23 |
| Calcium% ³ | 3.90 | 3.90 | 3.90 | 3.92 |
| Available phosphorus, % ³ | 0.36 | 0.36 | 0.36 | 0.36 |
| Methionine + Cystine, % ³ | 0.66 | 0.66 | 0.66 | 0.66 |
| Lysine, % ³ | 0.88 | 0.88 | 0.88 | 0.88 |
| Ash, % | 13.84 | 13.83 | 13.85 | 13.89 |

¹ The composition of dicalcium phosphate provided the following amounts per kilogram of diet: Ca 23% and P20%

² Vitamin-mineral premix provided per kg of diet; vitamin A, 15.000 IU; vitamin D₃, 5.000 IU; vitamin E, 50 mg; vitamin K₃, 10 mg; thiamine, 4 mg; riboflavin, 8 mg; pyridoxine, 5 mg; vitamin B₁₂, 0.025 mg; niacin, 50 mg; Ca-pantothenate, 20 mg; folic acid, 2 mg; biotin, 0.25 mg; ascorbic acid, 75 mg; choline, 175 mg; Mg, 35 mg; Mn, 56 mg; Zn, 140 mg; Fe, 56 mg; Cu, 10.5 mg; I, 1 mg; Co, 0.28 mg; Se, 0.28 mg; Mo, 0.7 mg

³ Based on NRC 1994 values for wheat, DDGS, corn, sunflower seed meal, soybean meal and vegetable oil

⁴ Based on analysis of wheat, DDGS, corn, sunflower seed meal and soybean meal

The number of eggs laid by each bird was recorded daily. Also, eggshell defects including broken, cracked, leaking, soft-shelled eggs and misshapen eggs were determined daily for each cage throughout the experiment. Average daily feed consumption was determined by weighing feed in the morning of the first day, and weighing back in the next morning for each replicate every two weeks, considering the number of birds.

Similarly to feed consumption, egg weights were measured gravimetrically and recorded for each bird every two weeks. Egg mass (percentage egg production x average egg weight) and feed conversion ratio (average daily feed intake/egg mass) were also calculated to better evaluate overall hen performance. In addition, for the internal egg quality, twenty four randomly selected eggs from each treatment groups were collected every 4 weeks, and egg quality characteristics were determined

24 hours after collection of the eggs. Eggshell thickness was measured after peeling off the membrane under the shell with Mitutoyo digital micrometer gauge (digital 395 series with 0.001 mm sensitivity, Kawasaki, Japan) on three locations (broad, equator and sharp end) from the equatorial region of each egg, and calculated as an average value. Eggshell breaking strength, egg albumen height and haugh unit were measured by using Futura 3/A egg quality measuring system (Futura, Lohne, Germany). Yellowness of egg yolk was determined by CR-10 Konica Minolta Color Reader (Osaka, Japan). Body weight of the birds was measured individually at the beginning and at the end of the experiment, and then body weight gain was calculated. Moreover, mortality of each replicate was daily determined during the study for each cage.

For the viscosity analysis, the intestinal content of 4 hens from each group was taken into tubes at the end of the

experiment. While awaiting analysis, samples were placed on ice blocks within ice buckets. The samples were centrifuged at 3500 rpm when they reached room temperature. The liquid accumulating on top was taken by pipette, and the viscosity values were determined as centipoise (cPs) by Brookfield Viscosimeter (Model LVDVII + CP) with spindle No 42 at 40°C [20]. Crude fiber intakes were calculated from feed consumptions and from values obtained from analysis of raw materials (wheat, DDGS, corn, sunflower seed meal, soybean meal).

Statistical analyses of data were performed as a randomized block design, with a factorial arrangement of 4×2, taking into consideration main effects of DDGS levels (0, 10, 20 and 30%) and enzyme supplementation (with or without) with an equal number of 6 replicates for each treatment by using statistical software Minitab R Release 16.1.0. Significant differences were tested further using a Tukey's Honestly Significant Difference multiple range tests to determine the differences among treatments.

RESULTS

The results for egg production, egg mass, egg weight,

feed intake, feed conversion ratio, body weight gain and livability of birds fed different levels of DDGS in diet with or without enzyme supplementation during the experiment are shown in [Table 2](#). Significant interaction was not found between DDGS and the enzyme supplementation ($P>0.05$), therefore, primary attention was directed to the main effects of DDGS and the enzyme supplementation on egg production, egg mass, egg weight, feed intake, feed conversion ratio, body weight gain and livability.

The results for eggshell breaking strength, eggshell thickness, eggshell ratio, egg albumen height, haugh unit and egg yolk color of birds consuming different level of DDGS in diet with or without enzyme supplementation during the experiment are shown in [Table 3](#). There was no significant interaction between DDGS and the enzyme supplementation, therefore main attention was directed to the effects of DDGS and the enzyme supplementation on eggshell breaking strength, eggshell thickness, eggshell ratio, egg albumen height, haugh unit and egg yolk color.

The results for gut viscosity of birds is shown in [Table 4](#). There was no significant interaction between DDGS and enzyme supplementation for gut viscosity.

Table 2. Effect of enzyme supplementation in laying hens diets including different levels DDGS on performance

| 14 Weeks Period | Enzyme | | SEM | DDGS Levels | | | | SEM | P | | |
|--------------------------------------|-------------------|-------------------|-----|-------------------|--------------------|--------------------|-------------------|------|-----|-----|-----|
| | Without Enzyme | With Enzyme | | 0 | 10% | 20% | 30% | | E | D | ExD |
| Egg production (%) | 86.0 ^a | 87.3 ^b | 0.4 | 87.1 | 87.2 | 86.3 | 86 | 0.6 | 0.0 | 0.1 | 0.5 |
| Egg weight (g) | 63.0 | 62.9 | 0.3 | 63.6 | 62.9 | 62.7 | 62.7 | 0.4 | 0.8 | 0.1 | 0.5 |
| Egg mass (g/hen/day) | 54.2 | 54.9 | 0.4 | 55.4 ^a | 54.8 ^{ab} | 54.2 ^{ab} | 53.9 ^b | 0.5 | 0.2 | 0.0 | 0.7 |
| Feed intake (g/hen/day) | 119.9 | 120.7 | 0.6 | 120.3 | 121.7 | 120.6 | 118.7 | 0.7 | 0.3 | 0.1 | 0.2 |
| Feed conversion ratio (g feed/g egg) | 2.2 | 2.2 | 0.0 | 2.2 | 2.2 | 2.2 | 2.2 | 0.0 | 0.6 | 0.3 | 0.8 |
| Body weight gain (g) | 249 | 262 | 15 | 272 | 224 | 287 | 240 | 20.8 | 0.5 | 0.8 | 0.1 |
| Livability (%) | 90.1 | 90.2 | 0.1 | 90.1 | 90.2 | 90.2 | 90.1 | 0.1 | 0.3 | 1.0 | 0.8 |

SEM values are pooled standard errors of mean for enzyme (n=24 replicate) and dietary (n=12 replicate) treatments

^{a,b} Means in a row within the same treatment with different superscripts differ significantly ($P<0.05$)

Table 3. Effect of enzyme supplementation in laying hens diets including different levels DDGS on eggs quality characteristics

| 14 Weeks Period | Enzyme | | SEM | DDGS Levels | | | | SEM | P | | |
|--|----------------|-------------|------|-------------------|-------------------|-------------------|-------------------|------|------|------|------|
| | Without Enzyme | With Enzyme | | 0% | 10% | 20% | 30% | | E | D | ExD |
| Eggshell breaking strength (Newton) | 38.5 | 38.9 | 0.48 | 40.1 | 38.0 | 38.1 | 38.7 | 0.66 | 0.45 | 0.17 | 0.14 |
| Eggshell thickness (10 ⁻² mm) | 319.4 | 319.1 | 1.58 | 322.3 | 316.3 | 318.6 | 319.7 | 2.18 | 0.87 | 0.51 | 0.07 |
| Eggshell ratio % | 8.9 | 9.0 | 0.03 | 9.0 | 9.1 | 8.9 | 8.9 | 0.05 | 0.98 | 0.85 | 0.40 |
| Egg albumen height | 7.5 | 7.5 | 0.07 | 7.4 | 7.6 | 7.4 | 7.5 | 0.09 | 0.83 | 0.72 | 0.49 |
| Haugh Unit | 85.4 | 85.3 | 0.42 | 84.8 | 85.9 | 85.0 | 85.6 | 0.59 | 0.93 | 0.52 | 0.70 |
| Egg yolk color | 12.2 | 12.1 | 0.06 | 12.0 ^a | 12.2 ^b | 12.3 ^b | 12.2 ^b | 0.06 | 0.36 | 0.01 | 0.11 |

SEM values are pooled standard errors of mean for enzyme (n=24 replicate) and dietary (n=12 replicate) treatments

^{a,b} Means in a row within the same treatment with different superscripts differ significantly ($P<0.05$)

Table 4. Effect of enzyme supplementation in laying hens diets including different levels DDGS on intestinal viscosity, crude fiber intake

| 14 Weeks Period | Enzyme | | SEM | DDGS Levels | | | | SEM | P | | |
|--------------------------------|----------------|-------------|------|-------------------|-------------------|-------------------|-------------------|------|------|------|------|
| | Without Enzyme | With Enzyme | | 0% | 10% | 20% | 30% | | E | D | ExD |
| Intestinal viscosity cPs | 1.53 | 1.38 | 0.05 | 1.53 | 1.44 | 1.40 | 1.46 | 0.07 | 0.04 | 0.42 | 0.81 |
| Crude fiber intake (g/hen/day) | 5.85 | 5.89 | 0.11 | 5.34 ^a | 5.73 ^b | 5.68 ^b | 6.73 ^c | 0.04 | 0.82 | 0.00 | 0.17 |

SEM values are pooled standard errors of mean for enzyme (n=24 replicate) and dietary (n=12 replicate) treatments
^{a,b} Means in a row within the same treatment with different superscripts differ significantly (P<0.05)

DISCUSSION

Our study showed that there were no significant effects of adding 0, 10, 20 or 30% DDGS into laying hen diets on egg production during the 14-week of the experimental period. This result is in agreement with earlier findings. Pineda et al.^[21] examined the effect of increasing DDGS level (0, 23, 46, or 69%) on egg production, and reported that laying hens could be fed DDGS levels as high as 69% without adverse effects on egg production, but advised that all nutrients (e.g. amino acids) should be considered when formulating diets containing DDGS. Similar results were obtained by Masa'deh^[22] who found that increasing DDGS level up to 25% did not affect negatively egg production. In contrast, Deniz et al.^[23] found that the inclusion of 20% DDGS significantly (P<0.05) depressed egg production of layers. More recent studies showed that rising DDGS level up to 22% decreased egg production^[12,17].

The overall egg production of birds with and without enzyme supplementation was 87.3% and 86.0%, respectively, representing a significant (P<0.05) difference of 1.3%, a result independent from DDGS levels. These results agree with the findings of Nelson^[24] who stated that laying performance was improved by adding enzyme preparations containing a variety of enzyme. In our study, the beneficial effect of enzyme which has the high level of xylanase and low amounts of hemicellulase, α -amylase and protease activity- may have no effect on the added DDGS but work to improve the nutritive value of the constant ingredients (5% wheat and 5% sunflower seed) of the diet for laying hens. In addition, its cellulase content may be improve fiber digestibility.

Differences in egg weight in association with the different levels of DDGS content with or without enzyme supplementation to diet were not significant.

Egg mass production is defined as hen-day production multiplied by average egg weight for each replicate. In contrast to egg weight, the significant difference observed in egg mass between the groups fed the control or 30% DDGS diet was due to the numeric reduction in egg weight and rate of lay. However, there were no significant differences in egg mass between the groups with or

without enzyme supplementation. This result is partly in agreement with the observation by Lumpkins et al.^[25] who found that 15% DDGS in the basal diet has no significant impact on egg weight. Similarly, Roberson et al.^[26] claimed that DDGS has no effect on egg weight and egg mass. In contrast, some studies found significant decrease in egg weight and egg mass due to the inclusion of 20% DDGS into the diet^[8,27].

The results of the current study (Table 2) indicate that increasing DDGS level up to 30% and supplementing enzyme has no significant effect on feed intake and feed conversion ratio. Similarly to these findings, earlier publications showed that the inclusion of various levels (15%, 20% or 25%) of DDGS in diet causes no significant difference in feed intake of hens^[8,23,25,26]. In contrast, Deniz et al.^[22] reported that the addition of 20% DDGS into the layers' diet significantly depresses feed intake (P<0.05). Some studies suggest that feed conversion is also not affected negatively by DDGS^[26] while contradictive results were also reported showing reduced feed conversion (P<0.05) by the inclusion of 20% of DDGS compared to lower levels^[22]. The authors attributed this reduction to the decreased percentage of laying rate and egg weight at this inclusion level of DDGS. However, in the present study differences observed in egg production and egg weight in association with the different levels of DDGS in diets were not significant among the groups. In addition, enzyme supplementation increased overall egg production (P<0.05). Body weight changes were calculated from values measured before and after the experiment. The results did not demonstrate a pattern with respect to increasing DDGS level and enzyme supplementation. Mean changes in body weight were positive but the relatively high standard error of means at the same time suggests that the body weight of many of the birds responded differently in each treatment. Wide variations in body weight change are, however, not surprising for mature laying hens^[28]. Findings in this experiment on body weight change are in accord with the literature^[17,23,25,29-31]. More recently noticed that inclusion of various levels of DDGS in the diet did not exert any detrimental (P>0.05) effect on final body weight and body weight change^[8]. In addition, there were no significant differences of livability as a result of the inclusion of DDGS with or without enzyme supplementation.

Both the findings of this study and the literature suggest that DDGS does not affect shell strength. Differences observed in eggshell breaking strength, eggshell thickness, eggshell ratio in association with the increasing level of DDGS and with or without enzyme supplementation were not significant among the DDGS and enzyme groups. Several authors reported similar results [25,27,32,33], although some reported increased shell thickness [34], while others observed negative effect on shell thickness [35] in association with increasing DDGS level in the diet.

Similar to eggshell quality parameters, no significant differences were observed in egg albumen height and haugh unit in association with the increasing level of DDGS and with or without enzyme supplemented diet consumed by birds. Masa'deh [23] suggested that high inclusion levels of DDGS caused no significant differences in haugh units among the different treatments. Furthermore, Deniz et al. [22] stated that feeding laying hens on DDGS up to 15% with or without enzyme supplementation had no adverse effects on both exterior and interior egg quality criteria.

In contrast to eggshell breaking strength, eggshell thickness, eggshell ratio, egg albumen height and haugh unit, there were significant differences in egg yolk color between the control and the DDGS groups regardless to enzyme supplementation. Intensified yolk color was expected. Because DDGS contains high concentrations of xanthophylls, which is responsible for the yellow color of yolk. However, the increase in yolk color was not linear and there were no significant differences among the DDGS groups. This finding was partly in agreement with the finding of Masa'deh [23] who explained that egg yolk color was linearly increased ($P < 0.001$) as dietary level of DDGS increased throughout the study. Also, El-Hack and, Mahgoub [17] pointed out that yolk color density increased as the increasing DDGS level. (basal diet or diets including 5%, 10% and 15% DDGS). In contrast, Roberts et al. [29] noted that 10% DDGS had no effect on yolk color, and Lumpkins et al. [25] found no improvement in yolk color when 15% DDGS was used in the diet. Xanthophyll pigments are susceptible to light and heat damage, thus the observed different effects on yolk color from different studies might have been related to the different xanthophyll content in DDGS sources. Xanthophyll content can vary in DDGS because of heat demolition during drying [36].

Differences observed in gut viscosity in association with the increasing level of DDGS and with or without enzyme supplemented diet consumed by birds were not significant among the DDGS ($P > 0.05$) but in enzyme groups ($P < 0.05$). Viscosity reducing effects of xylanase based enzymes in the gut have been well documented in laying hens. Increased nutrient absorption because of decreased gut viscosity by enzyme supplementation could be possible

reason for the improvement in the egg production ($P < 0.05$) in the present study (Table 2). This study showed that inclusion of 20% DDGS in a diet could be the upper feeding limit for laying hens. As most of the starch is removed from corn during ethanol production, the resultant co-product, dried distillers grains with solubles (DDGS), contains concentrated levels of protein, minerals, and fiber [37-39]. DDGS is linked to the high level of indigestible fiber components present in corn DDGS. Our results (Table 4) show that when DDGS inclusion reaches 30% in the feeds, the amount of calculated fiber intake increased by 26% (6.73 g/hen/day) compared to the ($P < 0.05$) hens received the diets without DDGS (5.34 g/hen/day). The increase in fiber intake is also accompanied with a decrease in egg mass. It appears that the use of specific enzyme preparations in this study containing mainly xylanase to target the DDGS diet and its NSP (non-starch polysaccharides) components has been partly successful. This could be due to the fact that in ethanol plants "viscosity-reducing" enzymes are used to facilitate the fermentation process. In this context, the "viscosity-reducing" enzyme preparation would contain appreciative amounts of xylanase, glucanase and cellulase activities which would contribute to a significant NSP depolymerization during the fermentation process. Thus the clear effects of enzyme supplementation in DDGS diets for all studied parameters other than gut viscosity and egg production could be hidden by presence of some enzyme activities originated from the fermentation process in ethanol production. As DDGS is a corn co-product and, as mentioned earlier, the majority of commercial enzyme products are typically added to diets containing barley or wheat, to date there is little indication of success from the development of enzyme preparations specific to corn/soybean diets [40]. Therefore, targeting the indigestible components specific to DDGS with the correct blend of supplemental carbohydrase enzymes may allow for greater inclusion of DDGS into poultry diets and thus overall profitability is improved significantly.

This experiment investigated the effects of increasing level of DDGS in diet with and without the addition of an enzyme complex on production parameters, egg quality and intestine viscosity. The potential effects of enzyme and maximum inclusion level of DDGS for laying hen diets have been demonstrated. The data suggest that the use of DDGS up to a level of 20% in the diet of brown egg hens is possible without causing any problem on the performance and egg quality parameters, and additional contribution can be arises from the use of enzyme. The results of the present study showed that xylanase based enzyme supplementation could improve egg production and decrease intestinal viscosity regardless of the inclusion level of DDGS. However, layers' performance can be negatively affected (reduction in egg mass) with inclusion levels of 30% DDGS in diets due to the high fiber

content. In addition, the use of DDGS in the diets of laying hens offers the possibility of improvement in yolk colour at even the lowest level applied in this experiment. Therefore, the inclusion of DDGS in layers' diets might be cost saver to producers if attention is given to nutrient balance.

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