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Mob: +92 300 3008585, Fax: +92 41 8815544
E-mail: editorijps@gmail.com

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Effects of Phytase and Vitamin D_3 addition to Diets Containing Distillers Dried Grains with Solubles (DDGS) on Performance and Some Egg Traits in Laying Hens†

Bekir Hakan Koksal[1], Pinar Sacakli[2] and Ahmet Ergun[2]
[1]Department of Animal Nutrition and Nutritional Diseases, Faculty of Veterinary, Adnan Menderes University, 09016, Isikli, Aydin, Turkey
[2]Department of Animal Nutrition and Nutritional Diseases, Faculty of Veterinary, Ankara University, 06110, Diskapi, Ankara, Turkey

Abstract: The effects of feeding diets containing 15% Distillers Dried Grains with Solubles (DDGS) with or without phytase or vitamin D_3 on egg production and some quality parameters were evaluated in laying hens. At 40 wks of age, 324 Lohmann Brown hens were randomly assigned to 6 treatments (two positive and two negative controls and two treatments) with 9 replicate groups of 6 hens each. The diets were formulated to be isocaloric and isonitrogenous (17% CP and 2700 kcal/kg ME, respectively). Dietary treatments consisted of two positive control which contain with or without 15% DDGS (3.5 g Available Phosphorus (AP)/kg and 3.58 g Ca/kg diet) in diets; two negative control which contain with/without 15% DDGS (1.9 g AP/kg and 3.33 g Ca/kg diet) in diets; negative control diet containing 15% DDGS supplemented with 300 FTU phytase/kg diet or phytase + 0.06% vitamin D_3, respectively. At the end of the experiment period, dietary treatments did not significantly affect egg production, feed intake, feed efficiency and some egg quality parameters. However, hens fed with 15% DDGS consumed significantly higher yolk color then other treatment groups ($p<0.001$). As a result, 15% DDGS addition had no adverse effects on performance and egg quality parameters in laying hens.

Key words: DDGS, egg yield, phytase, vitamin D_3

INTRODUCTION

Distiller's Dried Grains with Soluble (DDGS) is a by-product of the distilling industry and is a source of energy, protein, water soluble vitamins and minerals (Jensen, 1978; Waldroup *et al*., 1981; Parsons *et al*., 2006) same as a source of xanthophylls (Runnels, 1957) and linoleic acid (Scott, 1965) for poultry. This by-product has been accepted feed ingredients in poultry diets for long years (Waldroup *et al*., 1981). Especially, last two decades, there were numerous of research conducted with DDGS to replace corn, soybean meal in poultry feeds (Choi *et al*., 2008). Reports form different studies indicated that laying hens could be fed diets with up to 20% DDGS without any negative effect on egg production and egg weight (Harms *et al*., 1969; Jensen, 1978; Matterson *et al*., 1966; Lumpkins *et al*., 2005; Roberson *et al*., 2005). However there are some contradictions about recommendable levels of DDGS in laying hens between different studies. Roberson *et al*. (2005) concluded that DDGS could be fed to laying hens at levels as high as 15%, whereas Lumpkins *et al*. (2005) recommended a DDGS inclusion level of no more than 10% to 12%. Similarly, Roberts *et al*. (2007) found that using 10% DDGS in laying hens diets had no negative effects on egg production or egg quality parameters. Pineda *et al*. (2008) evaluate to effects of graded levels (between 0 to 69%) of DDGS on egg production and egg quality in white leghorn-type laying hens. Researchers noticed that feed consumption increased with increasing dietary DDGS content, but FCR did not change. Similarly, egg quality which was measured as Haugh units, egg composition and specific gravity was not affected by the DDGS inclusion. On a contrary Scheideler *et al*. (2008) noticed that egg weights were lower when the diets contained more then 20% DDGS. Moreover Shalash *et al*. (2009) notice that 5% level of DDGS resulted with increase of egg production and egg mass while 15 or 20% level of DDGS level supplementation improve yolk color and shell thickness in laying hens. It has been reported that inclusion of DDGS into diets had increased yolk color index in laying hens diets (Roberson *et al*., 2005; Roberts *et al*., 2007; Pineda *et al*., 2008).

DDGS is higher in Non-Starch Polysaccharides (NSP), than in the parent grain (Batal and Dale, 2003) and monogastrics do not digest feedstuffs efficiently which include high rate of NSP (Barrera *et al*., 2004). The addition of exogenous phytase to monogastric diets

Corresponding Author: Bekir Hakan Koksal, Department of Animal Nutrition and Nutritional Diseases, Faculty of Veterinary, Adnan Menderes University, 09016, Isikli, Aydin, Turkey

increases the bioavailability of phosphorus in grains (Augspurger *et al*., 2003). Swiatkiwicz and Koreleski (2006) reported that addition of enzymes in diets with 20% DDGS at 44 to 68 weeks of laying period showed better laying rate and improved daily egg mass compared with control group (no DDGS addition) in laying hens. Because of high level P content of DDGS, vitamin D_3 and its metabolites are considered alternative feed additives for poultry rations.

Vitamin D_3 stimulates Phosphor (P) transport mechanisms in the intestine (Biehl and Baker, 1997) and also appears to enhance phytase activity. Supplemental P has been reduced by feed additives such as phytase and vitamin D_3 (Angel *et al*., 2005; Yan *et al*., 2003). Nowadays there are new efforts about replacing of conventional ingredients with low-cost by products such as DDGS for economic profit in poultry industry. However, because of low nutrient digestibility of these by-products, supplementation of some enzymes (such as phytase) or other additives (such as vitamin D_3) is become necessary process for poultry nutrition. So the objective of present study was investigated the effects of phytase and vitamin D_3 addition in diets which contain DDGS, on some performance and egg traits in laying hens.

MATERIALS AND METHODS

Experiment design and diets: A total of 324 laying hens (Lohmann Brown), 40 wks old, were used in this study. Hens were randomly allotted into 6 equal groups of 54 birds. Each group was divided into 9 replicates as subgroups, containing 6 hens each. Hens were housed in 54 laying cages (50 x 59 x 60 cm) in a windowed poultry house at a light regimen of 16 h light. They were kept at 23±2°C during the experiment. Feed in mash form and water were provided *ad libitum* during the entire 12 wks long experimental period. Dietary treatments consisted of two positive control contain with/without 15% DDGS (3.5 g Available Phosphorus (AP)/kg and 3.58 g Ca/kg diet); two negative control with/without 15% DDGS (1.9 g AP/kg and 3.33 g Ca/kg diet); negative control diet containing 15% DDGS supplemented with 300 FTU phytase/kg diet or phytase + 0.06% vitamin D_3 in diets, respectively.

Data collection: The ingredients and calculated composition of the diets are presented in Table 1. The diets were formulated to be isocaloric and isonitrogenous. As shown in Table 1, the amount of corn and soybean meal slightly decreased and the amounts of DDGS increased in the diets. Mortality was recorded as it occurred. Eggs were collected daily and egg production was calculated as a hen-day basis. All the eggs laid during the last two consecutive days of every week were collected and weighed individually to determine the egg weight. Feed intake was biweekly recorded and calculated as g per hen per day. The value of feed efficiency was calculated as kg feed per kg egg. To determine the egg traits, 18 eggs were collected randomly from each group on wks 40, 44, 48 and 52

Table 1: Ingredients and chemical composition of the diets

	Treatment groups					
Feed ingredients	PC-Corn*	NC-Corn**	PC-DDGS	NC-DDGS	Phytase	Phytase + Vit.-D_3
Corn	44.15	45.45	36.90	39.20	39.10	39.04
Barley	13.00	13.00	13.00	13.00	13.00	13.00
Soybean meal	28.00	28.25	20.00	20.00	20.00	20.00
DDGS	-	-	15.00	15.00	15.00	15.00
Vegetable oil	3.00	2.50	3.50	2.20	2.20	2.20
DCP	1.20	0.15	1.00	-	-	-
Limestone	10.00	10.00	10.00	10.00	10.00	10.00
DL-Methionine	0.20	0.20	0.20	0.20	0.20	0.20
Salt	0.25	0.25	0.20	0.20	0.20	0.20
Vitamin premix***	0.10	0.10	0.10	0.10	0.10	0.10
Mineral premix***	0.10	0.10	0.10	0.10	0.10	0.10
Phytase	-	-	-	-	0.10	0.10
Vit-D_3	-	-	-	-	-	0.06
Chemical composition (calculated)						
Crude protein	16.80	17.00	16.90	17.10	17.10	17.10
Metabolically energy	2712.00	2714.00	2712.00	2716.00	2712.00	2710.00
Calcium	3.58	3.33	3.53	3.33	3.33	3.33
Phosphor	0.57	0.41	0.58	0.41	0.41	0.41
Available phosphor	0.35	0.19	0.35	0.19	0.19	0.19

*PC: Positive Control, **NC: Negative Control. ***Vitamin[1] and mineral[2] premix provided per kilogram of diet: 1; vitamin A, 15000 IU; vitamin D_3, 5000 IU; vitamin E, 50 mg; vitamin K_3, 10 mg; vitamin B_1 4 mg; vitamin B_2, 8 mg; vitamin B_6, 5 mg; vitamin B_{12}, 0.025 mg; niacin, 50 mg; pantothenic acid, 20 mg; folic acid, 20 mg; biotin, 0.25 mg; choline, 175 mg, [1]canthaxanthin 250 mg; 2: manganese, 100 mg; zinc, 150 mg; iron, 100 mg; cupper, 20 mg; iodine, 1.5 mg; cobalt, 0.5 mg; selenium, 0.2 mg; molybdenum, 1 mg; magnesium, 50 mg

after the beginning of the experiment (a total of 72 eggs per group was collected during the experiment). Each egg was weighed and evaluated for the egg quality within 24 hrs after egg collection. The collected eggs were classified as normal, damaged and dirty; the latter included the flowing; broken eggs (an egg with broken shell and destroyed membrane (Gurbuz *et al*., 2011), cracked eggs (an egg with a broken shell but intact membrane (Gurbuz *et al*., 2011) and dirty eggs (an egg is affected with numerous contaminants including fecal material, dust, blood and the contents of other eggs, (Gupta, 2008).

Each egg was weighed and egg shell breaking strength was measured by using an egg breaking tester (static compression device, Dr Ing. GeorgWazau Mess Pruftechnick, Berlin, Germany). The egg content was broken onto a glass-topped table. Egg shell thickness was measured in three different parts (upper and lower ends and middle) using a micrometer (Mitutoya, No. 1044N, 0.01-5 mm; Kawasaki, Japan).

Statistical analyses: Statistical analyses were done using SPSS program (SPSS Inc., Chicago, IL, USA). One way ANOVA was performed to examine differences among groups. The significance of mean differences between groups was tested by Duncan. Values were given as mean ± standard error. Level of significance was taken as $p<0.05$.

RESULTS

It was summarized that egg performance and egg weight results showed no significant differences between all treatment and control groups any time period of trial in Table 2. Similarly, feed intake and feed efficiency (Table 2) results also did not altered between groups with DDGS, phytase or vitamin D_3 addition when consider of all trial period. In parallel, DDGS supplementation had no significant effect on egg characteristics in present trial which was shown in Table 3. Damaged egg results were showed nearly equal values for positive and negative controls with other treatment groups. Also, DDGS addition resulted with no damaged egg ratio in present trail. Similarly this result, breaking strength and shell thickness values (Table 4) showed no alterations among groups while yolk color index changed by DDGS addition ($p<0.001$). These results indicated that phytase and vitamin D_3 addition to laying diets which contain DDGS at level of 15% had no significant effect on performance and egg quality parameters for laying hens.

DISCUSSION

In present trail it was indicated that DDGS could be successfully fed at levels up to 15% in laying hen diet which agrees with previous researches (Lumpkins *et al*., 2005; Shalash *et al*., 2009). Roberts *et al*. (2007) also found similar results about that using 10% DDGS in laying hens diets had no effects on egg production. On the other hand, Swiatkiwicz and Koreleski (2006) noted that inclusion of 20% DDGS in laying hen diets negatively affected egg production, weight, number, mass and feed conversion. Similarly, Pescatore *et al*. (2010) noticed that even tough feed conversion ratio did not altered between experiments groups, feed intake significantly decreased with dietary DDGS inclusion in brown laying hens. Moreover Roberson *et al*. (2005) noticed that as inclusion level of DDGS increased there were linear decreases were observed in egg weight, egg mass and specific gravity. However researchers found that egg production parameters were not different at most ages (between 48 to 67 wks age) for laying hens. In present study showed that enzyme supplementation to diets showed similar results for performance in layers. This showed a contradiction with

Table 2: Effects of phytase and vitamin D_3 addition to diets containing DDGS on performance of hens

Treatments	EP	EW	FI	FE
PC-Corn	92.36	63.93	108.63	1.84
PC-DDGS	93.34	63.82	112.51	1.89
NC-Corn	91.01	63.71	111.65	1.93
NC-DDGS	89.78	64.53	111.47	1.90
NC-Phytase	94.32	64.04	110.56	1.83
NC-Phytase + Vit.D_3	91.37	63.87	111.93	1.92
P	NS	NS	NS	NS
SEM	1.25	0.65	1.31	0.03

Differences between treatment groups are not statistically significant ($p>0.05$), NS: Non-statistically important. EP = Egg Performance; EW = Egg Weight; FI = Feed Intake; FE = Feed Efficiency

Table 3: Effects of phytase and vitamin D_3 addition to diets containing DDGS on some egg traits of hens

Treatments	Cracked egg (%)	Damaged egg (%)	Dirty egg (%)
PC-Corn	0.92	0.07	2.35
PC-DDGS	1.16	0.00	1.55
NC-Corn	0.67	0.17	2.27
NC-DDGS	0.87	0.00	3.33
NC-Phytase	0.74	0.11	1.64
NC-Phy+ Vit.D_3	1.17	0.03	2.00
P	NS	NS	NS
SEM	0.36	0.07	0.81

^{a,b}Means on the same column with different superscript differ significantly

Table 4: Effects of phytase and vitamin D_3 addition to diets containing DDGS on yolk color and egg shell traits of hens

Treatments	Yolk color index	Shell thickness	Breaking strength
PC-Corn	4.74^{b}	44.15	2.29
PC-DDGS	5.56^{a}	44.23	2.49
NC-Corn	4.83^{b}	44.47	2.56
NC-DDGS	5.85^{a}	44.02	2.40
NC-Phytase	5.76^{a}	43.66	2.35
NC-Phy + Vit.D_3	5.50^{a}	44.34	2.46
P	***	NS	NS
SEM	0.12	0.40	0.13

NS: Non-statistically important. (***): $p<0.001$

Shalash *et al.* (2009) trail which was detected enzyme supplementation to diets containing DDGS increased egg production and egg numbers. Moreover, Yakout *et al.* (2003) reported that egg mass was significantly improved by enzyme addition. Pescatore *et al.* (2010) found that DDGS inclusion to diets up to 23% had no adverse affect on feed conversion and with a help of enzyme inclusion shell quality could be improved. Results from different reports had indicated that the inclusion of DDGS did not shell quality, as indicated by the shell breaking or specific gravity of the eggs (Lumpkins *et al.*, 2005; Roberson *et al.*, 2005; Pineda *et al.*, 2008). The differences about results between previous studies could be cause of different enzyme combination or age differs of layer.

DDGS addition into diets with level of 15% resulted with significantly higher yolk color index ($p<0.001$) especially in DDGS and phytase supplemented groups. This may due to dietary pigmentation released from cell wall contents (Graham, 1991). These results showed positive match with Shalash *et al.* (2009) results. They also noticed that egg yolk color affected from DDGS and enzyme addition to diets of layers. Similarly, Roberson *et al.* (2005) found that yolk color was increased linearly ($p<0.01$) as DDGS was increased in the diet throughout in their experiment. They concluded that egg yolk was visually changed when dietary DDGS inclusion level 10% or higher. Similarly, Pescatore *et al.* (2010) noticed that yolk color was improved with a dietary DDGS inclusion in their trail.

Cheon *et al.* (2008) also concluded that the use of DDGS up to 20% resulted in linearly yolk color increase in their experiment ($p<0.05$). On a contrary, in another experiment (30) dietary DDGS inclusion levels 15 or 23% resulted with lower yolk lightness compared with control treatment. Similarly, Rossi *et al.* (2011) declared that feeding 15 or 23% level of DDGS with or without enzymes decreased yolk lightness, while feeding level of 23% DDGS increased yolk redness and yellowness compared with other treatment groups.

As a conclusion, dietary DDGS inclusion at level of 15% had no adverse effects on performance in laying hens. The results did not show any differences about any performance and egg quality parameters (except yolk color) between groups. Besides, this by-product inclusion into layer diets had been brought out better yolk color index which is important factor for consumer choose for egg industry in Turkey.

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[†]Some results of this "Trail" have been presented in 18th European Symposium on Poultry Nutrition, 31 October 31-November 04, 2011, Cesme, Izmir, Turkey.