Mitigating harmful emissions from laying hens manure and enhancing productive performance through feeding on DDGS with or without *Bacillus* spp

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Abstract:

A study included some inclusion rates of distillers dried grains with solubles **(**DDGS) with or without dried *Bacillus subtilis* and *Bacillus licheniformis* supplementation to Hisex brown laying hen diets was performed to evaluate the effects on nitrogen and phosphorus excretion in the manure as well as productive performance, egg quality criteria and some blood metabolites. A total number of 216 Hisex Brown laying hens 22 weeks old were randomly divided into eight treatment groups in a 4×2 factorial design experiment, included four levels of DDGS (0, 5, 10 and 15 %) and two levels of additives (0.0 and 1.5×10^8 CFU/g of dried *B. subtilis* and *B. licheniformis* fermentation product) through 22-34 weeks of the age. Results of excreted nitrogen and phosphorous were, by any standards, unique. Excreted nitrogen decreased by 8.62 and 4.31 % in hens fed 5 or 10 % DDGS, respectively. Excreted phosphorous declined by 3.33, 7.22 and 10.56 % in hens fed 5, 10 or 15 % DDGS, respectively when compared to the control group which fed diet without DDGS. The manure content of nitrogen and phosphorous decreased by 14.4 and 5.14 %, respectively from hens fed diets supplemented by dried *B. subtilis* and *B. licheniformis* comparing with those fed unsupplemented diets. Increasing DDGS level up to 15 % enhanced ($P \leq 0.01$) values of feed intake, egg weight, egg shape index and yolk color. Blood content of total protein increased by 9.88 % in hens fed on diets supplemented by probiotic bacteria. It appeared that increasing DDGS inclusion level in the diet up to 10 % and the addition of probiotic bacteria enhanced productive performance of laying hens and mitigated the harmful emissions from chicken manure such as ammonia; this means better production under environmentally friendly conditions.

Keywords: Harmful emissions, hens manure, *Bacillus* spp., dietary manipulation

Introduction:

Dried distillers grains with solubles (DDGS) are a co-product of corn obtained during the dry-milling process of corn to produce ethanol after the fermentation of cornstarch by selected yeasts. Dried distillers grains with solubles have been available for poultry for many decades and come primarily from the beverage industry. Dried distillers grains with solubles are a valuable source of energy, protein, and amino acids for poultry (Jensen, 1978, 1981; Parsons and Baker, 1983; Wang et al., 2007). Several researches had shown that DDGS are an acceptable ingredient in laying hen diets. It can contribute as much as one-third of the protein needed in the laying hen (Roberson *et al*., 2005). Roberson *et al.* (2005), Lumpkins *et al.* (2005), and Świątkiewicz and Koreleski (2006) all recommend a usage rate of up of 15 % DDGS in laying hen diets to maintain egg production. Feeding higher levels of DDGS could have a significant effect on feed costs for poultry producers because of higher availability of DDGS for livestock usage and the current price fluctuations of feed ingredients (Schilling et al., 2010).

Probiotics are several approaches that have potential to reduce enteric disease in poultry and subsequent contamination of poultry products (Gibson and Fuller, 2000). Probiotic has been defined as live microbial feed supplement which beneficially affects the host animal by improving its intestinal balance (Fuller, 1989). *Bacillus subtilis* group traditionally comprises four species: *B. amyloliquefaciens*, *B. licheniformis*, *B. pumilus* and *B. subtilis* itself. These bacteria have ability to produce enzymes may help animals in effective digestion and utilization of various feed ingredients and improve feed efficiency. This may be of help when animals are under stress, when endogenous enzyme production could be compromised or feed ingredients are of lower quality. Additionally, poultry manure is one of the major sources of N pollution (Song et al., 2012), in which NH_3 is a major aerial pollutant (Kristensen and Wathes, 2000) with adverse effects on the production of broilers (Miles et al., 2004). Dietary supplementation of *B. subtilis* has been found to reduce NH_3 emission in poultry by improving the activity of enzymes and the utilization of N (Santoso et al., 1999; Tanaka and Santoso, 2000). However, the efficacy of probiotic could be influenced by many factors, such as age of animals, strain of microorganism, and inclusion level (Chen et al., 2006). Therefore, this experiment was conducted to evaluate the effects of dried *B. subtilis* and *B. licheniformis* (1.5×10^8 CFU/kg) and feeding on DDGS on NH_3 emission and phosphorus excretion in the manure as well as productive performance, egg quality criteria and some blood metabolites within the period of 22-34 weeks of age.

Materials and methods:

This study was conducted at Poultry Research Farm, Department of Poultry, Faculty of Agriculture, Zagazig University, Egypt. All the experimental procedures were carried out according to the Local Experimental Animal Care Committee, and approved by the ethics of the institutional committee. Birds were cared for using husbandry guidelines derived from Zagazig University standard operating procedures.

Birds, experimental design and diets

A total number of 216 Hisex Brown laying hens 22 weeks old were randomly divided into eight treatment groups in a 4×2 factorial design experiment with nine replications of three hens each. The experiment included four levels of DDGS (0, 5, 10 and 15 %) and two levels of additives (0.0 and 1.5×10^8 CFU/g of dried *Bacillus subtilis* and *Bacillus licheniformis* fermentation product) through 22-34 weeks of the age. Three birds were housed per (50× 50×45 cm) wire cage. The cages were equipped with a nipple drinker and trough feeders. The bird's house was provided with programmable lighting and adequate ventilation. The lighting program at the start of the trial was 14 h of light and was increased by 15 min each week to 17 h of light. The diets and water were provided *ad libitum* throughout the experiment. Each experimental diet was formulated to meet nutrients recommendation of Hisex Brown management guide which met or exceeded the NRC (1994) recommendations. Experimental diets were isocaloric (2800 kcal of ME/kg) and isonitrogenous (18 % CP). The formulation and composition of basal and experimental diets are shown in Table 1.

Data collection and egg parameters

Feed intake was recorded and calculated as grams of feed disappearance over 7d divided by the number of bird days, adjusted for mortalities, while feed conversion ratio (g feed /g egg) was calculated as the egg mass value divided by the amount of feed consumed. Eggs were collected daily and egg number was calculated on a hen-day basis. Egg weight and egg number were recorded daily.

Egg quality criteria

Eggs were examined for interior and exterior quality. Egg components were monthly measured using three eggs from each treatment replicate. Egg shape index was computed as the ratio of egg width to the length (Awosanya *et al.,* 1998). Yolk index was calculated according to Funk *et al.* (1958), as average yolk height divided by yolk diameter (mm) following removal of the yolk from the albumen. The eggs were

Table 1. Composition and chemical analysis of the experimental diets

Items	**DDGS inclusion levels (%)**			
	0	**5**	**10**	**15**
Ingredients (%)				
Yellow Corn (8.5 %)	60.58	59.57	54.40	55.51
Soybean meal (44%)	22.00	16.89	13.70	10.00
DDGS (27.40 %)	0.00	5.00	10.00	15.00
Corn gluten meal (62%)	4.92	6.39	6.60	7.15
Di calcium phosphate	1.85	1.83	1.75	1.73
Limestone	8.17	8.19	8.25	8.27
Vitamins premix [1]	0.15	0.15	0.15	0.15
Minerals premix [2]	0.15	0.15	0.15	0.15
Salt	0.30	0.30	0.30	0.30
DL Methionine	0.12	0.10	0.10	0.09
L-Lysine HCl	0.04	0.13	0.18	0.24
Soybean oil	1.72	1.30	1.42	1.41

Total	100	100	100	100
Calculated chemical analysis[3]				
Crude protein (%)	18.00	18..04	18..01	18.00
ME (kcal/kg diet)	2850	2850	2850	2850
Calcium (%)	3.64	3.64	3.64	3.64
Nonphytate P (%)	0.45	0.45	0.45	0.45
Lysine (%)	0.84	0.84	0.84	0.84
Methionine+Cystine (%)	0.75	0.75	0.75	0.75
Crude fiber (%)	2.94	3.03	3.22	3.38

1. Layer vitamins Premix: Each 1.5 kg consists of: Vit. A, 12000.000 IU; Vit. D3, 2000.000 ICU, Vit.E 10 g; Vit. K, 328 mg; Vit. B1, 1000 mg; Vit. B2, 5000 mg; Vit. B6, 1500 mg, Vit. B12,10 mg ; Biotin 50 mg; Pantothenic acid , 10 g; Niacin, 30 g; Folic acid, 1000 mg.
2. Layer Minerals Premix: Each 1.5 kg consists of Mn, 60 g; Zn, 50 g; Cu, 10g; I, 1000 mg; Si, 100 mg; Co.1000 mg.
3. Calculated according to NRC (1994).

examined for shell quality by shell thickness (with shell membrane) of the eggs was measured by micrometer. Shell thickness was a mean value of measurements at three locations on the eggs (air cell, equator, and sharp end). Eggs were examined for yolk color using a Minolta Chroma Meter CR-310 (Minolta Corporation, Ramsey, NJ) calibrated with a white calibration plate as described by Roberts *et al.* (2007). The Chroma Meter measures Hunter L*, a*, and b* values, where L* is a measure of dark to light, with a greater value indicating a lighter color; a* is a measure of green to red, with a greater value indicating a redder color; and b* is a measure of blue to yellow, with a greater value indicating a more yellow color.

Blood sampling and laboratory analyses

Blood samples were randomly collected from six birds per each treatment from wing vein into sterilized tubes that closed with rubber stoppers. Samples were let to coagulate and centrifuged at 3500 rpm for 15 minutes to obtain serum, and the serum samples were kept in Eppendorf tubes at -20°C until analyzed. The following serum biochemical parameters viz., total protein (g/dl), triglyceride (mg/dl), calcium (mg/dl) and phosphorus (mg/dl), levels were determined spectrophotometrically using commercial diagnostic kits as described by the manufacture companies (Spectrum Diagnostics, Egypt, Co. for Biotechnology, S. A. E.) (Akiba *et al.*, 1982). Serum ammonia (mg/100mL) concentration was determined using coupled enzymatic assay (Ishihard *et al*., 1972).

Statistical Analysis

Data were statistically analyzed on a 5×3 factorial design basis according to Snedecor and Cochran, (1982) using SPSS® software statistical analysis program (SPSS, 1999).

Results and discussion

Productive performance

The effect of DDGS inclusion levels on the performance of laying hens during the experimental period is shown in Table 2. Results showed that DDGS levels significantly

(P≤0.01) affected all productive and egg quality traits studied except feed conversion during the experimental period (22-34 weeks of age). It is clear that DDGS levels of 5 and 10 % did not produce a significant depression in feed intake. In agreement with our results, Romeo *et al*. (2012) found that feed intake of laying hens fed on 112 g DDGS/day per hen, on average, was not affected. Also, Masa'deh (2011) revealed that increasing levels of DDGS from 0 to 25 % for White Leghorn-type hens did not have negative effects on feed intake. The level of 15 % DDGS accompanied with a significant (P≤0.01) decrease in feed intake as presented in Table 2; it could be due to the decrease in the diet palatability which may be resulted from the high content of fibers in DDGS. This result is in agreement with those reported by Myer and Cheeke (1975), Pescatore *et al.* (2010) and Deniz *et al.* (2013). Data of feed conversion did not show any significance, but it could be noticed that higher levels of DDGS up to 15 % resulted in the

Table 2. Productive performance and Egg quality traits as affected by DDGS levels, probiotic supplementation and their interactions.

Items		Productive performance				Egg quality traits					
						Egg shape index	Yolk index	Eggshell thickness	Yolk color		
		Feed intake	Feed conversion	Egg weight	Egg number				L[1]	a[2]	b[3]
DDGS impact											
0 %		114.40^{a}	1.75	70.17^{a}	27.96^{a}	80.08	49.65^{b}	0.378^{a}	61.37^{a}	9.70^{d}	41.03^{a}
5 %		113.35^{a}	1.79	69.69^{a}	27.42^{a}	80.38	49.65^{b}	0.375^{a}	60.73^{ab}	10.39^{c}	40.05^{b}
10 %		114.07^{a}	1.79	70.65^{a}	27.15^{a}	80.18	49.85^{b}	0.375^{a}	60.26^{b}	11.52^{b}	38.94^{c}
15 %		108.62^{b}	1.87	67.56^{b}	25.85^{b}	79.31	50.90^{a}	0.346^{b}	60.12^{c}	12.04^{a}	38.37^{d}
Probiotic impact											
Without		111.97	1.86	68.40	26.53	79.83	49.95	0.368	60.92	10.38	38.58
With		113.25	1.74	70.64	27.65	80.15	50.07	0.369	60.32	11.44	40.62
Interaction impact											
DDGS	Probiotic										
0 %	Without	111.26	1.75	69.80^{b}	27.36	80.06^{b}	49.61^{cd}	0.370^{b}	62.13^{a}	8.88^{d}	38.85^{c}
	With	117.54	1.75	70.54^{b}	28.55	80.11^{b}	49.68^{cd}	0.385^{a}	60.60^{bc}	10.53^{c}	43.22^{a}
5 %	Without	112.52	1.89	66.93^{c}	26.80	79.23^{bc}	49.90^{c}	0.381^{a}	60.92^{b}	10.30^{c}	38.82^{c}
	With	114.19	1.69	72.45^{a}	28.03	81.53^{a}	49.40^{cd}	0.370^{b}	60.55^{bc}	10.49^{c}	41.29^{b}
10 %	Without	114.53	1.86	69.38^{b}	26.69	80.07^{b}	51.11^{b}	0.371^{b}	60.84^{b}	10.78^{c}	38.88^{c}
	With	113.61	1.72	71.93^{a}	27.61	80.29^{b}	48.59^{d}	0.378^{ab}	59.68^{d}	12.27^{ab}	39.00^{c}
15 %	Without	109.57	1.93	67.51^{c}	25.28	79.95^{b}	49.18^{cd}	0.351^{c}	59.80^{cd}	11.59^{b}	37.78^{d}
	With	107.67	1.81	67.61^{c}	26.42	78.67^{c}	52.62^{a}	0.341^{d}	60.44^{bcd}	12.49^{a}	38.97^{c}
SEM		0.83	0.02	0.38	0.23	0.18	0.25	0.00	0.15	0.21	0.30
P value:											
DDGS		0.035	0.123	0.000	0.003	0.052	0.012	0.000	0.000	0.000	0.000

Probiotic	0.392	0.003	0.000	0.004	0.253	0.674	0.883	0.003	0.000	0.000
DDGS× Probiotic	0.227	0.232	0.000	0.989	0.001	0.000	0.000	0.002	0.021	0.000

[1] yolk whiteness, [2] yolk redness and [3] yolk yellownes

worst feed conversion (1.87) as compared with the control diet (1.75). Jiang *et al.* (2013) found similar results. They reported that hens fed 20 % DDGS gave the worst feed conversion compared with the basal diet (DDGS free). Results of egg number and egg mass gave the same trend. Hens fed the basal diet or diets incorporated with 5 or 10 % DDGS resulted in the best values of egg number and egg mass compared with those fed the highest level of DDGS (15 %) which laid less and light eggs. Consistent with previous reports of Roberson *et al*. (2005) and Bregendahl, Roberts (2006) and Deniz *et al.* (2013). On the contrary, Wu-Haan *et al.* (2010) and Świątkiewicz and Koreleski (2006) affirmed that laying hen diets including up to 20 % DDGS did not have any impact on laying rate from 21 to 26 and from 26 to 43 weeks of age, respectively.

Probiotic supplementation had a significant influence on all productive traits studied except feed intake. Values of feed conversion, egg weight and egg number improved by 6.45, 3.17 and 4.05 %, respectively compared with the control diet. Miles *et al.* (1981) and Mahdavi *et al.* (2005) showed that feeding live Lactobacillus acidophilus culture resulted in significant increase in egg production of laying hens.

Interaction effect between DDGS levels and probiotic was only significant ($P \leq 0.01$) on egg weight. The heaviest eggs (72.45 g) produced from hens fed 5 % DDGS plus probiotic, while the lowest egg weight (66.93 g) resulted from hens fed diet with 5 % DDGS without probiotic addition.

Egg quality criteria

Inclusion levels of DDGS had a significant ($P \leq 0.01$) impact on all egg quality criteria studied in the present study excepting egg shape index (Table 2). Eggs produced from hens fed the basal diet or diets included 5 and 10 % DDGS gave the best yolk index and shell thickness compared with eggs from hens fed 15 % DDGS. Our findings disagree with those reported by Świątkiewicz and Koreleski (2006) and Ghazalah *et al.* (2011) who found that the inclusion level of DDGS had no effect on yolk index values. In partially agreement with our results, Deniz *et al*. (2013) found that eggshell thickness or shell breaking strength did not depend on the level of corn DDGS. Similar results were obtained by Jensen *et al*. (1978), Lumpkins *et al*. (2005) and Swiatkiewicz and Koreleski (2006) who reported no significant differences in eggshell thickness or shell breaking strength between hens fed a commercial diet with 0 or different inclusion levels of corn DDGS. All yolk color parameters were affected significantly ($P \leq 0.01$) due to DDGS levels. Yolk color density increased ($P \leq 0.01$) as the level of DDGS increased; this suggests that xanthophylls in the DDGS were highly available. Dried distillers grains with solubles provide more xanthophylls than corn with approximately 34 mg/kg (Sauvant and Tran, 2004), which is 3 times the corn xanthophyll content of (10.62 mg/kg; NRC, 1981). Roberson *et al*. (2005) reported very similar results, indicating that dietary DDGS can make the yolk color denser. Removal of starch through ethanol fermentation raises the various nutrient contents including xanthophyll.

Probiotic supplementation had a significant positive impact only on yolk color parameters. In the same time, the interaction between DDGS levels and supplemental probiotic was significantly ($P \leq 0.05$ or $P \leq 0.01$) affected all egg quality traits as shown in Table 2. Mahdavi *et al.* (2005) reported an improvement in egg shell thickness when feeding laying hens with live Lactobacillus acidophilus culture.

Nitrogen and phosphorous excreted

As shown in Table 3, grams of daily excreted nitrogen declined when feeding hens on 5 or 10 % DDGS, but the opposite was found with increasing DDGS level up to 15 %. Excreted nitrogen decreased by 8.62 and 4.31 % in hens fed 5 or 10 % DDGS, respectively when compared to the control group which fed diet without DDGS. Our results are in line with those reported by Roberts *et al.* (2007) and Abd El-Hack *et al.* (2015) who reported a reduction in ammonia emission when laying hens provided with 10 % corn DDGS. In addition, Wu-Haan *et al.* (2010) reported a decrease in daily ammonia emission as the amount of DDGS increased from 0 to 20%. A linear depression in daily excreted phosphorous was noticed along with increasing DDGS inclusion level (Table 3). Excreted phosphorous declined by 3.33, 7.22 and 10.56 % in hens fed 5, 10 or 15 % DDGS, respectively when compared to the control group which fed diet without DDGS. It is well known that DDGS is high in total phosphorous (0.72 %) compared to corn grains (0.28 %) (NRC, 1994). While, the phosphorous in corn grains is only approximately 30 % bioavailable (Lumpkins and Batal, 2005), the bioavailability of

Table 3. Nitrogen and phosphorous excretion and some blood metabolites as affected by DDGS levels, probiotic supplementation and their interactions.

Items		Minerals excretion		Blood metabolites			
		Excreted nitrogen (g/day)	Excreted phosphorous (g/day)	Total protein (g/dl)	Triglycerides (mg/dl)	Ca (mg/dl)	P (mg/dl)
DDGS impact							
0 %		1.16^{b}	0.180^{a}	8.53^{a}	173.62^{c}	11.23^{a}	9.60
5 %		1.06^{d}	0.174^{b}	8.67^{a}	264.98^{b}	11.48^{a}	8.77
10 %		1.11^{c}	0.167^{c}	6.38^{b}	336.87^{ab}	11.24^{a}	10.96
15 %		1.32^{a}	0.161^{d}	7.21^{b}	345.37^{a}	8.78^{b}	11.49
Probiotic impact							
Without		1.25	0.175	7.30	311.56	11.28	9.76
With		1.07	0.166	8.10	248.86	10.08	10.64
Interaction impact							
DDGS	**Probiotic**						
0 %	**Without**	1.22^{b}	0.182^{a}	8.46	179.51^{d}	12.21^{a}	10.89^{ab}
	With	1.10^{c}	0.178^{c}	8.60	167.73^{d}	10.24^{ab}	8.32^{b}
5 %	**Without**	1.05^{cd}	0.181^{b}	8.56	204.44^{d}	11.37^{ab}	9.12^{b}
	With	1.06^{cd}	0.168^{d}	8.78	325.52^{bc}	11.59^{ab}	8.43^{b}
10 %	**Without**	1.18^{b}	0.169^{d}	5.51	449.89^{a}	11.16^{ab}	11.02^{ab}
	With	1.03^{d}	0.165^{e}	7.25	223.85^{cd}	11.33^{ab}	10.89^{ab}
15 %	**Without**	1.53^{a}	0.168^{d}	6.67	412.39^{ab}	10.39^{ab}	8.03^{b}

With	1.11[c]	0.154[f]	7.76	278.34[cd]	7.17[c]	14.94[a]
SEM	0.03	0.00	0.24	21.18	0.32	0.57
***P* value:**						
DDGS	0.000	0.000	0.000	0.000	0.000	0.208
Probiotic	0.000	0.000	0.022	0.022	0.007	0.374
DDGS× Probiotic	0.000	0.000	0.277	0.000	0.015	0.011

phosphorous in DDGS is much greater due to heat mediated destruction of phytate during drying (Martinez-Amezcua *et al*., 2007) and fermentation (Lumpkins and Batal, 2005). Hence, a gradual decrease in dicalcium phosphate level and total phosphorous content in the experimental laying hen diets were observed as the DDGS level increased (Table 1). The reason for the significant ($P \leq 0.01$) decrease in manure total phosphorous content for the highest inclusion level of DDGS (22 %) may be due to the lowest dietary total phosphorous content.

The manure content of nitrogen and phosphorous decreased by 14.4 and 5.14 %, respectively in hens fed diets supplemented by probiotic comparing with those fed unsupplemented diets. The interaction effect between DDGS levels and probiotic supplementation was highly significant ($P \leq 0.01$) on both nitrogen and phosphorus excreted. Hens consumed the diet of 10 % DDGS supplemented with probiotic recorded the lowest nitrogen excretion (1.03 g/day) compared with other treatment groups. The lowest amount of phosphorous excreted in the manure (0.154 g/day) associated with the highest level of DDGS (15 %) with the addition of probiotic.

Blood metabolites

Biochemical blood parameters are usually related to the health status. These parameters are vital indicators of nutritional and physiological status of birds and animals. The effect of DDGS levels, probiotic supplementation and their interactions are shown in Table 3. Serum content of total protein, triglycerides and calcium were significantly ($P \leq 0.01$) affected by DDGS levels. Serum total protein did not have a trend. Ghazalah *et al.* (2011) reported that the main effect of DDGS level at 75% (the highest DDGS substitution level in their study) substitution for soybean meal significantly decreased total protein compared to the control group. Our data conflict with Bor-Ling *et al*. (2011) who reported that plasma total protein was not impacted by DDGS levels (0, 6, 12 and 18 %) in laying hens diet. Results indicated that increasing DDGS level significantly ($P \leq 0.01$) increased serum triglycerides. Bor-Ling *et al*. (2011) disagreed with our results when reported that serum triglycerides were not affected by DDGS levels. In the present study, serum calcium increased by 2.18 and 0.09 % when feeding hens on 5 or 10 % DDGS, respectively. Bor-Ling *et al*. (2011) reported that plasma calcium and phosphorous contents were increased when 12% DDGS was used in the diet of laying hens.

Feeding laying hens on diets supplemented with probiotics enriched its blood by total protein and phosphorous and declined serum content of triglycerides and calcium. The interaction between DDGS levels and probiotic supplementation had a significant effect on all blood metabolites studied except total protein as shown in Table 3.

Conclusion

It appeared that increasing DDGS inclusion level in the diet up to 10 % and the addition of probiotic bacteria enhanced productive performance of laying hens and mitigated the harmful emissions from chicken manure such as ammonia; this means better production under environmentally friendly conditions.

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