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Performance, egg quality and serum biochemical parameters of laying hens fed corn distillers' grains with soluble diets with or without probiotic supplementation

Продуктивност, качество на яйцата и серумни биохимични параметри на кокошки носачки, хранени с изсушен спиртоварен остатък от царевица с или без пробиотик

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ABSTRACT

This study investigated the effects of feeding corn distillers' dried grains with solubles (corn DDGS) with or without probiotic supplementation on performance, egg quality and serum biochemical parameters of laying hens aged 22 to 33 weeks. A total of 240 brown-egg laying hens (RIR × RIW) were allocated to four dietary treatments in a 2 × 2 factorial design, with two levels of DDGS (0% and 15%) and two levels of probiotic (0% and 0.05% B-act®). Hens fed diets containing 15% DDGS showed reduced feed intake by 3.22%, final body weight by 2.91%, average egg weight by 2.34% and egg mass output by 9.67% compared to those fed 0% DDGS (*P*<0.05). The inclusion of DDGS significantly (*P*<0.05) improved internal egg quality by increasing Haugh units and yolk yellowness (b*), while probiotic supplementation influenced yolk colour parameters L* (lightness) and b* (yellowness). Although probiotics alone did not affect (*P*>0.05) laying performance, their combination with DDGS significantly improved eggshell thickness and yolk pigmentation at 32 weeks of age (*P*<0.05). Serum parameters were unaffected by DDGS and/or probiotic (*P*>0.05), except for a synergistic interaction that increased serum albumin levels in hens receiving both (*P*<0.05). These findings indicate that the addition of 15% DDGS enhances egg quality but may negatively affect overall performance. Probiotic supplementation complements DDGS by improving eggshell and yolk quality. Further research is needed to optimise the use of DDGS and probiotics in laying hen diets to maximise productivity.

Keywords: corn DDGS, feed additive, layers, production, egg quality, blood serum

РЕЗЮМЕ

Това проучване изследва ефектите от хранене с изсушен спиртоварен остатък от царевица (царевично DDGS) със или без добавяне на пробиотик върху продуктивността, качеството на яйцата и серумните биохимични параметри на кокошки носачки на възраст от 22 до 33 седмици. Общо 240 кокошки носачки снасящи яйца с кафява черупка бяха разпределени в четири хранителни режима при 2 × 2 факторен дизайн: две нива на DDGS (0% и 15%) и две нива на пробиотик (0% и 0,05% B-act®). При кокошките, хранени с фураж, съдържащ 15% DDGS, се наблюдава намалена консумация на фураж (3,22%), крайно живо тегло (2,91%), средно тегло на яйцата (2,34%) и добив на яйчна маса (9,67%) в сравнение с тези, хранени с 0% DDGS (*P*<0,05). Включването на DDGS значително (*P*<0,05) подобрява вътрешното качество на яйцата, повишавайки Хаф единиците и жълтеенето (b*) на жълтъка, докато пробиотичната добавка влияе върху светлостта (L*) и жълтеенето (b*) на жълтъка. Въпреки, че пробиотикът самостоятелно не повлиява яйчната продуктивност (*P*>0,05), комбинирането му с DDGS значително подобрява дебелината на черупката и пигментацията на жълтъка (*P*<0,05). Серумните параметри не са повлияни от DDGS и/или пробиотика (*P*>0,05), с изключение на синергичното взаимодействие, увеличаващо нивата на серумния албумин при комбинираната употреба (*P*<0,05). Тези констатации показват, че добавянето на 15% DDGS подобрява качеството на яйцата, но може да окаже негативно влияние върху продуктивността. Пробиотикът допълва ефекта на DDGS, като подобрява качеството на черупката и жълтъка. Необходими са допълнителни изследвания за оптимизиране на употребата на DDGS и пробиотици в диетите на кокошки носачки за постигане на максимална продуктивност.

Ключови думи: царевично DDGS, хранителна добавка, носачки, продуктивност, качество на яйцата, кръвен серум

INTRODUCTION

Alternative feed ingredients in poultry diets are gaining increasing popularity due to the need for economically viable and environmentally sustainable solutions. Notably, distillers' dried grains with solubles (DDGS) is an accessible and popular alternative feed resource, which is a co-product of ethanol production (Pont et al., 2023), obtained after the fermentation of starch from corn and other cereals using selected yeasts (Abd El-Hack et al., 2015).

Based on its high protein, lipid and mineral content, DDGS could be an attractive, low-cost ingredient to replace some of the more expensive feed components in poultry rations (Iram et al., 2020; Mohammadi et al., 2021). Corn DDGS also contains relatively high concentrations of tocopherols and xanthophylls, which are known to be potent antioxidants (Shin et al., 2018). Moreover, the presence of significant amounts of yeast residues and their cell wall components (β-glucans, mannans, and nucleotides) may promote health in animals (Shurson, 2018). Various studies have confirmed the advantages of including DDGS in monogastric diets (Świątkiewicz et al., 2016) and its positive impact on the production of high-quality animal products (Mohammadi et al., 2021). However, the potential challenges associated with feeding DDGS limit its inclusion in poultry diets to specific levels. Compared to whole grain, corn DDGS has a higher concentration of certain components, particularly non-starch polysaccharides (NSP) (Świątkiewicz et al., 2016). Monogastric animals do not effectively digest feed with high NSP content, due to reduced overall digestibility and nutrient absorption (Bederska-Łojewska et al., 2017; Pont et al., 2023), as well as increased intestinal permeability (Tellez et al., 2015), which negatively affects the gut microbiome (Langhout, 2000).

Some researchers concluded that the inclusion of DDGS at levels of 10%-12% in the diet of laying hens had no significant negative effects on their performance and egg quality (Lumpkins et al., 2005; Shalash et al., 2010; Jiang et al., 2013; Abd El-Hack et al., 2015; Abd El-Hack et al., 2019). Other studies recommend a maximum inclusion level of 15% (Roberson et al., 2005; Swiatkiwicz and Koreleski, 2006; Deniz et al., 2013). Despite these findings, DDGS inclusion can be increased up to 20% in a well-balanced diet without compromising hen performance and egg quality (Świątkiewicz et al., 2013; Yildiz et al., 2018; Attia et al., 2024). To improve the nutritional value of diets high in DDGS, various feed additives have been tested, such as enzymes (xylanase and phytase), sodium butyrate, probiotic bacteria (*Lactobacillus salivarius*), a mixture of herbal extracts (*Taraxaci siccum*, *Urticae siccum* and *Salviae siccum*), inulin or chitosan (Świątkiewicz et al., 2013). The results demonstrated a beneficial effect of enzyme supplements, as well as inulin and chitosan, on the performance of laying hens.

The use of probiotics has increased due to their remarkable beneficial effects on gut health, digestion and nutrient utilization and overall performance of birds (Abd El-Hack et al., 2020; Ding et al., 2021; Khan and Chousalkar, 2021). *Bacillus* species, the most extensively studied probiotics for monogastric animals, are particularly noteworthy due to their stability as spore-forming bacteria and their ability to produce antimicrobial peptides and enzymes (Luise et al., 2022). Among these, *Bacillus licheniformis* is recognised as a valuable probiotic candidate for laying hens, especially when relatively inexpensive, lower-quality feed ingredients are used (Ceylan et al., 2022). Supplementation with various strains of *B. licheniformis* has been shown to improve egg production and egg quality (Lei et al., 2013; Wang et al., 2017; Ceylan et al., 2022; Pan et al., 2022).

The inclusion of corn DDGS in the diet at levels up to 100 g/kg, combined with *Bacillus subtilis*, has demonstrated a synergistic effect, enhancing laying hen performance and reducing harmful emissions from litter (Abd El-Hack et al., 2017). This combination provides an opportunity to optimise laying hen diets. However, information on the efficacy of probiotic supplementation in DDGS-containing diets for laying hens remains scarce. In this context, the objective of this study was to evaluate the effects of including corn DDGS in laying hen diets, with or without probiotic supplementation, on productive performance, egg quality, and serum biochemical parameters during the period from 22 to 33 weeks of age.

MATERIALS AND METHODS

Experimental design, birds, and diets

The present study was conducted at the Poultry Farm of the Agricultural Institute, Stara Zagora, Bulgaria. A total of 240 brown egg-laying hens (22 weeks of age), crosses between Line T (Rhode Island Red; RIR) and Line N (Rhode Island White; RIW) with similar body weights, were randomly divided into four dietary groups (n = 60), each with four replicates of 15 birds. A 2 × 2 factorial design experiment was performed, including two levels of corn DDGS (0 and 15%) and two levels of probiotic (0 and 0,05%) over the period from 22 to 33 weeks of age. Birds from each replicate were kept in deep-litter floor pens. Each pen was equipped with a common trough feeder (10 cm feeding front), drinker (2,5 cm drinking front), laying nests (1 nest/5-6 hens), and perches (15 cm/hen). A photoperiod of 16 h of light, using incandescent lighting with an intensity of 15 lux, and 8 h of darkness was applied. All groups were housed under identical management and hygienic conditions, with free access to fresh water via the continuous drinking trough constant water flow.

Hens were fed the following diets: 1) 0% DDGS (based on wheat, sunflower and soybean meals); 2) 0% DDGS with 0.05% B-act®; 3)15% DDGS; and 4) 15 % DDGS with 0.05% B-act®. Diets were formulated to contain similar levels of energy and protein according to NRC recommendations (National Research Council, 1994). The diets were fed in mash form on an *ad libitum* basis. The main ingredients and calculated nutritional composition of the diets are presented in Table 1.

The corn DDGS used in the experiment contained 89.80% dry matter, 26% crude protein, 11.50% ether extract, 6.90% crude fiber, 5.40% ash, 0.30% sulphur, 0.80% phosphorus, and <0.020% aflatoxin B_1. The probiotic product (trade name: B-Act®; HuvePharma N.V.) contains a unique, single-strain spore-forming *Bacillus licheniformis* DSM 28710, and was added at the recommended level of 0.5 g/kg feed (equivalent to 1.6 × 10^9 CFU/kg feed). The additive was incorporated during the mixing process along with the premix of minerals and vitamins.

Data collection

Hens were weighed at the beginning and the end of the study, and body weight per replicate was recorded. The age at 50% egg production was calculated for each pen. Eggs were collected twice a day, and egg production was expressed as average hen-day production. Feed intake was recorded weekly on a pen basis. Eggs from each replicate were weighed weekly, and the average weight was recorded. Egg mass was calculated using egg production (%) and egg weight (g). Data on feed intake and egg mass were used to calculate the feed conversion ratio. Hens' health was monitored daily, and liveability was recorded for each replicate.

Table 1. Composition and nutritive value of the experimental diets

Ingredient,%	Diets	
	0% DDGS	15% DDGS
Wheat	57.23	46.72
Sunflower meal	15.00	15.00
Corn DDGS*	0.00	15.00
Soybean meal	11.00	5.00
Sunflower oil	5.00	6.50
Limestone	9.00	9.30
Dicalcium phosphate	1.52	1.20
Salt	0.30	0.30
DL-Methionine	0.14	0.09
L-Lysine	0.11	0.19
Vitamin-mineral premix[1]	0.20	0.20
Salgard	0.20	0.20
Mycotox	0.20	0.20
Optizym	0.10	0.10
Calculated nutritive value:		
Metabolizable energy (kcal/kg)	2758	2752
Crude protein,%	16.52	16.60
Crude fat, %	6.45	9.40
Crude fiber, %	4.90	5.21
Lysine, %	0.84	0.83
Methionine, %	0.43	0.44
Ca, %	3.86	3.85
Available P, %	0.42	0.43

[1] The premix provided the following per kilogram of diet: Vit. A: 6,000,000 IU; Vit D3: 2 500 000 IU; Vit. E: 45,000 mg; Vit B1: 2,000 mg; Vit B2: 4,500 mg; Vit B6: 2,500 mg; Pantothenic acid: 10,000 mg; Biotin: 125 mg; Vit. K3: 2 000 mg; Folic acid: 1 100 mg; Nicotinic acid: 32 500 mg; Vit. B12: 10 mg; Selenium: 150 mg; Manganese: 60,000 mg; Iron: 12,500 mg; Zinc: 45,000 mg; Copper: 7,500 mg; Iodine: 500 mg; ž

* Distillers dried grains with solubles

Egg quality

A total of 240 eggs (30 eggs per group) were randomly selected at 28 and 32 weeks of age to determine some internal (albumen and yolk percentages, Haugh unit, yolk color) and external (shape index, eggshell percentage and thickness) egg quality traits. Eggs were stored at room temperature up to 24 hours after collection, and then measurements were performed.

Egg width and length were measured using a digital calliper, and the egg shape index was calculated as the ratio of egg width to length.

Egg weight, as well as the weights of the egg yolk and eggshell, were measured on a digital scale with an accuracy to the nearest 0.01 g. Albumen weight was calculated by subtracting the yolk and shell weights from the whole egg weight. Eggshells were washed, dried at room temperature for three days, and then weighed. The proportions of yolk, albumen and shell in each egg were calculated. Eggshell thickness was defined as the average of measurements taken at three points on the egg (air cell, equator and sharp end) using a micrometer. Albumen height was measured using a tripod micrometer. Haugh units were calculated using the formula:

$$HU = 100 \log (H + 7.57 - 1.7W^{0.37}),$$

where H is the height of the albumen and W is the weight of the egg (Haugh, 1937).

Egg yolk colour was measured with a Konica Minolta Chroma Meter CR- 410 (Minolta, Tokyo, Japan) using the CIE (Commission Internationale d'Eclairage) Lab scale after calibration with a D65 white plate (Y=83.2; X=0.3208; y=0.3379). The L*, a* and b* values reflect brightness (0 = black, 100 = white), redness (−a = green, a = red) and yellowness (−b = blue, b = yellow), respectively. The Chroma Meter was positioned perpendicularly to the egg yolk surface in a Petri dish. CIE Lab measurements of each sample were performed in triplicate.

Blood samples

At the end of the experiment, six hens per group were randomly selected for blood collection from the wing vein. Blood samples were allowed to clot at room temperature and were then centrifuged for 10 minutes at 3000 × g before harvesting the serum. Serum samples were analyzed for total protein (TP), albumen (ALB), globulin (GLOB), total bilirubin (TB), amylase activity (AMY), creatinine (CREA), glucose (GLU), triglycerides (TG), blood urea nitrogen (BUN), calcium (Ca) and phosphorus (P) using an Automatic Biochemical Analyzer SMT-120VP (Chengdu Seamaty Technology Co., Ltd., China) and commercial reagent.

Statistical Analysis

Data were statistically analysed using a General Linear Model in SPSS (version 19.0; SPSS Inc., Chicago, USA). A two-way ANOVA was applied to evaluate the effects of corn DDGS inclusion, probiotic supplementation, and their interaction (2 × 2 factorial design). The following model was used:

$$Y_{ijk} = \mu + D_i + P_j + (DP)_{ij} + e_{ijk}$$

where

Y_{ijk} = observation,

μ = overall mean,

D_i = effect of DDGS,

P_j = effect of probiotic,

$(DP)_{ij}$ = interaction effect between DDGS and probiotic,

e_{ijk} = experimental random error.

The normal distribution of variables was assessed prior to performing the two-way ANOVA. Duncan's multiple range test was used to determine differences among means when treatment effects were significant. Statistical significance was considered at $P<0.05$.

RESULTS

No cases of mortality were observed during the study. The inclusion of corn DDGS in laying hen diets had no significant effect on the age at 50% egg production ($P>0.05$; Table 2). However, there was a trend toward reduced daily feed intake between 22 and 24 weeks of age ($P=0.076$; Table 2) and reduced egg production ($P=0.064$; Table 3). From 25 to 33 weeks of age (Table 3), DDGS inclusion led to reductions in feed intake by 3.22% ($P=0.015$), final body weight by 2.91% ($P=0.011$), average egg weight by 2.34% ($P=0.015$), and egg mass output by 9.67% ($P=0.017$). Feed conversion efficiency was also negatively affected, with a near-significant trend observed ($P=0.052$). Probiotic supplementation did not influence hen performance, nor was any interaction observed between DDGS and probiotic (Table 2 and Table 3; $P>0.05$).

Egg quality traits at 28 weeks of age, as influenced by DDGS, probiotic, and their interaction, are shown in Table 4.

DDGS inclusion did not significantly affect egg weight, egg shape index, shell thickness, or the proportions of egg white, yolk, and shell ($P>0.05$). However, the DDGS significantly impacted internal egg quality, increasing b* values (yellow/blue) ($P<0.001$) and Haugh units ($P=0.002$), while reducing negative a* values (red/green) ($P<0.001$). The negative a* values indicate a less pronounced red hue and a more pronounced yellowish tint in the yolks. Probiotic supplementation had no significant effect on egg quality parameters ($P>0.05$); however, significant interaction effects ($P<0.001$) were observed for yolk lightness (L) and greenish hue (a).

At 32 weeks of age, DDGS inclusion significantly affected egg quality (Table 5), with reductions in egg weight ($P=0.003$), increases in Haugh units ($P=0.019$). Changes in yolk colour included decreased L* values (darker coloration), reduced negative a* values (less green), and increased b* values (greater yellowness) ($P<0.01$). Probiotic supplementation significantly altered yolk colour by increasing L* and b* values while maintaining lower a* values ($P<0.05$). Interaction effects between DDGS and probiotic were significant for eggshell quality and yolk colour characteristics ($P<0.05$), leading to increased shell thickness ($P=0.004$), shell percentage ($P=0.047$), yolk lightness (L*), and yellowness (b*), along with diminished green hue (negative a*).

No significant changes were observed in serum biochemical parameters due to DDGS or probiotic supplementation (Table 6). However, a significant interaction was noted for serum albumin (ALB) levels ($P=0.012$).

Table 2. Effect of corn DDGS inclusion, probiotic supplementation and their interactions on hen performance (22-33 weeks of age)

Treatment		Initial body weight (g)	Final body weight (g)	Feed intake at 22-24 weeks of age (g/h/day)	Age at 50 % egg production (day)
Main effects					
DDGS[1] level (%)					
0		1548	1992 [a]	102.62	168.25
15		1524	1934 [b]	98.15	169.25
B-act (%)					
0		1538	1962	99.70	168.88
0.05		1534	1964	101.07	168.63
DDGS	B-act				
0	0	1555	1984	101.19	168.00
0	0.05	1540	2000	104.04	168.50
15	0	1520	1940	98.20	169.75
15	0.05	1527	1928	98.11	168.75
SEM		29.51	19.34	2.30	1.90
P - value					
DDGS		0.436	0.011	0.076	0.607
B-act		0.901	0.924	0.560	0.897
DDGS x B-act		0.710	0.471	0.533	0.699

[1] Distillers dried grains with solubles;

[a, b] different letters in each column indicate statistically significant differences at $P < 0.05$

Table 3. Effect of corn DDGS inclusion, probiotic supplementation and their interactions on overall performance of laying hens (25-33 weeks of age)

Treatment		Egg production (%)	Average egg weight (g)	Egg mass (g/h/day)	Feed intake (g/h/day)	Feed conversion (g feed/g egg)
Main effects						
DDGS[1] level (%)						
0		72.06	56.30 [a]	40.55 [a]	119.02 [a]	2.95
15		66.63	54.98 [b]	36.63 [b]	115.19 [b]	3.16
B-act (%)						
0		69.18	55.79	38.58	116.45	3.03
0.05		69.51	55.49	38.60	117.76	3.07
DDGS	B-act					
0	0	70.87	56.49	40.02	117.99	2.97
0	0.05	73.26	56.11	41.09	120.06	2.93
15	0	67.49	55.09	37.15	114.91	3.10
15	0.05	65.77	54.88	36.11	115.46	3.21
SEM		2.66	0.47	1.42	1.34	0.10
P - value						
DDGS		0.064	0.015	0.017	0.015	0.052
B-act		0.902	0.543	0.993	0.349	0.744
DDGS x B-act		0.454	0.860	0.472	0.583	0.471

[1] Distillers dried grains with solubles;
[a, b] different letters in each column indicate statistically significant differences at $P < 0.05$

Table 4. Effect of corn DDGS inclusion, probiotic supplementation and their interactions on egg quality traits of laying hens at 28 weeks of age

Treatment		Egg weight (g)	Egg shape index (%)	Shell thickness (mm)	Shell (%)	Albumen (%)	Haugh Units	Yolk (%)	*L**	*a**	*b**
Main effects											
DDGS[1] level (%)											
0		55.49	78.60	0.363	10.02	65.79	86.44 [b]	25.01	68.00	-7.32 [b]	28.07 [b]
15		54.62	78.63	0.356	9.98	65.02	90.74 [a]	24.99	69.08	-6.20 [a]	47.24 [a]
B-act (%)											
0		54.44	78.55	0.362	10.06	65.83	88.56	24.94	68.30	-6.88	37.12
0.05		55.66	78.68	0.357	9.95	64.99	88.62	25.06	68.72	-6.63	38.19
DDGS	B-act										
0	0	55.00	78.75	0.371	10.12	66.91	85.97	24.61	66.44 [b]	-7.09 [c]	27.23
0	0.05	55.98	78.45	0.356	9.92	64.67	86.92	25.41	69.56 [ac]	-7.55 [d]	28.90
15	0	53.88	78.36	0.354	9.99	64.74	91.15	25.27	70.16 [a]	-6.68 [b]	47.01
15	0.05	55.35	78.90	0.359	9.97	65.31	90.32	24.72	68.00 [bc]	-5.72 [a]	47.47
SEM		0.97	0.64	0.006	0.16	0.89	1.32	0.43	0.68	0.13	0.98
P - value											
DDGS		0.371	0.965	0.220	0.813	0.394	0.002	0.969	0.120	< 0.001	< 0.001
B-act		0.213	0.847	0.433	0.495	0.350	0.964	0.776	0.484	0.065	0.282
DDGS x B-act		0.799	0.518	0.096	0.585	0.120	0.503	0.130	< 0.001	< 0.001	0.541

[1] Distillers dried grains with solubles;
[a, b] different letters in each column indicate statistically significant differences at $P < 0.05$

Table 5. Effect of corn DDGS inclusion, probiotic supplementation and their interactions on egg quality traits of laying hens at 32 weeks of age

Treatment		Egg weight (g)	Egg shape index (%)	Shell thickness (mm)	Shell (%)	Albumen (%)	Haugh Units	Yolk (%)	*L**	*a**	*b**
Main effects											
DDGS[1] level (%)											
0		58.81 [a]	78.84	0.345	9.35	64.78	87.26 [b]	25.88	69.16 [a]	-7.62 [b]	31.05 [b]
15		56.94 [b]	78.53	0.344	9.36	64.15	89.38 [a]	26.50	67.83 [b]	-6.27 [a]	45.60 [a]
B-act (%)											
0		57.84	78.82	0.343	9.27	64.65	88.71	26.08	67.93 [b]	-7.15 [b]	36.99 [b]
0.05		57.91	78.55	0.346	9.43	64.28	87.94	26.29	69.07 [a]	-6.75 [a]	39.51 [a]
DDGS	B-act										
0	0	58.81	78.69	0.351 [ac]	9.42 [ab]	64.96	87.99	25.62	69.34 [a]	-7.63 [c]	31.35 [c]
0	0.05	58.81	78.99	0.339 [bc]	9.27 [ab]	64.59	86.53	26.13	68.98 [a]	-7.62 [c]	30.74 [c]
15	0	56.88	78.94	0.335 [b]	9.12 [b]	64.34	89.42	26.55	66.51 [b]	-6.67 [b]	42.93 [b]
15	0.05	57.01	78.12	0.354 [a]	9.59 [a]	63.96	89.34	26.45	69.16 [a]	-5.88 [a]	48.27 [a]
SEM		0.61	0.86	0.005	0.15	0.39	0.88	0.38	0.54	0.10	0.91
P - value											
DDGS		0.003	0.716	0.991	0.950	0.116	0.019	0.104	0.017	< 0.001	< 0.001
B-act		0.910	0.759	0.501	0.288	0.350	0.387	0.583	0.039	< 0.001	0.012
DDGS x B-act		0.916	0.516	0.004	0.047	0.989	0.436	0.421	0.007	< 0.001	0.002

[1] Distillers dried grains with solubles;
[a, b] different letters in each column indicate statistically significant differences at $P < 0.05$

Table 6. Effect of corn DDGS inclusion, probiotic supplementation and their interactions on serum biochemical parameters of laying hens

Treatment		TP (g/L)	ALB (g/L)	GLOB (g/L)	A/G	TB (umol/L)	AST (U/L)	AMY (U/L)	CREA (umol/L)	BUN (mmol/L)	GLU (mmol/L)	TG (mmol/L)	Ca (mmol/L)	P (mmol/L)
Main effects														
DDGS[1] level (%)														
0		45.57	20.37	25.20	0.81	1.50	235	305	19.52	1.19	11.48	8.00	4.26	2.31
15		44.97	20.68	24.83	0.87	1.95	293	259	22.98	1.29	12.43	8.22	3.76	2.41
B-act (%)														
0		45.20	19.88	25.30	0.80	1.58	238	225	20.03	1.12	12.28	9.58	4.07	2.61
0.05		45.33	21.17	24.18	0.88	1.87	289	338	22.47	1.28	11.63	6.64	3.94	2.11
DDGS	B-act													
0	0	46.37	20.90 [ab]	25.47	0.83	1.30	216	289	19.60	1.11	11.35	9.56	4.29	2.58
0	0.05	44.77	19.83 [b]	24.93	0.80	1.70	235	320	19.43	1.11	11.60	6.43	4.22	2.04
15	0	44.03	18.87 [b]	25.13	0.78	1.87	261	162	20.47	1.12	13.20	9.60	3.85	2.64
15	0.05	45.90	22.50 [a]	23.43	0.96	2.03	325	356	25.50	1.45	11.65	6.84	3.66	2.18
SEM		1.83	0.73	1.84	0.07	0.27	27.37	67.42	2.61	0.15	1.22	1.44	0.28	0.33
P - value														
DDGS		0.751	0.675	0.632	0.440	0.133	0.065	0.519	0.221	0.279	0.457	0.883	0.110	0.779
B-act		0.944	0.116	0.561	0.285	0.323	0.098	0.133	0.379	0.314	0.609	0.076	0.643	0.169
DDGS x B-act		0.371	0.012	0.759	0.165	0.676	0.635	0.263	0.349	0.296	0.482	0.902	0.843	0.900

[1] Distillers dried grains with solubles;
[a, b] Different letters in each column indicate statistically significant differences at $P<0.05$;
SEM: Standard error of mean; TP: Total protein; ALB: Albumin; GLOB: Globuline; A/G: Albumin/globulin ratio; TB: total bilirubin; AST: Aspartate aminotransferase; AMY: Amylase; CREA: Creatinine; BUN: Blood urea nitrogen; GLU: Glucose; TG: Triglycerides; Ca: Calcium; P: phosphorus.

DISCUSSION

This study investigated the effects of including corn DDGS in laying hen diets, with and without probiotic supplementation, on production parameters, egg quality, and serum biochemistry. The results revealed that incorporating 15% DDGS in the diet significantly reduced feed intake, egg weight, egg mass, and final body weight of laying hens (*P*<0.05). The reduced feed intake observed in hens fed DDGS diets may be attributed to the high levels of indigestible components and low palatability of DDGS. Antinutritional factors, such as the high levels of insoluble fibers (e.g., arabinoxylans) in DDGS, have been reported to retain water and increase intestinal bulk, which can reduce feed intake and subsequently impair performance (Paloheimo et al., 2011). Additionally, DDGS can alter the taste, odor, and texture of feed, potentially making it less palatable to hens (Deniz et al., 2013), especially when the quality of DDGS varies due to differences in production or storage processes. Reduced feed intake likely limited the hens' ability to meet their amino acid and energy requirements, thereby negatively affecting egg weight and production in this study. In agreement with our findings, Shalash et al. (2010) reported that increasing DDGS levels to 15% or 20% in laying hen diets significantly decreased egg production, egg weight, and egg mass, while worsening feed conversion compared to diets containing 0%, 5%, or 10% DDGS, although body weight gain and feed consumption remained unaffected. Similarly, Castiblanco et al. (2021) observed that including 15% or 20% DDGS in laying hen diets reduced egg production and impaired both feed conversion and egg weight. Abd El-Hack et al. (2017) also noted that a 15% DDGS inclusion reduced feed intake and impaired feed conversion, which aligns with the findings of the present study. Lumpkins et al. (2005) found slightly reduced egg production from 26 to 34 weeks of age in hens fed a low-density diet containing 150 g/kg DDGS (17.0% protein and 2805 kcal/kg ME), although this effect was not observed after 34 weeks of age. Roberson et al (2005) reported inconsistent effects of DDGS on egg production over different trial periods and suggested that lower levels of DDGS should be used when first introduced to diets. In contrast, some previous studies reported no adverse effects of 15% DDGS on hen performance parameters (Świątkiewicz and Koreleski, 2006; Masa'deh et al., 2011; Deniz et al., 2013). These inconsistencies may be attributed to variations in the energy content and digestible nutrients of DDGS from different sources, as well as differences in diet formulation and management practices.

Dietary probiotic supplementation did not significantly affect hen performance in the present study (*P*>0.05). However, in a study by Ceylan et al. (2022), the addition of a probiotic strain, *Bacillus licheniformis* DSM 28710, at 0.5 g/kg to a barley-sunflower meal-based diet significantly improved feed conversion ratio and egg mass. Similarly, supplementation with other strains of *B. licheniformis* has been shown to increase egg production and egg mass, although no significant effects on egg weight, feed consumption or feed conversion were observed (Lei et al., 2013). Pan et al. (2022) also reported enhanced feed utilisation efficiency following probiotic supplementation.

The interaction effects between DDGS and probiotics on hen performance were not significant in the current study (*P*>0.05). In contrast, Abd El-Hack et al. (2017) reported a significant interaction between DDGS levels and *Bacillus subtilis*, specifically in egg weight and egg mass, with the highest values observed in hens fed 50 g/kg DDGS in combination with probiotic.

The inclusion of DDGS in the diet significantly improved yolk colour (*P*<0.05). This result was expected, as corn is a rich source of xanthophylls, the primary pigments influencing yolk colour. Corn DDGS, which is a more concentrated source of xanthophylls than whole corn grain, may further increase pigment content during the fermentation process (Sun, 2011). The results of this study indicate that the xanthophylls in DDGS were efficiently absorbed and utilised by the hens, making DDGS a viable alternative to synthetic products for enhancing yolk colour. The positive effect of DDGS on yolk pigmentation observed in this study aligns with previous research (Shalash et al., 2010; Cortés-Cuevas et al., 2015; Shin et al., 2016; Trupia et al., 2016; Abd El-

Hack et al., 2017; Abd El-Hack et al., 2019; Castiblanco et al., 2021; Attia et al., 2024). However, Lumpkins et al. (2005) and Deniz et al. (2013) reported no significant effect of a diet containing 15% corn DDGS on yolk colour, suggesting variability due to differences in DDGS composition, source or experimental conditions. Haugh units, an important indicator of egg freshness and quality, were also significantly improved in this study. Higher Haugh unit values indicate better albumen quality and a longer shelf life, further demonstrating the potential benefits of DDGS. These results are consistent with those of Abd El-Hack et al. (2017) and Abd El-Hack et al. (2019), who reported a significant increase in Haugh units with the inclusion of 15% DDGS in laying hen diets. Conversely, other studies, such as those by Pineda et al. (2008) and Deniz et al. (2013), found no effect of DDGS on Haugh units, eggshell weight, shell thickness, or egg components.

Regarding the effect of probiotic supplementation on egg quality, the results of this study are consistent with those of Lei et al. (2013), Tang et al. (2015) and Abd El-Hack et al. (2017), who reported enhanced yolk coloration when hen diets were supplemented with *Bacillus licheniformis* and *Bacillus subtilis*, respectively. Several potential mechanisms may explain the influence of probiotics on egg yolk colour. Probiotics could enhance gut health and promote the absorption of carotenoids, as suggested by Darboe et al. (2023). Additionally, certain bacterial strains may produce enzymes that hydrolyse carotenoid esters, thereby increasing carotenoid bioavailability (Eroglu et al., 2023). In contrast to our results, other studies have reported effects of probiotics on eggshell weight, eggshell thickness, eggshell strength and Haugh units (Fathi et al., 2018; Ceylan et al., 2022; Liao et al., 2023; Liu et al., 2024). In the present study, significant interaction effects between DDGS and probiotics were observed, improving yolk coloration and eggshell quality ($P<0.05$).

Similarly, Abd El-Hack et al. (2017) found significant interactions between DDGS levels and *Bacillus subtilis* supplementation on egg quality traits, although the combination of 150 g/kg DDGS and 1000 mg/kg *Bacillus subtilis* resulted in thinner eggshells. Other studies have also reported beneficial effects of *Bacillus subtilis* and *Bacillus licheniformis* on eggshell thickness (Lei et al., 2013; Ceylan et al., 2022; Liao et al., 2023). This improvement may be attributed to enhanced gut health, including a reduction in gut pH due to the production of antibacterial compounds and organic acids by probiotic bacteria (Al-Khalaifa et al., 2019). Improved gut health likely facilitates the absorption of key minerals such as calcium and phosphorus, which are essential for eggshell formation (Abdelqader et al., 2013; Guo et al., 2017; Wang et al., 2021).

In our study, the inclusion of 15% corn DDGS in diets did not affect blood serum metabolites ($P>0.05$), indicating no adverse effects on the health status of the hens. These results are consistent with those reported by Wickramasuriya et al. (2020), who found no significant effect of DDGS inclusion level on plasma concentrations of total protein, ALT, and AST. However, Abd El-Hack et al. (2017) observed increased levels of total protein, albumin, triglycerides, cholesterol, and calcium at higher dietary DDGS inclusion levels. Similarly, Jiang et al. (2013) reported increased serum phosphorus concentrations in hens fed diets containing 100 and 200 g/kg DDGS.

In the present study, no significant differences were observed in any of the monitored serum biochemical parameters due to probiotic supplementation ($P>0.05$). This aligns partially with the findings of Pan et al. (2022), who reported only a reduction in glucose levels following the inclusion of *Bacillus licheniformis*. Other studies have associated *Bacillus* spp. supplementation with increased serum protein levels, suggesting a possible role in host protein metabolism (Wang et al., 2023; Ogbuewu et al., 2024).

The only significant interaction between corn DDGS and probiotic supplementation observed in this study was on serum albumin levels ($P<0.05$). In contrast, Abd El-Hack et al. (2017) reported significant interaction effects for most of the blood metabolites studied, except total protein. The increase in serum albumin observed in our study may indicate improved protein metabolism, potentially directed toward maintaining physiological homeostasis (e.g., immune response or tissue repair) rather than directly supporting egg production.

CONCLUSION

The inclusion of 15% corn DDGS in laying hen diets negatively affected performance by reducing feed intake, final body weight, egg weight, and egg mass output. However, it altered internal egg quality, demonstrating potential for improving yolk pigmentation and albumen quality. Probiotic supplementation alone did not significantly affect laying performance but showed beneficial interactions with DDGS, improving eggshell quality and yolk pigmentation. Serum biochemical parameters were largely unaffected, except for increased serum albumin levels observed with the combined use of corn DDGS and probiotics. These findings highlight the need for further research to explore the potential synergistic effects of probiotics in diets containing DDGS and their implications for productivity.

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