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DISTILLER'S DRIED GRAINS SUPPLEMENTED WITH ENZYME COCKTAIL AND YEAST AFFECT EGG QUALITY, REPRODUCTIVE PERFORMANCE AND BLOOD PROFILE OF BREEDING HENS

Y. A. Attia[1,2*], R. A. Alhotan[3*], N. F. Addeo[4], F. Bovera[4], R. A. Hassan[5], A. D. Al-Qurashi[1], A. E. Abd-El-Hamid[2], W. S. Selim[2] and K. A. Asiry[1]

[1]Agriculture Department, Faculty of Environmental Sciences, King Abdulaziz University, Jeddah, Saudi Arabia,
[2]Animal and Poultry Production Department, Faculty of Agriculture, Damanhour University, Damanhour 22516, Egypt, https://orcid.org/0000-0001-6505-3240
[3]Department of Animal Production, College of Food and Agricultural Sciences, King Saud University, Riyadh, Saudi Arabia, https://orcid.org/0000-0002-7789-4722;
[4]Department of Veterinary Medicine and Animal Production, University of Napoli Federico II, via F. Delpino 1, 80137 Napoli, Italy
[5]Deperatment of Poultry Nutrition, Animal Production Research Institute, Agricultural Research Center, Giza, Egypt
Corresponding author's email: ralhotan@ksu.edu.sa; yaattia@kau.edu.sa

ABSTRACT

This experiment aimed to examine the influences of distiller's dried grains with solubles (DDGS) in breeding hen's nutrition (0, 10, and 20%, respectively) with or without enzyme cocktail blend and yeast (*Saccharomyces cerevisiae*, SC) supplementation on the sustainability of eggs and semen quality, fertility and hatchability, and blood biochemistry. In total, 360, twenty weeks old indigenous Inshas breeding hens and 36 Inshas cocks (for natural mating) were used in the experiment. Inshas is a crossing breed that originated from mating Sinai and White Plymouth Rock breeds. Hens and cocks of all experimental groups had approximately similar initial body weights (1,560 ± 12.3 g) at the start of the experiment and were divided into 12 groups, each containing three replicates (10 hens + 1 cock). Moreover, 60 cocks were divided into 12 groups of five cocks each and housed individually in cages for semen quality estimation using the artificial collection technique. Increasing DDGS up to 20% in diets of breeding hens decreased ($p \leq 0.05$) eggshell thickness compared to 0 and 10% DDGS, but it elevated ($p \leq 0.05$) color of yolk and Haugh unit score during the storage period. Dietary treatments did not significantly affect the percentage fertility, hatchability, and abnormality of embryos and body weight of day-old chicks. Semen quality was not significantly affected by dietary DDGS. Yeast supplementation increased ($p \leq 0.05$) sperm concentration per ejaculate by 5.21% and total normal sperm/ejaculate and sperm concentration by 5.87%. Increasing DDGS levels up to 10% and 20% decreased blood plasma total cholesterol by about 4.1%. Enzyme cocktail supplementation increased ($p \leq 0.05$) blood plasma Ca by 5.4% of 20% DDGS diets, but other blood plasma parameters were unaffected. Enzyme cocktail addition to DDGS diets also decreased plasma cholesterol by 3.63%. Feeding a 10% and 20% DDGS diet increased ($p \leq 0.05$) plasma creatinine by 9.75% and decreased the uric acid/creatinine ratio by about 11.1%. In conclusion, corn DDGS could be included in dual-purpose breeding hen's diet up to 20% without adverse effects on the sustainability of the quality of eggs and semen, fertility and hatchability, and blood biochemistry. Furthermore, enzyme cocktail at 500 gm/ton feed and yeast at 1 kg/ton feed supplementations significantly and similarly improved eggshell thickness by 1.44-1.72%, and yeast increased sperm concentration and total sperm output per ejaculate.

Key words: Distillers dried grains with soluble; Breeding hens; Multi-enzymes; Yeast; Fertility; Hatchability; Semen quality.

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INTRODUCTION

The recent global crisis, such as Russia-Ukraine war and Covid-19 pandemic, together with the increased demand, increased the price of some imported ingredients such as soybean meal and corn (Hafez and Attia, 2020; USDA 2020). To lower production costs, it is vital to investigate newly available food sources (Valdivié-Navarro *et al*. 2020), such as distillation grains with solubles which have been proposed as a diet option for poli- and monogastric animals (Abd El-Hack *et al*. 2017). The increased ethanol production for biofuel increases

the dried distillers grain with solubles (DDGS) production (Zhu *et al*. 2018). For example, the USA produces around 44 million metric ton/year of DDGS (US Grains Council 2021).

In developing nations, poultry protein is crucial, where the average daily intake is far lower than the required limits (Onyimonyi and Onu., 2009; Hafez and Attia, 2020). Soybean meal is the traditional protein source in the poultry diet, and external fluctuations affect its availability and cost. Feed is the most expensive of the poultry industry and frequently makes up 60–65% of overall costs. Developing diets with alternative and locally accessible ingredients is the best way to lower feed costs. Rising ingredient prices continue to be the most significant single factor influencing profit margins in chicken production (Ghazalah *et al*., 2011; Shirisha *et al*., 2021; Attia and Serina, 2022). After the fermentation of corn for ethanol generation, DDGS are generated in significant quantities for the biofuel industry. A well-known and reasonably priced source of protein for chicken diets is corn DDGS (Harpster, 2007; Prasad, 2022). Notably, distillers dried grains with solubles (DDGS) exhibit an elevated concentration of non-starch polysaccharides (NSPs) compared to their parent grain counterparts. Despite the inherent inefficiency of monogastric animals in fully digesting NSP-rich diets, various research endeavors have corroborated the beneficial effects of enzyme supplementation on enhancing feed intake and egg production in laying hens. These studies, including those conducted by Scheideler et al. (2005) and Jones et al. (2022), underscore the potential of enzyme supplementation in optimizing the nutritional value of NSP-rich feedstuffs for monogastric animals.

The sustainability of laying performance, fertility, hatchability, and semen quality is crucial for the profits of breeding hen farming. Dietary DDGS demonstrated no deleterious impact on laying hen egg quality whether internally (Haugh unit and albumen height) or externally (shell strength and specific gravity) (Lumpkins *et al*., 2005; Pineda *et al*., 2008; Wittkiewicz and Koreleski, 200 and 2008). They also that including 15% DDGS in laying hen diets did not change laying rate. The Midwest's laying hen industry uses between 5 and 20% DDGS, primarily due to feeding cost reductions, according to Bregendahl (2008); however, the author increased the ratio up to 20% DDGS as a maximal inclusion rate. Moreover, it was demonstrated that up to 69% of DDGS may be supplied without affecting the total laying rate (Pineda *et al*., 2008). Increased DDGS inclusion favored egg color (Roberson *et al*., 2005; Roberts *et al*., 2007; Witkiewicz, and Koreleski, 2006 & 2008). When brown leghorn layers were provided 15% or 23% DDGS, Pescatore *et al*. (2010) noticed a considerable decrease in feed intake. Moreover, Hassan *et al*. (2013a) demonstrated that corn DDGS in a quail layer diet up to 18% with enzymes and up to 12% with citric acid could be advised without hurting egg quality and blood parameters to enhance economic efficiency.

Moreover, no harmful influences on the quality and hatchability of quail were cited (Hassan *et al*., 2013b). In light of recent disruptions to the global feed supply chain, the optimization of by-product utilization as alternative feed resources is crucial for enhancing laying hen productivity, product quality, and physiological health. The ongoing conflict between Russia and Ukraine has exacerbated global feed shortages, leading to significant price increases for key feed ingredients such as yellow corn and soybean meal. Additionally, extended shipping times have compromised the nutritional value of these components, necessitating the exploration of unconventional feed alternatives. Consequently, this investigation sought to test the impacts of graded doses of maize DDGS for soybean meal and yellow corn, along with or without enzyme cocktail and yeast, on the sustainability of egg quality, semen, fertility, hatchability, and blood biochemistry in laying hens.

MATERIALS AND METHODS

The DDGS was purchased from the Cairo Poultry Company, and the by-product was golden in color, suggesting reasonable heating during processing. The other feedstuffs used in diet formulations were purchased from the local market. The additives used were Kemzyme and yeast; Kemzyme PLUS Dry is an enzyme cocktail produced in Kemin Europe N.V., Kemin Industries, Inc., USA (Kemzyme® PLUS Dry, euro code E 1620, contains five different enzymatic activities, 400 U/g alpha-amylase,4,000 U/g endo-1,4-beta-glucanase, 2,350U/g endo-1,3(4)-beta-glucanase, 450 U/g bacillolysin, and 20,000 U/g endo-1,4-beta-xylanase, in addition to Saccharomyces cerevisiae (containing active yeast: 7×10^8, CFU/g); the yeast was a Saccharomyces cerevisiae (SC) preparation produced by Alltech, Nicholasville, Kentucky, USA.

Experimental birds: A total of 360 local dual-purpose breeding hens of Inshas strain (mean body weight 1488.7±6.49), established by crossing between Sinai and White Plymouth Rock breeds, (Bakir*et al*., 2002), and 36 Inshas cocks (for natural mating with 1613±14.6) aged 28 weeks old were taken at random and divided into 9 experimental groups, with each group containing three replicates (10 hens + 1 cock). The profile of diets is reported in Table 1.

The experimental design was two treatments factors with three DDGS levels (0, 10, and 20%), and three feed additives (unsupplemented, enzyme cocktail and yeast supplementation) and their interactions.

The additives tested were Saccharomyces cerevisiae (supplemented at 1 kg/ton feed), and commercial enzyme cocktail preparations Kemzyme PLUS Dry® (supplemented at 500 g/ton feed). Birds of each replicate were kept in floor pens (2.8 long × 2.2 m wide) furnished with rice hulls as bedding material. Birds were offered 16 h light and 8 h of dark daily. All groups of birds were reared under the same hygienic and administrative guidelines. Fresh water and food were always available over the experiment's 28–48 week duration.

Table 1: Composition and calculated analysis of experimental diets.

Ingredients (%)	Control diet	10% DDGS	20% DDGS
Yellow corn	64.00	60.69	57.00
Soybean meal (44% CP)	23.70	18.30	12.76
Wheat bran	1.85	0.70	0.00
distiller's dried grains with solubles	0.00	10.00	20.00
Limestone	8.00	8.00	8.06
Dicalcium phosphate	1.63	1.53	1.41
Sodium cholride	0.45	0.33	0.21
Vitamin and mineral premix*	0.30	0.30	0.30
DL-methionine	0.07	0.06	0.05
L-lysine-HCl	0.00	0.09	0.21
Calculated analyses (%)			
Crude protein	16.04	16.01	16.01
Metabolizeable energy (kcal/kg)	2,700	2,706	2,704
Crude fiber	4.64	4.93	5.25
Methionine	0.36	0.36	0.37
Methionine + Cystine	0.62	0.62	0.62
Lysine	0.79	0.79	0.79
Calcium	3.46	3.44	3.42
Available phosphorus	0.43	0.43	0.42
Sodium	0.19	0.19	0.19
Determined analyses (%)			
Dry matter	89.80	89.56	89.68
Crude protein	16.54	16.48	16.41
Ether extract	2.70	3.52	4.38
Crude fiber	4.78	4.82	5.00

*Supplied per kg of diet: Vit. A, 12000 IU; D_3, 2200 IU; Vit. E, 10 mg; Vit. K_3, 2 mg; Vit. B_1, 1 mg; Vit. B_2, 5 mg; Vit. B_6, 1.5 mg; Vit. B_{12}, 10 mcg; niacin, 30 mg; pantothenic acid, 10 mg; folic acid, 1 mg; biotin, 50 µg; choline, 260 mg; copper, 10 mg; iron; 30 mg; manganese, 60 mg; zinc, 50 mg; iodine, 1.3 mg; selenium, 0.1 mg; cobalt, 0.1 mg.

Egg quality: Five eggs per replicate per treatment were collected during 44, 46, and 48 weeks of age, which amounted to 60 eggs per group during the experimental time. Eggs of each treatment were used to estimate the egg quality of fresh eggs; the other eggs were stored at room temperature for 7, 14 and 21 days to establish the egg quality of stored eggs. Each egg was weighed separately before measuring its length and width with a compass with a 0.01 mm resolution. The eggs were then cracked on a glass surface, and the height of the yolk was measured using a micrometer with a resolution of 0.01 mm. A compass with a 0.01 mm resolution was used for measuring the yolk's breadth. Each egg's albumen and yolk were detached, and the albumen's and yolk's weights in grams and percentage in relation to the egg's weight were calculated. The yolk/albumen ratio was estimated by dividing the yolk's weight by the albumen's weight. Each egg's shell was rinsed in softly running water to eliminate any remaining albumen, and it was then left to dry for 24 hours outside. For each egg, the combined weight of the shell and membrane was measured in grams and reported as relative to the egg weight. The thickness of three eggshell locations (the top, medial, and base) was estimated and averaged using a micrometer. The formula for the Haugh unit was $HU=100 \log (H+ 7.37-1.7 EW^{0.37})$, where H stands for albumen height (mm) and EW for egg weight (g).

Additionally, the egg shape index was equal to (Egg width in mm/ egg length in mm) x 100. The egg yolk index (YI) was equal to (yolk height/yolk diameter) × 100. The Roche Yolk Color Fan was utilized for yolk color score estimation.

Semen quality: The semen quality was estimated utilizing an artificial collection approach. Adult cocks (n=60) were allocated to 12 groups of five cocks per each

and kept separately in cages, and semen samples were artificially gathered by abdomen massaging. Ejaculated volume (ml) was estimated using a 2 ml calibrated pipette. Sperm motility was measured just after semen collection by microscopic examination. Abnormal sperm and dead spermatozoa and sperm concentration were evaluated using a hemocytometer, as reported by Attia *et al*. (2015).

Fertility and hatchability of eggs: Throughout the trial, measurements of fertility and hatchability were evaluated at 44, 46, and 48 weeks of age. Eggs were collected (n = 100 per treatment/period) representing all treatment replicates of around 33 eggs each. Eggs were gathered for seven days and kept in a chamber at 70% relative humidity (RH) levels and 18.5°C dry bulb. They were incubated at 60% RH and 37.5 °C, and hatched at 70% RH and 37.2 °C in an automatic Chick Master machine and hatcher. Eggs were candled on the 18th day of incubation to test fertility. On day 21, all unhatched eggs were broken to distinguish infertile eggs from those having dead embryos. Fertility and hatchability were calculated as outlined by Attia *et al*. (1995).

Blood constituents: Blood samples (n=3) of each treatment representing all replicates were collected at the end of the trial, and the plasma was separated by centrifugation at 2,000 rpm for 20 minutes. The biochemical constituents of blood plasma were determined following the methods by Attia *et al*. (2014; 2015; 2020) utilizing the commercial kits (Diamond Company, Cairo, Egypt), including plasma total protein and albumin total cholesterol total lipids, Ca++, inorganic P, creatinine, and alanine aminotransferase (ALT), and aspartate aminotransferase (AST) were evaluated. Total antioxidant capacity was obtained using available commercial kits (Spectrum diagnostics, Germany), and malondialdehyde (MDA) was estimated using a spectrophotometer at 532 nm absorbance at 2-TBA (Hitachi, Japan).

Data analysis: Data were examined statistically using two treatments factors with three DDGS levels (0, 10, and 20%), and three feed additives (unsupplemented, enzyme cocktail and yeast supplementation) and their interactions using the General Linear Model procedure of SAS (2008), according to the following model:

$$Yijk= \mu + Di+ Aj+ D*Aij+ Eijk,$$

Where Yijk is the measured characteristic, μ is the overall mean, Di represents the influence of DDGS levels (i=1, 2, 3), Aj represents the effect of feed additives (j=1, 2, 3), (D*A)ij represents the interaction between DDGS and additive type, and Eijk represents the experimental error.

The effect of storage on egg quality traits was studied using three treatments factors with three DDGS levels (0, 10, and 20%), three feed additives (unsupplemented, enzyme cocktail and yeast supplementation) and two storage time fresh vs. storage time) their interactions according to the following model:

$$Yijkt= \mu + Di+ Aj+St+ D*Aij+ D*Sit+ A*Sjt+ D*A*Sijt+Eijtk,$$

Where Yijkt is the measured characteristic, μ is the overall mean, Di represents the influence of DDGS levels (i=1, 2, 3), Aj represents the effect of feed additives (j=1, 2, 3), St, represents the effect of storage time, (D*A)ij represents the interaction between DDGS and additive type, (D*S)it represents the interaction between DDGS and storage time, (A*S)jt represents the interaction between additive type and storage time, (D*A*S)ijt represents the interaction between additive type, DDGS and storage time and Eijk means the experimental error.

The egg quality traits were presented when the storage time effect was significant. The mean differences at $p \leq 0.05$ were compared using the Student-Newman-Keuls test. The experiment unit was the replicate.

RESULTS AND DISCUSSION

Egg quality traits: Table 2 reports the physical quality of eggs as influenced by DDGS levels, additive supplementation and their interaction. The DDGS level affects only shell thickness (p=0.001) and yolk color (p=0.0001). Thus, DDGS at 20 % did not harm egg quality traits maintaining its sustainability.

Only the interaction between dietary additives and DDGS levels significantly impacted weight (%) (p= 0.018) and thickness of shell (p=0.001). Feeding 20% DDGS diets without enzyme cocktail and SC supplementation significantly decreased shell weight (%) and thickness compared to the other groups, showing the negative effect of high levels of DDGS on calcium metabolism by laying hens.

In addition, enzyme cocktail or SC supplementation similarly restored (p=0.003) shell quality to the level of the other groups. Thus, the negative effect of high levels of DDGS was utterly diminished. Because DDGS includes sulfur, which may prevent dietary Ca from the small intestines from being absorbed, this diet's detrimental effects on eggshell quality may occur. The drop-in plasma calcium (3.5%) supports these Ca levels for the 20% DDGS-supplemented diet, as seen in Table 9. These findings concur with those made by Pineda *et al*. (2008), Saeed *et al*. (2017), and Abd El-Hack *et al*. (2017), who found that DDGS concentrations between 15 and 20 percent were detrimental to eggshell quality. Previous results show that high quantities of crude fibre in poultry diets enhance mineral restriction and impact the absorption of the minerals like calcium and magnesium and, thus the quality of the eggshell (Savón *et al*., 2007). In addition, Sun and Kim (2020) demonstrated that increasing phytic P intake decreases Ca

absorption and, consequently, eggshell thickness in laying hens because of the imbalance in the Ca: P ratio.

Our research, however, showed that feeding a 10% DDGS diet had no adverse effects on the characteristics of shell quality. These findings are consistent with those from Lumpkins *et al*. (2005), Roberts *et al*. (2007), and Pineda *et al*. (2008). The laying hen industry uses specific gravity to indicate good egg quality. In this regard, a dietary increase of DDGS decreased ($p<0.005$) specific gravity, showing poorer eggshell quality. Instead, enzyme cocktail supplementation significantly increased linearly ($p\leq0.05$) egg-specific gravity of diets containing 15 or 23% DDGS (Pescatore *et al*., 2010). Similarly, we found that a positive effect of enzyme cocktail and SC supplementation on eggshell quality found herein, particularly when added to 20% DDGS diets, indicated an increase in minerals, mainly Ca, for eggshell formation, as shown by Abd El-Hack *et al*. (2017), with the rise in eggshell (%) and plasma Ca concentration.

DDGS at 10% and 20% significantly ($p=0.0001$) impacted yolk color compared to the control diet (Table 2). According to Abd El-Hack *et al*. (2015), the carotenoids in the yellow corn used to make ethanol give DDGS its golden hue. Because of the enhancing process, high levels of lutein and zeaxanthin in DDGS lead to a more intense egg yolk color (Shin *et al*., 2016). This finding was anticipated, given that corn contains a significant amount of xanthophyll, a major factor affecting yolk color. This suggests that xanthophyll was readily available in the DDGS. According to Sauvant and Tran (2004), dried distiller's grains containing solubles have a xanthophyll concentration of about 34 mg/kg, which is three times more than that of maize (10.62 mg/kg) (NRC, 1994). The present findings concur with those obtained by Pineda *et al*. (2008) and NRC (1994). Similarly, adding 15, 20, or 25% DDGS to laying hens' diets modified the egg yolk color (Shalsh *et al*., 2010; Masa'deh *et al*., 2011; Cuevasa *et al*., 2012). In addition, DDGS up to 25% in laying hen diets boosted egg yolk pigmentation compared to the control group. Nevertheless, laying hens fed diets containing 10-15% DDGS did not influence egg yolk color (Lumpkins *et al*., 2005; Roberts *et al*., 2007; Deniz *et al*., 2013).

Table 2: Egg quality as affected by distiller's dried grains with solubles, Kemzyme, and Saccharomyces cerevisiae Interaction between DDGS and additive.

Dietary treatments		Egg quality traits								
		YW (%)	AW (%)	Y/A	SW (%)	YI (%)	ST (µm)	ES (%)	HU	YC
Effect of distiller's dried grains with soluble										
DDGS	0	33.8	54.5	0.620	11.7	46.4	354^{a}	76.5	87.4	5.60^{b}
(%)	10	33.8	54.5	0.620	11.8	46.3	354^{a}	76.5	87.4	6.20^{a}
	20	33.7	54.5	0.615	11.7	46.3	348^{b}	76.4	87.3	6.40^{a}
Effect of additives										
Additive	0	33.7	54.7	0.616	11.6	46.3	348^{b}	76.4	87.3	6.06
	Kemzyme	33.8	54.5	0.619	11.8	46.4	353^{a}	76.5	87.4	6.04
	SC	33.8	54.4	0.620	11.8	46.4	354^{a}	76.5	87.4	6.13
DDGS (%)	**Additive**									
	0	33.7	54.5	0.618	11.7^{a}	46.3	355^{a}	76.5	87.4	5.60
0	Kemzyme	33.8	54.4	0.622	11.7^{a}	46.5	354^{a}	76.5	87.4	5.60
	SC	33.8	54.4	0.621	11.7^{a}	46.4	355^{a}	76.5	87.5	5.73
	0	33.8	54.5	0.619	11.7^{a}	46.3	353^{a}	76.4	87.4	6.20
10	Kemzyme	33.9	54.4	0.623	11.7^{a}	46.4	354^{a}	76.5	87.6	6.20
	SC	33.7	54.5	0.617	11.8^{a}	46.4	355^{a}	76.5	87.3	6.26
	0	33.6	55.0	0.611	11.4^{b}	46.2	339^{b}	76.4	87.2	6.40
20	Kemzyme	33.5	54.7	0.613	11.8^{a}	46.3	353^{a}	76.4	87.4	6.33
	SC	33.8	54.4	0.622	11.8^{a}	46.3	353^{a}	76.4	87.4	6.40
SEM		0.313	0.366	0.009	0.187	0.851	42.5	0.618	0.530	0.328
P value s										
DDGS		0.455	0.210	0.327	0.393	0.929	0.0001	0.956	0.769	0.0001
Additive		0.856	0.133	0.469	0.011	0.929	0.003	0.955	0.818	0.743
DDGS × additive		0.341	0,262	0.394	0.018	1.000	0.001	1.000	0.956	0.994

YW: yolk weigh; AW: albumen weight; Y/A: yolk/albumen ratio; SW: shell weight; YI: yolk index; ST: shell thickness; ES: egg shape; HU: Haugh unit; YC: yolk color. Different superscripts (^{a,b}) within rows differ at $P\leq0.05$; SEM: standard error of the mean, DDGS: distiller' dried grains with solubles, SC: *Saccharomyces cerevisiae*.

Partially supporting the present findings, laying hens fed DDGS up to 15% with or without additives showed no negative influences on the shell quality, egg yolk, or Haugh units (Deniz et al. 2013). Similarly, Saeed et al. (2017) and Abd El-Hack et al. (2017) demonstrated that DDGS did not significantly impact the egg shape index and that supplementing with enzymes and DDGS did not influence the yolk index and shell percentage. However, the Haugh unit score increased substantially due to including DDGS in laying hens' diets at 16.5%, while the albumen percentage decreased. Moreover, enzyme supplementation significantly decreased yolk (%) and egg shape index but increased albumen percentage and did not affect shell quality, Haugh unit score, and yolk index.

This study's findings that SC improves eggshell quality could be explained by better gut health and yeast's phytase enzyme contents, which increase calcium absorption and retention (Bradley and Savage, 1995). According to Park et al. (2001), hens fed yeast-containing diets produced fewer soft shells and broken eggs than those in the control group. Probiotics have been shown to affect gut health (Aghaei et al. 2010; Mikulski et al. 2012; Youssef et al. 2013). Probiotic bacteria create a favorable environment and produce short-chain fatty acids (SCFAs) that lower luminal pH faster in the gut (Mohan et al., 1995; Scholz-Ahrens et al., 2007; Panda et al., 2008; Mikulski et al., 2012).

Calcium solubility and absorption are both increased by low luminal pH. (Van den Heuvel et al., 1999). Moreover, SCFAs promote villus height and proliferation of intestinal epithelial cells (Garcia et al., 2007), improving absorption effectiveness (Scholz-Ahrens et al., 2007). Consequently, greater nutrients, including calcium, may be absorbed, enhancing the eggshell's quality.

In our investigation, SC had a comparable impact as an enzyme cocktail and did not appreciably affect the internal egg quality characteristics such as yolk weight (%), color and index, or albumen quality (albumen percentage and Haugh unit score). Furthermore, neither Xu et al. (2006) nor Zhang et al. (2012) observed any modifications in yolk pigmentation due to probiotics. Several authors have reported that probiotics administered to laying hens induced a deeper egg yolk color (Mikulski et al., 2012; Youssef et al., 2013). Other trials did not reveal this effect, according to Zhang et al. (2012), who discovered that probiotics had a favorable impact on albumen quality (Xu et al., 2006; Panda et al., 2008; Mikulski et al., 2012; Youssef et al., 2013). The enhancement in albumen quality in the group receiving microbial supplements, reported by Mahdavi et al. (2005), has no logical justification. One could assume that carotenoids can be absorbed and moved into the egg yolk, enriching the yolk pigmentation. Low serum cholesterol is linked to diets high in carotenoids (Yeum and Russell, 2002).

Thus, DDGS did not adversely impact interior egg quality. Still, a high level (20%) DDGS significantly decreased eggshell quality, and supplementation with enzyme cocktail and SC similarly restored eggshell quality to the normal level. Thus, 20% DDGS with enzyme cocktail or SC supplementation could be fed to breeding hens from 28 to 48 weeks of age without adverse or influences on eggshell quality.

Haugh unit at different storage times: No significant variations were recorded due to the enzyme cocktail, SC, and the interaction between DDGS and enzyme cocktail and SC on the Haugh unit score (Table 3). However, time significantly (p=0.0001) affected Haugh unit score measurements after 7, 14, and 21 days of storage at room temperature, showing a progressive decrease in Haugh Unit with increasing storage period. The results indicated that 20% of DDGS significantly changed the Haugh unit during storage times compared to the control group. In addition, 10% DDGS improved Haugh unit score during the third week compared to the control group (Table 3). DDGS may provide a potential solution to maintain the shelf life of albumen quality. Similarly, researchers Lilburn and Jensen (1984), Saeed *et al*. (2017), and Abd El-Hack *et al*. (2017) found that adding DDGS between 10% to 20% increased Haugh units. Instead, the researchers Swiatkiwicz and Korleski (2006), Roberson *et al*. (2005), and Pineda *et al*. (2008) observed no impact of 0% to 20% DDGS on Haugh units.

Table 3: Haugh unit score at different storage times as affected by distiller's dried grains with solubles, Kemzyme, and Saccharomyces cerevisiae.

Dietary treatments		Haugh unit score
Time effect		
Days	0	80.95[a]
	7	78.80[b]
	14	75.70[c]
	21	73.10[d]
SEM		0.312
Probability value		
Time		0.0001
DDGS × additive		0.986
DDGS × time		0.502
Additive × time		0.999
DDGS × additive × time		0.998

Different superscripts ([a,b,c,d]) within rows differ at P≤0.05; SEM: standard error of the mean, DDGS: distiller's dried grains with solubles

Chemical analysis of egg yolk; DDGS concentrations with or without enzyme cocktail and SC had no

discernible impact (p≥0.05) on the fat, moisture, protein, and ash, content of egg yolks (Table 4). Nevertheless, Sun and Kim (2020) showed that while there were no variations in the dry matter content of egg yolks between treatments, the protein and fat content of egg yolks in the group on 50% DDGS were considerably lower than those in the 0, 17, and 35% groups. Moreover, Saeed *et al*. (2017) and Abd El-Hack *et al*. (2017) discovered that DDGS to laying hen diets from 0, 5.5, 11, and 16.5% significantly enhanced total egg solids, protein, and lipids while progressively lowering nitrogen-free extract.

Table 4: Chemical components of egg yolk as affected by distiller's dried grains with solubles, Kemzyme, and Saccharomyces cerevisiae.

Dietary treatments		Yolk chemical composition (%)			
		Fat	**Moisture**	**Protein**	**Ash**
Effect of distiller's dried grains with soluble					
	0	32.3	48.6	16.6	2.5
DDGS (%)	10	32.2	48.5	16.6	2.7
	20	32.2	48.4	16.7	2.9
Effect of additives					
	0	32.2	48.4	16.5	2.9
Additive	Kemzyme	32.3	48.6	16.6	2.6
	SC	32.3	48.5	16.6	2.6
Interaction between DDGS and additive					
DDGS (%)	Additive				
	0	32.2	48.5	16.6	2.73
0	Kemzyme	32.3	48.8	16.7	2.23
	SC	32.3	48.6	16.6	2.46
	0	32.2	48.3	16.5	2.93
10	Kemzyme	32.2	48.6	16.6	2.60
	SC	32.3	48.5	16.6	2.56
	0	32.2	48.5	16.4	2.90
20	Kemzyme	32.3	48.3	16.5	2.96
	SC	32.3	48.4	16.5	2.83
SEM		0.170	0.293	0.188	0.347
P values					
DDGS		0.983	0.589	0.575	0.352
Additive		0.855	0.852	0.861	0.617
DDGS × additive		0.910	0.931	0.996	0.935

SEM: standard error of the mean; DDGS: distiller' dried grains with solubles, SC: *Saccharomyces cerevisiae*.

Reproductive performance: The effects of different DDGS levels with or without additives on fertility (%), hatchability traits (%), abnormality (%), and body weight of day-old chick (g) are shown in Table 5.

Data indicated no effects (p≥0.05) of DDGS and/or supplementation with enzyme cocktail and SC on fertility, hatchability traits, abnormal chicks, and weight of day-old chicks. These findings revealed that the reproductive efficiency of laying hens was unaffected by DDGS and DDGS diets provided adequate nutrients to maintain the reproductive performance of breeding hens. Also, El-Deek *et al*. (2003) reported no impact of enzyme addition on either hatchability or fertility. According to Shalash *et al*. (2010), dietary DDGS at 5, 10, 15, and 20% had no appreciable impact on hatchability compared to the control diet. In addition, adding enzymes to diets that included 5, 10, 15, and 20% dietary DDGS did not affect hatchability compared to the equivalent control diets. Incorporation of DDGS alone or in conjunction with enzymes into the diets of laying hens did not exert any significant influence on fertility rates, overall egg production, proportion of fertile eggs, or chick body weight at hatching (Ghazalah et al., 2011)

Semen quality traits: The findings in Table 6 showed up to 20% DDGS did not harm the semen quality of cocks and DDGS diets provide adequate nutrients for maintaining sufficient quantity and qualitative semen production. Nevertheless, additive fortification resulted in a considerable rise in sperm concentration and total sperm/ejaculate compared to the unsupplemented control. These outcomes are consistent with those established by El-Deek *et al*. (2003), who discovered that the addition of enzymes had no harmful impact on total egg hatchability (%) or fertility (%). Nevertheless, Shalash *et al*. (2010) found no appreciable impacts of DDGS inclusion at 0–

20% on semen quality, fertility (%), hatchability (%), and chicks' weight in the hatch. Moreover, according to Ghazalah *et al*. (2011), adding DDGS and/or enzymes had no appreciable impact on the semen quality parameters.

Table 5: Reproductive performance as affected by distiller's dried grains with solubles, Kemzyme, and Saccharomyces cerevisiae.

Dietary treatments		Traits				
		Fertility (%)	Total egg hatchability (%)	Fertile eggs hatchability (%)	Abnormality (%)	Hatched chicks BW (g)
Effect of distiller's dried grains with soluble						
DDGS (%)	0	90.2	81.4	90.2	0.955	34.8
	10	90.0	81.0	90.0	1.06	34.7
	20	89.7	80.5	89.7	1.17	34.7
Effect of additives						
Additive	0	89.9	80.7	89.8	1.09	34.7
	Kemzyme	90.1	81.2	90.1	1.07	34.7
	SC	89.9	81.0	90.1	1.02	34.8
Interaction between DDGS and additive						
DDGS (%)	Additive					
0	0	90.1	81.0	89.9	1.00	34.7
	Kemzyme	90.2	81.8	90.7	1.00	34.8
	SC	90.2	81.3	90.1	0.866	34.8
10	0	89.9	80.9	90.0	1.03	34.8
	Kemzyme	90.0	81.0	90.0	1.07	34.7
	SC	90.0	81.1	90.1	1.07	34.8
20	0	89.5	80.0	89.4	1.23	34.6
	Kemzyme	90.0	80.7	89.7	1.13	34.7
	SC	89.7	80.8	90.1	1.13	34.7
SEM		0.349	0.663	0.492	0.684	0.129
P values						
DDGS		0.301	0.646	0.126	0.076	0.583
Additive		0.784	0.746	0.419	0.737	0.890
DDGS × additive		0.981	0.942	0.895	0.888	0.960

BW: body weight. SEM: standard error of the mean, DDGS: distiller's dried grains with solubles, SC: *Saccharomyces cerevisiae*.

The B vitamins of SC and/or mannan-oligosaccharides (MOS) of SC cell walls may be responsible for the significant (p=0.024) increase in sperm concentration per ejaculate and total sperm per ejaculate of cocks fed SC diets. These findings support a prior study by Mc-Daniel (1991), which found that breeder males fed diets supplemented with yeast culture had enhanced sperm concentration. The increased availability of nutrients made possible by more effective nutrient absorption at the gastrointestinal tract may have contributed to the observed improvement in sperm concentration in the male MOS-fed animals.

Moreover, studies have revealed increased antioxidant activity in piglets and birds fed diets supplemented with MOS (Zhou *et al*., 1991). The potential for sperm maturation and antioxidant activity, such as glutathione peroxidase and superoxide dismutase, to improve in MOS-fed birds should be carefully considered from this perspective (Shashidhara and Devegowda, 2003). High amounts of glutathione peroxidase in the tests are an effective antioxidant for developing spermatids and spermatozoa (Ursini *et al*., 1999). The current findings concur with those of Shashidhara and Devegowda (2003), who claimed that the MOS, a cell wall component, increases fertility and hatchability in older breeder females by enhancing the quality of the eggshell and sperm production in male breeders.

Blood plasma constituents*:* Blood plasma proteins were estimated to show the protein quality from the standpoint of the metabolic status of laying hens fed different DDGS levels without or with additive supplementations (Table 7). Results showed no significant differences among other dietary treatments, such as DDGS, enzyme cocktail, and SC ($p \geq 0.05$) in plasma total protein and its fractions,

suggesting that DDGS had good protein quality and did not adversely affect protein metabolism.

The present results are in harmony with those by Gabr *et al*. (2008) and Awad *et al*. (2011), who observed that DDGS up to 30% had no significant influence on the total protein of chickens and ducks. Furthermore, the lack of substantial effect of enzyme cocktail and SC on plasma total protein agrees with those by Abou El-Wafa *et al*. (2002), Abu Suliman (2012), and Attia *et al*. (2014), who reported that enzyme-supplemented diets did not significantly impact blood plasma total protein and its fractions. Moreover, Wakwak *et al*. (2003) and Mateova *et al*. (2009) found that probiotics did not influence the blood biochemistry of laying hens.

Table 6: Semen quality of Inshas cockerels as affected by distiller's dried grains with solubles, Kemzyme, and Saccharomyces cerevisiae.

Dietary treatments		Semen characteristics									
		Volume, ml	Sperm concentration, million/mm³	Motility, %	Normal sperm %	Abnormal Sperm, %	Dead sperm, %	Live Sperm, %	Sperm concentration/ejaculate, million	Total normal sperm/ejaculate, million	Total functional sperm, million
Effect of distiller's dried grains with soluble											
DDGS (%)	0	0.343	2.58	82.4	85.3	9.67	5.11	94.9	0.883	0.753	0.621
	10	0.340	2.54	82.3	85.3	9.22	5.44	94.6	0.866	0.739	0.608
	20	0.336	2.51	82.2	85.1	9.56	5.33	94.7	0.845	0.719	0.591
Effect of additives											
Additive	0	0.336	2.51	82.1	84.9	9.89	5.22	94.8	0.844^{b}	0.716^{b}	0.588
	Kem	0.340	2.53	82.4	85.4	9.22	5.44	94.6	0.861^{ab}	0.736^{ab}	0.607
	SC	0.343	2.59	82.4	85.4	9.33	5.22	94.8	0.888^{a}	0.758^{a}	0.625
Interaction between DDGS and additive											
DDGS (%)	Add										
	0	0.340	2.57	82.3	85.0	9.67	5.33	94.7	0.872	0.741	0.340
0	Kem	0.340	2.57	82.7	85.3	9.67	5.33	94.7	0.871	0.743	0.340
	SC	0.348	2.60	82.3	85.7	9.67	4.67	95.3	0.905	0.775	0.348
	0	0.337	2.53	82.0	85.0	9.67	5.33	94.7	0.853	0.725	0.337
10	Kem	0.341	2.50	82.3	85.7	9.00	5.33	94.7	0.852	0.730	0.341
	SC	0.343	2.60	82.7	85.3	9.00	5.67	94.3	0.892	0.761	0.343
	0	0.331	2.43	82.0	84.7	10.3	5.00	95.0	0.806	0.682	0.331
20	Kem	0.340	2.53	82.3	85.3	9.00	5.67	94.3	0.861	0.735	0.340
	SC	0.338	2.57	82.3	85.3	9.33	5.33	94.7	0.867	0.739	0.338
SEM		0.005	0.052	1.22	0.942	0.703	1.18	1.18	0.019	0.019	0.021
P values											
DDGS		0.396	0.321	0.975	0.946	0.726	0.940	0.940	0.092	0.132	0.254
Additive		0.319	0.203	0.928	0.710	0.475	0.965	0.965	0.044	0.056	0.128
DDGS × additive		0.909	0.709	0.999	0.997	0.905	0.985	0.985	0.622	0.718	0.860

Different superscripts (^{a,b}) within rows differ at P≤0.05; SEM: standard error of the mean; DDGS: distiller's dried grains with solubles; Add: additive; Kem: Kemzyme; SC: *Saccharomyces cerevisiae.*

The impact of DDGS and the interaction between DDGS and additives were insignificant (p≥0.05) for total lipids and cholesterol/total lipid ratio for plasma, showing that DDGS did not negatively affect lipid metabolism in laying hens showing the safety of DDGS as feedstuffs for feeding laying hens. In addition, feed additives did not significantly affect the plasma cholesterol/total lipid ratio. However, the impact of feed additives was near significant (p=0.056) on plasma total lipids and significant (p=0.024) for total plasma cholesterol. There is a trend (p=0.115) toward a decrease in plasma lipid with increasing DDGS levels, whereas this effect was significant in plasma cholesterol with no difference between 10% and 20% DDGS levels. On the other hand, the inclusion of DDGS did not affect the cholesterol/total lipids ratio (p≥0.05).

These findings conflict with Saeed et al. (2017) and Abd El-Hack et al. (2017), who claimed that adding 11% and 16.5% DDGS to laying hens' diets considerably raised their plasma triglycerides and total cholesterol,

respectively. Nasir Akbar (2017) discovered that broiler chicken plasma triglycerides and total cholesterol were unaffected by 20% DDGS. The increase in dietary fiber, which prevents lipids from being absorbed, may be responsible for the decrease in total lipids and cholesterol in the DDGS-fed groups (Al-Harthi et al., 2010). DDGS components of essential oils affect the activity of hepatic 3-hydroxy-3-methylglutaryl CoA (HMG-CoA) reductase and consequently decrease the plasma cholesterol content (Crowell, 1999). The previous assumption could support the reduction in plasma lipids and cholesterol due to DDGS and enzyme cocktail or SC fortification. Like this, Gabr et al. (2008) discovered that feeding meals containing 10, 15, and 20% DDGS did not significantly influence cholesterol; however, giving 20% DDGS reduced meaningly total lipid compared to the control.

In the literature, raising dietary DDGS level by up to 18% in comparison to those fed the control diet for female ducklings resulted in a negligible ($p \leq 0.05$) drop in plasma cholesterol (Awad et al., 2011). Similarly, dietary supplementation with yeast or yeast products decreased the cholesterol found in egg yolks (Yalçn et al., 2008; Yousefi and Karkoodi, 2007; Yalçn et al., 2010). The reduction in yolk cholesterol could be attributed to a decrease in cholesterol production, absorption, or both in the digestive tract (Mohan et al., 1995). High-density lipoprotein (HDL), the beneficial type of cholesterol, is of interest in this context.

Table 7: Plasma protein and lipid profiles as affected by distiller's dried grains with solubles, Kemzyme, and Saccharomyces cerevisiae.

Dietary treatments		Blood plasma metabolites						
		Total protein, (mg/dl)	A, (mg/dl)	G, (mg/dl)	A/G ratio	Total lipids, (mg/dl)	Cholesterol, (mg/dl)	Cholesterol/ total lipid ratio
Effect of distiller's dried grains with soluble								
	0	5.42	3.38	2.03	1.69	569.0	152.3[a]	0.267
DDGS (%)	10	5.35	3.33	2.02	1.65	560.7	147.3[b]	0.262
	20	5.42	3.34	2.07	1.62	558.6	146.1[b]	0.261
Effect of additives								
	0	5.33	3.33	2.00	1.68	570.2	151.7[a]	0.266
Additives	Kemzyme	5.44	3.35	2.08	1.62	559.8	146.2[b]	0.261
	SC	5.42	3.37	2.04	1.66	558.2	147.9[ab]	0.264
Interaction between DDGS and additive								
DDGS (%)	Additive							
	0	5.33	3.36	1.96	1.75	570.0	155.3	0.272
0	Kemzyme	5.50	3.36	2.13	1.58	572.0	150.0	0.262
	SC	5.43	3.43	2.00	1.74	565.0	151.7	0.268
	0	5.33	3.30	2.03	1.63	572.0	150.7	0.263
10	Kemzyme	5.30	3.33	1.96	1.70	553.3	144.3	0.260
	SC	5.43	3.36	2.06	1.63	556.7	147.0	0.264
	0	5.33	3.33	2.00	1.68	568.7	149.0	0.261
20	Kemzyme	5.53	3.36	2.16	1.56	554.0	144.3	0.260
	SC	5.40	3.33	2.06	1.62	553.0	145.0	0.262
SEM		0.730	0.068	0.116	0.125	6.10	2.23	0.004
Probability value								
DDGS %		0.451	0.577	0.826	0.802	0.115	0.008	0.222
Additive		0.173	0.727	0.649	0.771	0.056	0.024	0.395
DDGS × additive		0.382	0.955	0.785	0.857	0.527	0.993	0.867

Different superscripts ([a,b]) within rows differ at $P \leq 0.05$. SEM: standard error of the mean, DDGS: distiller's dried grains with solubles, SC: *Saccharomyces cerevisiae*, A: albumin, G: globulin.

In contrast, low-density lipoprotein (LDL) is the real villain to health because of its excess forms' plaques on the arteries walls, making them thicker, which could even block the passage of blood to the tissues and heart, causing cardiovascular problems. Elevated levels of LDL and triglycerides (TRG) are associated with several human diseases, such as coronary heart disease, stroke, atherosclerosis, which predispose to heart attacks and strokes, and diabetes. The combination of high levels of

TGR and LDL and low HDL levels further accelerates the process of atherosclerosis (Ma, 2004).

Compared to the control group, adding multienzymes and SC fortification to 10% and 20% DDGS diets appears to reduce total plasma lipid by 1.8 and 2.1%, respectively. Furthermore, adding enzyme cocktail decreased beneficially (p=0.024) plasma cholesterol by 1.8% compared to the control group. On the contrary, it was reported by Attia *et al*. (2014), Saeed *et al*. (2017), and Abd El-Hack *et al*. (2017) that enzyme supplementation did not appreciable impact triglycerides and total cholesterol in plasma,

Yeast SC had no discernible impact on total plasma lipids, cholesterol, or the cholesterol/total lipid ratio (Table 8). Similarly, Yalçin *et al*. (2008) showed that adding yeast culture had no impact on serum levels of total protein, triglycerides, cholesterol, AST, or ALP. Furthermore, Zek (2012) revealed that serum triglyceride and total cholesterol concentrations were unaffected pointedly by MOS fortification. Alternatively, it has been demonstrated that adding SC to chicken diets reduces blood cholesterol (Mohan *et al*., 1995) and the cholesterol in egg yolks (Abdulrahim *et al*., 1996). According to research by Krasowska *et al*. (2007), baker's yeast SC appears to be the ideal organism for lowering cholesterol in the gut system. Furthermore, Nicolasi *et al*. (1999) noted that the yeast-derived -glucan considerably reduced total cholesterol concentrations in men with hypercholesterolemia. According to a recent study, prebiotics reduced broiler chicken's belly fat and serum cholesterol (Yusrizal, 2003). Prebiotic administration may have increased the Lactobacillus count (Gilliland *et al*., 1985), lowering plasma cholesterol, according to (Mohan et al., 1995; Attia *et al*., 2008). Nelson and Gilliland (1984) and Gilliland *et al*. (1985) proposed that some probiotic microbes may metabolize the gut cholesterol, lowering the absorbed cholesterol. Probiotics have been proposed to block the action of hydroxymethyl-glutaryl-coenzyme A in the digestive system, lowering cholesterol levels (Fukushima and Nakano, 1995). Absorbing dietary cholesterol into bacterial cells and deconjugating bile salts in the gut prevents them from portraying as precursors in production of cholesterol. Probiotic bacterial strains can also alter the enterohepatic cycle and lower cholesterol (Abdulrahim *et al*., 1996; St-Onge *et al*., 2000; Kalavathy *et al*., 2003). Consequently, probiotic bacteria lowered cholesterol levels in blood plasma and yolk lipids (Mahdavi *et al*., 2005; Xu *et al*., 2006; Panda *et al*., 2008; Ramasamy *et al*., 2009; Capcarova *et al*., 2010; Mikulski *et al*., 2012). The effect of SC was not substantially different from that of the enzyme cocktail, which demonstrated a significantly lessening influence than the control, according to the current data, which partially agreed with these noticed by abovementioned authors.

Index of liver function and Renal Function: Table 8 shows the indices of liver and renal functions. The present results showed that dietary DDGS, enzyme cocktail, and SC did not negatively affect (p≥0.05) liver leakage indices, AST, ALT, AST/ALT ratio, and alkaline phosphatase. These results reveal that DDGS, enzyme cocktail and SC did not harm liver function.

The AST is a glutamic oxalacetic transaminase (GOT) enzyme, which catalyzes the rescindable transamination of aspartate and α-ketoglutarate into oxalacetate and glutamate. At high AST also suggests hepatic steatosis and lesions in the heart muscle, erythrocytes, liver, and skeletal and cardiac muscles (González & Silva, 2006).

ALT also is an enzyme; it is present in higher quantities in the liver and is used for the evaluation of hepatic damage than AST (González & Silva, 2006; Bovera *et al*., 2007). ALT and AST are leakage enzymes found in the liver and many other intestinal cells, impairing cell function (Attia *et al*., 2014). These results support those obtained by Gabr *et al*. (2008), who observed that GPT and GOT were not significantly influenced by feeding diets containing DDGS at 10%, 15%, and 20%. Similarly, Salem *et al*. (2008) showed slight AST and ALT levels change although differences were insignificant due to adding enzymes to Golden Montazah male chicken's diets.

The results showed that adding DDGS, enzyme cocktail, and/or SC did not significantly affect (p≥0.05) uric acid. Still, creatinine (p=0.007) and uric acid/creatinine ratio (p=0.002) in plasma were affected considerably by DDGS only (Table 8). These results and plasma total protein confirmed that DDGS had a good and safe protein quality for laying hens.

Creatine (CRE) is a non-protein nitrogenous compound that is formed through the biosynthesis of creatine, a substance composed of amino acids (Murray et al., 2003). Creatine synthesis involves the combination of glycine and arginine, followed by the transfer of methyl groups from S-adenosylmethionine (Lehninger& Cox, 2014).

Creatine is produced in the liver and enters the bloodstream, where the muscle immediately takes up, acting on energy production for muscle contraction. Creatine can be stored after being phosphorylated by creatinine kinase as phosphocreatine, whose degradation generates CRE, which is excreted by the kidneys (Murray *et al*., 2003). Plasma CRE levels reflect the renal filtration rate, and its high concentration suggests a deficiency in renal function, but it may also indicate high protein intake (Tangri *et al*., 2011). The blood concentrations of CRE are proportional to the muscular mass of the animal, occurring in cases of muscular atrophy and other related diseases, decreasing its

concentration in the blood serum (González and Silva, 2006).

Plasma CRE level indicates harmed kidney function or renal disease. As the renal become damaged for any reason, the creatinine concentration in the blood will increase due to poor renal clearance of creatinine. Abnormally high levels of creatinine show possible malfunction or failure of the renal. Even though there is a significant increase of 9.52% and 9.98% in the plasma due to feeding DDGS at 10% and 20%, respectively, compared to the control group, the changes are in the normal physiological range; however, sulfuric acid in the DDGS caused slight sulfur toxicity or increased ingestion of aflatoxin. These results match those made by Shalash *et al*. (2009), who claimed that eating a diet that contained 12% DDGS compared to the control had no appreciable impact on plasma creatinine. Moreover, according to Saoud and Daghir (1980) and Attia *et al*. (2014), single-cell protein and enzyme supplementation did not influence the renal function of broiler chickens.

Plasma calcium, phosphorus, and antioxidants status: Excluding the enzyme cocktail fortification, which led to a significantly elevated (p=0.037) plasma Ca level compared to the control diet, dietary DDGS, enzyme cocktail, and SC did not have a statistically significant (p≥0.05) effect on most plasma P, Ca, and Ca/P values (Table 9). The results indicate that increasing the DDGS content in the diets of laying hens to 10% and 20% did not cause a significant change in the levels of P, Ca, or the Ca/P ratio. The data presented here suggest that SC and, to a lesser extent, enzymes enhanced Ca availability by liberating Ca from NSP molecules. Attia et al. (2014) and Panda et al. (2003) also observed increased blood calcium levels with enzymes and probiotics, respectively.

Table 8: Indices of liver and renal functions as influenced by distiller's dried grains with solubles, Kemzyme, and Saccharomyces cerevisiae.

Dietary treatments		Blood plasma constituents						
		Liver function indices				Renal function indices		
		AST (U/ml)	ALT, (U/ml)	ALT/AST	ALP (U/ml)	Uric acid (mg/dl)	Creatinine (mg/dl)	Uric/creatinine
Effect of distiller's dried grains with soluble								
	0	45.6	13.3	3.4	25.0	4.78	0.441[b]	10.9[a]
DDGS (%)	10	45.9	13.0	3.5	24.7	4.72	0.483[a]	9.78[b]
	20	45.7	13.4	3.4	25.5	4.70	0.485[a]	9.69[b]
Effect of additives								
	0	45.9	13.2	3.5	24.9	4.75	0.477	9.99
Additive	Kemzyme	45.7	13.2	3.5	25.2	4.70	0.471	10.0
	SC	45.6	13.3	3.4	25.1	4.75	0.461	10.4
Interaction between DDGS and additive								
DDGS (%)	Additive							
	0	45.7	13.0	3.53	24.7	4.66	0.453	10.4
0	Kemzyme	45.7	13.0	3.52	25.2	4.83	0.440	11.0
	SC	45.3	14.0	3.24	25.0	4.86	0.430	11.3
	0	45.7	13.0	3.55	24.6	4.80	0.486	9.87
10	Kemzyme	46.0	13.0	3.54	24.6	4.66	0.486	9.59
	SC	46.0	13.0	3.55	25.0	4.70	0.476	9.88
	0	46.3	13.7	3.42	25.3	4.80	0.493	9.73
20	Kemzyme	45.3	13.7	3.34	25.9	4.60	0.486	9.47
	SC	45.3	13.0	3.50	25.3	4.70	0.476	9.87
SEM		0.571	0.726	0.188	0.473	0.084	0.017	0.195
P values								
DDGS		0.770	0.741	0.663	0.154	0.423	0.007	0.002
Additive		0.770	0.977	0.908	0.652	0.654	0.488	0.445
DDGS × additive		0.741	0.774	0.795	0.848	0.211	0.994	0.647

Different superscripts ([a,b]) within rows differ at P≤0.05; SEM: standard error of the mean, DDGS: distiller's dried grains with solubles, SC: *Saccharomyces cerevisiae*, AST: aspartate aminotransferase, ALT: alanine aminotransferase, ALP: alkaline phosphatase.

Addeo et al. (2021) reported that MDA, a soluble degradation product of lipids, could be used to monitor the extent of lipid peroxidation. Malondialdehyde and total antioxidant capacity were

unaffected (p≥0.05) by the addition of DDGS, enzyme cocktail, and SC (Table 9). Our findings indicate that DDGS maintains the antioxidant balance of laying hens, and this concurred with those of Lin *et al*. (2005), Song (2013), and Jiang *et al*. (2013), who discovered no significant differences in antioxidant capacity between meals containing DDGS at three different levels (0, 10, and 20%). The antioxidant theory states lipid peroxidation rises in tissues and plasma levels as antioxidant concentrations fall, damaging cell membranes (Gallo-Torres, 1980; McDowell, 1989). *Saccharomyces cerevisiae's* free radical quenching property stops the chain reaction of lipid peroxidation. This action of SC affects the polyunsaturated fatty acids of bio-membranes and stops the free radical attack at an early stage (McDowell, 1989). Despite being a rich source of polyunsaturated fatty acids (PUFAs) (NRC, 1994) and increasing egg yolk levels of UFAs (Abd El-Hack et al., 2017), our findings did not reveal any adverse effects of DDGS supplementation on total antioxidant capacity (TAC) or malondialdehyde (MDA) levels. As a result, Kemzyme and/or SC did not improve (p≥0.05) the antioxidant capacity of laying hens' blood plasma.

Table 9: Plasma calcium, phosphorus, total antioxidants capacity, malondialdehyde, and response to SRBCs as affected by distiller's dried grains with solubles, Kemzyme, and Saccharomyces cerevisiae.

Dietary treatments		Blood plasma metabolites						
		Ca (mg/dl)	P (mg/dl)	Ca/ P ratio	TAC (nmol/ml)	MDA (nmol/ml)	Antioxidants balance	SRBCs (10^3/mm^3)
Effect of distiller's dried grains with solubles								
	0	22.9	8.68	2.63	1.53	0.332	4.61	4.49
DDGS (%)	10	23.0	8.97	2.56	1.53	0.335	4.57	4.40
	20	22.7	8.82	2.59	1.51	0.339	4.45	4.31
Effect of additives								
	0	22.6[b]	8.68	2.60	1.51	0.341	4.43	4.32
Additive	Kemzyme	23.1[a]	9.02	2.56	1.53	0.334	4.58	4.46
	SC	22.9[ab]	8.77	2.61	1.53	0.332	4.61	4.43
Interaction between DDGS and additive								
DDGS (%)	Additive							
	0	22.9	8.80	2.60	1.51	0.341	4.43	4.31
0	Kemzyme	22.9	8.66	2.64	1.55	0.328	4.73	4.64
	SC	22.9	8.60	2.66	1.54	0.327	4.71	4.53
	0	22.7	8.80	2.58	1.52	0.339	4.48	4.35
10	Kemzyme	23.1	9.13	2.53	1.53	0.337	4.54	4.39
	SC	23.1	9.00	2.56	1.54	0.331	4.65	4.45
	0	22.1	8.46	2.63	1.50	0.342	4.39	4.29
20	Kemzyme	23.3	9.26	2.51	1.52	0.336	4.52	4.34
	SC	22.9	8.73	2.62	1.52	0.337	4.51	4.31
SEM		0.195	0.193	0.062	0.047	0.004	0.412	0.102
Probability value								
DDGS %		0.610	0.214	0.338	0.862	0.397	0.562	0.133
Additive		0.037	0.120	0.567	0.766	0.162	0.357	0.239
DDGS × additive		0.250	0.233	0.712	0.994	0.850	0.857	0.597

Different superscripts ([a,b]) within rows differ at P≤0.05; SEM: standard error of the mean, DDGS: distiller's dried grains with solubles. SC: *Saccharomyces cerevisiae*, CA: calcium, P: phosphorus, TAC: total antioxidant capacity, MAD: malondialdehyde, antioxidants balance = TAC/MDA; SRBCs: sheep red blood cells.

Conclusion: DDGS can be a highly acceptable ingredient for laying hens up to 20% with 500 g/ton feed of enzyme cocktail or 1 kg/ton feed of yeast fortification. The enzyme cocktail and yeast fortification significantly improved the eggshell thickness by 1.44-1.72%, and yeast increased sperm output and total sperm production per ejaculate by 5.21 and 5.87%, respectively.

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Data Availability Statement: The data supporting this study's findings are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

Conflicts of Interest: None.

Authors' contribution: YAA and RAA conceived and designed the study. RAA, AEA, and WSS executed the experimental data and analyses the samples. KAA, NFA, and FB analyzed the data. RAA and YAA funding acquisition. All authors interpreted the data, critically revised the manuscript for important intellectual contents, and approved the final version.

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