EFFECT OF DIETARY INCLUSION OF CORN DISTILLERS DRIED GRAINS WITH SOLUBLES ON PRODUCTIVE PERFORMANCE, NUTRIENT DIGESTIBILITY, IMMUNE STATUS, ILEAL HISTOMETRIC AND MICROBIOTA OF BROILER CHICKENS

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SUMMARY

The high cost of protein and energy sources in the poultry diet, especially during the last three years due to climate changes and the COVID-19 pandemic around the world, has become a high challenge to poultry producers. Distillers dried grains with solubles (DDGS) have shown potential as an alternative source for both protein and energy. The experiment was conducted in the summer of 2021 to investigate the effect of dietary inclusion of DDGS in broiler diets on performance, digestibility, immune status, and both ileal histometrics and microbiota. A total of 600 un-sexed one-day-old Ross 308 broiler chicks were randomly distributed into four groups as follows; control, fed a corn-soy diet, other groups were fed diets inclusion with 10, 20, and 30 % DDGS replaced for corn and soybean meal. Results showed that the inclusion of DDGS up to 20 % had non-significant (p> 0.05) effects on performance, carcass traits, and immunological parameters compared with control group. Adverse effects were recorded with 30% DDGS. Body weight gain, digestibility coefficient of crude protein, and production efficiency factor decreased, but feed conversion ratio, mortality rate, and E. coli counts of ileum were deteriorated with 30% DDGS inclusion. In conclusion, the replacement of dietary corn and soybean meal with DDGS at 10 and 20 % had no significant effects on performance, lipid profile, nutrient digestibility, immunological traits, and ileum development.

Key words: *DDGS, broiler, performance, nutrient digestibility, ileum development***.**

INTRODUCTION

Poultry feed mostly depends on grains and oil extraction residues, which are associated with the efficiency of poultry utilization of these nutrients. In recent years, nutritionists have begun searching for inexpensive, non-conventional alternatives to feedstuffs to be used as feed sources for broilers, compared with the high cost of conventional grains (corn and soy). Dried distillers' grains with solubles (DDGS) is the ethanol by-product from corn that is characterized by higher quality components (protein, vitamins, fats, and available phosphorous) than the original resource (corn) due to starch removal (Belyea et al., 2010; Pederson et al., 2014). In addition, ethanol is produced through the fermentation process, which indicates that the by-product may contain yeast residues that may have nutritional and health benefits for performance. Some studies showed different effects on the productive performance of broiler chickens fed a diet containing different levels of DDGS (Loar et al. 2010; Zhang et al. 2013; Elbaz et al., 2022). This necessitated further research on the level of DDGS that may negatively affect the health and performance of the bird, its causes, and how to treat it. Therefore, the present study aimed to investigate the effects of dietary inclusion of high levels of DDGS on performance, some blood parameters, immune competence, as well as ileum histomorphology and microbiota of broilers.

MATERIALS AND METHODS

Diets, Experimental Design, and Management:

The experimental procedures were performed according to the Experimental Animal Care Committee, Desert Research Center. The experiment was conducted on the farm of Siwa Research Station of the Desert Research Center from June to July 2021 (summer season). Six hundred unsexed broiler chicks (Ross 308) were purchased from a commercial hatchery and distributed randomly into four experimental treatments (6 replicates, each containing 25 birds). The control group (DG0) was fed a basal diet (corn and soybean), and the other three groups were fed diets including 10, 20, and 30% DDGS (DG10, DG20, and DG30 respectively). All the experimental diets were installed to be isocaloric and isonitrogenous according to NRC (1994) as shown in Table 1. Chicks were reared in plastic wire-floor pens (2.50 × 1.50 m), feed and water were provided ad libitum. The temperature was set at 34 and the relative humidity was 65% during the first day, and then the temperature gradually decreased at a rate of half a degree per day until the 10thday. The birds were exposed to the natural environment until the end of the experiment. The lighting program during the first 3 days was 24 L: 0 D and during the rest of the experiment were 22 L: 2 D. All chicks were vaccinated against Newcastle disease (NDV, Clone 30) at 1, 10, and 20 days of age, and against influenza (AIV, H9N2) at 7 days of age to determine the total antibody titers as humoral immunity.

Table 1. Composition for the starter and grower experimental diets.

Ingredient (%)	Phase							
	Starter 1-21d				Grower 22-35d			
	DG0	DG10	DG20	DG30	DG0	DG10	DG20	DG30
Yellow Corn	53.3	50.3	47.8	44.6	61.7	58.9	55.9	52.5
Soybean meal (44%)	34.8	28.7	22.2	16.1	26.5	20.1	13.9	7.70
DDGS	0.00	10.0	20.0	30.0	0.00	10.0	20.0	30.0
Corn Gluten Meal	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00
Soybean oil	2.60	1.70	0.70	000	2.75	1.55	0.55	000
Calcium Carbonate	1.20	1.10	1.10	1.10	1.15	1.20	1.20	1.20
Di-Calcium Phosphate	2.20	2.30	2.20	2.20	2.00	2.10	2.10	2.10
Premix*	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30
NaCl (salt)	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30
L-lysine HCl	0.10	0.10	0.20	0.20	0.10	0.25	0.45	0.60
DL-methionine	0.20	0.20	0.20	0.20	0.20	0.30	0.30	0.30
Price / ton	8965	8895	8830	8790	8590	8535	8495	8450
Calculated composition								
ME (kcal.kg)	3000	3000	3000	3000	3100	3100	3100	3100
CP (%)	23	23	23	23	20	20	20	20
Ca%	1.04	1.04	1.04	1.04	0.95	0.95	0.95	0.95
AP %	0.52	0.52	0.52	0.52	0.47	0.47	0.47	0.47
Meth %	0.56	0.56	0.56	0.56	0.51	0.51	0.51	0.51
SAAs %	1.08	1.08	1.08	1.08	0.99	0.99	0.99	0.99
Lys %	1.44	1.44	1.44	1.44	1.29	1.29	1.29	1.29

**Each 2.5 kg Vitamins and minerals premix contains :vitamin A, 7700,000 IU; vitamin D3, 3300,000 IU; vitamin E, 6,600 mg; vitamin K3, 550mg; thiamine, 2200 mg; riboflavin, 4400 mg; vitamin B , 4400 mg; Ca Pantothenate, 550 mg; nicotinic acid, 200 mg; folic acid, 110 mg; choline chloride, 6275,000 mg; biotin, 55 mg; vitamin B12, 8.8 mg; Trace mineral (milligrams per 2.5 kilogram of diet): Mn, 66000; Zn, 66000; Fe, 33000; Cu, 8800; Se,300; and I, 900.*

Performance and carcass traits:

Body weight gains (BWG) and feed intake (FI) were recorded at 21 and 35 days. Feed conversion ratio (FCR), and European Production Efficiency Factor (EPEF) were calculated, as presented in the following equation:

FCR= the feed intake (g) / body weight gain (g)

EPEF= [((Livability (%) X BW (g)) / ((age (days) X FCR))] X 100

Twenty-four chicks were selected and slaughtered at 35 days (6 birds/group) to estimate the relative weight of the carcass yield. Breast and thigh muscle, liver, and abdominal fat were calculated. Also, the relative weight of lymphoid organs (spleen, bursa of Fabricius, and thymus) was calculated.

Nutrient digestibility:

At 35 days, 6 broiler chickens were taken from each group and weighed (representing the average weight of each group), placed in individually digestion battery, and were fasted for 12 hours to empty their digestive tracts; excreta samples were collected 3 times/day for 4 consecution days, removing feathers and the rest of the impurities, and then dried in the oven at 70 C for 24 hours. Dry matter (DM), crude protein (CP), ether extract (EE), and nitrogen-free extract (NFE) were determined according to AOAC (2000).

Plasma Biochemical:

At day 35of age, blood samples were obtained via the puncture of jugular vein in heparinized tubes (6 birds / group), centrifuged (3000 rpm for 10 min) to obtain plasma, and then stored at -20o C till analysis. Commercial kits were used to determine plasma concentrations of total cholesterol (CHO), low-density lipoprotein (LDL), high-density lipoprotein (HDL), triglycerides (TRI), total protein (TP), albumin (ALB), aspartate aminotransferase (AST), and alanine aminotransferase (ALT) with an automatic biochemical analyzer (Beckman Coulter Inc., Fullerton, Canada). Plasma total antibody titers for secondary immune response were determined against Newcastle disease (ND) and avian influenza viruses by using a hemagglutination inhibition (HI) test according to Shahzad Munir et al., (2012).

Histological examination of ileal microbial counts:

Contents of the ileum were emptied after eviscerated into sterilized glass vials at a rate of 1g / bird/ replicate (6 birds from the group). Samples were diluted, plated on the agar of each microbe, and incubated at indicated temperature and time. Total coliforms, Lactobacillus, and Escherichia coli were enumerated. For histological examination, 2 cm of the medium part of ileum was excised, rinsed with saline, and fixed in 12% formalin saline solution, then embedded in paraffin wax to prepare glass slides and finally stained with H&E satins for examination as described by Bancroft et al. (1990). Villus height (VH) and crypt depth (CD) were measured by using an electron light microscope (ZEISS Axio Imager A2, Germany) and the percentage of villus height/crypt depth (VH/CD) was calculated.

Statistical analysis:

All data were analyzed by one-way analysis of variance (ANOVA) using SPSS v. 20.0 Statistical Package (SPSS Inc., Chicago, IL, USA). Differences in the mean values among the dietary treatments were performed using Duncan's multiple (a significance level of 0.05) range test (Duncan, 1955).

RESULTS AND DISCUSSION

Growth performance:

This study was carried out to complete a previous study that showed that the inclusion of DDGS up to 15% had no negative impact on broiler chicken's performance (Elbaz *et al.*, 2022). Therefore, further studies were urgently needed on the effects of including higher levels of DDGS (10, 20, and 30%) on growth performance, plasma parameters, immune responses, and intestinal growth of broiler to reduce the cost of the diet. Data showed that replacing up to 20% of the broiler diet with DDGS did not affect growth performance, as shown in Table 2. However, the inclusion of DDGS at 30% in the diet led to negative effects on production performance, as BWG and FCR deteriorated. BWG decreased linearly, while FCR showed inferior values as DDGS inclusion increased from 20% to 30%. Furthermore, the mortality rate increased significantly, and the European Production Efficiency Factor (EPEF) decreased with the addition of DDGS (30%) to the diet compared with those that received low levels of DDGS or a control ($p < 0.05$). Feed intake was not affected by the inclusion of DDGS during the experimental periods. Our results agree with several studies which indicate that DDGS cannot be included in the diet in large quantities without affecting broiler growth performance (Oryschak *et al.,* 2010; Alizadeh *et al.,* 2016). Kim *et al.* (2018) also confirmed that the substitution of 20% of broiler diets with DDGS did not affect the growth performance indices. The reason for poor broiler performance can be due to the high level of anti-nutritional substances such as fiber (nonstarch polysaccharides (NSP)) when high levels of DDGS are included, which negatively affects the digestion and absorption of nutrients (Choct *et al.*, 2010). This observation is reinforced by the increased mortality rate and the decrease in EPEF with an increase of DDGS in the diet of broiler chickens. Our results showed that the inclusion of DDGS up to

20% could replace part of soybean meal and corn without any negative effect on productive performance in broiler diets. These results agree with Wang *et al.* (2007), who observed that inclusion of DDGS up to 20% had no effect on BWG and FCR in broiler diets. In contrast, Loar *et al.* (2010) concluded that DDGS seemed safe at levels of 8% during the starter phase. While the high levels of DDGS in the diet caused pellet quality and bulk density to decrease at grower phase. Also, DDGS may be advantageous to broilers in the starter diet to condition the digestive system to this by-product before being exposed to even higher levels in the grower phase.

Table (2): Effect of dietary inclusion of DDGS on the performance of broiler chickens.

Parameters	DG0	DG10	DG20	DG30	SEM	P-value
			Days 1-21			
BWG (g)	834^{a}	861^{a}	812^{b}	797^{b}	2.801	0.031
FI (g)	971	958	964	968	4.005	0.710
FCR	1.163^{ab}	1.113^{b}	1.188^{ab}	1.216^{a}	0.127	0.023
			Days 22-35			
BWG (g)	1218^{b}	1270^{a}	1190^{b}	1055^{c}	3.251	0.017
FI (g)	2610	2611	2602	2528	5.304	0.245
FCR	2.141^{b}	2.055^{c}	2.184^{b}	2.396^{a}	0.255	0.041
			Overall Days 1-35			
BWG (g)	2054^{b}	2132^{a}	1997^{b}	1850^{c}	2.661	0.015
FI (g)	3581	3569	3562	3496	8.224	0.266
FCR	1.744^{b}	1.673^{c}	1.783^{b}	1.887^{a}	0.157	0.024
Mortality rate	4.65	4.00	5.33	6.67	-	-
EPEF	314	348	303	263	-	-

$^{a–c}$ Means with different superscripts within the same row differ significantly (P <0.05); SEM standard error of means; BWG, body weight gain; FI, feed intake; FCR, feed conversion ratio; EPEF, European Production Efficiency Factor.

Carcass traits:

Data of carcass traits presented in Table (3) showed that no significant effects on the carcass yield and organ weights (thigh muscle, breast muscle, or abdominal fat) with including DDGS in the diet compared with the control, except for the reduction of the relative weight of the liver with an increase ($p < 0.05$) in the inclusion level of DDGS in the broiler diet, as displayed in Table 3. Similar results were reported by Wang *et al.* (2007), who reported that birds fed diets with 15% DDGS did not significantly differ in carcass characteristics from broilers fed the control diet (no DDGS). Also, Ghazalah *et al.* (2012) showed no significant effect of DDGS levels on carcass characteristics. In addition, the current results agree with Loar *et al.* (2010), who reported that a significant decrease in the relative liver weight of broilers was observed when including more than 22.5% DDGS in the diets. The decrease in the weight of the liver may be due to a defect in hepatic metabolic disorders, which is related to feeding on a diet that includes DDGS, which may be contaminated with mycotoxins (Loar *et al.*, 2010; Kim *et al.*, 2021).

Nutrient digestibility:

The inclusion of different levels of dietary DDGS had no significant effect on the nutrient digestibility of DM, EE, and NFE ($p < 0.05$) in broilers at 35 d. (Table 3), whereas there was a significant increase in protein digestibility with the inclusion of DDGS at 10 and 20%, but 30% of DDGS inclusion had a negative effect. The most likely significant decrease in digestibility of protein with the increased of DDGS level in the broiler diet may be due to the drying process that occurs during the extraction of ethanol, which affects the digestibility of amino acids due to thermal treatment (Bandegan *et al.*, 2009; Olukosi *et al.*, 2010), and thus leads to a detrimental effect on protein digestion and the bird's performance.

Table (3). Effect of dietary inclusion of DDGS on the carcass traits and nutrient digestibility of broiler chickens at 35 days of age.

Parameter	DG0	DG10	DG20	DG30	SEM	P-value
			Carcass traits (%)			
Carcass yield	76.7	77.3	76.9	76.1	0.518	0.197
Breast	24.3	25.1	24.8	24.6	4.260	0.126
Thigh	16.7	16.3	16.8	16.6	1.701	0.094
Liver	3.90[a]	3.57[a]	3.42[a]	2.85[b]	0.108	0.038
Abdominal fat	5.21	5.48	5.30	5.94	0.017	0.071
			Nutrient digestibility (%)			
DM	77.2	76.8	76.5	75.9	0.235	0.122
CP	68.4[b]	72.6[a]	70.9[ab]	66.5[c]	0.144	0.020
EE	66.1	65.8	66.4	65.6	0.256	0.151
NFE	80.8	82.4	81.9	81.6	0.135	0.092

[a–b] Means with different superscripts within the same row differ significantly (P <0.05); SEM standard error of means; DM, dry matter; CP, crude protein; EE, ether extract; NFE, nitrogen-free extract.

Immunological parameters:

In Table (4), the main relative weights of lymphoid organs and plasma total antibody titers against ND and AI viruses are presented. These parameters were estimated as a measure of the health status of birds fed a diet including DDGS. The relative weights of lymphoid organs (bursa of Fabricius) were significantly reduced with 30% dietary inclusion of DDGS, whereas the spleen, as a secondary lymphoid organ and thymus, had no effect. On the contrary, inclusion of DDGS at 10, 20, and 30% in the diets had no significant effect on antibody titers against ND and AI viruses. DDGS contains high levels of unsaturated fatty acids and yeast components that have an immunostimulating role, such as mananoligosaccharides and B-glucan. These compounds may improve growth performance and increase immune response (Fathi *et al.,* 2012). In this regard, Cheng *et al.* (2004) concluded that the relative weight of the bursa of Fabricius was reduced with the increase in E. coli of the broiler. This was confirmed by our results, which will be clarified later, according to the increase in the number of E. coli in the ileum with an increase in dietary DDGS levels.

Table (4): Effect of dietary inclusion of DDGS on the relative weight of immune organs and humoral immune response of broiler chickens at 35 days of age.

Parameter	DG0	DG10	DG20	DG30	SEM	P-value
			Immune organs (%)			
Thymus	2.71	2.54	2.38	2.51	0.087	0.092
Spleen	1.80	1.72	1.76	1.84	0.129	0.151
BF	3.29[a]	3.31[a]	3.40[a]	2.82[b]	0.036	0.040
			Humoral immune			
AIV	3.05	2.94	3.06	3.11	0.131	0.211
NDV	5.02	5.19	5.34	5.28	0.118	0.192

[a–b] Means with different superscripts within the same row differ significantly (P <0.05); SEM standard error of means; BF, Bursa of Fabricius; AIV, Avian influenza virus (H9N1); NDV, Newcastle disease virus.

Plasma Biochemical constituents:

Plasma parameters are studied to know the effect of adding DDGS on the metabolism of nutrients and the physiological state of birds. Plasma parameters indicate that dietary DDGS did not impact hepatic biomarkers (evidence of the metabolism of proteins) and lipid profile ($p < 0.05$), as displayed in Table 5. Different levels of dietary DDGS had no impact on plasma concentrations of TRI, HDL, LDL, TP, or ALB ($p < 0.05$). In addition, the plasma concentrations of ALT and AST were insignificantly affected when broilers were fed different levels of dietary DDGS ($p < 0.05$). As there was a significant decrease in plasma cholesterol in broilers fed on a different level of DDGS. The same result was also shown by Elbaz *et al.* (2022) that the inclusion of DDGS led to decreased serum cholesterol concentration in the broiler at 35 days of age. Feed compositions play an important role in the level of cholesterol in the blood. Therefore, the reason for the decrease in plasma cholesterol may be due to the high content of DDGS from fiber in this study. The high content of fiber leads to the absorption of bile acids, which reduces the level of bile acids and thus reduces the concentration of cholesterol in the plasma (Regar *et al.*, 2019).

Table (5): Effect of dietary inclusion of DDGS on plasma lipid profile and hepatic biomarkers of broiler chickens at 35 days of age.

Parameter	DG0	DG10	DG20	DG30	SEM	P-value
			Lipid profile (mg dl^{-1})			
TRI	197	188	194	193	2.007	0.082
CHO	237^{a}	214^{ab}	194^{b}	218^{ab}	4.255	0.025
HDL	60.9	62.5	66.8	65.1	2.063	0.061
LDL	98.5	94.2	102.4	89.4	1.301	0.117
			Hepatic biomarkers			
TP1	5.06	4.82	5.34	5.19	0.026	0.070
ALB1	2.34	2.57	2.41	2.65	0.061	0.233
AST2	226	230	209	216	2.715	0.065
ALT2	26.5	25.8	26.1	26.7	0.522	0.101

$^{a-b}$ Means with different superscripts within the same row differ significantly (P <0.05); SEM standard error of means; TRI, Triglycerides; CHL, Cholesterol; HDL, high-density lipoprotein; LDL, low-density lipoprotein; TP, Total Proteins; ALB, Albumin; AST, aspartate aminotransferase; ALT, alanine aminotransferase; 1, (g dl−1); 2, (U L−1).

Ileal histometric and microbial counts:

In the current study, ileum histometric (including villus height and crypt depth) was not affected by the inclusion of DDGS in the broiler diets, as shown in Table 6. Despite that, a harmful effect on the intestine microbes appeared, with the increase in the number of E. coli. As reported by Elbaz *et al.* (2022), the results showed an increase in E. coli counts of the ileum with the increased inclusion of DDGS in the diet. These results are reinforced by Apajalahti *et al.* (2001), who showed that the composition of the feed and the source of raw materials affect the microbial communities. The reasons for the high number of E. coli in the population may be due to the conditions of transportation, fertilization of crops with natural organic manure (the waste of sick animals), or poor storage (Maciorowski *et al.*, 2007).

Table (6): Effect of dietary inclusion of DDGS on ileal microbial count (Log10 CFU g^{-1}) and histomorphological parameters of broiler chickens at 35 days of age.

Parameter	DG0	DG10	DG20	DG30	SEM	P-value
			Histomorphological (μm)			
VH	819	827	806	851	29.4	0.722
CD	245	237	241	248	5.47	0.528
VH/CD	3.34	3.48	3.35	3.42	2.50	0.188
			Microbial count (Log10 CFU g−1)			
E. coli	2.52^{b}	2.40^{b}	2.76^{ab}	3.16^{a}	1.08	0.030
T. coliform	2.64	2.17	2.31	2.62	1.25	0.144
Lactobacillus	4.70	4.82	4.35	4.59	2.09	0.109

$^{a-b}$ Means with different superscripts within the same row differ significantly (P <0.05); SEM standard error of means; VH, villus height; CD, crypt depth; VH/CD, villus height/crypt depth; E. coli, Escherichia

CONCLUSION

Inclusion of DDGS up to 20% in broiler diets had no negative effect on growth performance, carcass traits, plasma parameters, immune responses, and ileum development. The addition of 30% DDGS, on the other hand, had some negative effects on performance and intestinal development. So, we need more future studies to determine how to treat the negative effects of high levels of DDGS through applying some biological additives to increase its levels in broiler diets.

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تأثير إضافة النواتج الثانوية لتقطير الذرة على الأداء الإنتاجي و معاملات الهضم والحالة المناعية والصفات النسيجية للأمعاء و الحمل الميكروبي في علائق دجاج التسمين

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أصبح أمام منتجي الدواجن تحدياً كبير نظرا للتكلفة العالية لمصادر البروتين والطاقة في علائق الدواجن ، خاصة خلال السنوات الثلاث الماضية بسبب التغيرات المناخية ووباء COVID-19 في جميع أنحاء العالم . أظهرت النواتج الثانوية لتقطير الذرة (DDGS) إمكانية كبيرة في استخدامها كمصدر بديل لكل من البروتين والطاقة. أجريت التجربة في صيف عام 2021 لدراسة تأثير إضافة الـ DDGS لعلائق دجاج التسمين على الأداء ، والهضم ، والحالة المناعية ، والصفات النسيجية للأمعاء والعد الميكروبي .
تم توزيع عدد 600 كتكوت من دجاج التسمين روس 308 غير مجنس عمر يوم بشكل عشوائي على أربع مجموعات على النحو التالي: مجموعة المقارنة وتم تغذيتها على عليقة الكنترول بينما غذيت المجموعات الأخرى على علائق تحتوي على 10 و 20 و 30٪ DDGS كإستبدال من الذرة وفول الصويا . أظهرت النتائج أن إضافة DDGS حتى 20٪ ليس له تأثيرات ضارة (p <0.05) على الأداء ، وصفات الذبيحة ، والقياسات المناعية مقارنة مع مجموعة الكنترول. تم تسجيل تأثيرات ضارة لإضافة DDGS عند مستوى 30% حيث حدث إنخفاض لوزن الجسم المكتسب ومعامل الهضم للبروتين الخام ومعامل كفاءة الإنتاج وحدث تدهور لمعامل التحويل الغذائي وزيادة لكل من معدل الوفيات و زيادة محتوى اللفائفي (P <0.05) من بكتيريا E-coli.
وبصفة عامة إضافة الـ DDGS بمستوى 10 أو 20٪ لم يحدث أي تأثير معنوي على الأداء وخصائص الدهون ومعاملات الهضم والصفات المناعية وتطور اللفائفي لدجاج التسمين.