Asian Journal of Poultry Science

ISSN 1819-3609

Academic Journals Inc.

www.academicjournals.com

Asian Journal of Poultry Science 7 (2): 41-54, 2013
ISSN 1819-3609 / DOI: 10.3923/ajpsaj.2013.41.54

The Impact of Feeding Graded Levels of Distillers Dried Grains with Solubles (DDGS) on Broiler Performance, Hematological and Histological Parameters

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ABSTRACT

An evaluation of Distillers Dried Grains with Soluble (DDGS) as feed ingredients for broilers have been done. It is valuable source of energy, protein, water soluble vitamins and minerals in poultry diets. The present study was carried out during the summer season to study the impact of feeding graded levels of DDGS on broiler performance, hematological and histological parameters. A total number of 160-one day old, unsexed cobb broiler chicks were randomly divided into four groups. Birds of each group were subdivided into four replicates of ten birds each. The different experimental diets (Starter, grower and finisher) contain DDGS at levels of (0, 5, 10 and 15%). Diets were iso-caloric and iso-nitrogenous. Feed intake, body weight gain, feed conversion ratio, carcass characteristics were recorded. Values for pH and length of Gastrointestinal Tract (GIT), glucose concentration, hematological parameters (Hb, Ht% and H/L ratio) and histological study of small intestine, liver and pancreas were obtained. The result showed that DDGS insignificantly ($p \leq 0.05$) affected productive performance, carcass characteristic and Ht%, DDGS inclusion in the diet significantly ($p \leq 0.05$) decreased glucose concentration, H/L ratio and pH value and improved the histology of small intestine, liver and pancreas. This study concluded that the graded levels of DDGS up to 15% showed no adverse effects when used in broiler starter, grower and finisher diets on growth performance and carcass characteristics. The hematological and histological parameters were improved suggesting that DDGS can be used up to 15% in broiler diets.

Key words: DDGS, broiler performance, carcass characteristics, hematology, digestive tract parameters, histology

INTRODUCTION

Distiller's Dried Grain with Solubles (DDGS) is one of three types of residual co-producted from the production of ethanol from grain. DDGS is common commercially available product produced after fermentation of corn starch to produce ethanol.

Feed prices have increased sharply due increase in the feed ingredient prices. However, corn Distillers Dried Grains with Soluble (DDGS) corn supply energy, protein (amino acids), linolic acid and phosphorus in poultry diets often at a competition price and may lower feed cost.

A vast increase in ethanol production over last 10 year has led to an increase supply of DDGS that is available for live stock feed (Noll *et al*., 2007).

New generation of DDGS (built after 1990) from U.S. Med west ethanol plants has higher levels of apparent ileal digestible amino acids (Whitney *et al*., 2000), metabolizable energy (Sphiehs *et al*., 1999) and available phosphorus (Whitney *et al*., 2001), than published by NRC (1994).

Amani *et al*. (2009) evaluated DDGS as feed stuff in poultry diets and found that DDGS could be considered a poultry feed stuff as alternative source of protein in poultry ration with other protein source or after dietary supplementation with lysine.

Early studies demonstrated that higher levels could be used in nutritionally balanced diets. Waldroup *et al*. (1981) found that DDGS could be used in broiler diets up to level 25% with no adverse effect on broiler performance if dietary energy was held constant. Adding lysine to DDGS improve protein quality with no difference with dehulled soybean meal (Parsons *et al*., 1983).

Recent research about new generation (DDGS) has centered on nutrient content and variability (Robinson, 2005; Behnke, 2007).

The increasing supply of DDGS encourages the use of higher percentages of DDGS in broiler diets to the acceptable level, Dale and Batal (2003) used 0, 6, 12 and 18% DDGS in a 24 day grow out study and reported that 12 and 18% DDGS resulted in a slight decrease in performance during the starter period. While 12% DDGS had no negative effect over 42 day period. Lumpkins *et al*. (2004) concluded that new generation of DDGS could be safely used as feed ingredient for broiler diets at level up to 6% in the starter period and 12 to 15% in grower and finisher periods. Choi *et al*. (2008) showed that DDGS supplementation (0, 5, 10 and 15%) did not significant influence in the growth performance during the starter and grower periods. Also, Wang *et al*. (2008) reported that the inclusion of up to 20% DDGS known composition can be used in broiler starter, grower and finisher diets formulated on the basis of digestible amino acid with no adverse effects on live performance of carcass characteristics.

On the other hand, only few data are available on the hematological and histological responses of broiler chicks supplemented with DDGS.

Due to increased interest and availabihty of this feed stuffs, the present study were conducted to evaluate the effect of using different levels of DDGS on the broiler performance, carcass characteristics and the hematological and histological parameters.

MATERIALS AND METHODS

The experimental work of the present study was carried out at the Poultry Nutrition Experimental Farm, Department of Poultry Production, Faculty of Agriculture, Ain Shams University, during the period from July to September (in the summer season).

A total number of 160-one day old, unsexed cobb broiler chicks were wing banded, individually weighted to the nearest gram and randomly divided in to four equal groups of approximately similar initial body weight. Birds of each group were subdivided into four replicates of ten birds each (10 birds x 4 replicate x 4 treatments groups). These birds were kept in previously cleaned and fumigated batteries of wire floored cages in an open-system house. Feed and water were offered *ad-libitum* and artificial light was provided 23 h daily all over the experimental period, which lasted for 6 weeks. Chicks of all treatment groups were kept under similar hygienic and environmental conditions and vaccinated against common disease.

The different experimental diets (Starter, grower and finisher) were formulated according to recommended requirements of "cob" strain used .all experimental diets per each period were containing DDGS at levels of (0, 5, 10 and 15%) and were iso-caloric and iso-nitrogenous. The birds

Table 1: The composition of experimental diets

	Starter				Grower				Finisher			
Ingredients (%)	T1	T2	T3	T4	T1	T2	T3	T4	T1	T2	T3	T4
Yellow corn	61.800	59.700	57.300	54.90	70.00	67.800	65.700	63.400	72.000	69.900	67.700	65.600
Soybean meal 48%	23.200	20.500	17.900	15.40	15.65	12.900	10.000	7.600	13.100	10.300	7.700	5.050
Corn gluten meal 62%	9.000	9.000	9.000	9.00	8.60	8.700	8.900	8.800	8.850	9.000	9.000	9.000
DL-Methionine	0.205	0.205	-	0.20	-	0.250	0.250	0.245	0.220	0.215	0.215	0.210
Lysine –HCl	0.480	0.525	0.600	0.65	-	0.705	0.775	0.830	0.580	0.650	0.705	0.770
Bone meal	3.900	3.100	3.100	3.05	3.015	3.020	2.965	2.870	2.900	2.850	2.800	2.800
Oil	1.400	1.200	1.100	1.00	1.000	0.850	0.620	0.500	1.500	1.250	1.100	0.900
Premix*	0.300	0.300	0.300	0.30	0.300	0.300	0.300	0.300	0.300	0.300	0.300	0.300
Colin	0.100	0.100	0.100	0.10	0.100	0.100	0.100	0.100	0.100	0.100	0.100	0.100
Salt	0.285	0.220	0.170	0.20	-	0.225	0.220	0.165	0.250	0.210	0.205	0.165
Sodium bicarbonate	0.150	0.150	0.230	0.10	0.180	0.150	0.100	0.050	0.200	0.200	0.100	0.050
DDGS 28%	-	5.000	10.000	15.00	-	5.000	10.000	15.000	-	5.000	10.000	15.000
Lime stone	-	-	-	-	-	-	0.070	0.140	-	0.025	0.075	0.055
Chemical composition**												
Crnde protein (%)	23.03	23.000	23.000	23.020	20.000	20.000	20.000	20.010	18.99	19.010	19.01	19.000
ME %(K.cal\Kg. diet)	3152.000	3151.00	3151.000	3152.000	3200.000	3202.000	3200.000	3200.000	3253.00	3250.000	3251.00	3251.000
Calcium (%)	1.000	1.001	0.997	1.016	0.962	0.958	0.963	0.958	0.921	0.911	0.911	0.898
Available phosphorns (%)	0.501	0.505	0.510	0.508	0.479	0.485	0.482	0.475	0.461	0.460	0.458	0.463
Methionine (%)	0.613	0.618	0.618	0.624	0.607	0.617	0.624	0.624	0.571	0.573	0.578	0.578
Methionine+cystine (%)	1.002	1.003	1.000	1.003	0.960	0.958	0.962	0.959	0.903	0.902	0.904	0.901
Lysine (%)	1.352	1.353	1.364	1.359	1.270	1.270	1.271	1.272	1.150	1.153	1.149	1.152
Na (%)	0.175	0.170	0.196	0.197	0.170	0.172	0.181	0.170	0.171	0.180	0.179	0.170
EE (%)	4.238	4.440	4.737	5.031	4.067	4.320	4.498	4.773	4.617	4.774	5.026	5.231
CF (%)	2.381	2.545	2.706	2.870	2.262	2.423	2.581	2.751	2.210	2.372	2.537	2.702

T1: Control, T2: 5% DDGS, T3: 10% DDGS, T4: 15% DDGS, *Supplied per kg of diet: Vit A, 12000 I.U; Vit D_3, 2200 I.U, Vit E, 10 mg, Vit K_3, 2 mg, Vit B_1, 1 mg, Vit B_2, 4 mg, Vit B_6, 1.5 mg, Vit B_{12}, 10 µg, Niacin, 20 mg, Pantothenic acid, 10 mg, Folic acid, 1 mg, Biotin, 50 µg, Choline chloride, 500 mg, Copper, 10 mg, Iodine, 1 mg, Iron, 30 mg, Manganese, 55 mg, Zinc, 50 Mg and Selenium, 0.1 mg, **Calculated values based on feed composition tables of NRC (1994)

from 1-12 days fed starter diets containing 3150 kcal ME/Kg feed and 23%CP; from 13-23 days of age they were fed grower diets containing 3200 kcal ME/Kg feed and 20%CP and from 24-42 days of age they were fed finisher diets containing 3250 kcal ME/Kg feed and 19%CP. The experimental diets and their calculated analysis are shown in Table 1.

Birds were weighted at 1, 12, 23 and 42 days of age, feed conversion ratio were obtained by divided total feed intake by body weight gain for each replicate at the end of each period.

At the end of the experiment (6 weeks of age) five birds near to average live body weight of each replicate were sacrificed to study carcass traits. The assigned birds were derived of feed for 12 h, after which they were individually weighed, slaughtered to complete bleeding, followed by plucking the feathers. The organs were removed and weight. Weights of such organs were expressed relative to live body weight of birds.

Biochemical and hematological parameters: Blood samples were taken at the end of the experimental period from birds at slaughtering time. Five samples per each treatment group were collected in heparinized tubes, centrifuged (4000 rpm) for 10 min and plasma was decanted in eppendorf tubes and stored at -20°C until biochemical analysis. Few drops of fresh blood samples

were taken to determine blood hemoglobin (Hb) and packed cell volume (hematocrit, Ht%). Blood smears were done, stained with Wright's stain procedure and used to calculate the number of lymphocytes (L) and heterophils (H) in 100 white blood cells and then the H/ L ratio was calculated. Plasma glucose (mg dL^{-1}) was spectrophotometrically determined by using available commercial kits as described by the manufacturer companies (Bio Med-Diagnostics, Egypt. Co. for Biotechnology).

The intestinal length (cm) was considered. Values of pH in different parts of the Gastrointestinal Tract (GIT) were measured immediately by using a digital pH meter. Ten gram of gut content from all parts were collected aseptically in 90 mL sterilized physiological saline (1: 10 dilution) and pH was determined (Al-Natour and Alshawabkeh, 2005).

Histological observations: At the end of the experimental period representative tissue samples from ileum, liver and pancreas were taken to study the histological changes associated with the experimental treatments. Samples were fixed in a 10% formalin-saline solution before preparing the histological sections by using paraffin method technique. All sections were dehydrated in ascending grades of ethanol, cleared in zylene and then embedded in paraffin wax. Transverse sections (4-5 microns, thickness) were taken, mounted on glass slides and stained with haemotoxyline and eosin (H and E) stains. All sections were examined under electric microscope provided with computerized Camera.

Statistical analysis: Data were subjected to the analysis of variance by using the General Linear Models Procedure (GLM) of the Statistical Analysis System (SAS, 1994), Differences among treatment means were detected by using Duncan's multiple range test (Duncan, 1955).

RESULTS AND DISCUSSION

Productive performance and carcass characteristics: Table 2 shows the productive performance of broiler chicks fed the different dietary treatments demonstrated as a live body weight, body weight gain, feed consumption and feed conversion ratio. The results revealed that, during the periods of starter (1-12 day), grower (13-23) and finisher (24-42 day) as well as the whole period (1-42 day), the addition of DDGS at any studied levels resulted in no significant differences in growth performance parameters. During the experimental period (1-42 day) weight gain ranged from 1530.7 (T2) to 1585.2(T3) with no significant differences. Feed conversion ranged from 1.74(T1) to 1.85(T4) with no significant differences. These results are expected based on equal dietary protein, energy and c/p ratio; moreover, no alteration in diet composition was induced.

Generally, these results showed that feeding graded levels of DDGS (5, 10, 15%) resulted in no significant differences among treatments in weight gain, feed intake and feed conversion ratio values comparing to that given to the control group .These results are in agreement with those reported by Waldroup *et al.* (1981) who reported that up to 25% DDGS could be used in broiler diets if dietary energy was held constant. Parsons *et al.* (1983) reported that protein quality of DDGS was comparable to that of dehulle soybean meal when supplemented with lysine also, Lumpkins *et al.* (2004) concluded that DDGS from modern ethanol plants was an acceptable feed ingredient for broiler diets and no difference in growth performance during 0-42 days of age when they were fed iso-caloric and iso-protein diets containing 12% DDGS. Choi *et al.* (2008) investigated the effects of the addition of DDGS (at levels of 0, 5, 10 and 15%) to broiler diets on growth performance. They found no significant difference in growth performance among the four

Table 2: Effect of using Distiller's Dried Grain with solubles (DDGS) on broiler performance

	DDGS levels				
Item	T1	T2	T3	T4	SE*
1-12 days of age					
Initial weight	45.57	44.29	43.71	43.57	0.50
Final weight	255.30	258.00	257.00	249.57	4.00
Weight gain	209.70	213.70	213.30	206.00	3.52
Feed intake	249.90	250.10	256.40	252.90	3.53
Feed conversion	1.19	1.18	1.21	1.27	.02
13-23 days of age					
Initial weight	255.30	258.00	257.00	249.57	4.00
Final weight	748.20	756.30	761.30	743.67	-
Weight gain	492.90	498.30	504.30	494.10	10.53
Feed intake	833.90	840.30	839.30	828.00	17.15
Feed conversion	1.68	1.68	1.66	1.67	.01
24-42 days of age					
Initial weight	748.20	756.30	761.30	743.70	4.00
Final weight	1618.00	1575.00	1629.00	1615.00	21.81
Weight gain	870.00	818.70	868.00	871.30	16.37
Feed intake	1671.00	1666.00	1814.00	1846.00	33.31
Feed conversion	1.91	2.06	2.09	2.04	0.03
1-42 days of age					
Initial weight	45.57	44.29	43.71	43.57	0.50
Final weight	1618.00	1575.00	1629.00	1615.00	21.81
Weight gain	1572.40	1530.70	1585.20	1571.40	-
Feed intake	2734.00	2755.00	2909.00	2903.00	39.07
Feed conversion	1.74	1.81	1.83	1.85	0.02

T1: Control, T2: 5% DDGS, T3: 10% DDGS, T4: 15% DDGS, *SE: Standard error

treatments. It was shown that the use of DDGS in broiler diets up to 15% could replace. part of corn and soybean meal without any negative effect on growth performance. Similar results were reported by Wang *et al.* (2007) who observed that, inclusion of up to 20% of traditional DDGS in broiler diets did not affect body weight or feed: gain ratio.

On the other hand, Ghazalah *et al.* (2012) showed that feeding broiler chicks on diets containing deferent levels of DDGS up to 60% replacement for soya bean meal with or without enzymes supplementation improve body weight, body weight gain and feed conversion value the effect of different treatments on carcass characteristics (as a percentage is shown in Table 3.

The levels of DDGS used did not exert any significant effect on dressing%, heart, liver, gizzard, thigh and breast%. Carcass% ranged from 72.8(T4)to 73.65(T3)with no significant differences. The highest breast% (24.57) was in T3 and the lowest was (18.72)in T4. These results are in agreement with Wang *et al.* (2007) who reported that birds fed diets with 15% DDGS from 1 day of age or from 14 to 42 day of age did not differ significantly in dressing percentage from birds fed the control diet with no DDGS. Also Choi *et al.* (2008) reported that, there was no negative effect of DDGS supplementation up to 15% on meat qualities. Similar results were reported by El-Alaly *et al.* (1981) concerning carcass characteristics and composition of broiler chicks fed rations based on different protein sources. Gamal (1989), Mady *et al.* (1991) and Ismaiel (1992) showed that carcass

Table 3: Effect of using Distiller's Dried Grain with solubles (DDGS) in carcass quality

	DDGS levels				
Item	T1	T2	T3	T4	SE
Live body weight	1618.000	1575.000	1629.000	1615.000	-
Carcass (%)	73.090	73.140	73.650	72.080	0.402
Heart (%)	0.369	0.363	0.351	0.379	0.017
Liver (%)	1.713	1.500	1.583	1.627	0.040
Gizzard (%)	2.160	2.190	2.090	2.040	0.044
Giblets (%)	4.343	4.023	4.000	4.043	0.070
Breast (%)	22.800^{a}	24.260^{a}	24.570^{a}	18.720^{b}	0.612
Thigh (%)	32.740	32.590	31.770	31.900	0.301
Total edible parts (%)	77.510	76.970	77.690	76.400	0.361

Means within rows with no common superscripts differ significantly, T1: Control, T2: 5% DDGS, T3: 10% DDGS, T4: 15% DDGS

Table 4: Effect of DDGS on glucose and hematological parameters of broilers

Treatment	Glucose (mg dL^{-1})	HB	Ht%	H/L ratio
T1	254.24±5.41^{a}	0.26±0.01^{c}	36.77±3.36	0.40±0.01^{a}
T2	222.15±3.46^{b}	0.39±0.03^{a}	34.95±0.71	0.18±0.01^{c}
T3	244.30±9.15^{a}	0.43±0.01^{a}	38.97±1.98	0.16±0.02^{c}
T4	217.29±4.37^{b}	0.33±0.01^{b}	35.37±0.66	0.28±0.02^{b}
Significance	**	**	NS	**

** p≤0.01, NS: Non-significant, Means within columns with no common superscripts differ significantly, T1: Control, T2: 5% DDGS, T3: 10% DDGS, T4: 15% DDGS

characteristics did not change among both sexes of broilers due to different protein sources. The current results coincide with those cited above. Also, Ghazalah *et al.* (2012) showed that no significant effect of DDGS levels on average values of carcass characteristics.

Biochemical and hematological parameters: The effect of using DDGS on glucose and hematological parameters of broiler are presented in Table 4. Plasma glucose concentration was significantly decreased in T2 and T4 groups (5 and 15% DDGS) compared with T1 (control) and T3 (10% DDGS). This effect is due to improve the carbohydrate metabolism and then decrease glucose concentration in blood. This concept was supported by the fact that there is a need for glucose to provide energy for different tissues in the body. Because glucose is the easiest substrate for cells to obtain energy, since its level in blood is decreased (Rajgude *et al.*, 2005).

Hematological parameters serve as indicators of the physiological state of bird (Chowdhury *et al.*, 2005; Saied *et al.*, 2011).

Hemoglobin (Hb) concentration was significantly increased by using DDGS at all levels compared to control group. This increase in hemoglobin concentration may due to higher oxygen consumption associated with more hemoglobin saturation and dissociation rates (Yahav *et al.*, 1998). Hematocrite (HT%) was insignificantly changed by using DDGS in the summer season although the T3 group (38.97) has numerically increased Ht% compared with T1 (36.77). this may indicate higher blood viscosity associated with a pronounced increase in blood corpuscular volume. Which concomitant with an increased erythrocytes count or may reflect be chronic macrocytosis related to heat stress exposure which marked in this experiment. These responses were supported by the finding of Zhou *et al.* (1999) who found that blood packed cell volume of heat stressed birds

Table 5: Effect of DDGS on digestive tract length (cm) of broiler

Treatment	GIT	Prov.	Gizzard	Duodenum	Jejunum	Ileum	Cecum	Colon
T1	98.33±2.19^{b}	4.25±0.25	5.63±0.24	27.17±0.60	87.67±1.33^{a}	68.33±1.76	17.15±0.10^{a}	9.00±2.420
T2	111.50±4.50^{a}	4.95±0.05	5.95±0.45	25.50±0.50	68.00±2.00^{bc}	51.00±5.00	14.38±0.63^{b}	9.27±0.120
T3	87.67±0.88^{c}	4.50±0.23	5.50±0.29	25.67±0.33	74.00±2.65^{b}	57.33±5.24	15.67±0.88^{ab}	9.50±0.290
T4	106.67±1.20^{a}	4.76±0.23	6.50±0.29	26.67±0.33	63.67±2.67^{c}	64.33±4.67	14.37±0.32^{b}	11.70±0.35
Significance	**	ns	ns	ns	**	ns	**	ns

**p≤0.01, NS: Non-significant, Means within columns with no common superscripts differ significantly, T1: Control, T2: 5% DDGS, T3: 10% DDGS, T4: 15% DDGS

are significantly affected by environmental temperature, water consumption, dietary contents and the size of red blood cells. Moreover, the higher Hb content in treatment (T3) may reflect an increase in RBC's counts which support the findings (HT%).

Heterophils to lymphocytes (H/L) ratio appears to be a more reliable indicator for determining stress in poultry, H/L ratio was significantly decreased by using DDGS in summer season compared by the control group, the lowest value was recorded for T3 (0.16) followed by T2 (0.18) then T4 (0.28). This is a good response of broiler under heat stress may be a result of highly oil content of DDGS (Behnke, 2007; Belyea *et al*., 2010; Lamsal *et al*., 2012) which enhance the immunity and alleviate the deleterious effect of high temperature during the whole experimental period. Also this my a result to an increase in small intestine goblet cells, which secretes mucous and other substances responsible for reducing pH of the intestinal tract (the present study) and help in production of antibodies and lymphocytes.

Digestive tract parameters: Effect of DDGS on digestive tract length (cm) of broiler is presented in Table 5. DDGS did not affect the length of proventriculus, gizzard, duodenum, ileum and colon but significantly affect the Gastrointestinal Tract (GIT), jejunum and cecum length.

GIT length was significantly increased by using DDGS in T2 (111.50 cm) and T4 (106.67 cm) while decrease significantly in T3 the length of GIT (87.67 cm) compared to T1 (98.33 cm). These conflicting findings are ambiguous and remain the subject of farther investigation. Jejunum length was significantly decreased by using DDGS treatments, the lowest length was recorded for T4 (63.67 cm) followed by T2 (68.00 cm) then T3 (74.00 cm) compared to T1 (87.67 cm). Also, the cecum length was significantly decreased in T4 and T2 (14.37 and 14.38 cm, respectively) followed by T3 (15.67 cm) compared to T1 (17.15 cm).

The small intestine densities (weight/ length) has considered as an indication of the intestinal villi dimension of mucosa layer (Palo *et al*., 1995). Furthermore, many researchers largely ignore the dynamics of intestinal turnover and presume intestinal absorptive capacity based on simple morphometric measures of villi height and crypt depths. Transient changes in villus height, however, do not always affect nutrient utilization. Michael and Hodges (1973) reported that the reduction in villi height did not significantly influence nutrient utilization, this is in agreement with the histological observation of small intestine in this study (the villi height was decreased in T2, T3 and T4 groups while the vilh diameter of T2, T3 and T4 groups were larger than T1 group, this may explain the decrease in the number of villi per unit area. Moreover, the number of Crypts of LieberKuhn was apparently greater in T3 and T4 treatment groups than both T2 and T1 groups). These results supply that help improving absorption of nutrients and used as indicator for digestive tract health (Jamroz *et al*., 2006; Karcher and Applegate, 2008).

Effect of DDGS on digestive pH of broiler are presented in Table 6. DDGS did not affect the pH value of glandular stomach and ceca but significantly affect crop, muscular stomach, duodenum, ileum and bile pH value.

Table 6: Effect of DDGS on digestive pH in broiler

Treatment	Crop	Glandula stomach	Muscular stomach	Duodenum	Ileum	Ceca	Bile
T1	5.03±0.19^{a}	4.80±0.06	4.97±0.07^{a}	6.30±0.15^{a}	6.63±0.03^{a}	5.75±0.09	6.45±0.15^{a}
T2	4.47±0.09^{bc}	4.84±0.07	4.70±0.12^{b}	5.64±0.12^{b}	6.13±0.08^{b}	5.53±0.12	6.68±0.12^{a}
T3	4.81±0.10^{ab}	4.78±0.12	4.50±0.06^{b}	6.40±0.06^{a}	6.14±0.09^{b}	5.67±0.09	5.87±0.05^{b}
T4	4.42±0.04^{c}	4.97±0.12	3.80±0.06^{c}	6.13±0.12^{a}	6.25±0.05^{b}	5.74±0.03	6.71±0.05^{a}
Significance	*	ns	**	**	**	ns	**

**p≤0.01, NS: Non-significant, Means within columns with no common superscripts differ significantly, T1: Control, T2: 5% DDGS, T3: 10% DDGS, T4: 15% DDGS

Crop pH value was significantly decreased by using DDGS, the lowest value was recorded for T4 (4.42) followed by T2 (4.47) then T3 (4.81) compared to T1 (5.03). Moreover, the muscular stomach ph was significantly decreased especially in T4 (3.80) compared with T1 (4.97). While, the duodenum pH value was significantly decreased in T2 (5.64) compared to T1although the T3 (6.40) numerically but not significantly increased than T4 (6.13) and T1 (6.30). Furthermore, the ileum pH value was significantly decreased by using all levels of DDGS compared with control group. While the bile pH was significantly increased by using 15% and 5% DDGS in the diet (6.71 and 6.68, respectively) but 10% DDGS significantly increased the pH value of the bile (5.87) compared to the control group. DDGS reduced the pH value in most GIT segments compared to control group.

This may enhance the digestibility and absorption of most nutrients and increase the count of the beneficial microorganisms which reflect on the GIT health. This finding is in close agreement of Rahmani and Speer (2005), Rynsburger and Classen (2007) who reported that pH level affects the digestibility and absorptive value of most nutrients. Also, the beneficial microorganisms live in an acidic pH. Also, Perez *et al.* (2011) mentioned that Increasing concentrations of dietary DDGS had clear effects on changing bacterial populations in GIT.

Histological observation

Small intestine histology: Histological examination of the intestinal sections (Fig. 1a-d) showed that the villi height was increased in T1 treatment compared with the other groups. However, the villi diameter of T2, T3 and T4 groups were larger than T1 group. This may explain the decrease in the number of villi per unit area (microscopic field). Moreover, the number of Crypts of LieberKuhn was apparently greater in T3 and T4 treatment groups than both T2 and T1 groups. These Crypts are known to secrete fluids containing different vital substances essential for the internal micro- environment of the digestive tract segments (Hodges, 1974). The Crypts fluids are rapidly absorbed from the villi lumens, making a circulation from Crypts to the apex of villi. These results in a watery vehicle supply that help improving absorption of nutrients, elaboration and production of antibodies and lymphocytes along with an increase in goblet cells, which secretes mucous and other substances responsible for reducing pH of the intestinal tract. This may explain the improvements of body weight, weight gain and feed conversion ratio of DDGS-treated vs. control group. It is of interest to notice that the muscular layer in T2 and T4 sections were obviously decreased compared with the other treatments. This was explained by many workers to reflect better digestive tract peristaltic motility and (or) higher blood flow to the intestinal villi (Huisman *et al.*, 1990). This results point out that DDGS addition improves gut morphology and may play a role in processes of digestion and absorption of nutrients which is confirmed by the obtained data. In this respect, several possibilities for the gut to adapt or to react morphologically to change in diet composition or the intestinal microflora.

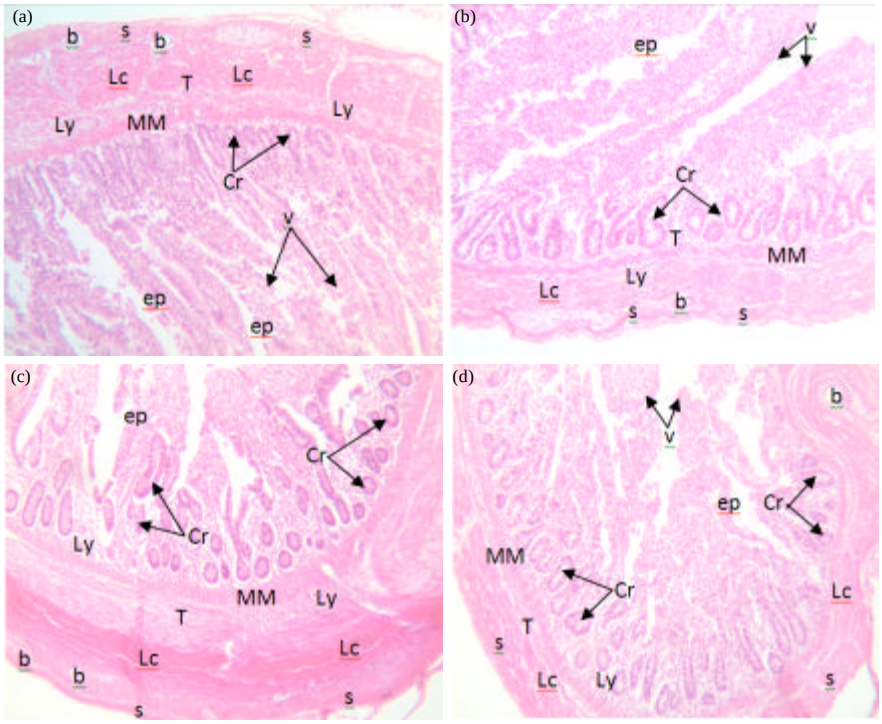

Fig. 1(a-d): The effect of using Distiller's dried grain with solubles (DDGS) on small intestine histology, T.S. in the ileum from, (a) T1 treatment group, (b) T2 treatment group, (c) T3 treatment group and (d) T4 treatment group (H&E×40), s: Serosa layer, b: Blood vessels, v: Vilh, ep: Epithehum of the mucosa membrane, MM: Muscularis mucosa, Cr: Crypts of LieberKuhn, T: Tunica propria, Lc: Longitudinal and circular muscle layer, Ly: Small and large lymphocytes

The intestine can change its surface area by growing to length and (or) by increasing or decreasing the height of the villi. In general, shortening and fusion of villi, reduction of Crypts depth (T1) or deeper Crypts (T2, T3 and T4) could be used as indicator for digestive tract heath this results are in close agreement with those obtained by some investigators who studies the morphological changes in gut morphology as related to different dietary supplementations (Huisman *et al*., 1990; Iji *et al*., 2001; Van Dijk *et al*., 2002; Jamroz *et al*., 2006; Karcher and Applegate, 2008).

Liver histology: Examination of liver sections (Fig. 2 a-d) showed moderate (T1) to sever changes (T4) associated with the experimental treatments. It is clear that liver section from T1 (control) treatment shows homogenous masses of liver cords with apparently normal hepatocytes (Fig. 2a).

The liver parenchyma was pale- stained with some necrotic areas, more fatty cirrhotic areas and normal blood vessels, bile ducts and blood sinusoids the previous observations was also observed in T2 and T3 (Fig. 2b and c), although some scattered fatty cirrhosis and infilterable fluids could be seen. The hepatic cells stained dark compared with T1 treatment.

These were many binucleated cells indicative of hyperplasia in T2 sections but the hepatocytes are larger in T3 indicative of hyper trophy of liver cells (Hodges, 1974; Loar II *et al*., 2010). This

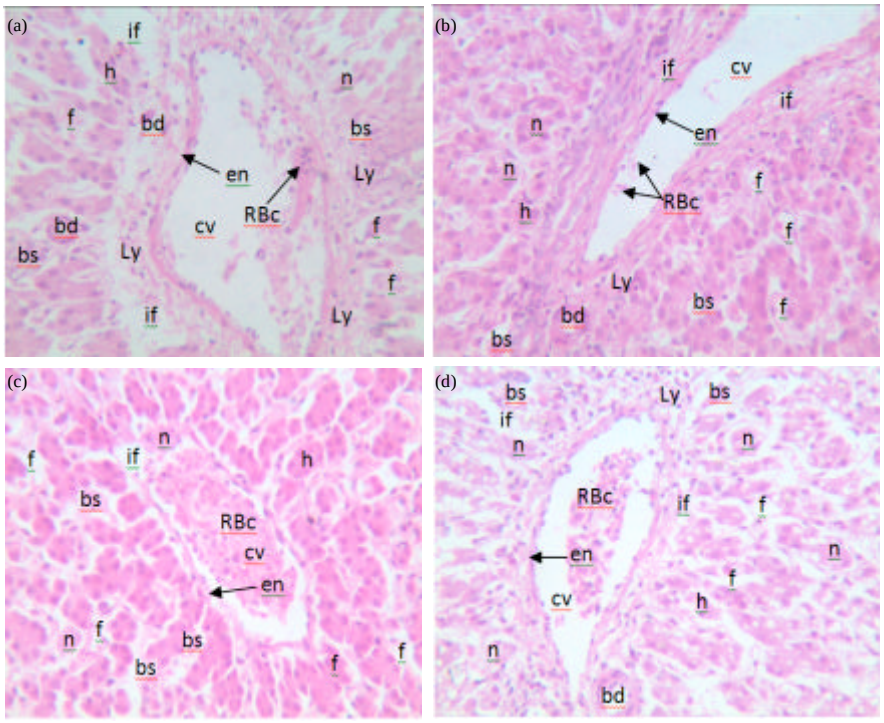

Fig. 2(a-d): The effect of using Distiller's dried grain with solubles (DDGS) on liver histology, T.S. in the liver from, (a) T1 treatment group, (b) T2 treatment group, (c) T3 treatment group and (d) T4 treatment group (H and E×40), en: Endothelial cells, cv: Central vein, h: Hepatocytes, if: Infiltrable fluid, Ly: Lymphocytes, f: Fatty cirrhotic area, bs: Blood sinuses, bd: Bile duct, n: Necrotic area, RBc: Red blood cells

was accompanied by vaculations of the hepatocytes and an obvious decrease in the endothelial linning of the central vein. These changes were more sever in the liver sections from T4 treatment (Fig. 2d), where a marked disruption in the arrangement of the hepatic cords (hepatocytes) and more infilterable fluids could be seen. Liver hepatocytes stained- pale which may reflect a tendency to fat droplets deposition and (or) liver glycogen storage. Although, differences in the liver histology between treatments could be seen, these variations did not show any histopathological changes. The observations may reflect hyperactivity of liver cells due to higher metabolism of nutrients associated with the genetic background of the fast growing broiler. It appears that DDGS-supplementation did not adversely affect liver histology although some signs of degeneration in T4 section could be seen, which does not affect the productive performance results.

Pancreas histology: The pancreas, in addition to its digestive function, secretes, mainly, two important hormones, insulin and glucagon. It is composed of two major types of tissues: (1) the acini, which secrete digestive juices (enzyme- secreting cells) and (2) the islets of Langerhans, which secrete hormones. The endocrine pancreas of birds caccupies considerably more tissue mass than it does in mammals and the distribution of cell types differs, although species differences are exist (Kern, 1970; Hodges, 1974; Swenne and Lundquist, 1980; Hazelwood, 1981a, b).

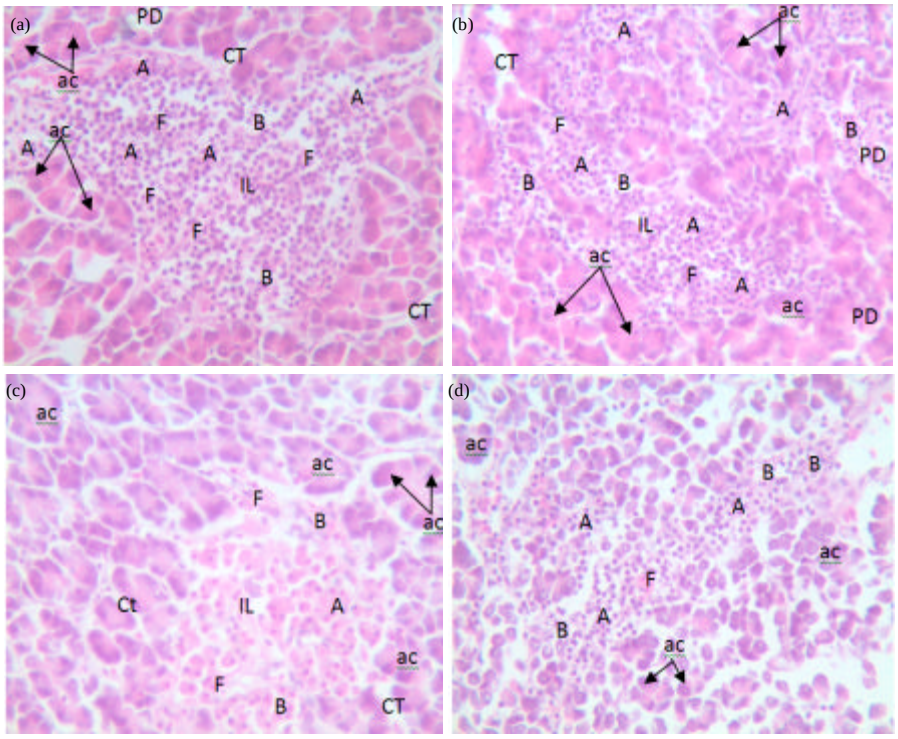

Fig. 3(a-d): The effect of using Distiller's dried grain with solubles (DDGS) on pancreas histology, T.S. in the pancreas from, (a) T1 treatment group, (b) T2 treatment group, (c) T3 treatment group and (d) T4 treatment group (H and E×40), IL: Islets of langerhans, as: pancreatic acini lined by pyramidal cells (arrows), A: Alpha cells (glucagon- secreting cells), B: Beta cells (insulin- secreting cells), F: pp cells (pancreatic polypeptide secreting cells), CT: Fine connective tissue septa, PD: Pancreatic duct

Examination of pancreas sections (Fig. 3a-d) showed considerable changes related to DDGS- addition. It is clear that the Islets of Langerhans occupies the most of the pancreatic parenchyma in the T1 sections (Fig. 3a). Alpha cells (glucagon- secreting) are abundant and they have a dark- stained and large nuclei. Moreover, beta cells (insulin-secreting) and F (pancreatic polypeptide (pp)-secreting) cells were scattered within the Islets. Pancreatic acini with their pyramidal cells are well developed and deeply stained indicative of their higher activity in secreting pancreatic digestive enzymes. This was also observed for the second treatment (T2) but the islets were scattered along the whole pancreatic parenchyma (Fig. 3b). The pyramidal cells tends to the shorter than that observed in T1 treatment. Moreover, the histological appearance of both T3 and T4 treatments showed some different changes (Fig. 3c, d). The islets of Langerhans are pale- stained (T3) with disturped arrangement of both alpha and beta cells in-between the pancreatic acini (T4). Also, the exocrine part of pancreas sections in both T3 and T4 treatments show some degenerative areas without any signs of pathological damage.

CONCLUSION

It can be concluded from this study, that the dietary treatments containing graded levels of DDGS up to 15% are acceptable in broiler starter, grower and finisher diets with no adverse affects on growth performance and carcass characteristics. While it improved hematological and histological parameters.

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