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Novel Method for Improving the Utilization of Corn Dried Distillers Grains with Solubles in Broiler Diets

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Abstract: An experiment was conducted with broiler chicks to study the possibility of improving the utilization of Dried Distillers Grains with Solubles (DDGS) in broiler diets. A total number of 150 day-old broiler chicks were randomly assigned to five groups received, diet containing 12% corn Dried Distillers Grains with Solubles (DDGS diet) without or with enzyme preparation (E), radish root extract (RRE, as source of peroxidase enzyme) or E plus RRE, in addition to the control diet .The addition of RRE improved numerically Body Weight (BW) at 28 days and significantly at 42 days by 3.42 and 3.11%, respectively compared to birds fed DDGS diet alone. Enzyme preparation failed to improve performance for broiler fed DDGS diet. While using DDGS diet significantly decreased plasma antioxidants capacity by 56.11% compared to control birds, addition of RRE significantly increased it by 515% compared to the birds fed DDGS diet alone. There were insignificant differences between experimental treatments in plasma cholesterol, lipids or creatinine content. The addition of RRE increased plasma phosphorus by 65.93% compared to the birds fed DDGS diet alone while the later decreased it by 6.22% compared to control diet. The control birds recorded significantly higher values of plasma uric acid compared to other treatments. It was concluded that using RRE as a source of peroxidase enzyme is a suitable feed additive for improving the utilization of DDGS.

Key words: Broiler diet, feed additive, corn dried distillers grains

INTRODUCTION

Increased emphasis on ethanol production as biofuel in the United States and other countries has and will continue to lead to significant increase in the amount of Distillers Dried Grains with Solubles (DDGS) available to the feed industry (Batal and Dale, 2003). Production of ethanol from 100 kg of corn using the dry-milling method produces approximately 34.4 kg of ethanol, 34.0 kg of carbon dioxide and 31.6 kg of distillers dried grains with solubles (Renewable Fuels Association, 2005). Traditionally, DDGS had been fed mainly to ruminants because of its high level of fiber and high variability in content and bioavailability of some nutrients, such as lysine.

Dale and Batal (2003) suggested a maximum level of 6% DDGS from ethanol production in starter diets and 12% in grower-finisher diets.

Also, Wang *et al*. (2007a,b) reported that broilers can be fed 15% DDGS without affecting carcass composition or growth. The manufacturing process of DDGS may damage a portion of the protein due to excessive heat during the drying that accompanies this process, thus making it unavailable to the animal. It is clear from previous studies (Parsons *et al*., 1992) that excessive heat applied during the drying process may cause Maillard reactions between the lysine residues and carbohydrate moieties, subsequently darkening the color of by-product. Maillard reaction may reduce the digestibility of lysine by competing with absorption of lysine (Sherr *et al*., 1989) or inhibit the release of protein bound lysine by inhibition of carboxypeptidases (Hansen and Millington, 1979). Finot (1990) offers a useful summary of physiological and pharmacological effects of Maillard products which may adversely affect protein, mineral and vitamin nutrition. Briefly, these effects include (a) inhibition of growth, protein and carbohydrate digestion, amino acid absorption and activity of intestinal enzymes including aminopeptidases, proteases and saccharidases and pancreatic enzymes such as chymotrypsin; (b) induction of cellular changes in the kidneys (karyomegaly and hypertrophy) and the liver (brownspots, hypertrophy and decrease in enzyme production); (c) adverse effects on mineral metabolism (Ca, Mg, Cu and Zn) and (d)variable effects on allergic response and cholesterol metabolism. Melanoidins are high molecular weight amino-carbonyl compounds produced by non-enzymatic browning reactions called as Maillard reactions during the food processing and preservation. On the other hand, low molecular weight of Maillard reaction products exhibit antioxidant effects in organism after they get absorbed in small intestine (Chandra *et al*., 2008). The enzymatic system responsible for the degradation of melanoidins (high molecular) consists mainly of sugar oxidases and peroxidases, manganese dependent and independent peroxidases (MnP and MIP) (Watanabe *et al*., 1982). Peroxidases (donor: hydrogen-peroxide oxidoreductase, EC1.11.1.7) are a group of heme-containing oxidoreductases that act on peroxide as electron donors. They are present in all known organisms and their

biological function is related to the removal of the toxic hydrogen peroxide which is a product of cell metabolism. Tsujiyama *et al.* (1993) compared alkaline treatment and phenoloxidase (Laccase and Horseradish peroxidase) treatments to cellulase-treated lignin-carbohydrate complex. They found that similar amounts and composition of monosaccharide being released from each treatment.

(Ali, 2002, 2005) described method for improving the utilization of high fiber feedstuffs using RRE as a source of peroxidase enzyme like wheat bran in broiler diets (Ali, 2002 and Ali *et al.*, 2008a), wheat bran in laying hens diets (Abaza *et al.*, 2004 and Ali *et al.*, 2006a) and Quail diets (Ali *et al.*, 2006b). They found from the previous experiments that RRE (as a source of peroxidase enzyme) either alone or with commercial enzyme preparation improved performance and digestibility of nutrients.

All cells of the vertebrate animal depend on a functional antioxidant capacity to provide protection against the harmful effects of free radicals and reactive oxygen species that are the inevitable consequences of aerobic life (Halliwell, 1999). It was found that plasma antioxidants capacity is accompanied with good hatch performance (Ali *et al.*, 2007) and best feed conversion (Ali *et al.*, 2008a).

In this study we examined the hypotheses that RRE (as a source of peroxidase enzyme), enzyme preparation (Polytec Binder Plus) or both can improve the utilization of broiler diet containing 12% DDGS in both starter and finisher diet. Also, given details about the effect of DDGS diets and tested additives on some plasma parameters.

MATERIALS AND METHODS

The experimental work was carried out at El-Takamoly Poultry Project, Research Unit, Fayoum, Egypt while the laboratory work was done at Poultry Nutrition Department, Animal Production Research Institute, Agricultural Research Center, Ministry of Agriculture, Egypt.

One hundred and fifty one day-old of unsexed Arbor Acres broiler chicks from Cairo Poultry Company were used in this experiment. They were given a control diet (Table 1) for the first week of age and then chicks were wing-banded, individually weighed and randomly distributed into 5 groups each in three replicates of 10 chicks each and caged in battery brooders.

Experimental diets and water were offered *ad-libitum* over the experimental period starting from 7 days old. Chicks in all treatments were kept under similar conditions of management. Artificial lightning was provided 24 h daily during the whole experimental period.

Corn Dried Distiller Grains with Solubles (DDGS) was provided by Cairo Poultry Company. Polytec Binder Plus (Enzyme Preparation, E) from Polytec Holland contained xylanase 1500 U, beta-glucanase 1500 U, protease 1500 U and amylase 1500 U. Radish roots were purchased from local market, Cairo. Radish Root Extract (RRE) was prepared by cutting the root of radish into chips and put the chips into carrot press and the juice was collected into glass cups. A sample of radish extract was taken to measure peroxidase activity according to method of Amako *et al.* (1994). The peroxidase activity of radish extract is expressed in a unit/milligram protein. Peroxidase activity for RRE was 0.81 U/mg protein.

Chicks were allotted on the following dietary treatments:

T1 = The control diet (Table 1).
T2 = Diet contained 12% DDGS in the starter and finisher diet.
T3 = T2+0.05% Polytec Binder Plus (Enzyme Preparation, E)
T4 = T2+1% RRE (as a source of peroxidase enzyme)
T5 = T2+0.05% (E) +1%RRE

The Body Weight (BW), Feed Intake (FI) and Weight Gain (WG) values were weekly recorded while Feed Conversion (FC) was calculated as a unit of FI per unit of WG.

At the end of experiment period (42 days) three birds were taken randomly from each treatment and slaughtered. The edible organs included heart, empty gizzard and liver were weighed. Carcass and organs weights percentage were calculated on the basis of live body weight. Individual blood samples were taken from birds within each treatment and collected into dry clean centrifuge tubes containing drops of heparin and centrifuged for 20 min (3000 rpm) for obtaining plasma. Antioxidant capacity in plasma was determined using commercial kit produced by Biodiagnostic Company. Plasma cholesterol, total lipid, creatinine, phosphorus and uric acid were determined by suitable commercial kits. The specific immunity against Avian Influenza Disease Virus (AIDV) in plasma were determined in National Laboratory for Veterinary Quality Control on Poultry Production, Egypt. The statistical analysis on data obtained was computed using analysis of variance procedure (SAS, 1990) and the significant mean differences between treatment means were separated by Duncan's multiple range test procedure (Duncan, 1955).

RESULTS AND DISCUSSION

Growth performance: At 28 days of age, there were significant differences ($p \leq 0.01$) between experimental treatments in BW and WG. The birds fed DDGS plus RRE recorded the highest value while birds fed DDGS plus E recorded the lowest value. The addition of RRE to DDGS increase BW by 3.42% compared to birds fed DDGS diet alone. The beneficial effect of RRE may be

Table 1: Composition and calculated analysis of the control and DDGS starter and finisher diets.

	Control		DDGS	
Items	Starter	Finisher	Starter	Finisher
Yellow corn	58.84	70.54	53.73	65.31
Soybean meal (44%)	24.50	13.23	17.77	6.70
Corn gluten meal (60%)	9.00	9.00	9.00	9.00
Soy oil	2.50	2.50	2.50	2.50
Limestone	1.73	1.55	1.73	1.54
Mono calcium phosphate	1.82	1.57	1.69	1.42
NaCl	0.49	0.55	0.35	0.36
Vitamin and Min.Mix*	0.30	0.30	0.30	0.30
DL-methionine	0.24	0.17	0.22	0.15
L-Lysine HCl	0.58	0.59	0.71	0.72
DDGS	--	--	12.00	12.00
Total	100	100	100	100
Calculated analysis**				
CP%	22.04	18.04	22.00	18.08
Kcal ME /kg	3091	3229	3071	3210
Crude fiber%	3.12	2.59	3.63	3.11
Crude fat %	5.15	5.51	5.98	6.33
Calcium %	1.01	0.88	1.00	0.86
Available phophorus %	0.50	0.43	0.50	0.42
Lysine %	1.35	1.09	1.35	1.09
Methionine %	0.62	0.50	0.62	0.511
Methionine + Cystine%	0.97	0.800	0.97	0.800
Sodium%	0.20	0.22	0.20	0.22

*Premix contain per 3 kg vit A 12 000 000IU, vit D_3 2000 000 IU, vit E 40 000 mg, Vit K_3 4000 mg , vit B_1 3000 mg, vit B_2 6000 mg, vit B_6 4000 mg, vit B_{12} 30 mg, pantothenic acid 12000 mg, Niacin 30000 mg, Biotin 80mg, Folic acid 1500 mg, Choline 350 g, Selenium 200 mg, Copper 10000 mg, Iron 40000 mg, Manganese 70000 mg, Zinc 70000 mg, Iodine 1500 mg, Cobalt 250 mg and $CaCO_3$ to 3000 g, **Calculated according to NRC (1994).

due to its effect on Maillard reactions and fiber matrix. It was reported that the enzymatic system responsible for the degradation of melanoidins (high molecular weight amino-carbonyl compounds produced by non-enzymatic browning reactions) consists mainly sugar oxidases and peroxidases manganese dependent and independent peroxidases (Watanabe *et al*., 1982). The DDGS diet contain also high content of fiber and peroxidase may be affect fiber matrix. Monties (1994) compared lignin peroxidase and horseradish peroxidase and indicated that horseradish peroxidase is a ligninolytic enzyme. These results confirm those found by previously by Ali (2002) who found with broiler diet contained 30% wheat bran that RRE (as a source of peroxidase enzyme) and enzyme preparation improved performance and digestibility of nutrients. In this respect, Abaza *et al*. (2004) with local hens found that diets contained 35% wheat bran and supplemented with enzyme preparation alone or with RRE improved feed conversion. With quails, Ali *et al*. (2006b) concluded that RRE alone or with enzyme preparation can be used to improve the utilization of 30% wheat bran in quail diets in both growing and laying phases. The RRE also, has other beneficial effect on performance for example, RRE was used to partial detoxification of aflatoxin B1 with growing chicks (Qota *et al*., 2005) or laying hens (Ali *et al*., 2006c).

At 42 days of age, there were significant ($p \leq 0.05$) differences between the experimental treatments in BW and WG (7-42 days). The birds fed DDGS alone had lower BW by 2.37% compared to the control.

The reduction in BW for birds fed DDGS alone may be due to reduction of lysine availability. The reduction in lysine digestibility in darker colored DDGS has also been reported by Ergul *et al*. (2003), who demonstrated a reduction in true lysine digestibility of approximately 20% from their lightest to darkest DDGS sources. The DDGS used in this study was medium between light and dark.

In this respect, Dale and Batal (2003) suggested a maximum level of 6% DDGS from ethanol production in starter diets and 12% in grower-finisher diets. Also, Lumpkins *et al*. (2004) stated that DDGS from modern ethanol plants could be safely used at 6% in the starter period and 12-15% in the grower and finisher periods. At 42 days of age, the birds fed DDGS plus RRE recorded the highest value of BW while the birds fed DDGS plus RRE+E recorded the lowest one. The promised feed additive RRE improve BW by 3.11% compared to birds fed DDGS diet alone. It is clearly that addition of E alone or with RRE is not beneficial to DDGS under the condition of this study. Mixed enzyme in E may be not combatable with DDGS. However, McNab (1993) showed that for a variety of reasons, microbial feed enzymes may not give a consistent response. The data present in Table 2 indicating that the differences between experimental diets in FI and FC during starter

Table 2: Effect of dietary treatments on broiler performance up to 42 days.

Item	Control	DDGS	DDGS +E	DDGS +RRE	DDGS +E+RRE
IBW (g)	106±0.05	106±0.05	106±0.08	106±0.14	106±0.16
BW 28 d (g)	968^{ab}±9.81	963^{ab}±20.7	920^{c}±7.79	996^{a}±4.04	933^{bc}±12.74
BW 42 d (g)	1939^{ab}±9.52	1893^{bc}±3.75	1896^{abc}±9.52	1952^{a}±29.44	1862^{c}±20.49
WG 7-28 (g)	861^{ab} ±9.84	856^{ab} ±20.75	814^{c} ±7.82	889^{a} ±3.95	826^{bc} ±12.89
WG29-42 d (g)	971±19.34	930±24.5	976±17.3	956±25.40	929±7.83
WG 7-42 d (g)	1833^{ab}±9.49	1787^{bc}±3.78	1790^{abc}±9.50	1845^{a}±29.3	1756^{c}±20.64
FI 7-28 d (g)	1364^{c}±25.6	1392^{bc}±1.45	1432^{ab}±4.33	1437^{a}±7.21	1439^{a}±12.12
FI 29-42 d (g)	2017 ±56.2	2031±36.6	2013±73.91	2025±40.12	1956±3.46
FI 7-42 d (g)	3382 ±81.9	3424 ±38.1	3446.3 ±96.5	3463 ±47.34	3395 ±15.58
FC 7- 28 d (g feed/g gain)	1.58^{a} ±0.04	1.62^{a}±0.03	1.76^{b} ±0.01	1.61^{a} ±0.01	1.74^{b} ±0.01
FC 29-42 d (g feed/g gain)	2.07 ±0.01	2.18±0.09	2.06 ±0.03	2.12 ±0.09	2.01±0.01
FC 7-42 d (g feed/g gain)	1.84±0.03	1.91±0.02	1.92±0.02	1.87±0.05	1.93±0.01

a-c Means in the same row with different letters, differ significantly ($p \leq 0.05$). IBW = Initial body weight FI = Feed Intake
BW = Body weight FC = Feed conversion WG = weight gain

Table 3: Effect of dietary treatments on carcass characteristics.

Item*	Control	DDGS	DDGS +E	DDGS +RRE	DDGS +E+RRE
Carcass %	72.26±1.75	69.45±0.82	71.22±1.28	71.67±0.40	69.39±0.76
Heart %	0.61±0.02	0.62±0.02	0.62±0.05	0.60±0.08	0.51±0.03
Liver %	2.44^{b}±0.04	2.39^{b}±0.17	2.91^{a}±0.16	2.65^{ab}±0.06	2.37^{b}±0.07
Gizzard %	1.66±0.11	2.04±0.18	2.07±0.21	1.95±0.22	1.69±0.10
Abdominal fat %	2.10±0.06	1.49±0.12	1.10±0.21	1.64±0.51	1.51±0.39
Giblets %	4.72±0.12	5.06±0.08	5.61±0.34	5.21±0.035	4.58±0.14
Spleen %	0.13±0.01	0.18± 0.01	0.19±0.03	0.18±0.02	0.15±0.02
Total edible parts %	76.98±1.74	74.52±0.75	76.83±1.33	76.89±0.48	73.97±0.90

a-b Means in the same row with different letters, differ significantly ($p \leq 0.05$). *Relative to BW at 42 days of age.

period were significant ($p \leq 0.01$). The control group recorded the lowest FI and best FC. However, the promised feed additive RRE improved FC all over the experimental period by 2.09% compared with birds fed DDGS alone. In this respect, Ali *et al.* (2006b) found with quail that addition of RRE, enzyme preparation each alone or in combination significantly improved FC of diet contained 30% wheat bran. Putting in mind that the problem in wheat bran was the high content of crude fiber and phenolic compounds while in DDGS the problem was the high content of crude fiber and Maillard reactions. Further studies are needed with high level of DDGS like 20% or 30%.

Carcass traits: There were insignificant differences in different carcass characteristics (Table 3) except liver ($p \leq 0.05$). We can not explain the increase in liver weight percentage in birds fed DDGS plus E. However, Lumpkins *et al.* (2004) found that feeding 0, 6, 12, or 18% DDGS to broiler chicks had no effect on carcass yield.

Plasma parameters: The data present in Table 4 indicating that the differences between the values of plasma antioxidants capacity recorded by different treatments were significant ($p \leq 0.0001$). The birds fed DDGS diet alone recorded lower value by 56.11% compared to control birds. The decrease in antioxidants capacity by DDGS may be due to Maillard reactions and its bad effect on enzyme activity. Finot (1990) showed that Maillard reactions inhibit several enzymes and has adverse effects on mineral metabolism (Ca, Mg, Cu and Zn). It is known that Cu and Zn have a role in free radical protection so, it is suggested that Maillard reactions may increase the free radical in intestinal tissues and increase damage in the cells especially it is known that oxidative damage is the major feature of intestinal inflammation in birds (Allen, 1997).

Another explanation for the decrease in plasma antioxidants capacity in broilers fed DDGS was suggested by Corzo *et al.* (2009) who found that Thiobarbituric Acid Reactive Substances, (TBARS) testing thigh meat of broilers fed 8% DDGS may be slightly more susceptible to oxidation than thigh meat of broilers fed the control diet. Also, Lancaster *et al.* (2007), suggested that greater inclusion rate of DDGS may adversely affect consumer acceptance of beef, which may be attributed to the level of Polyunsaturated Fatty Acids (PUFA). Increasing the PUFA level of meat increases the potential for oxidation, reducing shelf life and retail-display appeal to consumers especially, when plasma antioxidants decreased. The value of plasma antioxidants capacity recorded by birds fed DDGS not only lower than the control diet but also lower than the value reported by Maurice and Lightsey (2007) who found with adult leghorn that plasma total antioxidant capacity for female was 0.383 m mol ,while for male was 0.468 m mol. It was surprise that addition of RRE

Table 4: Effect of dietary treatments on some plasma parameters.

Item	Control	DDGS	DDGS+E	DDGS +RRE	DDGS +E+RRE
Total antioxidants capacity mmol/L	0.556^{c}±0.027	0.234^{d}±0.055	0.399^{cd}±0.023	1.448^{a}±0.106	0.901^{b}±0.09
Total Cholesterol mg/dl	117.31±10.40	148.67 ±6.09	130.09±5.96	105.03±9.87	93.77±10.20
Total Lipids mg/dl	952.92±48.96	828.62±57.46	1114.88±80.8	1032.02±34.87	1062.15±69.73
Phosphorus mg/dl	18.00^{b}±0.91	16.88^{b}±0.22	18.02^{b}±0.13	28.01^{a}±0.15	18.65^{b}±0.88
Creatinine mg/dl	0.85±0.10	0.85±0.05	0.80±0.01	1.02±0.11	0.95±0.13
Uric acid mg/dl	12.61^{a}±0.39	5.78^{b}±0.85	6.75^{b}±0.23	6.87^{b}±1.36	9.09^{b}±0.81
specific immunity against AIDV	4.00^{ab}±0.57	5.66^{a}±0.66	3.33^{bc} ±0.33	1.66^{c}±0.88	4.00^{ab}±0.57

a-d Means in the same row with different letters, differ significantly ($p \leq 0.05$).

significantly increased plasma antioxidants capacity by 515% compared to birds fed DDGS alone.

These results can be explained on the basis that peroxidase may degrade the higher Maillard molecules into low molecules weight which are exhibited as antioxidants. It appears that especially low molecular weight Maillard reaction products exhibit antioxidant effects in organisms after they get absorbed in the small intestine (Chandra *et al*., 2008). The beneficial effect of promised feed additive RRE on plasma antioxidants capacity in this study leads us to increase the level of DDGS in diets in the future and reevaluate it using RRE as an additive. It is known that peroxidase play a role in removing the toxic hydrogen peroxide which is a product of cell metabolism. So, we believe that RRE is a smart additive, taking hydrogen peroxide from intestine tissue and use it to detoxify toxic compounds. These results agree with those obtained by Sipos *et al*. (2002) who demonstrated that granule radish root extract protected cell membrane against lipid peroxidation in rats fed fat-rich diet. The addition of E to RRE decreased values of plasma antioxidants compared to the birds fed RRE alone, this confirmed with previously mentioned that E may be not compatible with DDGS. The birds fed DDGS recorded higher plasma cholesterol than other treatments while addition of RRE alone or with E numerically decreased it compared with those fed DDGS diet alone. The radish contains large amounts of glucosinolates (Reddy and Hayes, 1994) which resulted in enlargement of thyroid (hyperthyroidism), liver and kidneys (Van Etten and Tookey, 1983).

The RRE may play a role in metabolism of lipids and caused the reduction of its level in plasma. However, the pure components of essential oils inhibit hepatic 3-hydroxy-3-methylglutaryl coenzyme A (HMG-C0A) reductase activity (Crowell, 1999) which is a key regulatory enzyme in cholesterol synthesis. These results agree with those obtained by Abaza *et al*. (2004) and Ali *et al*. (2006a). There were insignificant differences between experimental treatments in total lipids.

The data in Table 4 indicated that there were significant differences ($p \leq 0.0001$) between plasma phosphorus values recorded by different treatments. The DDGS decreased the level of plasma phosphorus by 6.22% compared to the control diet without significant differences. The bad effect of DDGS on plasma phosphorus can be explained on the base that Maillard reactions inhibit many enzyme and pass way metabolism resulted in lower phosphorus metabolism. These result disagree with those obtained by Martinez-Amezcua *et al*. (2004) who noted a substantial variability in phosphorus bioavailability among nine samples, ranging from 69-102% relative to KH_2PO_4 and reported that increasing heat processing of DDGS may increase the bioavailability of phosphorus in DDGS. These result agree with those obtained by Leytem *et al*. (2008) who found that the apparent retention of both nitrogen and phosphorus decreased linearly with increasing DDGS inclusion (0-20%) in the diets. They found also, that at the greatest DDGS inclusion rate (20%), there was a corresponding decrease in nitrogen digestibility of 19% and phosphorus digestibility of 23% compared with the control diet (0% inclusion).

The addition of E alone numerically raise the plasma phosphorus compared to birds fed DDGS alone. It was surprise that RRE promised feed additive increased the level of plasma phosphorus by 65.93% compared to birds fed DDGS alone. Since the peroxidase is a ligninolytic enzyme (Monties, 1994), The peroxidase may increases the degradation of fiber matrix in whole diet and not DDGS alone and then increases liberation of phosphorus from fiber matrix. In this respect, Ismail-Beigi *et al*. (1977) showed that the fiber sources itselfes may reduce trace mineral availability by binding the mineral to the fiber matrix. On the other hand, it is known that Maillard reactions inhibit several enzyme and RRE as source of peroxidase affect this Maillard reaction and its bad effect on weight gain (Table 2) and plasma antioxidants capacity (Table 4) and consequently increase absorption and availability of phosphorus. Perhaps RRE may solve the problem of increasing excreta phosphorus content when chicks fed diet containing DDGS. In this connection, Leytem *et al*. (2008) stated that the water soluble phosphorus content of the excreta increased with increasing DDGS inclusion rates as well as the proportion of the total excreta phosphorus that was in soluble form, which indicates that this excreta would have a greater risk of off-site phosphorus losses once applied to land.

These results agree with those obtained by (Ali *et al*., 2006a) who found that the addition of enzyme

preparation and RRE to laying hen diet contained 50% wheat bran significantly increased phosphorus level in plasma by 64.32% compared to hens fed wheat bran diet alone while addition of enzyme preparation alone did not change the level of phosphorus in plasma. However, Viveros *et al*. (2002) found that Phytase supplementation to a low-nonphytate phosphorus diets increased plasma P level by 8%. Further studies are needed to compare between RRE and phytase in liberating the phosphorus in broiler diets. The addition of RRE+E did not increase plasma phosphorus as RRE alone which can be explained on the basis that E is not combatable with DDGS. The data in (Table 4) indicated that differences between values of plasma creatinine for different experimental treatments were insignificant. These results demonstrate that either DDGS diet or tested additives did not affect the kidney function under the conditions of this study.

Results in (Table 4) reveal that specific immunity against AIDV of broilers were significant ($p \leq 0.01$). The birds fed DDGS diet alone rerecorded the highest value while the bird fed DDGS plus RRE recorded the lowest one. Friedman *et al*. (1998) indicated that humoral immune responses are directly affected by vitamin E as an antioxidant and that excessive vitamin E intake has a detrimental effect on antibody production in chickens and turkeys. The birds fed DDGS diet plus RRE have the highest plasma antioxidants capacity (Table 4) and this may be decrease the humoral immune. Possibly, the addition of natural antioxidants in the diet may decrease the immune cost as a result of decreasing microorganism hosted. Sandberg *et al*. (2007) showed that the more virulent pathogen may have stimulating a greater immune response, rather than causing more damage to the host and through a larger resource requirement of the greater immune responses had greater reductions in growth. In this respect, Ali *et al*. (2008b) found with rabbit that addition of citric acid + *Curcuma longa* decreased both the harmful microorganisms in the caecum and values of plasma globulin. They indicated that this additive saved protein needed for immune cost which is directed towards growth. On the other hand, the addition of herbs is not necessary to increase the humoral immunity. Jafari *et al*. (2008) suggested that diet supplementation with garlic powder can not stimulate the humoral response of chickens against AIDV.

There were significant ($p \leq 0.01$) differences between experimental treatments in plasma uric acid. The control birds recorded the highest value while the birds fed DDGS recorded the lowest value.

However, Plasma uric acid is considered as the principal antioxidants in plasma based on mass and activity (Wayner *et al*., 1987). It is known that birds generally have several-fold higher levels of plasma uric acid than mammals, including humans (De Boeck and Stockx, 1978). This high level increases the likelihood that plasma uric acid will act as an antioxidant *in vivo* to reduce the effects of the products of oxidation, including free radicals. The lower values of plasma uric acid in birds fed DDGS alone or with additives may due to its bad effect on plasma antioxidants capacity (Table 4).

Conclusion: DDGS decreased BW in the finisher period (28 and 42 day), plasma antioxidants capacity and phosphorus while increase specific immunity against AIDV compared to control birds. Enzyme perpetration seemed to be not suitable for DDGS diet. Addition of RRE to DDGS diet increased BW (28 and 42 days), plasma antioxidants capacity and phosphorus while decreased specific immunity against AIDV compared to control birds. It was concluded that RRE as a source of peroxidase enzyme is a suitable additive for improving the utilization of DDGS.

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