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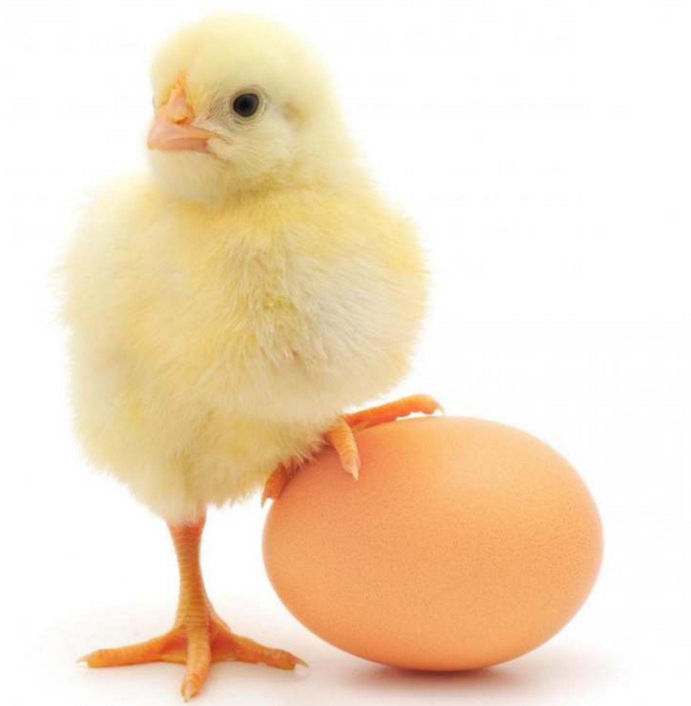

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Effect of Rovabio® Max AP on Performance, Energy and Nitrogen Digestibility of Diets High in Distillers Dried Grains with Solubles (DDGS) in Broilers[1,2]

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Abstract: This experiment was conducted to evaluate the effect of a commercial carbohydrase preparation (Rovabio® Max AP) on protein and energy utilization of diets with 0 or 30% DDGS. One hundred and ninety two, 18-day old male broiler chicks of a commercial strain (Cobb 500) were randomly distributed among six treatments in a 2 x 3 factorial arrangement. Each treatment was replicated four times, with six chicks per replicate. Treatments included two basal diets containing 0 or 30% of DDGS; each supplemented with or without an enzyme preparation fed at the level recommended by the manufacturer (1X), two (2X) and four times (4X) the recommended level. After a five-day adaptation period, excreta samples were collected for determination of AME and N retention (NR). Body weight, feed intake, feed conversion, fecal gross energy (GE) and N, AME, AMEn, GE digestibility and NR were determined. The results showed that weight gain, feed intake, feed conversion ratio and mortality rate were not significantly affected by level of DDGS or enzyme inclusion in the diet, or their interactions. Excreta N and GE were significantly increased by inclusion of 30% of DDGS in the basal diet. While AME and AMEn values were not affected by the addition of high level of DDGS in the diet, GE digestibility and NR were significantly affected. Supplementation of either basal diet with different levels of enzyme had no significant effects on excreta N content or AME, GE digestibility, or NR values. Moreover, the interaction between different levels of DDGS and enzyme levels on performance or nutrient utilization parameters were not significant. These data indicate that the addition of the enzyme preparation used in this trial was not effective in improving nutrient utilization of corn-soybean meal diets with or without DDGS.

Key words: Broilers, DDGS, metabolizable energy, enzyme, nitrogen retention

INTRODUCTION

The increasing trend in ethanol production from cereal grains in the United States and other developed countries results in large amounts of byproducts of corn fermentation, known as Distillers Dried Grains with Solubles (DDGS). While most of the previous DDGS products were a mix of residue of different grain fermentation produced by the beverage industry, modern DDGS products in the United States are mainly produced from corn fermentation (Cromwell *et al.*, 1993; Spiehs *et al.*, 2002).

Since the DDGS contains much lower quantities of starch and high levels of fiber than the original grain, the usable energy content is much lower. Consequently, based on its high fiber and low energy content, it seems that DDGS is more suitable for use in ruminant rather than single-stomach animals. However, due to the governmental subsides to increase ethanol production, more DDGS is available for the animal feed industry. At the same time, the competition for corn among the poultry and ethanol industries has resulted in more demand for corn, raising the price and increasing the shortage to supply the global demand (Donohue and Cunningham, 2009). Therefore, using a higher level of DDGS in broiler diet formulation is more economically justifiable. However, variability in the chemical composition of DDGS obtained from different ethanol production plants (Cromwell *et al.*, 1993; Spiehs *et al.*, 2002) and also the performance-suppressing effect of using high level of DDGS in broiler diets (Wang *et al.*, 2007a, 2007b), hinders broad and unrestricted inclusion of DDGS in diet formulation.

During recent years, several studies have been undertaken to estimate the nutrient availability of DDGS and improve its nutritional value for poultry (Batal and Dale, 2003; Lumpkins and Batal, 2005; Kim *et al.*, 2008). It has been shown that in addition to the reduced bioavailability of certain amino acids such as lysine, high concentration of the Non-Starch Polysaccharides component (NSP) is another limiting factor in utilizing of high level of DDGS in broiler diet (Choct, 2006). It has been proposed that addition of exogenous enzyme preparations to break down complex polysaccharides to less complicated components will improve the energy

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and nutrient availability of DDGS. Any increase in the digestibility of DDGS by use of exogenous enzymes should enable the inclusion of higher levels of DDGS in monogastric diets. However, little work has been published to assess the effect of exogenous enzymes to improve nutritional value of diets with high levels of DDGS (Swiatkiewicz and Koreleski, 2006, 2007; Shalash *et al.*, 2009). The objective of this study was to evaluate the effect of using a carbohydrase preparation (Rovabio® Max AP, Addiseo, Alphareta GA) on energy digestion and nitrogen utilization of diets containing high levels of DDGS.

MATERIALS AND METHODS

Animal, housing and management: One hundred and ninety two 18-d old male broiler chicks of a commercial strain (Cobb 500, Cobb-Vantress Inc., Siloam Springs AR) were placed in wire floored grower batteries. The chicks had been fed a nutritionally complete diet to 18 day of age. During the experimental period of 18 to 23 d, light was constant and house temperature was maintained under control. Chicks were given free access to mash feed and water for *ad-libitum* consumption. Care and management of birds in this experiment followed recommended guidelines (FASS, 1999). All procedures used were approved by the University of Arkansas Institutional Animal Care and Use Committee.

Treatment and experimental design: Two basal diets were prepared. The first was based on corn-soybean with no DDGS and the second contained 30% of DDGS of known moisture and protein content. The diets were formulated to meet minimum nutrient requirements for broilers (NRC, 1994). The level of DDGS was selected based on previous experiments from our laboratory (Wang *et al.*, 2007a, 2007b) to be marginal in maintaining optimum growth and feed conversion, but sensitive enough to show any improvement in nutritional value due to addition of enzyme to the diets. A dietary metabolizable energy level was selected that required approximately 1% supplemental poultry oil in the control diet; a higher level of poultry oil was needed for the DDGS diet to allow it to be isocaloric to the control diet. Requirements for amino acids were adjusted to the energy content of the diet. All diets were fortified with complete vitamin and trace mineral mixes. Celite™ (Celite Corporation, Santa Barbara CA) was added at 2% to all diets as an indigestible marker for use in estimating digestibility of nutrients. Composition and calculated analysis of two basal diets are shown in Table 1.

The enzyme preparation used in this experiment was Rovabio® Max AP (Adisseo, Alphareta GA). This product as described by the supplier as containing a naturally compatible combination of NSP enzyme activities produced by the non-genetically modified fungus *Penicillum funiculosum* combined with a 6-phytase produced from *Schizosaccharomyces pombe*. The enzymes contained in the product are said to include xylanases, β-glucanases, pectinases, mannanases and phytase as well as an α-galactosidase. Recommended level of supplementation of this enzyme is 200 g/ton. The product was obtained directly from the producer and maintained in a cool location until used within one week of arrival.

Table 1: Composition (g/kg) and calculated analysis of basal diets with and without Distillers Dried Grains with Solubles (DDGS)

Ingredient	Control	30% DDGS
Yellow corn	644.35	414.18
Poultry oil	10.22	26.92
Soybean meal	287.26	203.19
DDGS	0.00	300.00
Limestone	5.25	11.59
Defluorinated phosphate	18.39	11.70
Sodium chloride	3.03	2.37
Sodium bicarbonate	0.86	0.00
MHA 84[1]	2.48	1.14
L-Threonine	0.33	0.04
L-Lysine HCl	1.83	2.87
Vitamin premix[2]	5.00	5.00
Mintrex P_Se[3]	1.00	1.00
Celite[4]	20.00	20.00
Total	1000.00	1000.00
ME kcal/kg	3020.00	3020.00
Crude protein	19.63	22.25
Calcium	0.90	0.90
Total P	0.68	0.70
Nonphytate P	0.45	0.45
Methionine	0.55	0.52
Cystine	0.32	0.38
Lysine	1.14	1.20
Tryptophan	0.23	0.22
Threonine	0.75	0.80
Isoleucine	0.78	0.86
Histidine	0.51	0.58
Valine	0.88	1.04
Leucine	1.64	2.11
Arginine	1.25	1.25

[1]MHA-84 is a supplemental methionine product and contains 84% methionine activity (Novus International, Inc., St. Louis MO 63141).

[2]Provides per kg of diet: vitamin A (from vitamin A acetate) 7715 IU; cholecalciferol 5511 IU; vitamin E (from dl-alpha-tocopheryl acetate) 16.53 IU; vitamin B12 0.013 mg; riboflavin 6.6 mg; niacin 39 mg; pantothenic acid 10 mg; menadione (from menadione dimethylpyrimidinol) 1.5 mg; folic acid 0.9 mg; choline 1000 mg; thiamin (from thiamin mononitrate) 1.54 mg; pyridoxine (from pyridoxine HCl) 2.76 mg; d-biotin 0.066 mg; ethoxyquin 125 mg.

[3]Provides per kg of diet: Mn (as manganese methionine hydroxy analogue complex) 40 mg; Zn (as zinc methionine hydroxy analogue complex) 40 mg; Cu (as copper methionine hydroxy analogue complex) 20 mg; Se (as selenium yeast) 0.3 mg. Novus International, Inc., St. Louis MO 63141.

[4]Celite Corporation, Santa Barbara CA

Aliquots of the two basal diets were supplemented with different levels of enzyme. The levels of enzyme were 200 g/ton as suggested by the supplier (1X), two times (2X) and four times (4X) recommended level. The enzymes were carefully blended with a portion of the diet before being combined with the entire feed mix. In total, eight treatments including two basal diets unsupplemented with enzyme were prepared and each treatment was assigned to four pens of six male chicks stratified across tiers in the battery. All the experimental diets were fed as mash to avoid any possible heat damage from pelleting.

Measurements and laboratory analysis: Birds were weighed on the first day of the experiment (18d) and at end (23 d) on pen basis. Feed intake was also recorded at the same time points for determination of feed conversion ratio. Feed conversion ratio (FCR) was calculated as feed intake/ weight gain. Mortality was also recorded daily and feed conversion data corrected for body weight of mortality. Average body weight, weight gain, feed intake, feed conversion ratio were determined. At the end of five days of acclimation of the birds to experimental diets, excreta samples were collected on aluminum foil in the dropping pans, as free as possible from feathers or spilled feed. The excreta samples were freeze-dried until consistent weight was obtained. The gross energy and N contents of feed and excreta samples were analyzed using a Parr Adiabatic Bomb Calorimeter (Parr Instrument Company, Moline IL) and CHN-Analyzer (Na-2000 N-Protein, Fissons Instruments S.P.A., Strade Rivoltana, 20090 Rodano, Milan Italy), respectively. The acid insoluble ash contents of the feed and dried excreta samples were measured as described by Vogtmann *et al.* (1975).

Calculations: After chemical analysis of feed and excreta samples, the AME, AMEn and N retention in each diet were calculated as described by Vogtmann *et al.* (1975), Scott and Hall (1998) and Lammers *et al.* (2008) as follows:

$$AME = GE_{Diet} - \left[\frac{(GE_{Excreta} \text{ x } AIA_{Diet})}{AIA_{Excreta}}\right]$$

$$AME_n = GE_{Diet} - \left[\frac{(GE_{Excreta} \text{ x } AIA_{Diet})}{AIA_{Excreta}}\right] - 8.22 \text{ x } N_{Retained}$$

$$GE_{Digestibility} = \left(\frac{AME}{GE}\right) \text{x } 100$$

$$N_{Retained} = N_{Diet} - \left(\frac{N_{Excreta} \text{ x } AIA_{Diet}}{AIA_{Excreta}}\right)$$

$$N_{Retained} = 100 - \left[100 \text{ x } \left(\frac{AIA_{Diet}}{AIA_{Excreta}}\right) \text{x} \left(\frac{N_{Excreta}}{N_{Diet}}\right)\right]$$

Where AME (kcal/kg) = Apparent metabolizable energy; AMEn (Kcal/kg) = N-corrected apparent metabolizable energy content of the diet; GE_{Diet} and $GE_{Excreta}$ (kcal/kg) = GE of the diet and excreta, respectively; AIA_{Diet} and $AIA_{Excreta}$ (%) = Acid insoluble ash in the diet and excreta, respectively; N_{Diet} and $N_{Excreta}$ (%) = N contents of the diet and excreta, respectively; 8.22 (kcal/kg) = energy value of uric acid; $GE_{Digestibility}$ (%) = Gross energy digestibility, $N_{Retained}$ (g/kg) is the N retained by the birds per kg of diet consumed and NR (%) = Nitrogen retention.

Statistical analysis: Pen means served as the experimental unit for statistical analysis. Data were analyzed using the General Linear Models (GLM) procedure of SAS (SAS Institute, 1991) as a Completely Randomized Design, with 2 x 4 factorial arrangement. Analysis considered the main effects of DDGS inclusion and enzyme levels and the interaction of these two main effects. Mortality data were subjected to square root transformation prior to analysis; data are presented as natural numbers. Significant differences among treatments were determined at p<0.05 using Duncan's new multiple range tests.

RESULTS

The results of the present experiment on the effect of supplementation of a commercial carbohydrase preparation (Rovabio® Max AP) on performance, energy and nitrogen digestibility of corn or high DDGS basal diet are summarized in Table 2 and 3.

Performance: The results indicated that addition of the recommended level of Rovabio® Max AP (200 g/ton) or higher levels than recommendation level (two or four times) had no significant effect on broiler body weight and weight gain (Table 2). The feed intake, feed conversion ratio and mortality rate of the broilers were not significantly affected by supplementation of the diets with the different level of enzyme inclusion in the diet during the acclimation period.

The results also showed that the weight gain during adaptation period of five day was not significantly affected by inclusion of 30 % DDGS in the diet compared to the chicks fed the corn- soybean basal diet without DDGS. The results indicated the inclusion of high level of DDGS in the diet also had no significant negative effects on feed intake or feed conversion ratio during the 18 to 23d period of acclimation. In addition mortality rate also was not significantly influenced by feeding a diet contains 30% DDGS. As shown in Table 2, performance parameters such as body weight, weight gain, feed intake, feed conversion or mortality rate were not

Table 2: Effects of different levels of Rovabio® Max AP and DDGS level on live performance and mortality rate of male broiler chicks from 18 to 23 day of age

		Body weight 18d (g)		Body weight 23d (g)		Weight gain 18 to 23d (g)		FCR ($g.g^{-1}$)		Mortality* (%)	
Enzyme level**											
0		617		1038		421		1.694		2.08	
1x		626		997		371		1.729		2.08	
2x		619		1028		409		1.690		0.00	
4x		615		1010		395		1.699		0.00	
DDGS %											
0		618		1023		405		1.704		0.00	
30		620		1014		394		1.702		2.08	
DDGS %	**Enzyme**										
0	0	618		1050		432		1.683		0.00	
0	1x	621		1005		384		1.700		0.00	
0	2x	620		1024		404		1.730		0.00	
0	4x	615		1013		398		1.703		0.00	
30	0	615		1024		409		1.705		4.17	
30	1x	631		990		359		1.758		4.17	
30	2x	619		1033		414		1.650		0.00	
30	4x	616		1007		391		1.695		0.00	
		P > F	SEM	P > F	SEM	P > F	SEM	P > F	SEM	P > F	SEM
Enzyme		0.36	4.57	0.17	13.60	0.06	13.06	0.75	0.03	0.58	1.47
DDGS		0.65	3.23	0.54	9.62	0.42	9.24	0.94	0.02	0.17	1.04
DDGS x Enzyme		0.77	6.47	0.86	19.23	0.78	18.47	0.37	0.04	0.58	2.08
CV		2.09		3.77		9.26		4.67		1.99	

*Mortality data were subjected to square root transformation prior to analysis; data are presented as natural numbers.

**X = Recommended level of Rovabio® Max AP enzyme, 200 g/ton.

[ab]Means in column and under each main effects with common superscript do not differ significantly (p<0.05)

Table 3: Effects of different level of Rovabio® Max AP and DDGS level on nitrogen and energy utilization of male broiler chicks from 18 to 23 day of age

		N of excreta (%)		CP of excreta (%)		N retention (%)		GE excreta (kcal/kg)		AME (kcal/kg)		AMEn (kcal/kg)		GE digestibility (%)	
Enzyme level*															
0		3.47		21.67		69.34		3658.25		2952.79		2780.11		75.05	
1x		3.43		21.44		68.76		3696.88		2918.49		2747.40		74.19	
2x		3.47		21.66		68.31		3726.88		2904.48		2734.45		73.82	
4x		3.23		20.18		72.42		3701.13		2971.10		2790.72		75.55	
DDGS level (%)															
0		3.21[b]		20.07[b]		74.56[a]		3558.75[b]		2963.41		2786.89		78.52[a]	
30		3.59[a]		22.41[a]		64.85[b]		3832.81[a]		2910.02		2739.45		70.79[b]	
DDGS x Enzyme															
0	0	3.39		21.16		73.83		3606.75[bc]		2971.28		2796.50		78.73	
0	1x	3.14		19.63		74.31		3550.75[c]		2942.15		2766.24		77.96	
0	2x	3.20		20.02		73.24		3552.00[c]		2920.44		2747.05		77.38	
0	4x	3.12		19.47		76.86		3525.50[c]		3019.76		2837.80		80.02	
30	0	3.55		22.19		64.85		3709.75[b]		2934.30		2763.72		71.38	
30	1x	3.72		23.25		63.20		3843.00[a]		2894.82		2728.57		70.42	
30	2x	3.73		23.30		63.37		3901.75[a]		2888.53		2721.86		70.27	
30	4x	3.34		20.89		67.97		3876.75[a]		2922.44		2743.65		71.09	
		P > F	SEM	P > F	SEM	P > F	SEM	P > F	SEM	P > F	SEM	P > F	SEM	P > F	SEM
Enzyme		0.2118	0.09	0.2118	0.56	0.1107	1.24	0.4687	30.30	0.2812	26.33	0.3211	23.98	0.2457	0.65
DDGS		0.0003	0.06	0.0003	0.40	0.0001	0.88	0.0001	21.43	0.0538	18.62	0.0595	16.96	0.0001	0.46
DDGS x Enzyme		0.2814	0.13	0.2814	0.79	0.9149	1.76	0.0244	42.85	0.8077	37.23	0.7313	33.91	0.7588	0.92
CV		7.46		7.46		5.05		2.32		2.54		2.46		2.46	

*X = Recommended level of Rovabio® Max AP enzyme, 200 g/ton.

[ab]Means in a column and under each main effects with common superscript do not differ significantly (p<0.05)

significantly affected by the interaction between different levels of carbohydrase preparation and DDGS in the experimental diets. It should be noted, however, that the short period of feeding between 18 and 23 d of age may not be sufficient for any nutritional effect to be observed.

Nitrogen and energy digestibility: The results of this experiment showed that supplementation of diets containing different levels of the carbohydrase preparation had no significant effects on excreta nitrogen or protein content or on nitrogen retention. The results also demonstrated that the enzyme preparation at different levels had no significant effects on excreta gross energy, dietary AME, AMEn or GE digestibility during experimental periods.

As shown in Table 3, the results demonstrated that inclusion of high level of DDGS in the diet significantly increased excreta nitrogen and protein content. However, nitrogen retention during experimental period was significantly decreased by inclusion of 30% DDGS in the diet. The results clearly showed that excreta GE content was significantly higher in treatments fed the high level of DDGS, but dietary AME or AMEn contents were not affected by inclusion of DDGS in the experimental diets. This confirms that the metabolizable energy value assigned to the DDGS in formulation of the diets was accurate. The results showed that dietary GE digestibility was significantly decreased in birds fed diet with high level of DDGS compared to the control.

The interactions between different levels of enzyme and DDGS inclusion rate in the diet on excreta nitrogen and protein contents or dietary nitrogen retention, AME, AMEn and GE digestibility were not significant. However, GE content of excreta was significantly affected by the interaction of enzyme level and dietary DDGS content. The results indicated that while addition of enzyme had no effect on GE content of excreta of birds fed corn based diet; the excreta GE of birds fed diets high in DDGS were significantly increased by enzyme supplementation, regardless of enzyme level.

DISCUSSION

Lack of significant effects of different levels of Rovabio® Max AP on broiler chick performance and nutrient utilization in this experiment could be partially explained by the age of the birds used in this experiment. It has been shown that exogenous enzyme effects on broiler chick performance and nutrient utilization are generally more pronounced during the earlier part of the rearing period and substantially decreased or ineffective during grower or finisher periods (Scott *et al*., 2001; Olukosi *et al*., 2007).

However, a more plausible explanation for the ineffectiveness of enzyme in the present study may be related to the lack of appropriate substrate, soluble NSP, in the basal diets. Generally, corn has been considered as a high quality cereal and unlike wheat or barley, fewer successful works have been reported on the positive effects of carbohydrase preparations to improve digestibility or nutrient availability of corn based diets (Olukosi *et al*., 2007; Juanpere *et al*., 2005; Cowieson *et al*., 2006). These trials were primarily conducted during starter period and with marginally deficient diets. It has been shown that soluble mixed-linked non Starch Polysaccharides (NSP) components interfere in the absorption process of nutrients such as fat, protein and carbohydrates, increase viscosity of the intestinal digesta, enhance pathogenic bacteria proliferation and disturb the normal development of intestinal cell wall. Consequently, addition of proper exogenous carbohydrase to a diet high in NSP concentration to overcome their natural NSPase deficiency may improve nutrient digestibility and performance characteristics (Choct, 2006). It has been shown that the main beneficial effects of earlier generations of NSPase preparations are via partial, but not full depolymerization of soluble complex molecules of non starch polysaccharides and decreasing GIT digesta viscosity and to a lesser extent due to their effects on insoluble carbohydrate components of plant cell wall. Consequently, since the soluble NSP concentration of corn vs. other high NSP content diets is lower (Choct, 2006), insignificant effects of carbohydrase preparations in corn based diets in the present experiment are more likely to occur.

The failure of Rovabio® Max AP to improve nitrogen and energy digestibility of high DDGS contained diets is in agreement with our previous results (Min *et al*., 2009), in which no significant effects of two other commercial carbohydrase products were observed on the energy and N utilization of similar basal diets. It has been shown that during ethanol production, most of soluble and easy accessible carbohydrate undergoes fermentation by *Sacharomices cerevicea* and most of unfermentable nutrients residue are recovered in the final byproducts, dried distiller grains or DDG. Swiatkiewicz and Koreleski (2006) reported that maize DDGS contained 26.5% total NSP (3.05% soluble and 23.5 insoluble NSP).

During recent years, the potential of using enzymes to improve nutritional value of DDGS poultry diets has been investigated. Swiatkiewicz and Koreleski (2006, 2007) in studies with laying hens reported that inclusion of 5, 10 or 15 % of corn DDGS in the diet had no negative effects on nutrient digestibility, energy utilization or N, Ca and Zn balance; however, crude fat digestibility and metabolizable energy of the diet were decreased by a higher inclusion rate (20%) of DDGS in the diet. They reported that supplementation of the diet with an enzyme preparation containing xylanase and β-glucanase activity was effective in alleviating the negative effects of high level of DDGS on laying rate and daily egg mass and also increased nutrient utilization of the diets. Shalash *et al*. (2009) reported that inclusion of 12% corn DDGS in a broiler diet during starter and finisher periods decreased birds performance; supplementation of the diet with a commercial enzyme preparation containing xylanase, beta-glucanase, protease and amylase activity failed to improve bird's performance.

Lack of significant or consistent effects of carbohydrase preparation on diets containing DDGS may be related to the nature of fermentation process during ethanol production. During ethanol production, most of easy to access carbohydrate components are converted to ethanol and most of the carbohydrate products recovered after fermentation is in the form of insoluble NSP. Since the level of soluble NSP components in corn or DDGS of corn origin is lower than other cereals (wheat, barley or their DDGS) and the ability of the mature birds to overcome its negative influences is greater, the ineffectiveness of current carbohydrase products to improve performance or nutrient utilization of the diets based on corn or DDGS is understandable and indicates the need for more specific enzyme products targeting mainly insoluble non starch polysaccharide.

The interaction between DDGS and enzyme level on excreta GE contents in the present experiment is interesting. A possible explanation for this observation is that the enzyme preparation used in this experiment had some beneficial effects on releasing unavailable nutrients from the digesta, however, far from the absorption site in the gastrointestinal tract. More research is needed to clarify the actual reason for this observation.

Lack of significant effect of diets high in DDGS on performance during the 5 d adaptation period could be related to the length of adaptation period, which is probably not long enough to influence performance parameters. The other explanation for the lack of a significant impact of the high DDGS diet on performance might be related to the age of the birds (18 to 23 day). Data from previous work on the effects of high DDGS diets on broiler chick performance and the threshold of their tolerance to high level of DDGS in diet is inconclusive, depending on the quality of DDGS, the concentration of other nutrients in the diets and age of the birds (Cromwell *et al*., 1993; Wang *et al*., 2007a, 2007b; Lumpkins and Batal, 2005; Waldroup *et al*., 1981).

Waldroup *et al*. (1981) showed that broiler chicks can tolerate up to 25% of DDGS in the diet when the ME content of the diet was held constant, however, by decreasing of the energy level of the diet, the tolerance level was reduced to 15%. Cromwell *et al*. (1993) reported that variability in physical, chemical and nutritional value of different DDGS sources significantly influenced the weight gain, feed intake and feed conversion ratio of broiler chicks when used up to 20% of the diet. Lumpkins *et al*. (2004) reported that the effect of using a high DDGS diet is age related; using 18% DDGS in the diet during the starter period had negative effects on weight gain and feed efficiency, but performance was not negatively affected by using the same levels of DDGS during grower or finisher period. Wang *et al*. (2007a) showed that broiler chicks can tolerate up to 15% of DDGS in the diets formulated on digestible amino acids; however, inclusion of 30% of DDGS had negative effects on body weight, feed intake and breast meat yield. However, feeding diets with 30% DDGS alternating on a weekly basis with diets with no DDGS increased performance parameters to midway between control and 30% DDGS continuously up to 42 day or comparable to diets with 15% DDGS. Wang *et al*. (2007b) in another study demonstrated that feeding 30% of DDGS in the diet significantly depressed live performance and breast meat yield, but feeding diets with 15% DDGS had performance comparable to the control with no DDGS. In both experiments, increasing the level of the DDGS in the diet resulted in a decrease of the weight: volume ratio and pellet quality of the feed.

The age-related tolerance level of DDGS could be related to restriction in the gastrointestinal capacity of the broiler chicks during the starter period, which indicated that gut fill will remain as main obstacle in increasing in the level of DDGS from current recommendations.

The negative effects of inclusion of high level of DDGS in the diets on increasing excreta nitrogen and energy contents or decreasing dietary GE digestibility and nitrogen retention in this experiment is not surprising. Higher fiber and unavailable amino acid or generally the poor protein quality of DDGS compared to the original grain are the most important reasons for this observation. Widyaratne and Zijlstra (2007) in a comparative study on nutritional value of wheat and corn DDGS in pigs also reported higher N excretion in DDGS compared to the control treatment formulated using wheat, the original cereal. Leytem *et al*. (2008) reported that by increasing the inclusion rate of wheat-DDGS in broiler diets during the starter period from 0 to 25%, the apparent retention of N and P decreased linearly and at the same time the N and water soluble P content of excreta increased. Adeola and Ileleji (2009) also demonstrated that by increasing the corn DDGS level (0, 300 and 600 g/kg) in broiler diet from 14 to 21 day of age, dietary nitrogen retention decreased. They also demonstrated that energy retention, ME and MEn of the diet also linearly decreased from 78.6-58.6%, 3.615-2.753 kcal/g and 3.414-2.642 kcal/ kg, respectively, as DDGS increased from 0-600 g/kg. Applegate *et al*. (2009), in a study replacing soybean meal with high protein corn distillers dried grains in broiler diets, reported a substantial increase in excreta DM and nitrogen content.

One of the main reasons for higher nitrogen content of excreta of the group fed diets with 30% DDGS could be related to lower digestibility of amino acid and especially lysine in DDGS. During the ethanol production process, some of the amino acids, especially lysine, undergo the Maillard reaction because of binding of ε-amino group with reducing sugars. Consequently, reduced protein and amino acid digestibility and increased N excretion into the manure is predictable. Lumpkins and Batal

(2005) using cecectomized roosters estimated that the true digestibility of lysine in DDGS is about 75-80%. Batal and Dale (2006) reported that the average digestibility of DDGS amino acids were 81.7%, with the lowest digestibility for Lys, Cys and Thr, which indicates a negative effect of the drying process of DDGS on lysine bioavailability. Fastinger *et al.* (2006) in a study on five source of corn DDGS from several processing plants in the Midwestern United States, reported a high variability in amino acid digestibility and TME_n, in which darker colored DDGS sources had lower digestibility in amino acid and TME_n. In the Fastinger *et al.* (2006) study, the darker DDGS source contained nearly 600 kcal/kg less TME_n than lighter sources. Pahm *et al.* (2009) demonstrated that variability in digestible lysine (0.43-0.63%) in different sources of DDGS is greater than other amino acids. Their results indicated that a strong correlation existed between standard digestible lysine and reactive lysine, which is an indicator of higher digestibility of lysine when ε-NH_2 groups are not bound to sugars.

Lack of significant difference among the AME values between treatments, but lower GE digestibility in the diet with 30% DDGS indicates that the AME value we used for DDGS during formulation of the basal diet is consistent with the birds ability to extract metabolizable energy of diets high in DDGS (Waldroup *et al.*, 2007). The results of previous studies indicate that high variability exist between ME content of different DDGS samples. As an example, Batal and Dale (2006) in a study on eight representative samples of DDGS, reported a high variability among amino acid digestibility and TMEn values, with TMEn ranging from 2490-3190 kcal/kg. Consequently, this variability should be considered in practical diet formulation to avoid performance losses or inefficient utilization of dietary energy due to over or under estimation of ME value of DDGS samples.

The results of this study indicate that supplementation with up to four times the recommended level of the enzyme preparation used in this study was not effective in decreasing excreta N content or improving nitrogen retention, AME, AMEn or GE digestibility in corn-soybean meal diets or diets with 30% corn DDGS. The inclusion of 30% DDGS in the grower diet significantly increased excreta N and gross energy content and significantly decreased N retention and GE digestibility of the diet without affecting AME or AMEn values. The potential of exogenous enzymes to improve nutrient digestibility of corn or DDGS contained diets is enormous, however, more specific enzyme preparations are needed targeting insoluble NSP content of the diet. The potential environmental concerns of using high levels of DDGS in increasing excreta N and other nutrients should be considered in recommending the inclusion of high level of DDGS in poultry diets.

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