Effect of enzyme supplementation and acidification of diets on nutrient digestibility and growth performance of broiler chicks[1]

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ABSTRACT Two experiments were conducted to examine the effects of α-galactosidase supplementation and acidification of diets on nutrient digestibility and growth performance of broiler chicks. In experiment 1, dietary treatments consisted of feeding a low-energy basal diet (2.74 Mcal of ME/kg) alone, the basal diet supplemented with 1,724 units of α-galactosidase per kg, the basal diet supplemented with 2% citric acid, or the basal diet supplemented with both. α-Galactosidase significantly increased feed intake, weight gain, AME_n of the diets, and retention of CP and neutral detergent fiber (NDF) ($P < 0.05$). Citric acid significantly increased the retention of DM, CP, and NDF, but decreased feed intake and weight gain. The greatest values for DM and NDF retention and for AME_n were obtained with the combination of α-galactosidase plus citric acid. In experiment 2, chicks were fed diets with 2 levels of energy (2.74 or 3.11 Mcal/kg), 2 levels of citric acid (0 or 1.5%), and 2 levels of α-galactosidase (0 or 1,724 units/kg) in a 2 × 2 × 2 factorial arrangement of treatments. α-Galactosidase significantly increased the reducing sugar concentration in the crop content, whereas citric acid decreased the pH and increased the reducing sugar concentration in the crop content. Citric acid decreased the gain to feed ratio in the absence but not in the presence of α-galactosidase. The data from these studies indicate that acidification of diet improves the efficacy of α-galactosidase.

Key words: α-galactosidase, citric acid, enzyme, broiler, digestibility

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INTRODUCTION

Corn and soybean meal are the most important energy and protein sources in poultry diets. Although the gross energy of soybean meal exceeds the gross energy of corn, its metabolizable energy is less than that of corn (NRC, 1994). This is partly because of the content of α-galactosides (raffinose and stachyose) that cannot be digested in the small intestine of monogastric animals due to the lack of endogenous enzymes with α-galactosidase activity (Gitzelmann and Auricchio, 1965). The accumulation of these oligosaccharides in the alimentary tract results in fluid retention and an increased flow rate of digesta, which negatively affects the digestion and absorption of nutrients (Wiggins, 1984). Supplementation of poultry diets with exogenous α-galactosidase is one approach to help animals hydrolyze these oligosaccharides (Kidd et al., 2001; Graham et al., 2002). Products of reactions with this enzyme include galactose, fructose, and glucose. Thus, measurement of total reducing sugars is a common method of assaying this enzyme (Miller, 1959).

Studies with chicks and pigs have indicated a positive response in growth performance from the addition of various organic acids to diets (Vogt et al., 1981; Falkowski and Aherne, 1984; Giesting and Easter, 1985; Patten and Waldroup, 1988; Skinner et al., 1991). The mechanism by which organic acids improve performance in broiler chicks has not been clearly determined. However, several studies have suggested that organic acids influence the concentration of bacteria in the ceca and small intestine (Vogt et al., 1981), that they are bactericidal for salmonellae in the crop (Thompson and Hinton, 1997), and that they reduce the incidence of salmonellae on the carcass (van der Wal, 1979; Hinton et al., 1985; Rouse et al., 1988; Izat et al., 1990a,b). In recent years, there has been an increase in the use of acidifiers as substitutes for antibiotic growth promoters because of the concern of the development of microbial resistance (Radcliffe, 2000; Martin and Williams, 2002).

The inclusion of organic acids in the diet is known to lower the pH of the diet and the digesta (Giesting and Easter, 1985). The optimal pH level of fungal

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α-galactosidase is approximately 4.5 (Ademark et al., 2001), which is lower than the pH level (approximately 6.0) typically found in corn-soybean meal-based broiler diets. Previous studies in our laboratory (Ao et al., 2008) showed that the in vitro activity of α-galactosidase was substantially reduced as the pH was increased above 5.0. These findings suggested that the addition of an organic acid (e.g., citric acid) to the diet may increase the activity of exogenous α-galactosidase. The objective of these studies was to investigate the effects of simultaneous application of citric acid and α-galactosidase on nutrient digestion and growth performance of broiler chicks fed corn-soybean meal-based diets.

MATERIALS AND METHODS

Birds and Housing

Two experiments were conducted using male chicks of a female broiler breeder line (Avian Division, Cobb-Vantress, Monticello, KY). Chicks were 1 d of age at the start of each experiment and were housed in mesh wire-floored pullet starter cages (61 cm × 51 cm × 36 cm) in an environmentally controlled room. Both experiments used a randomized complete block design, with blocks based on physical location of the cages within the room. Twenty-two hours of light were provided daily. Each cage had one feeder that was removable for weighing and 2 adjustable nipple drinkers. Feed and water were supplied ad libitum. The temperature in the room was 31°C for the first week and then lowered to 27°C for the remainder of the study. If a bird died within the first 4 d, it was replaced by a bird of similar weight from an extra cage of chicks receiving the same diet. Birds and feed were weighed at the start of the study and then weekly. All procedures were approved by the University Institutional Animal Care and Use Committee.

Materials

A commercial fungal source of α-galactosidase was supplied by Alltech Inc. (Nicholasville, KY). The measured activity of α-galactosidase was 1,724 units (GalU) per gram. Food-grade anhydrous citric acid was purchased from Roche Vitamins Inc. (Parsippany, NJ). Chromic oxide (C333-3) was purchased from Fisher Scientific Inc. (Pittsburgh, PA).

Experimental Basal Diets

Two basal diets were used in these studies. A low energy basal diet was formulated to contain approximately 88% of the energy level in the normal energy basal diet (2.74 vs. 3.11 Mcal/kg). The latter diet contained an energy level that was similar to that recommended for commercial production (Hubbard LLC, 2008). The level of other nutrients met or exceeded NRC (1994) recommendations. The ingredient and calculated composition of nutrients are presented in Table 1. Chromic oxide (Cr_2O_3) was included in both experimental diets at 0.25% as an internal marker for the measurement of nutrient retention.

Experimental Design and Dietary Treatments

Experiment 1. One hundred forty-four broiler chicks were housed in cages of 6 birds each. Six replicate cages (2 within each of 3 blocks) were assigned to 1 of 4 dietary treatments. Treatments consisted of feeding 1) the low-energy basal diet alone, 2) the basal diet supplemented with 2% citric acid, 3) the basal diet supplemented with 0.1% α-galactosidase (providing 1,724 GalU/kg of diet), or 4) the basal diet supplemented with 2% citric acid and 0.1% α-galactosidase. On d 14,

Table 1. Ingredient and nutrient composition of the normal and low energy basal diets

Item	Normal energy, %	Low energy, %
Ingredient		
Corn	53.55	52.00
Soybean meal (48% CP)	38.50	36.00
Alfalfa meal (17% CP)	—	8.40
Corn oil	4.00	—
Salt	0.45	0.44
Limestone	1.32	1.06
Dicalcium phosphate	1.75	1.68
DL-Methionine	0.18	0.17
Vitamin-mineral mix[1]	0.25	0.25
Calculated nutrient concentration		
AME_n, Mcal/kg	3.11	2.74
CP, %	22.87	22.97
Ca, %	1.00	1.00
Available P, %	0.45	0.45
TSAA, %	0.90	0.89
Lysine, %	1.30	1.28
Sodium, %	0.20	0.20

[1]Supplied per kilogram of diet: 11,025 IU of vitamin A; 3,528 IU of vitamin D_3; 33 IU of vitamin E; 0.91 mg of vitamin K; 2 mg of thiamin; 8 mg of riboflavin; 55 mg of niacin; 18 mg of Ca pantothenate; 5 mg of vitamin B_6; 0.221 mg of biotin; 1 mg of folic acid; 478 mg of choline; 28 µg of vitamin B_{12}; 75 mg of zinc; 40 mg of iron; 64 mg of manganese; 10 mg of copper; 2 mg of iodine; and 0.3 mg of selenium.

excreta samples were collected from each cage for 24 h using plastic laminated boards suspended below the cages.

Experiment 2. Five hundred seventy-six chicks were assigned to 8 dietary treatments using a randomized complete block design. Each experimental unit consisted of 12 birds evenly divided among 2 cages (1 cage on the top tier of the battery and 1 cage on the bottom tier directly below). Six replicates (1 per block) were randomly assigned to each of the treatments.

The normal and low energy basal diets shown in Table 1 were used in this study. The treatment structure consisted of a 2 × 2 × 2 factorial arrangement with 2 levels of energy (low or normal), 2 levels of citric acid (0 or 1.5%), and 2 levels of α-galactosidase (0 or 0.1%). α-Galactosidase and citric acid were added in place of equivalent amounts of corn in the basal diet. The experiment lasted 3 wk. On d 18, excreta samples were collected from each cage for 24 h. On d 22, 6 birds from each treatment (a total of 48 birds) were randomly selected and not given feed for 12 h. Then, the birds were fed for 20 min before the feeders were removed. Thirty minutes after the removal of the feeder, 3 birds from each treatment (a total of 24 birds) were killed by asphyxiation with argon gas followed by cervical dislocation. Digesta from the crop were collected. The digesta pH was determined immediately after collection. Another sample was obtained from 3 more birds per treatment 60 min after removal of the feeder. The crop digesta samples were kept on ice until subsequent storage at −20°C for the measurement of reducing sugars.

Laboratory Analyses

Samples of feed and oven-dried excreta were ground to a fine texture. Dry matter was analyzed according to the AOAC (1995) method. Gross energy (**GE**) was determined by measuring the heat of combustion in the samples using a Parr 1281 Oxygen Bomb Calorimeter (Parr Instrument Co., Moline, IL). Nitrogen content was assayed using the Dumas methodology (method 990.03; AOAC, 1995) in a Leco FP-2000 Automated Analyzer (Leco Corporation, St. Joseph, MI). The determination of neutral detergent fiber (**NDF**) was based on the modified assay from Van Soest (1967). Chromium content in the samples (from Cr_2O_3) was analyzed using a procedure established by Williams et al. (1962). The pH of the crop content was measured with the method described by Radcliffe et al. (1998). A procedure proposed by Miller (1959) was used to determine the reducing sugar content of the crop digesta.

Statistical Analysis

Data were subjected to ANOVA for a randomized complete block design (Snedecor and Cochran, 1989) using linear models of Statistix V.8 (2003; Analytical Software, Tallahassee, FL). Mean differences were determined using Fisher's least significant difference test. Significance was declared when probability was less than 5%.

RESULTS

Experiment 1

The effects of α-galactosidase and citric acid on growth performance and retention of nutrients are listed in Table 2. Body weight gain and feed intake were significantly increased by α-galactosidase during the overall 21-d period and were significantly decreased by citric acid supplementation during d 1 to 14 and d 1 to 21. In the first 2 wk, but not during the entire 21-d period, there was a significant enzyme × citric acid interaction on weight gain and gain to feed ratio. The depression of both variables caused by the use of citric acid was corrected by α-galactosidase.

α-Galactosidase significantly increased AME_n of the diets and the retention of CP and NDF. Citric acid increased the retention of DM, CP, and NDF. There was a significant interactive effect of α-galactosidase and citric acid on the AME_n of the diets and the digestibility of DM and NDF. Significant improvements of these 3 variables were obtained by the combination of the 2 dietary factors.

Experiment 2

The interactive means and statistical significance of dietary treatments on the pH and the reducing sugar concentration of the crop digesta are presented in Table 3. Significant main effects of citric acid and enzyme supplementation but not energy level were observed. Citric acid significantly decreased the pH of the crop content (6.2 vs. 5.4). The crop contents from chicks fed citric acid also contained more reducing sugars than did the crop contents from chicks fed no citric acid (12.5 vs. 8.6 g/kg). The crop contents from chicks fed α-galactosidase contained significantly more reducing sugars than did crop contents from chicks fed no α-galactosidase (13.1 vs. 8.0 g/kg). There were no significant interactive effects on crop pH or reducing sugar content.

Table 4 shows interactive means and the statistical significance of treatments for growth performance and retention variables. Significant main effects of energy level were observed. Compared with the normal energy diets, feeding the lower energy diets significantly decreased BW gain (712 vs. 823 g/bird), increased feed intake (1,112 vs. 1,090 g/bird), decreased gain to feed ratio (0.68 vs. 0.75), decreased AME_n (3.03 vs. 3.44 Mcal/kg), and decreased DM retention (67.3 vs. 72.1%). No significant main effects of enzyme or acid supplementation on growth performance variables, DM and CP retention, and AME_n were detected. A significant interactive effect of enzyme × citric acid on gain

Table 2. Effects of α-galactosidase (GAL) and citric acid (CA) on growth performance and nutrient retention of broiler chicks (experiment 1)[1]

Treatment		Weight gain, g/bird		Feed intake, g/bird		Gain:feed ratio, g:g		Retention, %			AME_n, Mcal/kg of DM
GAL, %	CA, %	d 1 to 14	d 1 to 21	d 1 to 14	d 1 to 21	d 1 to 14	d 1 to 21	DM	CP	NDF	
0	—	344	714	524	1,123	0.65	0.63	67.6	62.2	13.5	3.07
0.1	—	361	766	537	1,164	0.67	0.66	68.2	64.3	18.3	3.12
—	0	369	775	545	1,173	0.68	0.66	67.0	62.2	12.6	3.07
—	2	336	706	515	1,114	0.65	0.63	68.8	64.4	19.2	3.12
0	0	376^a	769	542	1,166	0.69^a	0.66	67.2^a	61.5	11.9^a	3.07^a
0	2	313^b	659	505	1,081	0.62^b	0.61	68.1^a	62.8	15.1^b	3.07^a
0.1	0	362^a	780	548	1,180	0.66^a	0.66	66.9^a	62.8	13.3^{ab}	3.07^a
0.1	2	359^a	753	526	1,147	0.68^a	0.66	69.6^b	65.9	23.3^c	3.17^b
SEM[2]		10	23	7.2	12	0.013	0.018	0.46	0.78	0.81	0.019
Source of variation		Significance of treatment effect									
GAL		NS	*	NS	**	NS	NS	NS	*	**	*
CA		**	**	**	**	NS	NS	**	*	**	NS
GAL × CA		**	NS	NS	NS	**	NS	*	NS	**	*

[a–c]Means within a column that lack a common superscript letter differ.

[1]Six replicate groups of 6 birds were assigned to each of 4 treatments.

[2]Standard error of the mean for interactive effects.

$^*P < 0.05$; $^{**}P < 0.01$.

to feed ratio was noted. Chicks fed citric acid had a lower gain to feed ratio (0.705 vs. 0.723) than did the chicks fed both α-galactosidase and citric acid.

DISCUSSION

Effects of α-Galactosidase on Growth Performance, AME_n, and Retention of DM, CP, and NDF

Experiment 1 showed an increase of BW gain and feed intake by supplementation of α-galactosidase. This result was not repeated in experiment 2. One possible reason for this is that a greater concentration of citric acid was used in experiment 1 (2%) than in experiment 2 (1.5%). Weight gain and feed intake in experiment 1, but not in experiment 2, were either significantly or numerically decreased by adding only citric acid to the diet, but not by adding citric acid and α-galactosidase together. Dietary α-galactosidase increased NDF retention and reducing sugar concentration in crop contents, suggesting that hydrolysis of α-galactosides by α-galactosidase occurred. Improved retention of CP and AME_n by α-galactosidase was observed in experiment 1, but not in experiment 2. These results are consistent with findings by other investigators. Swift

Table 3. Effects of dietary energy level (EL), α-galactosidase (GAL), and citric acid (CA) on pH and reducing sugar concentration in crop content (experiment 2)[1]

	Factor				
Diet	EL	GAL	CA	pH	Reducing sugar,[2] g/kg
1	Low	−	−	6.2	6.4
2	Low	−	+	5.6	9.1
3	Low	+	−	6.2	10.6
4	Low	+	+	5.4	15.1
5	Normal	−	−	6.2	5.9
6	Normal	−	+	5.5	10.5
7	Normal	+	−	6.2	11.3
8	Normal	+	+	5.2	15.4
SEM				0.11	1.47
Source of variation				Significance of treatment effect	
EL				NS	NS
GAL				NS	**
CA				**	**
EL × GAL				NS	NS
EL × CA				NS	NS
GAL × CA				NS	NS
EL × GAL × CA				NS	NS

[1]Data presented are means of duplicates of 3 samples.

[2]Expressed as grams per kilogram of air-dried crop content.

$^{**}P < 0.01$.

et al. (1996) reported improved growth performance of broilers and improved digestibility of nitrogen and energy by dietary supplementation of an enzyme cocktail containing protease, amylase, and α-galactosidase activities of fungal origin. Zanella et al. (1999) showed that commercial carbohydrase products improved BW gain and feed conversion ratio in broilers fed a corn-soy diet as a result of increased ileal digestibility of protein and nonstarch polysaccharides. However, Marsman et al. (1997) found that only apparent ileal digestibility of CP and nonstarch polysaccharides, but not growth performance, of broilers was improved by treating soybean meal with protease and carbohydrase. Graham et al. (2002) also reported that the treatment of soybean meal with α-galactosidase reduced raffinose and stachyose levels by 65 and 50%, respectively, and increased TME from 2,974 to 3,328 kcal/kg. However, chick growth performance was not improved by enzyme treatment. Similar results were also reported by Kocher et al. (2002, 2003).

Effects of Citric Acid on Growth Performance, Digesta pH, AME$_n$, and Retention of DM, CP, and NDF

Either no effects or negative effects of dietary citric acid on chick growth performance were observed in these studies. These results are in agreement with the results reported by Cave (1984) and Brown and Southern (1985). However, Vogt et al. (1979, 1981) and Skinner et al. (1991) found a positive influence on chick growth performance by dietary inclusion of several organic acids at levels up to 2%. There are several possible reasons for the differing results among these trials. One is the level of acid used in the diets. Based on present trials, the negative effects of performance were caused by 2%, but not by 1.5%, of citric acid in the diet. Another possibility is variations in the specific acids used, which may have different impacts on the performance of the birds, especially feed intake. Citric acid, fumaric acid, propionic acid, and formic acid are commonly used acids in the poultry diets. Variations in feed ingredients and nutrient levels may also influence results. Based on the present results, the effect of acid on the performance of chicks is closely connected to the energy level of diet. The presence of microbial challenges in the various trials may also alter results. Because organic acids have antimicrobial activity, beneficial effects may have resulted from their use when these challenges were present.

Improved retention of DM, CP, and NDF by citric acid addition was observed in experiment 1. Few reports of similar results were found in the poultry literature. Giesting and Easter (1985) pointed out that the addition of organic acid to the diets of pigs increased gastric proteolysis and protein and amino acid digestibility.

Data from experiment 2 showed that the addition of 1.5% citric acid reduced the pH of the crop content by 0.8 units. This result supports the previous findings in this laboratory (Ao, 2005) and the findings reported by Hinton et al. (2000).

Interactive Effects of α-Galactosidase, Citric Acid, and Dietary Energy Level on Chick Growth Performance, Retention, and AME$_n$

Interactive effects of α-galactosidase and citric acid were found in both studies. In experiment 1, the depression of chick weight gain and gain to feed ratio in the

Table 4. Effects of dietary energy level (EL), α-galactosidase (GAL), and citric acid (CA) on growth performance and nutrient retention of broiler chicks (experiment 2)[1]

Treatment	Factor			Weight gain, g/bird	Feed intake, g/bird	Gain to feed ratio, g/g	Retention, %		AME$_n$, Mcal/kg of DM
	EL	GAL	CA				DM	CP	
1	Low	−	−	764	1,128	0.68	66.1	58.6	2.99
2	Low	−	+	750	1,113	0.67	67.0	60.7	3.00
3	Low	+	−	745	1,117	0.67	66.8	61.0	3.02
4	Low	+	+	748	1,091	0.69	69.3	61.7	3.10
5	Normal	−	−	827	1,080	0.77	72.2	64.1	3.45
6	Normal	−	+	800	1,086	0.74	72.0	63.2	3.44
7	Normal	+	−	833	1,099	0.76	72.9	64.2	3.47
8	Normal	+	+	832	1,094	0.76	71.4	62.1	3.41
SEM				14.4	13.9	0.008	1.23	2.41	0.057
Source of variation				Significance of treatment effects					
EL				**	*	**	**	NS	**
GAL				NS	NS	NS	NS	NS	NS
CA				NS	NS	NS	NS	NS	NS
EL × GAL				NS	NS	NS	NS	NS	NS
EL × CA				NS	NS	NS	NS	NS	NS
GAL × CA				NS	NS	*	NS	NS	NS
EL × GAL × CA				NS	NS	NS	NS	NS	NS

[1]Data presented are means from 6 groups of 12 birds.

*$P < 0.05$; **$P < 0.01$.

first 2 wk due to dietary inclusion of citric acid was corrected by the supplementation of α-galactosidase. Simultaneous supplementation of α-galactosidase and citric acid significantly increased the retention of DM and NDF, and the AME_n of the diets, compared with the use of the control diet or the control diet with either α-galactosidase or citric acid alone. In experiment 2, the chicks fed both α-galactosidase and citric acid had a greater gain to feed ratio than the chicks fed citric acid alone.

No interaction of dietary energy × α-galactosidase on AME_n of the diets was found in experiment 2. In contrast, Schang et al. (1997) observed that the addition of enzyme to a low nutrient density diet, but not to a high nutrient density diet, improved chick growth performance. Graham et al. (2002) reported that the TME of soybean meal was increased by 11.9% due to α-galactosidase treatment.

No significant interactive effect of citric acid by dietary energy level on the AME_n of the diets was noted in experiment 2 (Table 4). The results of Bolton and Dewar (1964) and Hume et al. (1993) suggested that many of the organic acids can serve as substrates in intermediary metabolism and can be used as energy sources.

Supplementation of α-galactosidase can benefit broiler chicks by improving the AME_n of the diets, which, in turn, may improve growth performance. Supplementation of the low energy diet with this enzyme improved AME_n by about 3% in experiment 1, but not experiment 2. Organic acids are widely used to inhibit pathogenic bacteria (e.g., *Salmonella* spp.) in poultry feeds, but they may negatively affect growth performance. However, these negative effects can be corrected by simultaneously adding α-galactosidase to the diets. The results from the present studies suggest that supplementing broiler diets with both α-galactosidase and an organic acid is more beneficial to nutrient retention and growth performance than using either α-galactosidase or organic acid alone.

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