Effects of Alpha-galactosidase Supplementation to Corn-soybean Meal Diets on Nutrient Utilization, Performance, Serum Indices and Organ Weight in Broilers

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ABSTRACT : Effects of alpha-galactosidase (GAL) on broiler corn-soybean meal diet was investigated. In experiment 1, sixty cockerels were allocated to five groups, including three enzyme treatments (GAL added at 0, 500, and 1,000 mg/kg diet), a nitrogen-free diet group and a fast group. The true nitrogen-corrected ME (TME_n) and true amino acid availability were determined. In experiment 2, 324 day-old chicks were used in a 2×3 factorial design consisting of two energy contents (high and low) and three GAL levels (0, 250, and 500 mg/kg). Three feeding phases, comprising 0-21 d, 22-35 d and 36-48 d, were involved. GAL addition improved TME_n and the availability of methionine and cystine ($p<0.05$). The apparent ME (AME) or nitrogen-corrected AME (AME_n) and digestibility of dry matter, organic matter, calcium, and phosphorus were improved significantly on d 21, so was crude protein and an interaction of energy and GAL on AME_n ($p<0.05$) was found on d 35. However, daily intake and daily gain were significantly improved with GAL addition ($p<0.05$) during 21 d. The small intestine relative weight decreased at 250 mg/kg GAL ($p<0.05$) on d 35, whereas presented an interaction between GAL and energy on d 21 ($p<0.05$). Likewise, this treatment increased breast muscle ratio ($p<0.05$). On d 21, triglycerides level of broilers showed interaction between energy and enzyme levels ($p<0.05$). Uric acid level in 500 mg/kg GAL declined linearly ($p<0.05$). On d 35, quadratic effects ($p<0.05$) were observed in total protein, albumin, globulin and cholesterol content for enzyme supplementation. And the interactive effects of energy and GAL on serum values showed more obviously. The study implies that GAL improved energy and nutrient availability of corn-soybean meal diet in broiler. The GAL supplementation to corn-soybean meal based diet can improve performance of broilers in early stages of growth. ***(Asian-Aust. J. Anim. Sci. 2005. Vol 18, No. 12 : 1761-1768)***

Key Words : Alpha-galactosidase, Broilers, Digestibility, Metabolizable Energy, Performance, Soybean Meal

INTRODUCTION

Rations based on corn and soybean meal (SBM) are commonly used in poultry for their high available energy and protein. However, some poly- and oligo-saccharides present in the feedstuffs are indigestible to monogastric animals (Iji and Tivey, 1998; Zhang et al., 2001; Park et al., 2003; Selle et al., 2003; Shim et al., 2004). Of these saccharides, the α-galactosidic oligosaccharides, such as raffinose, stachyose, verbascose and other α-galactosyl compounds, have received attention as potential anti-nutritional factors (Coon et al., 1990; Leske et al., 1995). Soybean meal contains 4.00-7.67% sucrose, 0.67-0.94% raffinose, 2.96-4.14% stachyose and trace amounts of verbascose (Kennedy et al., 1985). There is also about 0.3% raffinose and 1.5% sucrose in corn. Differences in their content and spectrum may be due to variety, cultivar, harvest time or climate (Kuo et al., 1988; Saini, 1989). Moreover, a few pectin and galactomannans substances containing α-galactosyl bonds also exist (Hartwig et al., 1997).

These compounds are heat stable and cannot be eliminated during processing. Poultry lack endogenous enzymes targeting α-1, 6-galactosyl bonds to digest them (Pluske and Linkemann, 1998). Especially, the α-galactosides have been implicated in reducing energy utilization, fiber digestion and feed retention in SBM-fed chicks (Coon et al., 1990), producing osmotic catharsis (Wagner et al., 1976) and flatus in man (Calloway et al., 1966) and animals (Leske et al., 1999). Hereby, substantial amounts of oligosaccharides present in diets may affect the nutrient digestibility and growth of broilers.

Various extraction methods and autolysis have been employed in the removal of the α-galactosides (Angel et al., 1988; Leske et al., 1993). However, these techniques are usually expensive and time consuming. Applying an exogenous enzyme preparation, mainly composed of α-1, 6-galactosidase, is an alternative to alleviate the detrimental effects of the saccharides, (Sugimoto and Van Buren, 1970; Pan et al., 2002). Therefore, this study was conducted to evaluate whether the α-galactosidase preparation improves the bioavailability of energy and nutrients of corn-soybean meal diets, and to investigate the interaction between dietary energy contents and the enzyme by broiler metabolic and performance tests.

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Table 1. Composition of experimental diet and nitrogen-free diet (Experiment 1)

Experimental diet		Nitrogen-free diet	
Ingredients	%	Ingredients	%
Corn (8.0% CP)	56.00	Cornstarch	45.50
Soybean meal (43.5% CP)	40.00	Sucrose	45.50
Dicalcium phosphate	2.70	Acetate cellulose	5.00
Sodium chloride	0.30	Dicalcium phosphate	2.70
Premix[1]	1.00	Sodium chloride	0.30
Alpha-galactosidase[2]	-/+	Premix[1]	1.00
Nutrient content[3]		Nutrient content[3]	
ME (kcal/g)	2.74	ME (kcal/g)	3.17
CP (%)	21.88	CP, %	-

[1] Provided the following per kilogram of diet: vitamin A (as retinyl acetate), 12,000 IU; vitamin D_3, 2,500 IU; vitamin E (as DL-α-tocopheryl acetate), 20 IU; vitamin K_3 (as menadione sodium bisulfite), 1.5 mg; D-pantothenic acid, 10 mg; Niacin, 20 mg; vitamin B_{12}, 0.02 mg; riboflavin, 5.5 mg; choline chloride, 500 mg; Mn, 75 mg; Zn, 75 mg; Cu, 9 mg; Fe, 80 mg; Se, 0.3 mg; I, 0. 5 mg.

[2] Alpha-galactosidase was added at 0, 500 or 1,000 mg/kg diet.

[3] Calculated values.

MATERIALS AND METHODS

Alpha-galactosidase preparation

The α-galactosidase (GAL) supplement was originally bio-synthesized by *Penicillium janczewskii* (Chinese General Microbiological Culture Central No. 0668) in solid-state fermentation. The primary active enzyme in this product was α-1, 6-galactosidase (E.C. 3.2.1.22). Enzyme activity added to the diets was analyzed. The final enzyme product, which provided 90.2 U/g of enzyme activity, was pilot-scale produced in ZhongnongBote Biotechnology Company (Beijing, China). One unit of α-galactosidase activity is the amount of enzyme that released one μmol of *p*-nitrophenol per minute from *p*-nitrophenyl-α-D-galacto-pyranoside within 10 min at 40°C and pH 5.5. Minor other hemicellulase and cellulase activities were also residual in the compounds.

Bird assays

Experiment 1 : Sixty intact Arbor Acres male broilers, averaging 2.2±0.1 kg, were used to determine ME and amino acid digestibility of corn-soybean meal added by GAL. The birds were housed in individual wire cages (50.3 cm×45.0 cm×37.2 cm), keeping the room temperature at 19-22°C. Before week of the test, all the birds were surgically sutured at the cloacae with a bottle to collect excreta. Thirty-six cockerels were assigned to three dietary treatments, with six replicate pens per treatment and two birds in each pen. Three dietary treatments comprised corn-soybean meal based diets (Table 1.) supplemented with GAL (0, 500, and 1,000 mg/kg diet). An additional 12 birds were fed nitrogen-free diet (Table 1.) while another 12 birds were fasted for a 48 h period in order to determine endogenous secretions of nitrogen and energy, respectively. Any feed was withdrawn from all birds for 48 h to ensure no diet residual in the gastrointestinal tract. These birds were precision-fed 40 g of tested diets or the nitrogen-free diet by gavage. The twelve fasted birds were starved continuously for another 48 h. Water was available *ad libitum* during this period.

Experiment 2 : A total of 324 day-old Avian male broilers, weighing 39.6±0.65 g, were wing-banded and assigned in completely randomized design into six treatments with six replicate pens of nine birds each. A 2×3 factorial design consisted of two ME content (high and low) and three supplemental levels of GAL (0, 250, and 500 mg/kg). GAL was mixed in the premix before complete formulation. The experiment was divided into three phases, with each phase having two energy levels (about 100-150 kcal/kg of discrepancy, Table 2). The diets, based on corn and soybean meal, were formulated to meet Feeding Standard of Chicken in China (ZB B 43005-86). All diets were fed as mash. Feed and water supplied *ad libitum*.

The birds were raised in three-tiered battery cages. Each of individual wired cages (61.2 cm×41.5 cm×35.3 cm) held three birds. The composite of the top, middle and bottom row of cages constituted one replicate pen. House was maintained at initial 33°C and gradually reduced to regular temperature (20°C). The vaccination program consisted of Marek's vaccine (day-old) and Newcastle and infectious bronchitis vaccine (at 14 and 24 d of age). The trial lasted for 48 d. Body weight was measured at d 1, 21, 35, and 48. Meanwhile, feed consumption was recorded as replicate units. Daily gain, daily feed intake and feed conversion were calculated.

Metabolic tests

In experiment 1, Excreta from each pen were collected for 48 h, and pooled to gain adequate sample size. The excreta were dried in a forced-draft oven at 65°C, equilibrated at ambient temperature for 24 h, and ground through 40-mesh sieve, were kept in sealed bag for analysis. The nitrogen-corrected metabolizable energy (ME_n), including the apparent ME_n (AME_n), true ME_n (TME_n), and true amino acid availability (TAAA) were calculated (Sibbald, 1979). In experiment 2, feed consumptions of the birds were recorded accurately between d 18 to 21 and d 33 to 35. Excreta from each pen were collected by placing a tray, fitted with nylon paper beneath the pen. They were weighed, homogenized and a 250 g sample dried for further analysis. The apparent ME (AME), AME_n, and apparent digestibility of DM, organic matter (OM), CP, calcium, and total phosphorous were calculated.

Chemical analysis

DM, OM, CP, calcium, and phosphorus in feed and

Table 2. Composition of the basal diets and nutrient levels (Experiment 2)[1]

Ingredients	Phase I (0-21 d)		Phase II (21-35 d)		Phase III (36-48 d)	
	H	L	H	L	H	L
	%					
Corn (7.9% CP)	53.00	55.00	56.00	57.50	60.00	59.50
Soybean meal (44.0% CP)	36.25	36.25	34.25	34.25	31.25	31.25
Soybean oil	4.00	2.00	4.70	3.00	4.80	3.80
Fish meal	2.00	2.00	1.20	1.00	0.00	0.00
Limestone	1.20	1.20	1.40	1.40	1.30	1.70
Dicalcium phosphate	2.00	2.00	0.90	1.30	1.25	2.35
Premix[2]	1.00	1.00	1.00	1.00	1.00	1.00
Sodium chloride	0.25	0.25	0.30	0.30	0.30	0.30
DL-methionine[3]	0.20	0.20	0.14	0.14	0.10	0.10
L-lysine-HCl[3]	0.10	0.25	0.11	0.11	0.00	0.00
Total	100.00	100.00	100.00	100.00	100.00	100.00
Nutrient content[4]						
ME (kcal/g)	2.93	2.82	3.00	2.88	3.05	2.90
CP (%)	21.23	21.09	19.97	19.90	18.12	18.04
Calcium (%)	1.15	1.17	0.89	0.93	0.83	0.86
Nonphytate phosphorus (%)	0.51	0.51	0.38	0.44	0.39	0.50
Lysine (%)	1.30	1.31	1.23	1.24	1.03	1.03
Methionine (%)	0.60	0.61	0.52	0.53	0.45	0.45
Methionine+cystine (%)	0.89	0.90	0.80	0.80	0.71	0.70

[1] H = high energy content. L = low energy content. the discrepancy is about 100-150 kcal/kg.

[2] Provided the following per kilogram of diet: vitamin A (as retinyl acetate), 12,000 IU; vitamin D_3, 2,500 IU; vitamin E (as DL-α-tocopheryl acetate). 20 IU; vitamin K_3 (as menadione sodium bisulfite). 1.5 mg; D-pantothenic acid. 10 mg; Niacin. 20 mg; vitamin B_{12}. 0.02 mg; riboflavin. 5.5 mg; choline chloride, 500 mg; Mn. 75 mg; Zn. 75 mg; Cu, 9 mg; Fe. 80 mg; Se. 0.3 mg; I. 0. 5mg.

[3] Supplied by Ajinomoto Corporation, Japan.

[4] Calculated except CP and calcium by chemical analysis.

excreta were analyzed according to AOAC (1995). Gross energy of diet and excreta were assayed in adiabatic oxygen bomb calorimeter (Automatic Energy Analyzer PARR 1281. Moline, IL.). Majority of AA were hydrolyzed by 6 mol/L hydrochloric acid at 110°C for 22 h. and measured with ion-exchange chromatography by an automatic amino acid analyzer (Shimadzu L-8800, Kyoto, Japan.), while methionine and cystine were treated with the mixture of 88% formic acid and 30% hydrogen peroxide (9:1 of volumetric ratio) prior to above-mentioned acid hydrolysis.

Bleeding and slaughter

On d 21 and 35. three chick was selected randomly from each replicate pen, blood taken by heart penetration with vacuum injector. and then euthanized by cervical dislocation. Broilers were eviscerated manually to take crop. gizzard. intestine. and immune organ such as thymus. spleen and Fabricius's bursa. The crop and gizzard were emptied and weighed. The small intestine including duodenum, jejunum and ileum was voided for determination of total weight. The immune organs were also weighted after drying by filter paper blotting. Relative organ weight was expressed as the ratio of the organ weight to the live body weight of the bird.

At the end of the feeding. six broilers per treatment were slaughtered by severing the jugular vein. Breast muscle, leg muscle and abdominal fat were dissected and weighted immediately. The proportion of the muscle or fat to the body weight of the bird was calculated.

Serum biochemical indices

Blood samples were centrifuged at 2,500 rpm for 15 min to separate serum. The samples were labeled and stored at -20°C until analysis. The serum indices. including total protein. albumin, globulin. triglycerides, uric acid. total cholesterol and glucose. were assayed by an automatic biochemical analyzer (TECHNICON RA-1000. Bayer Corporation. Diagnostics Division. NY.) with commercial reagent kits (Zhongsheng Beikong Bio-Technology and Science, Inc. Beijing. China.)

Statistical analyses

Data were analyzed by ANOVA and multivariate analysis of the General Linear Model Procedure of the SPSS system (SPSS Inc., 1998). For experiment 2. a factorial analysis was also conducted with the factors in the model being energy content. enzyme level. and their interactions. The differences among means were compared by Duncan's multiple-range test (Duncan, 1955).

RESULTS

ME and nutrient digestion

In experiment 1 (Table 3). the GAL addition to the corn-

Table 3. Nitrogen-corrected metabolizable energy and true amino acid availability of corn-soybean meal diet supplemented with α-galactosidase in broilers

Item	Alpha-galactosidase (mg/kg)			SEM[1]	p-value
	0	500	1,000		
AME_n (kcal/g)	2.73	2.77	2.81	0.040	0.717
TME_n (kcal/g)[c]	3.12[b]	3.27[a]	3.31[a]	0.023	0.017
Amino acid (%)					
Aspartic acid	86.7	86.9	85.6	1.698	0.848
Threonine	82.1	82.5	78.2	2.230	0.378
Serine	85.8	86.2	83.6	1.690	0.526
Glutamic acid	88.7	90.6	88.1	1.105	0.308
Proline	83.1	84.1	83.1	1.700	0.898
Alaine	78.6	79.7	79.2	2.483	0.955
Cystine[c]	81.7[b]	80.7[b]	90.2[a]	1.813	0.009
Valine	80.8	78.6	77.1	2.455	0.597
Methionine[c]	86.7[b]	90.2[a]	92.1[a]	1.068	0.017
Isoleucine	86.1	86.1	84.9	2.119	0.904
Leucine	89.2	89.3	88.0	1.456	0.812
Tyrosine	88.0	87.5	82.0	3.3371	0.413
Pheylalanine	90.6	90.4	87.7	1.477	0.365
Lysine	88.8	89.4	86.8	1.465	0.464
Histidine	79.7	81.7	77.2	2.190	0.392
Arginine	93.0	93.3	91.3	0.893	0.268
All amino acids	82.3	83.8	83.4	0.896	0.798

[a-b] Means within a row with no common superscript differ significantly ($p<0.05$). [c] Indicates a linear effect ($p<0.01$).
[1] Pooled standard error of the means.

Table 4. Effects of α-galactosidase supplementation on growth performance of broilers[1]

Item	Energy		SEM[2]	Alpha-galactosidase (mg/kg)			SEM[3]	p-value		
	High	Low		0	250	500		E	G	E×G
0-21 d										
21 d BW (g)[c]	612.3[a]	592.8[b]	6.370	587.8[b]	600.6[ab]	619.2[a]	7.801	0.039	0.027	0.716
ADFI (g)[c]	46.9	46.3	0.284	45.5[b]	46.9[a]	47.5[a]	0.348	0.077	0.009	0.795
ADG (g)[c]	28.5	27.6	0.304	27.2[b]	28.0[ab]	28.9[a]	0.373	0.052	0.001	0.149
Feed conversion	1.65	1.68	0.013	1.68	1.67	1.64	0.016	0.205	0.297	0.276
22-35 d										
35 d BW (kg)	1.53	1.48	0.017	1.47[b]	1.51[ab]	1.53[a]	0.021	0.089	0.076	0.680
ADFI (g)	124.9	126.7	1.803	124.6	124.2	128.6	2.208	0.477	0.317	0.870
ADG (g)	68.7	67.2	1.405	66.2	69.0	68.7	1.720	0.469	0.453	0.865
Feed conversion	1.83	1.89	0.038	1.89	1.81	1.88	0.046	0.214	0.429	0.714
36-48 d										
48 d BW (kg)	2.54	2.53	0.033	2.49	2.54	2.58	0.039	0.056	0.824	0.258
ADFI (g)	142.6	144.5	2.386	144.3	141.3	145.1	2.874	4.064	0.524	0.982
ADG (g)	79.5	82.4	2.073	81.5	79.7	81.8	2.498	3.533	0.399	0.383
Feed conversion	1.80	1.78	0.047	1.80	1.78	1.79	0.057	0.081	0.800	0.332

[a-b] Within main effects, means within a row with no common superscript differ significantly ($p<0.05$).
[c] Indicates a linear effect ($p<0.01$).
[1] E = Energy, G = α-galactosidase, E×G represents interaction (same as following tables).
[2] Standard error of the energy-effect means. [3] Standard error of the α-galactosidase-effect means.

SBM diets improved TME_n significantly (a linear effect, $p<0.01$). Compared to the control, TME_n increased by 4.8% and 6.1%, respectively, when 500 mg/kg and 1,000 mg/kg GAL was added. As for TAAA, the enzyme addition did not influence most of the TAAA except for the two sulfur amino acids. The availability of methionine and cystine showed a linear increase ($p<0.01$) due to enzyme supplementation.

The results in experiment 2 were summarized in Table 5. The AME and AME_n of the broilers fed a high energy content diet were greater than those of a low energy diet ($p<0.10$) for two stages. Similar trends were found in the CP at stage 2, but CP and phosphorus digestibility on stage 1 increased ($p<0.05$). At stage 1, there were quadratic effects ($p<0.05$) of GAL addition, especially 250 mg/kg, which significantly enhanced AME, AME_n and some nutrient digestibility except CP. At stage 2, it was found that

Table 5. Effects of α-galactosidase supplementation on metabolizable energy and nutrient digestibility in broilers

	Energy		SEM[1]	Alpha-galactosidase, mg/kg			SEM[2]	p-value		
	High	Low		0	250	500		E	G	E×G
Stage 1 (18-21 d)										
AME (kcal/g)[c]	3.05	2.95	0.027	2.90[b]	3.09[a]	3.01[ab]	0.033	0.073	0.045	0.457
AME_n (kcal/g)[c]	3.00	2.90	0.026	2.87[b]	3.04[a]	2.96[ab]	0.032	0.087	0.049	0.440
DM (%)[c]	71.92	70.94	0.365	69.38[b]	72.87[a]	72.13[a]	0.447	0.187	0.002	0.481
OM (%)[c]	76.76	75.91	0.322	74.54[b]	76.87[a]	77.68[a]	0.395	0.192	0.002	0.309
CP (%)	61.31[a]	56.46[b]	0.896	57.09	61.57	58.01	1.097	0.012	0.119	0.722
Calcium (%)[c]	49.99	51.62	0.909	45.58[b]	52.64[a]	54.42[a]	1.113	0.457	0.002	0.639
Phosphorus (%)[c]	45.14[a]	41.00[b]	0.907	36.98[b]	45.29[a]	46.97[a]	1.111	0.031	0.000	0.388
Stage 2 (33-35 d)										
AME (kcal/g)	2.96	2.83	0.031	2.89	2.88	2.92	0.038	0.060	0.833	0.478
AME_n (kcal/g)[c]	2.89	2.76	0.029	2.82	2.82	2.84	0.036	0.056	0.932	0.437
DM (%)	69.22	68.16	0.790	68.23	68.44	69.40	0.968	0.534	0.833	0.552
OM (%)	74.38	73.75	0.694	73.41	73.78	75.01	0.850	0.672	0.655	0.633
CP (%)[d]	58.66	54.74	1.003	54.59[b]	53.54[b]	61.96[a]	1.229	0.077	0.007	0.474
Calcium (%)	41.89	41.53	1.432	39.07	42.03	44.03	1.754	0.906	0.420	0.597
Phosphorus (%)	42.73	42.59	1.476	43.68	41.02	43.28	1.808	0.964	0.760	0.011

[a,b] Within main effects, means within a row with no common superscript differ significantly ($p<0.05$).
[c] Indicates a quadratic effect ($p<0.05$). [d] Indicates a linear effect ($p<0.05$).
[1] Standard error of the energy-effect means. [2] Standard error of the α-galactosidase-effect means.

Table 6. Effects of α-galactosidase supplementation on serum biochemical indices in broilers

Item	Energy			Alpha-galactosidase (mg/kg)			SEM[2]	p-value		
	High	Low	SEM[1]	0	250	500		E	G	E×G
At 21 d										
Total protein (g/dL)	3.27	3.35	0.137	3.31[ab]	3.04[b]	3.58[a]	0.168	0.675	0.098	0.600
Albumin (g/dL)	1.27	1.27	0.051	1.29	1.21	1.32	0.062	0.968	0.451	0.703
Globulin (g/dL)	2.00	2.08	0.096	2.02[ab]	1.83[b]	2.26[a]	0.118	0.564	0.050	0.448
Triglycerides (mg/dL)	32.50[a]	27.28[b]	1.457	28.58	30.50	30.58	1.784	0.017	0.673	0.025
Uric acid (mg/dL)[c]	6.08	5.09	0.431	5.96[a]	6.42[a]	4.38[b]	0.528	0.114	0.027	0.158
Total cholesterol (mg/dL)	116.78	108.94	4.544	116.83	115.67	106.58	5.565	0.232	0.388	0.307
Glucose (mg/dL)	241.58	249.76	9.020	244.11	251.43	241.47	11.047	0.526	0.805	0.709
At 35 d										
Total protein (g/dL)[d]	3.90	3.92	0.133	3.55[b]	4.37[a]	3.81[b]	0.163	0.899	0.004	0.016
Albumin (g/dL)[d]	1.47	1.54	0.057	1.36[b]	1.69[a]	1.48[b]	0.069	0.387	0.008	0.015
Globulin (g/dL)[d]	2.42	2.38	0.085	2.18[b]	2.69[a]	2.33[b]	0.104	0.702	0.005	0.033
Triglycerides (mg/dL)	71.00	69.50	7.484	63.25	74.83	72.67	9.166	0.888	0.641	0.584
Uric acid (mg/dL)[c]	5.12	6.32	0.443	4.93	5.89	4.84	0.542	0.745	0.328	0.583
Total cholesterol (mg/dL)[d]	126.67	135.33	5.705	116.58[b]	143.58[a]	132.83[ab]	6.987	0.291	0.034	0.048
Glucose (mg/dL)	325.92	337.81	19.093	312.29	362.85	320.46	23.384	0.663	0.275	0.036

[a,b] Within main effects, means within a row with no common superscript differ significantly ($p<0.05$).
[c] Indicates a linear effect ($p<0.05$). [d] Indicates a quadratic effect ($p<0.05$).
[1] Standard error of the energy-effect means. [2] Standard error of the α-galactosidase-effect means.

CP digestibility (a linear effect, $p<0.01$) increased at the 500 mg/kg GAL addition. An interaction on phosphorus digestibility ($p = 0.011$) was also detected.

Performance

The effects of energy and GAL supplementation on growth performance of broilers fed dietary treatments were presented in Table 4. The obvious effects occurred before 21 d of feeding period. GAL supplementation linearly improved BW, ADFI and ADG ($p<0.01$). The high energy diets notably improved BW of 21 d ($p<0.05$), and had a increasing daily intake and gain ($p<0.08$). No effects of GAL or energy on performance were observed during other stages of the trial.

The partial processing traits were also measured at the end of the feeding (Table 7). It implicated that GAL supplemented in diets improved the breast muscle proportion in live BW of broilers (quadratic, $p<0.05$), especially at 250 mg/kg.

Serum parameters

The serum biochemical indices are shown in Table 6.

Table 7. Main-effect means of partial carcass attributes for broilers fed dietary treatments[1]

Item	Energy		SEM[2]	Alpha-galactosidase (mg/kg)			SEM[3]	p-value		
	High	Low		0	250	500		E	G	E×G
Breast muscle (%)[c]	8.51	8.35	0.16	8.18[b]	8.81[a]	8.33[ab]	0.12	0.525	0.080	0.745
Leg muscle (%)	6.86	6.96	0.13	6.68	7.07	7.01	0.21	0.619	0.187	0.473
Abdominal fat (%)	1.59	1.69	0.19	1.39	1.95	1.59	0.24	0.640	0.287	0.718

[a,b] Within main effects, means within a row with no common superscript differ significantly ($p<0.05$).

[c] Indicates a quadratic effect ($p<0.05$).

[1] The values listed are expressed as the percentage of tissue weight to live body weight.

[2] Standard error of the energy-effect means. [3] Standard error of the α-galactosidase-effect means.

Table 8. Main-effect means of relative weight of digestive tract and immune organ for broilers fed dietary treatments

Item	Energy		SEM[1]	Alpha-galactosidase (mg/kg)			SEM[2]	p-value		
	High	Low		0	250	500		E	G	E×G
At 21 d (g/kg)										
Crop	4.77	4.62	0.110	4.98	4.41	4.68	0.135	0.589	0.160	0.619
Gizzard	31.16	31.39	0.629	31.61	31.35	30.88	0.770	0.856	0.885	0.826
Small intestine	43.41	42.63	1.008	42.12	44.41	42.65	1.234	0.780	0.599	0.028
Thymus	2.08	2.00	0.083	1.98	2.17	1.98	0.102	0.628	0.566	0.592
Spleen	1.23	1.11	0.054	1.14	1.23	1.14	0.066	0.322	0.750	0.223
Bursa	2.51	2.35	0.095	2.52	2.29	2.46	0.117	0.446	0.697	0.426
At 35 d (g/kg)										
Crop	3.90	4.05	0.140	4.03	3.87	4.02	0.171	0.562	0.799	0.239
Gizzard	22.34	21.71	0.574	21.88[ab]	20.30[b]	23.94[a]	0.703	0.804	0.081	0.813
Small intestine[c]	36.28	35.89	0.552	37.77[a]	33.97[b]	36.37[ab]	0.675	0.770	0.028	0.026
Thymus	2.87	2.32	0.170	2.16	2.67	3.02	0.208	0.188	0.277	0.188
Spleen	1.70	1.78	0.093	1.77	1.69	1.75	0.114	0.664	0.940	0.938
Bursa	1.38	1.27	0.117	1.55	1.17	1.23	0.143	0.586	0.393	0.945

[a,b] Within main effects, means within a row with no common superscript differ significantly ($p<0.05$).

[c] Indicates a quadratic effect ($p<0.05$).

[1] Standard error of the energy-effect means. [2] Standard error of the α-galactosidase-effect means.

On 21-d, triglycerides level of broilers fed high energy diet increased significantly ($p<0.05$), and showed an interaction with enzyme levels ($p<0.05$). Whereas uric acid level in 500 mg/kg GAL addition decreased compared to the other two (linear, $p<0.05$). On 35-d, no differences among groups occurred to energy effect. For enzyme addition, quadratic effects ($p<0.05$) were observed in total protein, albumin, globulin and cholesterol content, and the 250 mg/kg treatments showed increases ($p<0.05$). Along with the similar pattern, there also indicated evident interactions ($p<0.05$) of energy by GAL for these indices besides glucose.

Organ weight

The relative weights of various visceral organs were listed in Table 8. As for enzyme effect, the relative weight of small intestine at d 35 decreased in chickens fed 250 mg/kg GAL compared with the control, but the gizzard weight in 500 mg/kg increased significantly ($p<0.05$). From the two periods, no difference was found between two energy levels, but the intestine relative weight showed significant interaction of energy and GAL ($p<0.05$). At d 21, the relative weight of thymus and spleen in 250 mg/kg GAL had numerical increase.

DISCUSSION

As primary energy and protein sources met in poultry diets, corn and SBM should exert their potential at best to afford animal energy and digestible nutrients. However, few special enzymes for corn-soybean meal diet are available to improve their nutrient values. Previous researches have indicated that GAL may contribute to the objective. Alpha-galactosidase preparations have been successfully applied to corn-soybean meal diets in birds (Knap et al., 1996; Kidd et al., 2001a), but the reports on GAL to improve nutrient availability were inconsistent. Ghazi et al. (1997a) reported that GAL increased nitrogen retention and TME of SBM. Irish et al. (1995) indicated no improvement in the nutritive value of SBM for broilers under thermoneutral conditions. Our study found GAL significantly increased the TME_n and TAAA of methionine and cystine. Methionine is recognized as the first limit essential amino acid of poultry, so its high availability may benefit poultry growth performance. GAL tends to improve the apparent digestibility of most of nutrients to certain extent in early growing stage (before 21 d). But the CP digestibility is not so high as other reported (Ghazi et al., 1997b), as the value was calculated without correction of uric acid excretion. Low α-galactosides SBM

diet resulted in greater protein utilization and amino acid availability (Leske et al., 1995). By adding GAL to corn and legume diets, the α-1, 6-galactosidic linkages will be degraded into sucrose and galactose, and consequently their detrimental effects will be removed (Brenes et al., 1993a, b). Meanwhile, the α-galactosidic saccharides may be utilized to provide partial energy.

The present research showed notable improvement to GAL in early growth performance of chicks, but no interactions between energy and GAL. So the GAL action is inappreciable to compensate the vacant energy in SBM diet. Conceivably, the energy content of diet must be kept in a suitable level; otherwise, the GAL addition may give no effect. Our result of improving breast meat yield agreed with that of Lamptey et al. (2001). But Kidd et al. (2001b) demonstrated that diets containing GAL have no effect on carcass yield or breast meat yield. This discrepancy may be due to the different GAL characterization and rearing environment.

Alpha-galactosidase supplemented in SBM-based diet has been verified significant improvement on energy bioavailability, feed conversion ratio and weight gain of broilers (Pubols, 1993; Knap et al., 1996). Kidd et al. (2001a) also reported that Broilers fed diets supplemented with GAL improved energy utility of SBM and feed conversion at warm and thermoneutral environment. But Igbasan et al. (1997) observed no obvious growth response to GAL supplementation.

On the other hand, application of GAL combined with pectinase or protease may be more effective (Brenes et al., 1993a; Igbasan et al., 1997). But their experiments used a high inclusion of lupin or pea in diet for the chickens at d 21. Ghazi et al. (1997b) reported that GAL interacted with protease in SBM diet, but GAL had a more marked effect on TME_n than did protease treatment. GAL supplemented in SBM semi-purified diets from 7-28 d had a linear effect on weight gains in chickens. The improvement of meat yield may be interpreted by the utilization of nutrients liberated from the non-digestible compounds of corn and soybean meal diet with α-galactosidase (Fontana et al., 2001). From a nutritional viewpoint, the protein or amino acids liberation induced by GAL would have a sparing effect on supplemented levels of proteins and crystalline amino acid, which would decrease the cost of poultry diets.

The relative intestinal length and gizzard weight were reduced by enzyme treatment (Brenes et al., 1993a). As expected, alimentary canal turning thin may be beneficial to nutrient absorption. Our study indicated the similar results. It was found the relative intestine weight decreased significantly at 250 mg/kg GAL. But Kim et al. (2004) suggested that the length or weight of small intestine in early-weaned pigs was not affected by the enzyme addition to corn-SBM based diet. Cellular and humoral immunity had been evaluated (Kidd et al., 2001a, b), which implied continuous ingestion of GAL may stimulate immune response.

To date, no experiments have detected the changes of serum biochemical indices in broilers fed α-galactosidase. These values can be sensitive to reflect the status health and nutrient metabolism. GAL fed to broilers possibly ameliorated their immune function. The concentration of triglycerides on 21 d increased in high energy group, resulting from high energy easily causing fat deposit. Accelerating nitrogen retention in poultry body leads to descending uric acid excretion. Dietary GAL indicated an accumulative efficacy since the effects of GAL interaction appeared more markedly on d 35. It is speculated the enhancive globulin level may be indicative a reinforced immune status of the enzyme fed birds, though immune organ weight did not show any differences among treatments. The specific mechanism will be investigated further.

In conclusion, alpha-galactosidase preparation may be taken as a promising feed additive in corn-soybean meal diets of broilers with appropriate energy level. Research in progress should be addressed in view of different nutrient density and supplemental dose in practice.

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