Effect of a multicarbohydrase containing α-galactosidase enzyme on the performance, carcass yield, and humoral immunity of broilers fed corn–soybean meal–based diets of varying energy density

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Primary Audience: Broiler Growers, Nutritionists, Poultry Feed Companies, Poultry Extension Personnel and Researchers

SUMMARY

Nutrient availability is critical for the development of poultry digestive function and immune responses. Carbohydrase formulations, containing α-galactosidase (CAG) enzyme with specificity for galacto-oligosaccharides from soybean meal (SBM), have been shown to increase dietary ME in monogastric diets. This exploratory study aimed at evaluating the effect of CAG on the growth performance and humoral immunity of broilers fed corn–SBM diets with varying energy density. A total of 640 male 1-day-old chicks were allocated at random to one of 64 floor pens, with 10 birds each. Replicates were assigned to a 4 × 2 factorial experimental design. Four dietary energy density levels were formulated, namely control and with a −70, −100, and −120 kcal/kg of feed ME downspecification to the control. Each of these diets was treated with 2 levels of CAG supplementation, either none (−) or at a dose of 200 g/tonne (+). Growth performance was determined over a 35-d period. At the end of the study, carcass processing parameters, organ weight, and dimensions of the intestine were determined in 1 randomly selected bird per replicate. Humoral immunity was measured by quantification of antibody titers to Newcastle disease vaccination, in 1 bird per replicate, at 21 and 35 d of age. Dietary ME had a significant effect on BW and feed conversion ratio (FCR) ($P < 0.05$). Regression analysis confirmed differences in the linear response of CAG groups (− or +) in terms of elevation ($P < 0.05$) for BW gain and FCR. Significant interaction of the main effects confirmed that CAG maintained carcass dressing percentage equivalent to the control diet ($P < 0.05$). CAG increased antibody titers of broilers at 35 d, irrespective of the dietary energy density ($P < 0.001$). In conclusion, the impact on broiler growth performance from feeding low-energy-density diets can be ameliorated with a CAG enzyme.

Key words: α-galactosidase, carbohydrase, growth performance, energy density, humoral immunity

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DESCRIPTION OF PROBLEM

The nutritional benefits of using exogenous enzymes in poultry diets are well established. However, responses can vary depending on enzyme specificity and composition of the diet. The functionality and nutritional value of any exogenous enzyme are best measured, either directly or indirectly, by establishing its impact on metabolism and animal growth performance [1]. α-Galactosidase–based enzyme complexes, with specificity for galacto-oligosaccharides (e.g., raffinose, stachyose) found within soybean meal and other protein-rich oilcakes and legumes, have been shown to increase dietary ME [2]. The use of enzyme mixtures in diets formulated to give varying levels of ME allows the quantification of its nutritional contribution to the diet by comparing the relative growth performance of similar birds given diets formulated to fully meet their nutritional requirements for maintenance and growth [3].

The quantity and mix of individual nutrients supplied by the diet have a profound effect on digestive function and the immune response [4, 5]. Morphologic development of the gut epithelium will assist the production of endogenous digestive enzymes, its absorptive capacity, and associated mucosal immunity [6, 7]. Inflammatory reactions are nutrient demanding [8, 9]. It is possible that increasing the availability of ME via enzymatic degradation of nonstarch polysaccharides may play a role in reducing inflammatory responses associated with low-energy-density diets, which may hinder gut development. Antibodies are important biological agents prevalent in the healthy immune repertoire, which can be used as valid indicators of the humoral immunity of animals [10,11]. It has been suggested that enzymes may contribute to the in situ generation of components contributing to gut morphology, immune responses, and the overall health of broilers [12,13]. Therefore, evaluating whether exogenous enzymes can ameliorate the impact that suboptimal energy density may have on antibody production can help to discern plausible modes of action for enzymes beyond nutrient release. Previous research [14] has shown potential health benefits of α-galactosidase in broilers, although included as part of multienzyme carbohydrase formulations, which was demonstrated by reduced mortality. However, the same research group failed to show an effect of this enzyme preparation on humoral responses of chicken fed diets meeting the nutritional recommendations for the breed and age group [15]. The working hypothesis of this exploratory study is that a multicarbohydrase containing α-galactosidase (**CAG**) enzyme may be able to deliver growth performance improvements at graded dietary energy density and modulate humoral responses of broiler chickens.

MATERIALS AND METHODS

Birds and Housing

A total of 640 male 1-day-old broiler chickens (Arbor Acres Plus) were used in this study and allocated at random to one of 64 floor pens, containing 10 birds each. Each experimental treatment contained 8 replicate pens. Ten broilers were housed in each floor pen (stocking density, 10 birds/m^2), with similar starting BW. Broilers were offered the experimental diets on an ad libitum basis and had free access to fresh clean drinking water via nipple drinkers throughout the experimental period lasting 35 d. All experimental birds were vaccinated with Hitchner B1+H120 vaccine (intraocular route) [16] and avian influenza inactivated H5N2 vaccine (subcutaneous route) [16] on d 7 and d 10, respectively. Newcastle disease (ND) vaccine, LaSota vaccine [17] and 228E IBDV vaccine [16], was given via the intraocular route on d 14 and d 18, respectively. The experiment was conducted following the guidelines of the Cairo University Animal Ethics Committee.

Experimental Design, Diets, and Treatments

The experiment followed a factorial design with 4 different dietary energy density levels (ME) and 2 levels of supplementation with a CAG enzyme[1], resulting in 8 dietary treatments. The

[1] AGal-Pro 280P is a multienzyme system of 3 major activities: α-galactosidase derived from *Saccharomyces cerevisiae* and carbohydrases derived from *Aspergillus niger* and *Trichoderma citrinoviride*—Kerry Food Ingredients (Cork) Limited, Ireland.

Table 1. Ingredient and Nutrient Composition of Experimental Diets, With Varying Energy Density[1], Fed to Commercial Broiler Chickens Over a 35-d Study.

	Starter[3]				Grower[3]				Finisher[3]			
	PC	−70	−100	−120	PC	−70	−100	−120	PC	−70	−100	−120
Ingredients, %												
Yellow corn	55.1	56.05	57.54	56.55	59.51	60.44	60.83	61.02	65.78	66.32	66.9	67.02
Soybean meal, 46% CP	33.5	33.2	33.5	33.05	28.42	28.0	28.0	28.0	20.1	19.87	19.5	19.65
Corn gluten meal, 60% CP	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	7.15	7.15	7.15	7.15
Soybean oil	2.0	0.8	-	-	2.87	1.65	1.1	0.77	3.0	1.7	1.25	0.9
Dicalcium phosphate	1.9	1.9	1.9	1.9	1.68	1.68	1.68	1.68	1.45	1.45	1.45	1.45
Wheat bran	-	0.55	-	1.0	-	0.70	0.85	1.0	-	1.3	1.3	1.3
Limestone	1.3	1.3	1.3	1.3	1.3	1.3	1.3	1.3	1.3	0.9	1.2	1.3
Vit.+ Min. premix[2]	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3
DL-methionine	0.25	0.25	0.25	0.25	0.22	0.22	0.22	0.22	0.2	0.2	0.2	0.2
L-lysine HCl	0.25	0.25	0.26	0.25	0.30	0.31	0.32	0.31	0.32	0.33	0.32	0.33
Salt	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4
Nutrient specification												
Crude protein (%)	23	23	23	23	21	21	21	21	19	19	19	19
ME (kcal/kg)	3,000	2,930	2,900	2,880	3,100	3,030	3,000	2,980	3,200	3,130	3,100	3,080
Ca (%)	1.0	1.0	1.0	1.0	0.94	0.94	0.94	0.94	0.87	0.87	0.87	0.87
Av. P (%)	0.49	0.49	0.49	0.49	0.44	0.44	0.44	0.44	0.39	0.39	0.39	0.39
Lysine (total %)	1.4	1.4	1.4	1.4	1.3	1.3	1.3	1.3	1.1	1.1	1.1	1.1
M (total %)	0.67	0.67	0.67	0.67	0.61	0.61	0.61	0.61	0.58	0.58	0.58	0.58
M+C (total %)	1.04	1.04	1.04	1.04	0.95	0.95	0.95	0.95	0.90	0.90	0.90	0.90
Sodium (%)	0.18	0.18	0.18	0.18	0.18	0.18	0.18	1.8	0.18	0.18	0.18	0.18

Abbreviations: Av., available; Min., mineral; PC, positive control; Vit., vitamin.

[1]Energy density is quantified in 4 distinct levels, in which dietary ME met broiler nutrient recommendations (PC) or was reduced by −70, −100, or −120 kcal/kg of feed.

[2]Each 3 g of premix mixture contained: vitamin A (trans-retinyl acetate), 9,000 IU; vitamin D_3 (cholecalciferol), 2,600 IU; vitamin E (dl-α-tocopheryl acetate), 16 mg; vitamin B_1, 1.6 mg; vitamin B_2, 6.5 mg; vitamin B_6, 2.2 mg; vitamin B_{12} (cyanocobalamin), 0.015 mg; vitamin K_3, 2.5 mg; choline (choline chloride), 300 mg; nicotinic acid, 30 mg; pantothenic acid (d-calcium pantothenate), 10 mg; folic acid, 0.6 mg; d-biotin, 0.07 mg; manganese (MnO), 70 mg; zinc (ZnO), 60 mg; iron ($FeSO_4$ H_2O), 40 mg; copper ($CuSO_4$ $5H_2O$), 7 mg; iodine [$Ca(IO_3)_2$], 0.7 mg; and selenium (Na_2SeO_3), 0.3 mg.

[3]Target recoveries of α-galactosidase in all treatments and feeding phases supplemented with the test product were 1.6 u/kg of feed. Actual recoveries in starter feeds were 1.6, 1.7, 1.7, and 1.6 u/kg for the PC, −70, −100, or −120 kcal/kg ME diets, respectively. Actual recoveries in grower feeds were 1.8, 1.6, 1.7, and 1.7 u/kg for the PC, −70, −100, or −120 kcal/kg ME diets, respectively. Actual recoveries in the finisher feeds were 1.8, 1.7, 1.7, and 1.6 u/kg for the PC, −70, −100, or −120 kcal/kg ME diets, respectively.

Table 2. Effect of Dietary ME Density and Supplementation With a Multicarbohydrase Containing α-Galactosidase Enzyme (CAG) on the BW of Broiler Chickens.

Dietary ME[1]	CAG[2]	BW0	BW7	BW14	BW21	BW28	BW35
		g					
Main effects							
PC		39.6	154.8	389.1	866.7[a]	1,471.6[a]	2,143.6[a]
−70		39.7	151.3	383.9	858.3[ab]	1,441.0[ab]	2,110.0[a]
−100		39.6	152.8	392.7	831.9[b]	1,437.7[ab]	2,092.6[ab]
−120		39.7	152.9	389.1	834.1[b]	1,422.1[b]	2,026.7[b]
SEM		0.2	2.3	7.1	10.4	12.7	25.9
	−	39.6	153.1	386.3	823.8[b]	1,412.6[b]	2,059.8[b]
	+	39.6	152.8	389.5	871.6[a]	1,473.6[a]	2,126.7[a]
SEM		0.1	1.6	5.6	7.4	8.9	18.4
Treatment means							
PC	−	39.6	155.1	388.0	858.4	1,470.0[a]	2,149.6
−70	−	39.6	151.5	383.4	825.0	1,403.9[b]	2,053.8
−100	−	39.8	151.9	387.8	810.0	1,401.5[b]	2,046.1
−120	−	39.5	153.9	386.3	802.0	1,375.1[b]	1,989.6
PC	+	39.5	154.5	390.1	875.0	1,473.1[a]	2,137.5
−70	+	39.8	151.1	384.4	891.5	1,478.1[a]	2,166.3
−100	+	39.8	153.8	399.1	858.3	1,473.9[a]	2,139.1
−120	+	39.6	151.9	384.4	861.8	1,469.1[a]	2,063.8
SEM		0.2	3.3	11.2	14.7	17.9	36.7
P value	Diet ME	0.9232	0.7606	0.8716	0.0488	0.0559	0.0192
	CAG	0.8356	0.9030	0.6905	<0.0001	<0.0001	0.0126
	ME x CAG	0.7597	0.9467	0.9019	0.3462	0.0712	0.3482

Abbreviation: PC, positive control.

[a,b] means having different superscripts within a column are significantly different ($P < 0.05$).

[1]Dietary ME described as meeting broiler nutrient recommendations (PC) or being reduced by −70, −100, and −120 kcal/kg of feed.

[2]Feed supplementation with a multicarbohydrase containing α-galactosidase enzyme (CAG) at 0 (−) or 200 g/tonne (+).

four energy levels included a source of phytase (500 FTU/kg of feed [18]), which was applied with a corresponding available P release (0.1%). Diets were formulated on corn and soybean meal together with soybean oil and wheat bran, in order to achieve the desired energy density specifications (Table 1). CAG was added at 0 or 200 g/tonne of complete feed (− or +) and had a minimum α-galactosidase activity of 8 u/g [19], as well as 300 xylanase u/g [20] and 45 cellulase u/g [21]. Four basal diets were formulated to have varying energy levels: positive control (**PC**), being nutrient sufficient according to breed recommendations and age of the broiler, and 3 nutrient-deficient diets in which ME levels were reduced by −70, −100, and −120 kcal/kg relative to the PC diet. All experimental diets were manufactured through pelletization at 80°C and conditioning times of 30 s. Feed was offered as crumbs during the starter diet and as pellets in the growing and finishing stages. An ionophore anticoccidial program (semduramicin, 25 ppm) was included in all feeds used in the study. No antibiotics were used in either feed or water during the experiment.

Growth Performance and Carcass Yield Measurements

Individual BW of birds within the same pen was recorded at the start of the experiment (d 0) and weekly thereafter until completion of the study at 35 d of age. These BW were used to assess BW uniformity throughout the study. Feed consumption per pen was recorded weekly, and average BW gain (**BWG**), feed intake (**FI**), and feed conversion ratio (**FCR**) were calculated weekly and overall. Calculations of average daily FI were derived from weekly FI divided by the number of birds in each replicate and 7 d in each week. Processing parameters were determined in 1 randomly selected bird per replicate, with the weight of carcass measured as the percentage of live weight. Furthermore, weights of the front quarter, hind quarter, breast, thigh, and

Table 3. Regression Equations of BW Gain (BWG) and Feed Conversion Ratio (FCR) as a Dependent Variable of Dietary ME Density and Comparison Between Regression Equation for Unsupplemented (−) or Multicarbohydrase Containing α-Galactosidase Enzyme–Supplemented (+) Broiler Chickens Fed for a Period of 35 d.

		L regression[1]		Comparison[2], *P* value		
	Equation	r^2	*P* Value	V	S	E
BWG						
−	*y = 2109.7-1.2341x*	0.1103	0.0074	0.4351	0.1610	0.0127
+	*y = 2118.1-0.4264x*					
FCR						
−	*y = 1.55234+0.0002461x*	0.1054	0.0089	0.2492	0.0622	0.0094
+	*y = 1.54576+0.001188x*					

[1]Linear (L) regression.

[2]Comparison of regression lines based on enzyme supplementation and according to equality of variance (V), slopes (S), and elevations (E).

drumstick were calculated as a percentage of carcass weight. Meat was calculated as a percentage of carcass weight, after deboning the entire bird. Organ weights (e.g., the heart, gizzard, liver, giblets, spleen, bursa, and thymus), as well as the length and diameter of the intestine (from the Meckel's diverticulum to the cecal junction), were also measured.

Humoral Immunity Evaluation

Humoral immunity was determined by measurement of anti-ND vaccine antibody titers. These were measured in blood samples collected at 3 and 5 wk of age from the wing veins of 8 randomly selected bird in each treatment (1 bird per replicate). Serum samples were subjected to the hemagglutination inhibition assays using a method described by Swayne et al. [22]. The geometric means of serum hemagglutination inhibition titers obtained from each group were defined as the reciprocal logarithm in a base of 2 of the highest serum dilutions completely inhibiting agglutination.

Statistical Analysis

Data were statistically analyzed as a complete randomized design by 2-factor analysis using Statistix 10 [23]. The model included the main effects of dietary ME (PC, −70, −100, and −120 kcal/kg) and CAG supplementation (− or +), as well as the 4 × 2 interactions. The pen was used as the experimental unit for all growth performance measurements. Individual birds, one per replicate, were considered the experimental unit for all processing parameters and humoral immunity measurements. Mean differences were considered significant at $P < 0.05$, and trends were identified when $0.05 < P < 0.1$. When main effects or interactions were found significant, means were separated using least significant differences. Linear effects of

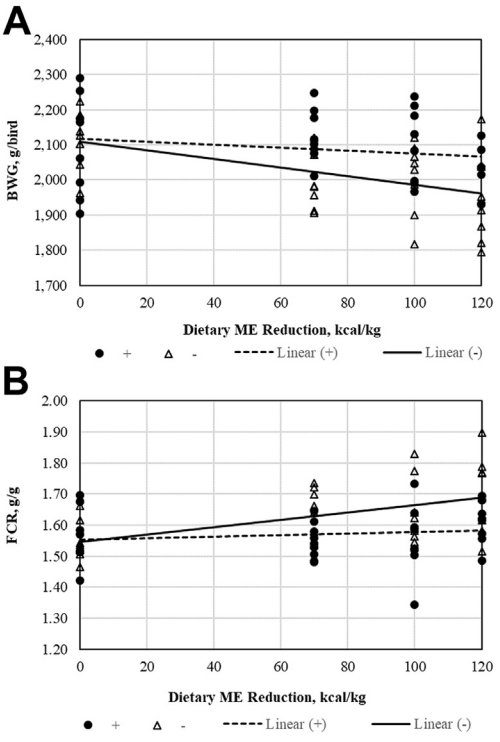

Figure 1. Effect of a multicarbohydrase containing α-galactosidase enzyme (− or +) on linear regression of (A) BW gain (BWG) and (B) feed conversion ratio (FCR) associated with dietary ME level in broiler chickens fed for a period of 35 d.

Table 4. Effect of Dietary ME Density and Supplementation With a Multicarbohydrase Containing α-Galactosidase Enzyme (CAG) on Average Daily Feed Intake (AFDI) at Weekly Intervals and Total Feed Intake (FI) Over a 35-d Study With Broiler Chickens.

		ADFI1	ADFI2	ADFI3	ADFI4	ADFI5	Total FI
Dietary ME[1]	CAG[2]			g/d			g
Main effects							
PC		23.6	51.5	99.2[b]	136.5	164.1[b]	3,324
−70		23.1	51.1	104.4[a]	133.9	168.1[ab]	3,362
−100		23.3	50.5	100.7[b]	133.1	168.9[a]	3,336
−120		23.1	51.6	100.5[b]	133.7	169.0[a]	3,344
SEM		0.5	0.5	1.2	1.5	1.5	18.6
	−	23.3	50.5[b]	101.0	136.0[a]	168.0	3,352
	+	23.2	51.8[a]	101.4	132.6[b]	167.1	3,332
SEM		0.4	0.3	0.8	1.1	1.1	13.2
Treatment means							
PC	−	23.6	50.8	98.1	138.9	163.3[b]	3,321
−70	−	23.3	50.4	103.1	136.0	166.9[ab]	3,356
−100	−	23.0	49.6	101.6	135.5	169.3[ab]	3,355
−120	−	23.5	51.4	101.1	133.6	172.5[a]	3,374
PC	+	23.5	52.3	100.3	134.1	165.0[b]	3,327
−70	+	22.9	51.8	105.6	131.9	169.3[ab]	3,368
−100	+	23.5	51.4	99.8	130.8	168.6[ab]	3,318
−120	+	22.8	51.9	99.9	133.8	165.5[b]	3,314
SEM		0.7	0.6	1.6	2.2	2.2	26.3
P value	Diet ME	0.8960	0.2895	0.0157	0.4165	0.0195	0.4483
	CAG	0.7077	0.0061	0.7453	0.0310	0.2719	0.1128
	ME x CAG	0.8404	0.7778	0.4157	0.6215	0.0666	0.2119

Abbreviation: PC, positive control.

[a,b] means having different superscripts within a column are significantly different ($P < 0.05$).

[1]Dietary ME described as meeting broiler nutrient recommendations (PC) or being reduced by −70, −100, and −120 kcal/kg of feed.

[2]Feed supplementation with a CAG at 0 (−) or 200 g/tonne (+).

decreasing dietary ME were tested for the CAG supplementation groups (− or +). A comparison of the regression equations was done by assessing the equivalence in variance (i.e., Bartlett's test), slopes, and elevation between these groups. These parameters were assessed in sequence until a response of statistical significance was found.

RESULTS AND DISCUSSION

Growth performance of broilers was influenced by the study main effects, dietary ME density, and CAG supplementation. Reducing the energy density of the diet had a detrimental effect on the BW of broilers, with the less-dense diets yielding lighter birds (Table 2). These effects were observable from 21 d of age, with differences evident at −100 kcal/kg or greater ME reduction on d 21 ($P < 0.05$) and at −120 kcal/kg on d 35 ($P < 0.05$). A previous study has also confirmed lower BW when reducing energy density of broiler formulations, with significant differences seen at −60 kcal/kg downspecification and proportional decreases with larger ME reductions to a maximum of −120 kcal/kg [3]. In the current study, the linearity of the BWG to dietary ME response was confirmed through regression evaluations ($r^2 = 0.1103$; $P < 0.01$; Table 3), which have also been established in previous studies [3,24]. CAG had a significant effect on broiler BW (Table 2). Broilers fed on diets supplemented with CAG had significantly higher BW on d 21 ($P < 0.0001$), d 28 ($P < 0.0001$), and d 35 ($P < 0.05$). Previous studies with α-galactosidase enzyme (as a monocomponent enzyme formulation or when factored as part of a multienzyme carbohydrase complex) have yielded varying responses in regard to its impact on broiler BW [15,25,26]. Jasek et al. [2] observed no effect of the same CAG enzyme on BW of broilers reared to 21 d. However, the objective of the

Table 5. Effect of Dietary ME Density and Supplementation With a Multicarbohydrase Containing α-Galactosidase Enzyme (CAG) on Feed Conversion Ratio (FCR) at Weekly Intervals (1-5) and Overall FCR Throughout the 35-d Study With Broiler Chickens.

		FCR1	FCR2	FCR3	FCR4	FCR5	FCR
Dietary ME[1]	CAG[2]			g/g			
Main effects							
PC		1.434	1.559	1.468^{y}	1.612	1.801	1.554^{b}
−70		1.454	1.544	1.554^{xy}	1.617	1.796	1.595^{ab}
−100		1.453	1.487	1.625^{x}	1.557	1.867	1.599^{ab}
−120		1.440	1.563	1.573^{xy}	1.599	2.003	1.655^{a}
SEM		0.044	0.046	0.041	0.046	0.092	0.023
	−	1.448	1.532	1.637^{a}	1.638	1.881	1.632^{a}
	+	1.443	1.544	1.473^{b}	1.555	1.853	1.570^{b}
SEM		0.031	0.032	0.029	0.032	0.065	0.016
Treatment means							
PC	−	1.434	1.536	1.483	1.636	1.754	1.547
−70	−	1.459	1.533	1.645	1.645	1.837	1.634
−100	−	1.452	1.503	1.744	1.609	1.899	1.644
−120	−	1.447	1.558	1.678	1.664	2.033	1.703
PC	+	1.434	1.582	1.454	1.589	1.849	1.561
−70	+	1.450	1.556	1.464	1.59	1.756	1.557
−100	+	1.454	1.472	1.505	1.505	1.834	1.555
−120	+	1.433	1.568	1.469	1.534	1.973	1.608
SEM		0.062	0.065	0.058	0.065	0.130	0.032
P value	Diet ME	0.9851	0.6220	0.0687	0.7797	0.3564	0.0278
	CAG	0.9102	0.7941	0.0002	0.0727	0.7632	0.0094
	ME x CAG	0.9991	0.9453	0.2888	0.9028	0.8948	0.3023

Abbreviation: PC, positive control.

^{a,b} means having different superscripts within a column are significantly different ($P < 0.05$); ^{x,y} means having different superscripts within a column tended to be significantly different ($0.05 < P < 0.1$).

[1]Dietary ME described as meeting broiler nutrient recommendations (PC) or being reduced by −70, −100, and −120 kcal/kg of feed.

[2]Feed supplementation with a CAG enzyme at 0 (−) or 200 g/tonne (+).

aforementioned study was primarily to assess its efficacy on nutrient digestibility, and therefore, the experimental design could have been limiting in regard to growth performance assessment. Comparison of the regression lines between the CAG groups (− or +) indicated an equivalence in terms of variance and slopes for BWG; however, lines differed in relation to elevation ($P < 0.05$; Table 3). This may suggest differences at the intercept level (Figure 1A), corresponding to the predicted BWG of birds fed the PC diets with no ME reduction. This "over-the-top" addition of an α-galactosidase enzyme mixture was shown to improve FCR, but not live weight, in a previous 49-d broiler evaluation [15].

In the current study, overall FI was not influenced by dietary ME content or CAG supplementation (Table 4). Although it is generally accepted that broilers still hold an ability to control feed consumption to normalize energy intake [27], neither the reductions in ME in the current study nor the supplementation with exogenous CAG was able to influence FI for the overall study period. Genetic advancements in the last 2 decades may have limited somehow this ability, and birds in this study may have reached their physiological capacity for uptake of feed. Nonetheless, the efficiency of these broilers to convert feed into BW was also influenced by the main factors in the experimental design of this exploratory trial. A significant effect of the energy content of the diet was determined for the overall FCR of broilers in this study ($P < 0.05$; Table 5), with clear detrimental effects on FCR observable in the group of birds consuming the less-energy-dense diet. Overall FCR responses to dietary ME content could be fitted to a linear response ($r^2 = 0.1054$; $P < 0.01$; Table 3), which is in agreement with previous findings [3,24,28]. Supplementation with CAG had a significant positive influence on overall FCR ($P < 0.01$;

Table 6. Effect of Dietary ME Density and Supplementation With a Multicarbohydrase Containing α-Galactosidase Enzyme (CAG) on Processing Yields of Broiler Chickens at 35-d of Age.

		Dressing	Front[3]	Hind[3]	Breast	Leg	Meat
Dietary ME[1]	CAG[2]	% Live weight			% Carcass weight		
Main effects							
PC		69.3[a]	38.4[a]	30.9	22.4[a]	15.5	38.0[a]
−70		68.8[b]	37.8[b]	31.0	21.9[b]	15.6	37.5[ab]
−100		68.5[b]	37.4[b]	30.8	22.0[b]	15.4	37.4[bc]
−120		68.6[b]	37.8[b]	31.1	21.4[c]	15.5	36.9[c]
SEM		0.1	0.1	0.1	0.1	0.1	0.2
	−	68.5[b]	37.7[b]	30.8	21.7[b]	15.6	37.3[b]
	+	69.1[a]	38.0[a]	31.0	22.2[a]	15.4	37.6[a]
SEM		0.1	0.1	0.1	0.1	0.1	0.1
Treatment means							
PC	−	69.4[a]	38.6[a]	30.6	22.5[a]	15.5	38.0
−70	−	68.6[bc]	37.8[ab]	30.8	21.5[c]	15.8	37.3
−100	−	68.1[c]	36.9[bc]	30.7	21.7[c]	15.5	37.2
−120	−	68.0[c]	37.4[c]	31.2	20.9[d]	15.6	36.5
PC	+	69.3[a]	38.2[ab]	31.1	22.4[a]	15.5	37.9
−70	+	69.0[ab]	37.9[ab]	31.1	22.3[a]	15.4	37.8
−100	+	69.1[ab]	38.2[ab]	30.9	22.3[ab]	15.3	37.6
−120	+	68.9[ab]	37.9[ab]	31.0	21.9[bc]	15.5	37.3
SEM		0.2	0.2	0.2	0.2	0.2	0.2
P value	Diet ME	0.0002	0.0001	0.4922	<0.0001	0.7061	0.0006
	CAG	0.0002	0.0078	0.1343	<0.0001	0.2259	0.0262
	ME x CAG	0.0351	0.0014	0.2735	0.0061	0.8312	0.2594

Abbreviation: PC, positive control.

[a-c] means having different superscripts within a column are significantly different ($P < 0.05$).

[1]Dietary ME described as meeting broiler nutrient recommendations (PC) or being reduced by −70, −100, and −120 kcal/kg of feed.

[2]Feed supplementation with a CAG enzyme at 0 (−) or 200 g/tonne (+).

[3]The front quarter included breast quarter muscles and the skin (without the wing) and the hind quarter (skinned) included the drumstick and thigh.

Table 5) and, specifically, broiler efficiency during wk 3 (d < 0.001) of the study. When comparing the regression lines of the CAG groups (− or +), no difference in variance was observed ($P > 0.1$). However, a strong tendency ($P = 0.0622$) indicated an effect of dietary ME reductions in nonsupplemented groups (Figure 1B). To the authors' knowledge, there are no other comparisons on prediction equations for FE in response to a CAG enzyme in broilers.

Dietary ME specification influenced the processing parameters of the broilers within the current study (Table 6). Dressing percentage was significantly reduced with the graded levels of dietary energy reductions ($P < 0.001$), with significant differences already observable at −70 kcal/kg ME downspecification. This was equally reflected in the proportion of the front quarter, breast, and meat ($P < 0.001$). At the same time, CAG had a positive influence on dressing percentage ($P < 0.001$) and proportions of the front quarter, breast, and meat ($P < 0.05$). Significant interactions between the main effects for dressing percentage ($P < 0.05$) and proportions of the front quarter and breast ($P < 0.01$) demonstrate that CAG can counter the negative

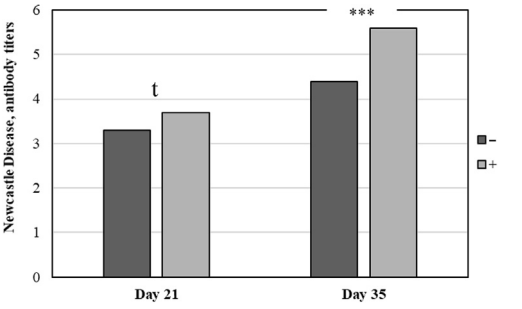

Figure 2. Effect of supplementation with a multicarbohydrase containing α-galactosidase enzyme (− or +) on the humoral immunity of broiler chickens after immunization against Newcastle disease (21 and 35 d of age). t, $P < 0.1$; ***$P < 0.001$.

effects of the dietary ME reduction. Even at the lowest dietary ME, CAG-supplemented birds (+) maintained processing parameters equivalent to those receiving the PC diet without CAG supplementation (−). Similar responses have been reported for other exogenous enzymes [28]. No effect of the main factors or their interaction was found in organ weight or dimensions of the intestine (data not shown).

Growth performance is tightly related to the health status of an animal [4,5]. Increased disease pressure and/or stress can trigger an immune response, which may lead to lower BW and poorer FE [29]. Nutritional deficiencies have been associated with impaired innate and acquired immunity [30]. Contrary to the results of this study, in which no significant antibody effect of diet energy density was reported (data not shown), Golian et al. [31] reported a reduction in antibody response to vaccination with increasing dietary ME content from 2,900 to 3,200 kcal/kg of feed. In the current study, CAG had an effect on antibody levels after ND vaccination (Figure 2). Increases in antibody production were found with CAG supplementation on 7 d ($P = 0.0677$) and 14 d ($P < 0.001$) after vaccination (21 and 28 d of age, respectively). These improvements in the humoral immunity status of CAG-supplemented birds could be associated with higher disease resilience [32]. These results may suggest the ability of CAG to influence dietary factors affecting specific immunity of broilers, as reported by other authors in response to phytase [33] and nonstarch polysaccharide–degrading enzymes [34]. Other authors have reported no impact on humoral immunity in response to dietary Lys levels and enzyme supplementation in broilers fed high–canola meal diets [35]. Because the research on the impact of exogenous enzymes on immunity is very limited, the results from the current study warrant caution, and additional investigation to confirm the effects and hypothesis on the plausible mode of action is needed.

CONCLUSIONS AND APPLICATIONS

1. The effects of reduced dietary ME on broiler growth performance and processing yields can be ameliorated with supplementation of a CAG enzyme.
2. Humoral immunity could be improved by a CAG enzyme, although supportive mode of action needs to be further researched.

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18. One FTU is the amount of enzyme that catalizes the release of 1 μmol of inorganic phosphate per min from 5.1 mM sodium phytase in pH 4.5 buffer at 37°C.

19. One alpha-galactosidase unit defined as the amount of enzyme that will produce one micromole of p-nitrophenol per min at 37°C.

20. One xylanase unit of activity is defined as the amount of enzyme required to release one micromole of xylose reducing sugar equivalents per minute under the defined assay conditions; Temperature 40°C, pH 4.7.

21. One cellulase unit of activity is defined as the amount of enzyme required to release one micromole of glucose reducing sugar equivalents per minute under the defined assay conditions: Temperature 40°C, pH 4.5.

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