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Efficacy and Metabolizable Energy Equivalence of an α-Amylase-β-Glucanase Complex for Broilers

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ABSTRACT

A trial was conducted to evaluate the effects of adding an exogenous α-amylase-β-glucanase complex produced from *Bacillus amyloliquefaciens* on the growth performance, carcass yield, and relative AME bioequivalence in broilers fed corn-soy diets from 1 to 40 d of age. One thousand seven hundred and fifty one-day-old Cobb x Cobb 500 slow-feathering male broilers were randomly allotted to seven treatments with 10 replicates of 25 birds each as follows: control diet (C); C diet with reduction of 60 (C-60), 90 (C-90), or 120 (C-120) kcal AME/kg; C diet with reduction of 120 kcal AME/kg and supplemented with 200 (C-120-200), 300 (C-120-300), or 400 (C-120-400) mg of the enzyme complex/kg. Each g of the enzyme complex corresponded to 200 kilo-Novo α-amylase and 350 fungal β-glucanase units. On d 40, eight birds were randomly taken from each pen and processed to evaluate carcass and commercial cuts yields. Percent mortality was not affected by the treatments ($p > 0.05$). Live performance, as indicated by BW gain (BWG) linearly decreased ($p < 0.05$) and FCR linearly increased with the reduction in AME. Birds fed diets supplemented with the enzyme complex showed weekly improvements in BWG and FCR. There were no effects of the treatments on the yield of the carcass or of commercial cuts; however, abdominal fat was significantly lower ($p < 0.0343$) in birds fed the C-120-400 compared to the C-120 feeding program (1.67% vs. 1.90%); all other treatments were intermediate. Average AME equivalence of the enzyme complex varied weekly. Estimations for the entire period were 40, 46, and 56 kcal for BWG and 58, 76, and 99 kcal AME/kg for FCR ($p < 0.001$) for the diets containing 200, 300, and 400 mg enzyme complex/kg, respectively.

INTRODUCTION

Studies evaluating the use of supplemental enzymes in poultry diets have presented a substantial increase in the last years. A wide variety of enzyme types targeting diverse substrates have been used in these studies, which include mono-component enzyme products with only one enzymatic activity and others having more than one enzyme.

Results from enzyme supplementation studies vary with the many circumstances involved in their evaluation, but having a target substrate in the feed is essential so that enzyme action can lead to an amount of released product that could be directly or indirectly measured through the evaluation of metabolism assays or animal performance (Olukosi & Adeola, 2008; Jozefiak *et al.,* 2010). It has been demonstrated that dietary supplementation of xylanase and glucanase improves the performance and nutrient digestibility of broilers fed diets containing high levels of grains rich in non-starch polysaccharides (NSP) (Gracia *et al.,* 2003), which have been frequently related to a decrease in intestinal viscosity (Petterson *et al.,* 1991; Lázaro *et al.,* 2003). However, total NSP content in corn and soy are low compared to wheat and barley, for instance (Willamil *et al.*, 2012), and therefore, diets having large proportions of corn are not expected to present important viscosity-related problems.

The post-hatching chick needs to adapt from maternal nutrition to exogenous feed, when nutrient supply changes from the high fat yolk sac to a high starch feed, and this process may take several days (Vieira & Moran, 1999). During this period, the secretion of endogenous enzymes also changes according to the composition of the exogenous feed, together with an increase in digestive organ size to rapidly accommodate the increase in feed intake (Noy & Sklan, 1995). Starch is the substrate that proportionally increases the most in corn-soy diets as birds age; therefore, amounts of secreted amylase may limit energy available for growth. The secretion of amylase seems to quickly stabilize even though starch intake is increased with time. Amylase secretion is low at 4 d of age (Noy & Sklan, 1995); however, increases in its pancreatic secretion seem to flatten between 7 to 21 d (Noy & Sklan, 1995; Uni *et al.,* 1995). The benefits of a β-glucanase in corn-soy diets are expected to be lower than in wheat- and barley-based diets due to a much smaller proportion of its substrate in that type of feed; however, there are few studies with this type of enzyme in corn-soy diets (Leslie *et al.,* 2007; Rutherfurd *et al.,* 2007; Cowieson *et al.,* 2010). It may be possible that the association with α-amylase could render measurable improvements when fed to broilers.

Performance and diet digestibility improvements were observed when blends of amylase, proteases, and xylanases were added to corn-soy diets (Zanella *et al.*, 1999; Douglas *et al.,* 2000; Café *et al.,* 2002). Gracia *et al.* (2003) reported positive digestibility and performance effects when including a mono-component α-amylase from *Bacillus amyloliquefaciens*. Similarly, Onderci *et al.* (2006) reported improvements in gut morphology, digestibility, and performance when birds were fed an α-amylase from *Bacillus stearothermophilus.* Enzyme supplementation with an enzyme blend containing α-amylase, β-glucanase, and xylanase increased the AME content of a corn-soy diet as well as the apparent and true ileal amino acid digestibility for all amino acids (Rutherfurd *et al.,* 2007).

The objective of the present study was to evaluate the effects of increasing supplementation levels of a commercial α-amylase-β-glucanase complex derived from *Bacillus amyloliquefaciens* fermentation on the growth performance and carcass and commercial cuts yields of broilers fed corn-soy diets from 1 to 40 d of age. An estimation of the equivalence in AME of this enzyme complex is also proposed.

MATERIALS AND METHODS

All procedures used in this study avoided unnecessary animal distress and were approved by the Ethics and Research Committee of Universidade Federal do Rio Grande do Sul, Porto Alegre, Brazil.

Broiler husbandry

A total of 1750 one-day-old Cobb × Cobb 500 slow-feathering male broiler chicks, vaccinated for Marek's disease, were obtained from a commercial hatchery and randomly assigned to seven treatments with 10 replicates of 25 birds each distributed in 70 floor pens measuring 1.65 x 1.65 m (9.18 birds per m²). Chick average weight was 45 ± 1 g at placement. Birds were housed in an open-sided broiler house with rice-hulls litter. Each pen was equipped with three water nipples and one 18-kg tube feeder.

Average house temperature was 32°C at placement and was maintained within a range to optimize animal comfort using heaters, fans, and foggers, when appropriate. Lighting was continuous until d 7 and a 16:8 h L:D schedule used thereafter until d 40. Feed and water were available for *ad libitum* consumption.

Experimental diets

Diets were based on corn and soybean meal and were formulated to meet or exceed the birds' nutrient and energy requirements (Rostagno *et al.,* 2011), with the exception of AME. Mash diets were fed in a 4-phase feeding program as follows: pre-starter (d 1 to 7), starter (d 8 to 21), grower (d 22 to 35), and finisher (d 36 to 40). Diets are presented on Tables 1 and 2. Dietary treatments were formulated for the pre-starter, starter, grower, and finisher phases: a control diet (**C**) with 2,950; 3,050; 3,100 and 3,150 kcal/kg of AME, respectively, and three corresponding reductions of 60 (C-60), 90 (C-90) and 120 (C-120) kcal AME/kg in all feeds.

The enzyme complex was supplemented to the diets with 120 kcal AME/kg reduction at 200 (C-120-200), 300 (C-120-300) and 400 (C-120-400) mg/kg of an exogenous α-amylase + β-glucanase complex (Ronozyme A, Novozymes S/A, Bagsvaerd, Denmark). The α-amylase + β-glucanase complex used is a granulated enzyme preparation produced by submerged fermentation of *Bacillus amyloliquefaciens*.

Each gram of the enzyme product corresponds to 200 kilo-Novo α-amylase units and 350 fungal β-glucanase units. The activity of one kilo-Novo α-amylase unit is defined as the amount of enzyme that hydrolyzes 1 mg of soluble starch at 60ºC at pH 6.0, per minute, whereas one fungal β-glucanase unit is the amount of enzyme that releases glucose or other reducing carbohydrates with a reduction power corresponding to 1 µmol glucose per minute at 30ºC and pH 5.0 (Rutherfurd *et al.,* 2007).

Measurements

Live performance was evaluated through weekly measurements of BW gain (BWG), feed intake (FI), and FCR corrected for the weight of dead birds from 1 to 40 d of age. At the end of the study birds were processed for evaluation of carcass yield as follows. Eight birds per pen (n = 560) were randomly selected, fasted for 8 h, and individually weighed previously to processing. Birds were then electrically stunned with 45V for 3s and then bled for 3 min after a jugular vein cut. Carcasses were scalded at 60ºC 45s, and the feathers being immediately mechanically plucked, manually eviscerated, and statically chilled in slush ice for approximately 3h. The eviscerated carcasses (without feet and neck but with lungs) were hung for 3 min to remove excess water prior to their individual weighing; abdominal fat was weighed separately.

Table 1 – Broiler pre-starter (1 to 7 d) and starter (8 to 21 d) diets with graded AME reductions and supplemented or not with α-amylase + β-glucanase complex.

Ingredients, %	Pre-starter				Starter			
	Control (C)	C - 60 kcal	C - 90 kcal	C - 120 kcal	Control (C)	C - 60 kcal	C - 90 kcal	C - 120 kcal
Corn 7.5%	50.12	51.53	52.22	52.84	56.44	57.87	58.57	59.20
Soybean meal 45%	41.54	41.28	41.16	41.04	35.10	34.84	34.72	34.60
Soybean oil	3.69	2.53	1.96	1.41	3.92	2.76	2.18	1.63
Dicalcium phosphate	1.88	1.88	1.88	1.88	1.84	1.83	1.83	1.83
Limestone	1.27	1.27	1.27	1.27	1.23	1.23	1.23	1.24
Salt	0.65	0.65	0.65	0.65	0.46	0.46	0.46	0.45
L-lysine HCL 78%	0.17	0.17	0.17	0.18	0.19	0.19	0.19	0.19
DL-methionine 99%	0.33	0.33	0.33	0.33	0.28	0.28	0.28	0.28
L-threonine 98.5%	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
Sodium Bicarbonate	0.04	0.05	0.05	0.05	0.20	0.20	0.20	0.20
Vitamin mineral mix[1]	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20
Choline chloride 60%	0.06	0.06	0.06	0.06	0.09	0.09	0.09	0.09
Kaolin (inert material)	---	---	---	0.04	---	---	---	0.04
Enzyme[2]	---	---	---	---	---	---	---	---
Total	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
Energy and nutrients, % or as noted								
AME, kcal/kg	2,950	2,890	2,860	2,830	3,050	2,990	2,960	2,930
CP	23.32	23.32	23.32	23.32	20.91	20.91	20.91	20.91
Digestible Lys	1.28	1.28	1.28	1.28	1.15	1.15	1.15	1.15
Digestible TSAA	0.96	0.96	0.96	0.96	0.86	0.86	0.86	0.86
Digestible Thr	0.83	0.83	0.83	0.83	0.75	0.75	0.75	0.75
Ca	1.00	1.00	1.00	1.00	0.96	0.96	0.96	0.96
Av.P	0.50	0.50	0.50	0.50	0.48	0.48	0.48	0.48
Na	0.23	0.23	0.23	0.23	0.21	0.21	0.21	0.21
Starch[3]	31.83	32.72	33.16	33.55	35.84	36.75	37.20	37.59

[1]Provided the following per kilogram of diet: vitamin A, 9,000 IU; vitamin D_3, 2,500 IU; vitamin E, 100 IU; vitamin K_3, 2.5 mg; vitamin B_{12}, 12 µg; thiamine, 1,5 mg; riboflavin, 6 mg; vitamin B_6, 3 mg; niacin, 25 mg; pantothenic acid, 12 mg; folic acid, 8 mg; biotin, 0.3 mg; selenium, 0.25 mg; iron, 100 mg; zinc, 100 mg; manganese, 160 mg; copper, 20 ppm; iodine, 2 mg; cobalt, 2 mg; Monensin sodium 110 ppm.

[2]α-amylase + β-glucanase complex produced by *Bacillus amyloliquefaciens* having a minimum activity of 200 kilo-Novo α-amylase units and 350 fungal β-glucanase units per gram obtained from Novozymes A/S (Bagsvaerd, Denmark).

[3]Estimated from FEDNA (2003).

Table 2 – Broiler grower (22 to 35 d) and finisher (36 to 42 d) diets with graded AME reductions and supplemented or not with an α-amylase + β-glucanase complex.

Ingredients, %	Grower				Finisher			
	Control (C)	C - 60 kcal	C - 90 kcal	C - 120 kcal	Control (C)	C - 60 kcal	C - 90 kcal	C - 120 kcal
Corn 7.5%	60.03	61.43	62.14	62.76	65.45	66.87	67.58	68.19
Soybean meal 45%	31.76	31.51	31.38	31.27	26.84	26.58	26.45	26.34
Soybean oil	3.98	2.83	2.25	1.70	3.76	2.60	2.02	1.47
Dicalcium phosphate	1.70	1.70	1.70	1.70	1.51	1.51	1.51	1.51
Limestone	1.22	1.22	1.22	1.22	1.17	1.17	1.17	1.17
Salt	0.44	0.44	0.44	0.44	0.40	0.40	0.40	0.40
L-lysine HCL 78%	0.20	0.20	0.21	0.21	0.22	0.22	0.22	0.23
DL-methionine 99%	0.26	0.26	0.25	0.25	0.23	0.23	0.23	0.23
L-threonine 98.5%	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03
Sodium Bicarbonate	0.08	0.08	0.08	0.08	0.09	0.09	0.09	0.09
Vitamin mineral mix[1]	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20
Choline chloride 60%	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10
Kaolin (inert material)	---	---	---	0.04	---	---	---	0.04
Enzyme[2]	---	---	---	---	---	---	---	---
Total	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
Energy and nutrients, % or as noted								
AME, kcal/kg	3,100	3,040	3,010	3,010	3,150	3,090	3,060	3,030
CP	19.67	19.67	19.67	19.67	17.87	17.87	17.87	17.87
Digestible Lys	1.08	1.08	1.08	1.08	0.98	0.98	0.98	0.98
Digestible TSAA	0.81	0.81	0.81	0.81	0.75	0.75	0.75	0.75
Digestible Thr	0.68	0.68	0.68	0.68	0.61	0.61	0.61	0.61
Ca	0.92	0.92	0.92	0.92	0.85	0.85	0.85	0.85
Av.P	0.44	0.44	0.44	0.44	0.40	0.40	0.40	0.40
Na	0.20	0.20	0.20	0.20	0.18	0.18	0.18	0.18
Starch[3]	38.12	39.01	39.46	39.85	41.56	42.46	42.91	43.30

[1]Vitamin, trace minerals, and growth promoters provided the following per kilogram of diet: vitamin A, 9,000 IU; vitamin D_3, 2,500 IU; vitamin E, 100 IU; vitamin K_3, 2.5 mg; vitamin B_{12}, 12 µg; thiamine, 1,5 mg; riboflavin, 6 mg; vitamin B_6, 3 mg; niacin, 25 mg; pantothenic acid, 12 mg; folic acid, 8 mg; biotin, 0.3 mg; selenium, 0.25 mg; iron, 100 mg; zinc, 100 mg; manganese, 160 mg; copper, 20 ppm; iodine, 2 mg; cobalt, 2 mg; Monensin Sodium 110 ppm.

[2]α-amylase + β-glucanase complex is produced by *Bacillus amyloliquefaciens* having a minimum activity of 200 kilo-Novo α-amylase units and 350 fungal β-glucanase units per gram obtained from Novozymes A/S (Bagsvaerd, Denmark).

[3]Estimated from FEDNA (2003).

Carcass yield was expressed relative to live weight, whereas commercial cuts and abdominal fat were expressed as a proportion of carcass weight.

Statistical analysis

Data were analyzed as a one-way analysis of variance using the GLM procedure of SAS (2009). Significance was accepted at 5% and mean differences were separated using the test of Tukey when the model effect was significant (Tukey, 1991). Linear and quadratic effects of decreasing AME were tested for the diets not supplemented with the enzyme. The corresponding AME for obtained BWG and FCR at each enzyme supplemental inclusion levels allowed estimations of added improvements in AME provided by the enzyme complex at any point of the curve. The regression equations of AME level intake and supplemental amylase intake (based on formulated values) for a particular response variable were equated and solved for x in quadratic or linear equations. Calculation of enzyme equivalence utilized in the present study followed the methodology reported by Adedokun *et al.* (2004) and Jendza *et al.* (2006), who estimated P equivalence from phytase.

RESULTS AND DISCUSSION

The analysis of the added enzyme complex in the experimental feeds showed that concentrations of α-amylase and β-glucanase were as expected (formulated: 40, 60, and 80 kilo-Novo α-amylase units/kg; analyzed: 42, 63, and 85 kilo-Novo α-amylase units/kg, respectively).

Treatments affected BWG ($p < 0.05$) and FCR ($p < 0.05$) of birds throughout all the evaluated weeks with performance losses as AME was reduced, but there were partial compensations by the enzyme supplementation (Table 3). Table 4 shows that BWG and FCR linearly fit to AME reductions in the diets without enzyme supplementation ($p < 0.05$), but no effects were observed on feed intake in all weekly evaluations. Figures 1 and 2 show the linear adjustments for BWG and FCR obtained from placement to the end of the study when birds were fed the different enzyme dietary concentrations. There were no effects of the treatments on mortality (overall grand mean was 3.52%).

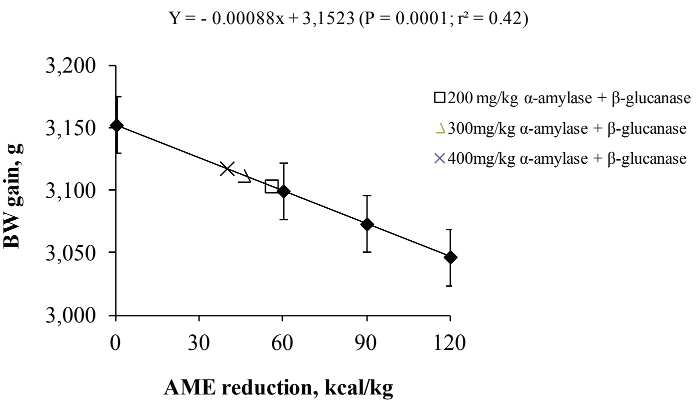

Figure 1 – BW gain of broilers fed diets with decreasing AME levels supplemented or not with an α-amylase + β-glucanase complex from 1 to 40 d, g.

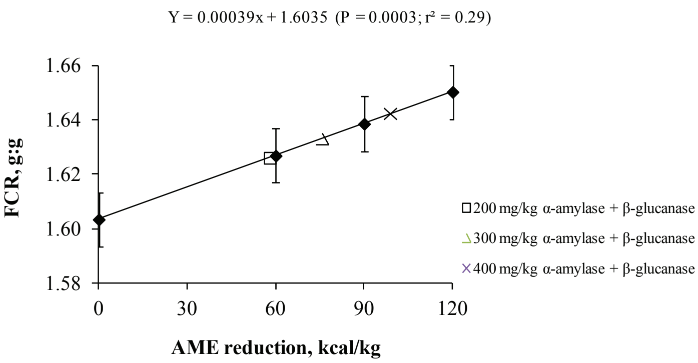

Figure 2 – Feed conversion ratio of broilers fed diets with decreasing AME levels supplemented or not with an α-amylase + β-glucanase complex from 1 to 40 d.

There were no effects of the treatments on carcass and commercial cuts yields. However, abdominal fat as proportion of the eviscerated carcass was significantly lower ($p < 0.0343$) in birds fed the C-120-400 compared with the C-120 feeding program (1.67% *vs*. 1.90%); all other treatments were intermediate (Table 5).

Apparent metabolizable energy equivalence was calculated using BWG and FCR of the birds fed the different enzyme levels compared with the responses obtained as AME was reduced in diets without enzyme supplementation. These equivalences varied with age and enzyme concentration in the feed, reaching the lowest values in the first week and highest in the fourth. Average AME equivalence for the enzyme complex varied weekly. Estimations for the entire period were 40, 46, and 56 kcal for BWG and 58, 76, and 99 kcal AME/kg for FCR when birds were fed 200, 300, and 400 mg/kg of the enzyme complex, respectively. Data for each week is presented on Table 4.

Research on the use of exogenous enzymes for broilers has been ongoing for decades; however, its commercial use is more recent. The practical use of phytase in chicken diets is presently well established in the commercial environment, but the use of enzymes targeting other substrates is less frequent. This is rapidly changing because of the increases in ingredient costs, especially those that supply energy because of their market connections with biofuels.

A diversity of explanations has been used to try to explain the improvements obtained using exogenous carbohydrases in poultry nutrition, and they are not always related to a greater degradation of the target substrate of these enzymes. Obviously, improvements in AME obtained with broilers fed corn-soy diets supplemented with amylases are immediately attributed to greater starch breakdown, and therefore, greater starch digestibility. However, the effects on AME improvements in broilers may also be related to the breakdown of non-starch polysaccharides and protein that can result from the supplementation with other enzymes (Zhou *et al.*, 2009).

The exposure of complex substrates (bearing different types of molecules) after their partial or total breakdown by the action of an enzyme can allow the access of other enzymes that otherwise would not be able to react with the original substrate. Therefore, degradation of a newly-presented substrate is frequently referred to explain these improvements (Cowieson *et al.,* 2010). Part of the plant nutrients, such as starch and protein, are trapped within the insoluble cell walls (this is sometimes referred as cage effect) such that poultry are unable to access these nutrients (Englyst, 1989). Also, soluble fibers dissolve in the gut, forming viscous gels that trap nutrients and slow down rates of digestion and feed rate passage through the gut (Bedford *et al.,* 1991; Veldman *et al.,* 1994).

Table 3 – Performance of broilers fed diets with graded AME reductions and supplemented or not with an α-amylase + β-glucanase complex from d 1 to 40.

Treatments	Enzyme, mg/kg[2]	BWG, g						
		1 – 7 d	8 – 14 d	15 – 21 d	22 – 28 d	29 – 35 d	36 – 40 d	1 – 40 d
Control (C)	-	120^{a}	296^{a}	524^{a}	657^{a}	851^{a}	707^{a}	3,155^{a}
C – 60 kcal/kg	-	118^{b}	282^{b}	513^{b}	641^{b}	842^{b}	701^{ab}	3,097^{c}
C – 90 kcal/kg	-	116^{b}	283^{b}	508^{c}	639^{bc}	827^{c}	695^{b}	3,068^{d}
C – 120 kcal/kg	-	113^{c}	285^{ab}	501^{d}	633^{c}	826^{c}	694^{b}	3,052^{e}
C – 120 kcal/kg	200	120^{a}	287^{ab}	513^{b}	637^{bc}	836^{b}	711^{a}	3,103^{bc}
C – 120 kcal/kg	300	120^{a}	292^{ab}	514^{b}	636^{bc}	837^{b}	712^{a}	3,111^{bc}
C – 120 kcal/kg	400	120^{a}	294^{ab}	513^{b}	639^{bc}	841^{b}	710^{a}	3,117^{b}
SEM[1]		0.70	1.22	1.70	2.36	3.36	3.14	6.21
P-value		0.0412	0.0042	0.0211	0.0171	0.0375	0.0493	0.0095
Treatments	Enzyme, mg/kg	Feed Intake, g						
		1 – 7 d	8 – 14 d	15 – 21 d	22 – 28 d	29 – 35 d	36 – 40 d	1 – 40 d
Control (C)	-	130	395	725	1,062	1,401	1,343	5,056
C – 60 kcal/kg	-	131	394	735	1,067	1,399	1,355	5,081
C – 90 kcal/kg	-	126	396	730	1,052	1,381	1,344	5,029
C – 120 kcal/kg	-	126	395	721	1,050	1,384	1,345	5,021
C – 120 kcal/kg	200	131	399	736	1,065	1,399	1,365	5,095
C – 120 kcal/kg	300	129	403	735	1,060	1,394	1,361	5,082
C – 120 kcal/kg	400	129	400	729	1,059	1,394	1,357	5,068
SEM		1.94	3.35	3.18	6.62	9.54	8.82	22.76
P-value		0.3431	0.2112	0.5791	0.9861	0.8586	0.9427	0.9999
Treatments	Enzyme, mg/kg	FCR[3]						
		1 – 7 d	8 – 14 d	15 – 21 d	22 – 28 d	29 – 35 d	36 – 40 d	1 – 40 d
Control (C)	-	1.080^{c}	1.336^{d}	1.383^{d}	1.617^{d}	1.646^{c}	1.900^{c}	1.602^{d}
C – 60 kcal/kg	-	1.114^{a}	1.396^{ab}	1.433^{ab}	1.665^{ab}	1.661^{ab}	1.933^{ab}	1.641^{ab}
C – 90 kcal/kg	-	1.087^{bc}	1.399^{a}	1.437^{ab}	1.646^{c}	1.670^{ab}	1.934^{ab}	1.639^{bc}
C – 120 kcal/kg	-	1.119^{a}	1.386^{b}	1.439^{a}	1.659^{abc}	1.676^{a}	1.938^{a}	1.645^{a}
C – 120 kcal/kg	200	1.096^{b}	1.392^{a}	1.434^{ab}	1.672^{a}	1.674^{a}	1.922^{b}	1.642^{ab}
C – 120 kcal/kg	300	1.098^{b}	1.381^{b}	1.430^{b}	1.667^{a}b	1.665^{ab}	1.911^{bc}	1.633^{c}
C – 120 kcal/kg	400	1.074^{c}	1.360^{c}	1.422^{c}	1.657^{abc}	1.657^{b}	1.911^{bc}	1.626^{cb}
SEM		0.004	0.003	0.003	0.009	0.004	0.006	0.003
P-value		0.0044	0.0001	0.0001	0.0006	0.0051	0.0437	0.0149

[a-e] Means within a column with no common superscript differ significantly ($p < 0.05$).

[1]Means of 10 replicates of 25 birds per treatment.

[2]α-amylase + β-glucanase complex is produced by *Bacillus amyloliquefaciens* having a minimum activity of 200 kilo-Novo α-amylase units and 350 fungal β-glucanase units per gram obtained from Novozymes A/S (Bagsvaerd, Denmark).

[3]Corrected for the weight of dead birds.

The use of α-amylase as part of an enzyme blend that also contained beta-glucanase and xylanase increased the AME content of a corn-soy broiler diet, as well as apparent and true ileal amino acid digestibility of all amino acids (Rutherfurd *et al.,* 2007). In the present study, field performance obtained with the diets with reduced energy and supplemented with an α-amylase-β-glucanase complex was improved, albeit not completely compensating for the performance losses induced by the reduction of 120 kcal/kg in a dietary feeding program fed to 40 d. The enzyme complex utilized in the current study has α-amylase and β-glucanase activities. Corn-soy diets have small contents of β-glucans, estimated as 0.1% in corn and 0.3% in soybean meal (Gracia *et al.,* 2003; Choct & Annison, 1990). However, even though improvements due the action of α-amylase on starch seem more likely because of the higher proportion of starch in

Table 4 – Regression equations of BWG and FCR as AME was reduced and relative AME equivalence estimates with the supplementation of an α-amylase + β-glucanase complex in the feeds from d 1 to 40.

Item	Regression Equations	r^2 [1]	*P*-value	Relative bioequivalence (kcal/kg AME_n)[2]		
				200 mg/kg	300 mg/kg	400 mg/kg
BWG, g						
1 to 7 d	Y = - 0.00005x + 0.1203	0.1851	0.0056	6	6	6
8 to 14 d	Y = - 0.00010x + 0.2931	0.1778	0.0067	9	11	61
15 to 21 d	Y = - 0.00019x + 0.5245	0.3109	0.0002	55	61	55
22 to 28 d	Y = - 0.00010x + 0.2931	0.2126	0.0028	95	85	100
29 to 35 d	Y = - 0.00010x + 0.2931	0.1138	0.0332	48	65	70
1 to 40 d	Y = - 0.00088x + 3,1523	0.4218	0.0001	40	46	56
FCR						
1 to 7 d	Y = 0.00025x + 1.0835	0.1128	0.0341	50	38	58
8 to 14 d	Y = 0.00046x + 1.3486	0.3063	0.0002	25	70	94
15 to 21 d	Y = 0.00048x + 1.3903	0.4643	0.0001	66	82	91
22 to 28 d	Y = 0.00031x + 1.6257	0.1732	0.0076	100	133	149
35 to 40 d	Y = 0.00032x + 1.9050	0.0983	0.0488	19	53	53
1 to 40 d	Y = 0.00039x + 1.6035	0.2891	0.0003	58	76	99

[1]Coefficient of determination (r^2) was obtained using data from all replicates.

[2]Determined based on response of the means to graded reduction of AME for each parameter. The relative bioequivalence was determined using the difference between the levels of AME (0, 60, 90 and 120 kcal/kg).

the compared with β-glucans, effects on the latter cannot be dismissed. Usually referred as having a highly negative impact on chicken performance due to their influence on gut content viscosity, β-glucans are usually considered an important anti-nutrient in diets containing rye, barley, or wheat. The contents of β-glucans (1.2% in rye, 7.6% in barley, and 0.7% in wheat) are low compared to their disproportional negative impact on poultry performance (Annison, 1991; Maisonnier *et al.,* 2001). Therefore, it possible that, despite in smaller proportions than in rye, barley and wheat, there may be a detectable impact of β-glucanase in corn-soy diets.

Since soybean meal is almost devoid of starch (Meng & Slominski, 2005), the bulk of the starch in the diets utilized in the current study derived from corn. Based on published data (De Blas *et al.,* 1999), the proportion of starch in the diets with 120 kcal AME/kg reduction

Table 5 – Carcass, abdominal fat, and commercial cuts yields of broilers fed diets with graded AME reductions and supplemented or not with an α-amylase + β-glucanase complex from d 1 to 40, %.

Treatments	Enzyme, mg/kg[2]	Carcass	Abdominal fat	Breast meat	Leg quarters	Wings
Control (C)		77.9	1.83[ab]	31.9	32.7	10.0
C – 60 kcal/kg	-	77.5	1.73[ab]	31.6	32.6	10.1
C – 90 kcal/kg	-	77.4	1.84[ab]	32.2	32.5	10.2
C – 120 kcal/kg	-	77.7	1.90[a]	32.0	32.3	10.2
C – 120 kcal/kg	200	77.6	1.79[ab]	32.2	32.3	10.1
C – 120 kcal/kg	300	77.6	1.79[ab]	32.0	32.5	10.2
C – 120 kcal/kg	400	77.4	1.67[b]	31.8	32.6	10.2
SEM[1]		0.110	0.019	0.086	0.063	0.026
P-value		0.8589	0.0346	0.4375	0.5509	0.2351

[a-b] Means with a column with no common superscript differ significantly ($p < 0.05$).

[1] Means of 10 replicates of 25 birds per treatment.

[2] α-amylase + β-glucanase complex is produced by *Bacillus amyloliquefaciens* having a minimum activity of 200 kilo-Novo α-amylase units and 350 fungal β-glucanase units per gram obtained from Novozymes A/S (Bagsvaerd, Denmark).

was estimated as increasing from 33.6% in the pre starter to 43.3% in the finisher diets. As shown in Table 3, improvements in performance were mostly evident as birds aged, indicating that the supplementation with the enzyme complex was more beneficial when birds were fed diets with higher proportions of starch. Starch digestibility was evaluated in the current trial; however, BWG and FCR consistently improved in birds fed the enzyme complex as starch increased as part of total daily intake. Therefore, improvements in starch digestibility and higher glucose availability, which was eventually utilized as energy, may have been generated by the α-amylase present in the enzymatic complex supplemented. Starch digestibility in poultry is typically considered high. For instance, Weurding *et al.* (2001) and Plavnik & Sklan (1995) found 97.4% and 97.3% starch digestibility using different evaluation methods. However, Noy & Sklan (1995) reported that cornstarch digestibility at the terminal ileum was as low as 85% and did not seem to increase with chicken age. Starch that escapes digestion in the small intestine, which is sometimes referred as resistant starch, may present an opportunity for the utilization of an exogenous α-amylase (Plavnik & Sklan, 1995). Chickens adapt well to starch-based diets (Svihus, 2011); therefore; the very high feed intake of the modern fast-growing broiler chickens may present physiological limitations for starch digestion, leaving part of the dietary starch undigested and available to react with a supplemental amylase.

Broiler performance improvements have been demonstrated with the use of many enzyme blends in a large range of environments and with feeds with very diverse ingredient composition. Based on the results of the present study, the dietary addition of the α-amylase-β-glucanase complex evaluated seems to be an alternative in the field of additives that may potentially improve energy utilization by broiler chickens.

CONCLUSIONS

The commercial α-amylase-β-glucanase complex tested in the present study had a beneficial impact on the BWG and FCR of broilers fed corn-soy diets. A reduction in abdominal fat was observed when 400 g/ton were supplemented to diets with 120 kcal AME/kg reduction. Apparent metabolizable energy estimates based on the improvements observed in BWG and FCR were of 49, 61, and 78 for 200, 300 and 400 g/ton of the enzyme complex, respectively.

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