Enzyme complex containing carbohydrases and phytase improves growth performance and bone mineralization of broilers fed reduced nutrient corn-soybean-based diets

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ABSTRACT One experiment was conducted to investigate the benefits of a multi-enzyme complex, containing carbohydrases (from *Penicillium funiculosum*) and phytase (bacterial 6-phytase) activities, on the performance and bone mineralization of broiler chickens fed corn-soybean meal diets. A total of 2,268 male broilers were allocated to 9 treatments, replicated 6 times, in a randomized complete block design from 1 to 43 d. A positive control (PC) diet formulated to be adequate in nutrients and 4 reduced nutrient diets (NC1 to NC4), with gradual decrease on AME, CP, and digestible amino acids (CP-dAA) and available P (avP) and Ca contents, with or without enzyme supplementation, were tested. The nutrient reductions applied were NC1 (−65 kcal/kg, −1.5% CP-dAA) and NC2 (−85 kcal/kg, −3.0% CP-dAA) both with −0.15 percent point avP and −0.12 percent point Ca and NC3 (−65 kcal/kg, −1.5% CP-dAA) and NC4 (−85 kcal/kg, −3.0% CP-dAA) both with −0.20 percent point avP and −0.16 percent point Ca. Supplementation of the NC diets with the enzyme complex increased ADFI ($P < 0.001$), ADG ($P < 0.001$), and reduced feed:gain ($P < 0.01$). The magnitude of the enzyme effect in increasing feed intake and weight gain was greater for the diets with greatest reductions in avP and Ca. Enzyme supplementation increased ($P < 0.001$) feed intake of birds fed on NC diets close to the level of feed consumption of the PC. Enzyme supplementation to NC diets resulted in all cases in lower ($P < 0.05$) feed:gain than the PC. Enzyme supplementation to NC1 and NC3 diets restored bone mineralization to that of the PC, whereas ash and Ca with NC2 and NC4 diets and P with NC4 diet remained lower ($P < 0.05$). These results suggest that the dietary supplementation with a multi-enzyme complex containing nonstarch polysaccharide enzymes and phytase is efficient in reducing the P, energy, protein, and amino acid specifications of corn-soybean meal diets.

Key words: nonstarch polysaccharide enzyme, phytase, broiler, formulation specification, growth

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INTRODUCTION

In commercial broiler production, combinations of different types of enzymes or multi-enzyme complexes are used to increase availability of nutrients and energy, especially combinations of different carbohydrases and phytase. The use of carbohydrases, targeted to hydrolyze soluble nonstarch polysaccharides (**NSP**), is widely and successfully implemented when viscous cereals (wheat, barley, rye, oats, or triticale) are used. Less extensive and more controversial is the use of enzymes in corn and soybean meal (**C-SBM**)-based diets, with the exception of phytase. Although both corn and soybean meal are considered highly digestible ingredients, there is some room for the improvement of their nutritional value (Zanella et al., 1999; Maisonnier-Grenier et al., 2004). Corn contains negligible amounts of soluble NSP, not yielding digesta viscosity problems, but it contains approximately 8% of insoluble NSP, mainly arabinoxylans (Choct, 2006). Soybean meal contains approximately 3% of soluble NSP and 16% of insoluble NSP (Irish and Balnave, 1993). Enzymes able to break down the cell wall matrix, especially the insoluble components, may facilitate the release of nutrients encapsulated in cell walls or incorporated into the cell wall itself, resulting in an easier access of digestive enzymes (Bedford, 1996; Cowieson, 2005; Choct, 2006). There is growing evidence suggesting that a combination of supplemental enzymes can improve the nutritional value of C-SBM-based diets. A summary of some published

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results presented by Cowieson (2005) reveals that the use of a combination of xylanase, protease, and amylase resulted in significant improvements in feed:gain (from 0.8 to 10.5%) and BW gain (from 1.9 to 6.9%). The same enzyme combination increased AME by 3% and nitrogen retention by 11.7% of both adequate and reduced energy and amino acid diets (Cowieson and Ravindran, 2008). Meng and Slominski (2005), using a multicarbohydrase preparation with a C-SBM diet, found an improvement in the total tract NSP digestibility, ileal protein digestibility, and AME_n. Also, a combination of xylanase, amylase, and glucanase increased dietary AME (by 2.2%) and true ileal amino acid digestibility for all amino acids (Rutherfurd et al., 2007). Such improvements in nutrient availability with carbohydrase supplementation often result in reduced feed intake, as a consequence of greater energy availability, leading to improved feed efficiency (Kocher et al., 2003).

The use of microbial phytase has increased remarkably since the early 90s and has become standard practice to reduce P levels in the environment and to compensate the growing cost of inorganic phosphates. The effects of phytase in poultry have recently been extensively reviewed by Selle and Ravindran (2007). They concluded that phytase increases P retention and tibia ash, but it has also positive effects on weight gain, feed intake, nitrogen retention, feed:gain, AME, Ca retention, and in reducing the endogenous loss.

Conversely to energy deficiency, which enhanced feed intake, P deficiency strongly reduces feed intake, which is restored by phytase supplementation. Most of the phytase effects result in restoring growth performance without much affecting feed efficiency. It thus appears interesting to investigate the combination of carbohydrases and phytase on their effect in feed intake, growth rate, and bone mineralization in broilers.

Moreover, there are 2 strategies to incorporate enzyme supplementation when formulating least-cost feeds for broilers. The formulation approach can be done either by reducing appropriately the nutrient specifications of the complete feed, assigning a nutritional value to the enzyme in the matrix based upon the main cereal used, or assigning specific ME improvements (uplift) to individual ingredients (Dalibard and Geraert, 2004). All approaches require accurate knowledge of which nutrients can be made more available by adding the enzyme to the diet (Shelton et al., 2004). However, the assessment of the nutrient specifications for the enzyme or the reduction in dietary nutrient concentration for a multi-enzyme complex, targeted for different substrates, with different modes of action, but sharing effects on performance and nutrient digestibility could be much more difficult to predict. Many factors can influence the response to an enzyme complex because it depends on the enzyme specificity, concentration of the substrate, doses of enzymes, interactions between enzymes, ingredient quality and type, level of nutrients in the diet, and age of animals (Bedford, 2002; Rosen, 2002; Cowieson and Adeola, 2005; Cowieson et al., 2006c).

The use of an enzyme complex containing carbohydrases and phytase should allow the reduction of dietary available P (**avP**) and energy, as well as CP, amino acids, and Ca concentrations in feed. Some attempts have been made to assess the magnitude of dietary nutrient reduction, which can be compensated by xylanase, amylase, protease, and phytase to maintain performance to that of the positive control, containing adequate nutrient concentrations (Cowieson et al., 2006b).

Another important point that has not yet received much attention is whether the use of carbohydrases and phytase in diets deficient in energy and digestible amino acids, in addition to reduced avP and Ca, would be able to restore bone mineralization to that of a positive control. It has been suggested that the P requirement for obtaining maximum mineral deposition in tibia ash is greater than the requirement needed for obtaining an optimal growth rate (Viveros et al., 2002).

The current study was designed to establish appropriate nutrient specifications for the application of a multi-enzyme complex, a combination of carbohydrases and phytase, in broiler chickens allocated on floor pens, fed pelleted C-SBM-based diets, from day-old to slaughter, focusing on performance and bone mineralization. The objective was also to identify the relative importance of nutritional characteristics (avP, ME, or digestible amino acids) in determining growth performance and bone mineralization in broilers.

MATERIALS AND METHODS

Enzyme

A multi-enzyme complex in liquid form was tested (Rovabio Max, Adisseo France SAS, Antony, France), containing carbohydrases produced from the fermentation of *Penicillium funiculosum* and bacterial 6-phytase (EC 3.1.3.2.6), derived from *Escherichia coli* and expressed in *Schizosaccharomyces pombe*. The multi-enzyme complex was applied after pelleting at a dose rate of 220 g/t of feed to provide a guarantee minimum of 1,100 visco-units of endo-β-1,4-xylanase, 100 azo-β-glucanase units of endo-1,3(4)-β-glucanase, and 500 phytase units (**FTU**)/kg of feed. One visco-unit of endo-1,4-β-xylanase activity is defined as the amount of enzyme reducing the viscosity of the solution, to give a change in relative fluidity of 1 dimensionless unit per minute per milliliter (or per gram) under the conditions of the assay (pH 5.5 and 30°C). One azo-β-glucanase unit of endo-1,3(4)-β-glucanase activity is defined as the amount of enzyme releasing oligomers, which are soluble in ethanol, to give an absorbance of 0.820 units at 590 nm under the conditions of the assay (20 min at pH 4.6 and 30°C). One FTU is defined as the amount of enzyme that liberates one micromole of inorganic orthophosphate from phytic acid per minute at pH 5.5 and 37°C (Engelen et al., 1994).

Diets and Experimental Design

The study was conducted as a 2 × 2 × 2 factorial arrangement of treatments [2 levels of AME, CP, and digestible essential amino acids (**CP-dAA**) reduction, 2 levels of avP and Ca reduction, with or without enzyme supplementation] plus a positive control. The positive control (**PC**) diet was formulated to be adequate or to exceed all nutrient requirements according to the Rhodimet Nutrition Guide (Adisseo France SAS, 2006). The reduced nutrient diets were **NC1** (−65 kcal/kg and −1.5% CP-dAA) and **NC2** (−85 kcal/kg and −3.0% CP-dAA), both with −0.15 percent point avP and −0.12 percent point Ca, and **NC3** (−65 kcal/kg and −1.5% CP-dAA) and **NC4** (−85 kcal/kg and −3.0% CP-dAA), both with −0.20 percent point avP and −0.16 percent point Ca. The PC diet provided 0.43 and 0.39%, NC1 and NC2 provided 0.28 and 0.24%, and NC3 and NC4 provided 0.23 and 0.19% of avP from 0 to 21 d and 22 to 43 d, respectively. Reduced nutrient diets were supplemented or not with Rovabio Max. There were a total of 9 dietary experimental treatments replicated 6 times each and allocated at random by blocks, according to the location in the experimental room. The feeding program consisted of 3 diets, starter diet supplied from 0 to 21 d, grower diet from 22 to 39 d, and withdrawal diet (same composition of grower diet without coccidiostat) from 40 to 43 d (Table 1). Celite was employed as a diluent in starter feeds. Feeds were supplied in pelleted form and the diameter of the pellets was 2.5 mm in starter, 3 mm in the grower, and 4 mm in the withdrawal diets, respectively. Enzyme was sprayed in liquid form after pelleting on to the cool pellets and at a dilution rate of 1/30. The anticoccidial program was as follows: 1 mg/kg of diclazuril from 0 to 21 d and 100 mg/kg of monensin from 22 to 39 d. The feed used in this trial did not contain any antibiotic growth promoter nor probiotic-prebiotic feed additives.

Phytase activity present in feeds was measured using the method of Engelen et al. (1994). Xylanase activity was measured with a dye-labeling method, using azo-xylan as substrate (Cosson et al., 1999).

Bird Housing and Management

Two thousand two hundred sixty 8-d-old male broiler (Ross 308) chicks were used and distributed into 54 floor pens of 4 m^2, at 42 chickens per pen. The house was a windowless house provided with artificial programmable lights, automated gas heating, and forced ventilation by depression. The temperature program was adjusted as follows: 0 to 2 d: 32 to 34°C; 3 to 7 d: 27 to 30°C; second week: 25 to 27°C; third week: 24 to 27°C; fourth week: 22 to 25°C. The lighting program was 23 h of light the first 4 d, 20 h of light until 10 d,

Table 1. Composition and calculated nutrient content of basal diets[1]

	0 to 21 d					22 to 42 d				
Item	PC	NC1	NC2	NC3	NC4	PC	NC1	NC2	NC3	NC4
Ingredients (%)										
Corn	57.5	59.0	58.9	59.0	58.9	61.0	64.9	66.4	65.4	66.8
Soybean meal, 48% CP	28.2	26.2	25.7	26.2	25.7	21.5	20.3	19.4	20.2	19.4
Full-fat extruded soybeans	8.60	10.00	9.80	10.00	9.80	9.00	9.00	9.00	9.00	9.00
Soybean oil	1.30	0.00	0.00	0.00	0.00	4.70	2.63	1.98	2.45	1.83
Calcium carbonate	1.00	1.25	1.26	1.33	1.33	1.00	1.26	1.26	1.34	1.34
Dicalcium phosphate	2.10	1.20	1.20	0.90	0.90	1.90	1.00	1.00	0.70	0.70
Salt	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30
Celite	0.00	1.05	1.84	1.27	2.07	—	—	—	—	—
Minerals and vitamins[2]	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40
DL-Methionine 99	0.35	0.35	0.35	0.35	0.35	0.19	0.19	0.19	0.19	0.18
L-Lysine HCl 98	0.25	0.25	0.25	0.25	0.25	0.05	0.05	0.05	0.05	0.05
Calculated nutrients[3]										
AME (kcal/kg)	3,006	2,937	2,915	2,937	2,915	3,204	3,142	3,120	3,141	3,120
CP (%)	21.1	20.8	20.5	20.8	20.5	18.1	17.8	17.5	17.8	17.5
Ether extract (%)	5.55	4.54	4.49	4.54	4.49	9.01	7.10	6.50	6.94	6.37
Ash (%)	5.67	5.20	5.17	5.34	5.30	5.17	4.71	4.67	4.56	4.82
Digestible lysine (%)	1.19	1.17	1.16	1.17	1.16	0.88	0.86	0.84	0.87	0.84
Digestible methionine (%)	0.65	0.65	0.65	0.65	0.65	0.45	0.45	0.45	0.45	0.45
Digestible methionine + cystine (%)	0.94	0.93	0.92	0.93	0.92	0.71	0.70	0.70	0.70	0.70
Digestible threonine (%)	0.70	0.69	0.68	0.69	0.68	0.61	0.60	0.59	0.60	0.59
Total Ca (%)	1.01	0.89	0.89	0.85	0.85	0.94	0.82	0.82	0.78	0.78
Total P (%)	0.74	0.58	0.58	0.53	0.52	0.68	0.52	0.52	0.46	0.46
Available P (%)	0.43	0.28	0.28	0.23	0.23	0.39	0.24	0.24	0.19	0.19

[1]PC = positive control; NC1 = −65 kcal/kg, −1.5% CP and digestible amino acids (CP-dAA), −0.15 percent point available P (avP), −0.12 percent point Ca; NC2 = −85 kcal/kg, −3.0% CP-dAA, −0.15 percent point avP, −0.12 percent point Ca; NC3 = −65 kcal/kg, −1.5% CP-dAA, −0.20 percent point avP, −0.16 percent point Ca; NC4 = −85 kcal/kg, −3.0% CP-dAA, −0.20 percent point avP, −0.16 percent point Ca.

[2]One kilogram of feed contains: vitamin A (all-*trans*-retinol), 12,000 IU; cholecalciferol, 60 μg; vitamin E (DL-α-tocopheryl acetate), 30 IU; menadione, 3 mg; thiamine, 2.2 mg; riboflavin, 8 mg; pyridoxine, 5 mg; cyanocobalamin, 11 μg; folic acid, 1.5 mg; biotin, 150 μg; calcium pantothenate, 25 mg; nicotinic acid, 65 mg; Mn, 60 mg; Zn, 40 mg; I, 0.33 mg; Fe, 80 mg; Cu, 8 mg; Se, 0.15 mg; and ethoxyquin, 150 mg.

[3]Based on tabulated values (INRA, 2002).

and 18 h of light afterward. Feed and water were provided ad libitum throughout the experiment.

Growth Performance

Chicks and feeds were weighed by pen at 21 and 42 d. Average daily weight gain, average ADFI, and feed:gain were calculated for the periods 0 to 21 d, 22 to 42 d, and for the overall experiment. Mortality was recorded daily. Production index was calculated with the equation

$$\text{Production index} = \{[\text{daily weight gain} \times (100 - \text{mortality})]/(10 \times \text{feed conversion})\}.$$

Bone Mineralization

At 21 and 43 d, 12 chickens per treatment (2 chickens per pen) were randomly selected and killed by cervical dislocation. To determine the percentage of tibia ash, the right leg was removed as drumstick and autoclaved, following the procedure described by Hall et al. (2003). After this, ash, total P (AOAC, 2000), and Ca concentration (by atomic absorption spectrophotometry after ashing the samples at 550°C for 5 h) were measured.

The experimental procedures used in the research were approved by the Animal Ethics Committee of the Institute for Food and Agricultural Research and Technology and were in compliance with the Spanish guidelines for the care and use of animals in research (Boletin Oficial del Estado number 252, October 21, 2005).

Statistical Analysis

To establish differences between PC and reduced nutrient diets, with and without enzyme supplementation, data were analyzed as a randomized complete block design with a 1-way ANOVA, by using the GLM procedure of SAS (SAS Institute, 2001), using pen as the experimental unit. These differences were compared by a set of contrasts. To determine the main effects of type and level of nutrient reduction and enzyme supplementation response and their interactions, data without the PC group were analyzed by a $2 \times 2 \times 2$ factorial analysis of the variance. Angular transformation was used to analyze data expressed as percentages. Data were assumed to be statistically significant when $P \leq 0.05$.

RESULTS

In-feed measured xylanase (expressed as grams of product/tonne of feed) and phytase (expressed as FTU/kg of feed) is shown in Table 2. Xylanase mean recovery averaged 101%, ranging from 76 to 114% of the intended value. Phytase mean recovery was 525 FTU/kg (105%) and variation within batches ranged from 83 to 120%. These recoveries were within acceptable limits, taking into account the relative SD of the analysis and the errors introduced by enzyme application and sampling.

The effect of diets and enzyme complex on performance from 0 to 21 d is presented in Table 3. All nutrient reduced diets decreased ($P < 0.01$) ADG compared with the PC diet. Feeding NC1, NC3, and NC4 diets reduced ($P < 0.05$) ADFI. Moreover, NC1, NC2, and NC4 diets impaired ($P < 0.05$) feed:gain. With enzyme supplementation, NC1 and NC2 diets resulted in a greater ($P < 0.05$), and NC3 and NC4 diets in a similar, weight gain to that of the PC diet. There were no significant differences in ADFI and feed:gain of birds fed reduced nutrient diets with supplemental enzyme and birds fed the PC. There were avP and Ca reduction × enzyme interactions ($P < 0.001$) on weight gain and feed intake. Birds fed NC1 and NC2 diets grew faster and ate more ($P < 0.001$) than those fed NC3 and NC4 diets. Enzyme supplementation increased ($P < 0.001$) ADG and ADFI in all cases, and the magnitude of the effects was greater with the most reduced avP and Ca nutrient diets. Performance of birds was unaffected by the level of AME and CP-dAA reduction.

From 22 to 42 d (Table 4), weight gain of birds fed NC3 and NC4 diets was lower ($P < 0.001$) than those fed the PC diet. With enzyme complex, there were no significant differences in the growth of birds fed reduced nutrient diets and PC diet. Feed intake of birds fed reduced nutrient diets were lower compared with PC ($P < 0.05$). With enzyme supplementation, feed intake of birds fed NC1 and NC3 diets was lower that of birds fed PC ($P < 0.05$). Overall, NC1, NC2, and NC3 diets resulted in a better ($P < 0.01$), and NC4 in a similar, feed:gain compared with PC and supplemental enzyme

Table 2. Recovery of xylanase and phytase activities in the experimental diets[1]

Item[2]		Xylanase[3] (g/t)	Phytase (units/kg)
Starter feeds	NC1	202	417
	NC2	200	476
	NC3	166	464
	NC4	226	505
Grower	NC1	250	511
	NC2	245	590
	NC3	223	540
	NC4	240	545
Withdrawal	NC1	221	539
	NC2	226	545
	NC3	240	600
	NC4	221	567

[1]The enzyme complex was added at 220 g/t of feed to provide a guaranteed minimum of 1,100 xylanase viscosimetric units and 500 phytase units/kg of feed.

[2]NC1 = −65 kcal/kg, −1.5% CP and digestible amino acids (CP-dAA), −0.15 percent point available P (avP), −0.12 percent point Ca; NC2 = −85 kcal/kg, −3.0% CP-dAA, −0.15 percent point avP, −0.12 percent point Ca; NC3 = −65 kcal/kg, −1.5% CP-dAA, −0.20 percent point avP, −0.16 percent point Ca; NC4 = −85 kcal/kg, −3.0% CP-dAA, −0.20 percent point avP, −0.16 percent point Ca.

[3]Xylanase activity is expressed as the quantity of product added in the feed.

did not affect it. There were avP and Ca reduction level × enzyme interactions on weight gain ($P < 0.001$), feed intake ($P < 0.001$), and mortality ($P < 0.05$). The growth rate and feed intake were higher in birds fed NC1 and NC2 compared with diets with a high reduction on avP and Ca, but supplemental enzyme greatly increased weight gain and feed intake of those fed NC3 and NC4 diets, by 9.2 g/d and 15.7 g/d for ADG and by 18.3 g/d and 25.4 g/d for ADFI, respectively. Mortality rate was higher ($P < 0.05$) in birds fed NC3 and NC4 diets, compared with PC, but there were no differences between groups with enzyme supplementation. Enzyme complex reduced mortality of birds fed NC4 diet from 6.9 to 0%.

The effects of treatments on the performance of birds over the entire trial period (0 to 42 d) are shown in Table 5. The growth rate and the final BW of birds fed NC1 and NC2 diets was not different from that of birds fed PC diet. Further avP and Ca nutrient reduction resulted in a lower ($P < 0.001$) weight gain, by 13% for NC3 and 16% for NC4 diet, and final BW compared with the PC. With enzyme supplementation, there were no significant differences in final BW and growth rate of birds fed on NC diets or on PC. Feed intake of birds fed on NC diets without enzyme supplementation was lower ($P < 0.05$) compared with PC, whereas fed intake of birds fed NC diets supplemented with enzyme was similar to that of PC. Without supplemental enzyme, feed:gain of birds fed on NC1, NC2, and NC4 was similar to that of PC, whereas feed:gain of birds fed on NC3 was better ($P < 0.05$) than that of the PC. Enzyme supplementation to NC diets resulted in all cases in better ($P < 0.05$) feed:gain than the PC. Mortality of birds fed NC3 and NC4 diets was higher ($P < 0.05$) compared with the PC (5.3 and 8.1% vs. 1.8%). Production index of birds fed NC3 and NC4 diets was lower than that of the PC. Enzyme supplementation to NC4 diet resulted in a higher ($P < 0.05$) production index compared with the PC. There were avP and Ca reduction level × enzyme interactions on final BW ($P < 0.001$), weight gain ($P < 0.001$), feed intake ($P < 0.001$), mortality ($P < 0.01$), and production index ($P < 0.001$). The magnitude of the enzyme effect in increasing ADG, ADFI, and productivity index was greater for the diets with the greatest reductions in avP and Ca (NC3 and NC4). Mortality was higher ($P < 0.05$) in birds fed NC3 and NC4 diets compared with those fed NC1. Enzyme supplementation reduced ($P < 0.05$) mortality from 8.1 to 0.4% in birds fed NC4 diet. There were also AME and CP-dAA reduction level × enzyme interactions on mortality ($P < 0.01$) and production index ($P < 0.05$).

Table 3. Effect of dietary nutrient reduction and enzyme supplementation on the performance of broilers (0 to 21 d)[1]

Item	Enzyme (g/t)	ADG (g/d)	ADFI (g/d)	Feed:gain (g/g)	Mortality (%)
Basal diet[2]					
PC	—	43.8	58.2	1.329	0.74
NC1	—	40.6	55.6	1.370	0.73
NC1	220	45.6	60.4	1.324	2.83
NC2	—	40.7	55.7	1.368	0.37
NC2	220	45.6	60.4	1.325	1.48
NC3	—	35.2	47.8	1.361	0.63
NC3	220	44.1	58.7	1.331	1.10
NC4	—	35.0	49.0	1.398	1.84
NC4	220	45.1	60.3	1.337	0.36
Pooled SEM		0.65	0.89	0.0116	0.712
P-value of contrast					
PC vs. NC1 no enzyme		**	*	*	NS
PC vs. NC2 no enzyme		**	†	*	NS
PC vs. NC3 no enzyme		***	***	†	NS
PC vs. NC4 no enzyme		***	***	***	NS
PC vs. NC1 enzyme		*	†	NS	†
PC vs. NC2 enzyme		*	†	NS	NS
PC vs. NC3 enzyme		NS	NS	NS	NS
PC vs. NC4 enzyme		NS	†	NS	NS
P-value of factorial analysis[3]					
EPA reduction[4]		NS	NS	NS	NS
PCA reduction[5]		***	***	NS	NS
Enzyme		***	***	***	NS
EPA × PCA		NS	NS	NS	NS
EPA × enzyme		NS	NS	NS	NS
PCA × enzyme		***	***	NS	NS
EPA × PCA × enzyme		NS	NS	NS	NS

[1]Data are means of 6 pens of 42 chickens.

[2]PC = positive control; NC1 = −65 kcal/kg, −1.5% CP and digestible amino acids (CP-dAA), −0.15 percent point available P (avP), −0.12 percent point Ca; NC2 = −85 kcal/kg, −3.0% CP-dAA, −0.15 percent point avP, −0.12 percent point Ca; NC3 = −65 kcal/kg, −1.5% CP-dAA, −0.20 percent point avP, −0.16 percent point Ca; NC4 = −85 kcal/kg, −3.0% CP-dAA, −0.20 percent point avP, −0.16 percent point Ca.

[3]Using negative control treatments with or without enzyme supplementation.

[4]EPA = AME, CP, and digestible amino acid reduction.

[5]PCA = available P and Ca reduction.

†$P < 0.1$; *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$; NS: $P > 0.1$.

Bone mineralization response is presented in Table 6. On d 21 and 43, tibia ash, P, and Ca concentrations of birds fed NC diets were lower ($P < 0.05$) compared with birds fed PC, except tibia P concentration of birds fed NC1 at 43 d ($P < 0.1$). Enzyme addition to NC1 and NC2 diets resulted in bone mineralization at 21 d that was not different ($P > 0.05$) from that of chickens fed the PC, whereas bone mineralization of birds fed NC3- and NC4-supplemented diets remained lower ($P < 0.05$). At 43 d, enzyme supplementation to NC1 and NC3 diets resulted in bone mineralization similar to that of the PC, whereas ash and Ca with NC2 and NC4 diets and P with NC4 diet remained lower ($P < 0.05$). There were avP and Ca reduction level × enzyme interactions on tibia ash ($P < 0.05$), P ($P < 0.01$), and Ca ($P < 0.05$) contents at 21 d and tibia ash, P, and Ca at 43 d ($P < 0.01$). Tibia ash, P, and Ca were higher in birds fed diets with the lowest reduction on avP and Ca (NC1 and NC2) compared with birds fed the highest reduction (NC3 and NC4 diets), at both ages. Enzyme supplementation increased ash, P, and Ca concentrations, but the greatest response to enzyme addition was obtained with the lowest dietary avP and Ca levels. At 43 d, there were energy and CP-dAA reduction level × enzyme interactions ($P < 0.001$) on ash and Ca concentrations.

DISCUSSION

The purpose of this experiment was to establish adequate nutrient specifications for the application of a multi-enzyme complex, a combination of carbohydrases and phytase, in broiler chickens fed on C-SBM-based diets. The responses were ADG, ADFI, feed:gain, mortality, and bone mineralization. A PC diet, containing adequate nutrient concentrations, and 4 NC diets, reduced by 65 kcal/kg and 1.5% CP-dAA or 85 kcal/kg and 3.0% CP-dAA, combined with −0.15 percent point avP and −0.12 percent point of Ca or −0.20 percent point avP and −0.16 percent point of Ca, were tested. These diets were supplied to the birds, with or without enzyme addition, to estimate which nutrients can be made more available by adding the enzyme complex to the diet.

From 0 to 21 d, both levels of dietary nutrient reduction decreased feed intake and weight gain; however, the highest reduction was obtained in birds fed diets with the lowest level of avP (0.23%) and Ca (0.85%), by 16.8 and 19.0% for ADFI and ADG, respectively. No differences between the reductions by 65 kcal/kg and 1.5% CP-dAA or 85 kcal/kg and 3.0% CP-dAA were detected, at both levels of avP and Ca tested. This might suggest that during this period, the avP

Table 4. Effect of dietary nutrient reduction and enzyme supplementation on the performance of broilers (22 to 42 d)[1]

Item	Enzyme (g/t)	ADG (g/d)	ADFI (g/d)	Feed:gain (g/g)	Mortality (%)
Basal diet[2]					
PC	—	96.9	186.7	1.928	1.19
NC1	—	96.2	179.0	1.861	0.79
NC1	220	94.7	178.8	1.889	2.74
NC2	—	96.6	179.2	1.857	3.18
NC2	220	96.3	180.8	1.878	0.79
NC3	—	87.5	160.9	1.841	4.98
NC3	220	96.7	179.2	1.855	2.34
NC4	—	83.2	157.0	1.888	6.86
NC4	220	98.9	182.4	1.845	0.00
Pooled SEM		1.76	2.27	0.0166	1.164
P-value of contrast					
PC vs. NC1 no enzyme		NS	*	**	NS
PC vs. NC2 no enzyme		NS	*	**	NS
PC vs. NC3 no enzyme		***	***	***	*
PC vs. NC4 no enzyme		***	***	NS	*
PC vs. NC1 enzyme		NS	*	NS	NS
PC vs. NC2 enzyme		NS	†	*	NS
PC vs. NC3 enzyme		NS	*	**	NS
PC vs. NC4 enzyme		NS	NS	**	NS
P-value of factorial analysis[3]					
EPA reduction[4]		NS	NS	NS	NS
PCA reduction[5]		**	***	NS	†
Enzyme		***	***	NS	*
EPA × PCA		NS	NS	NS	NS
EPA × enzyme		NS	NS	NS	*
PCA × enzyme		***	***	NS	*
EPA × PCA × enzyme		NS	NS	NS	NS

[1]Data are means of 6 pens of 40 chickens.

[2]PC = positive control; NC1 = −65 kcal/kg, −1.5% CP and digestible amino acids (CP-dAA), −0.15 percent point available P (avP), −0.12 percent point Ca; NC2 = −85 kcal/kg, −3.0% CP-dAA, −0.15 percent point avP, −0.12 percent point Ca; NC3 = −65 kcal/kg, −1.5% CP-dAA, −0.20 percent point avP, −0.16 percent point Ca; NC4 = −85 kcal/kg, −3.0% CP-dAA, −0.20 percent point avP, −0.16 percent point Ca.

[3]Using negative control treatments with or without enzyme supplementation.

[4]EPA = AME, CP, and digestible amino acid reduction.

[5]PCA = available P and Ca reduction.

†$P < 0.1$; *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$; NS: $P > 0.1$.

Table 5. Effect of dietary nutrient reduction and enzyme supplementation on the performance of broilers (0 to 42 d)[1]

Item	Enzyme (g/t)	42-d BW (g)	ADG (g/d)	ADFI (g/d)	Feed:gain (g/g)	Mortality (%)	Production index[2]
Basal diet[3]							
PC	—	2,992	70.3	121.8	1.733	1.84	399
NC1	—	2,912	68.4	116.9	1.709	1.47	395
NC1	220	2,986	70.2	118.8	1.694	5.32	393
NC2	—	2,923	68.7	116.9	1.704	3.34	390
NC2	220	3,019	71.0	120.0	1.692	2.22	410
NC3	—	2,614	61.3	103.8	1.693	5.25	344
NC3	220	2,997	70.4	118.4	1.681	3.27	406
NC4	—	2,523	59.1	102.3	1.731	8.08	315
NC4	220	3,062	72.0	120.9	1.679	0.36	427
Pooled SEM		41.2	0.98	1.30	0.0115	1.359	10.2
P-value of contrast							
PC vs. NC1 no enzyme		NS	NS	*	NS	NS	NS
PC vs. NC2 no enzyme		NS	NS	*	†	NS	NS
PC vs. NC3 no enzyme		***	***	***	*	*	***
PC vs. NC4 no enzyme		***	***	***	NS	*	***
PC vs. NC1 enzyme		NS	NS	NS	*	†	NS
PC vs. NC2 enzyme		NS	NS	NS	*	NS	NS
PC vs. NC3 enzyme		NS	NS	†	**	NS	NS
PC vs. NC4 enzyme		NS	NS	NS	**	NS	*
P-value of factorial analysis[4]							
EPA reduction[5]		NS	NS	NS	NS	NS	NS
PCA reduction[6]		***	***	***	NS	NS	**
Enzyme		***	***	***	**	NS	***
EPA × PCA		NS	NS	NS	NS	NS	NS
EPA × enzyme		NS	NS	NS	NS	**	*
PCA × enzyme		***	***	***	NS	**	***
EPA × PCA × enzyme		NS	NS	NS	NS	NS	NS

[1]Data are means of 6 pens of 42 (0 to 21 d) and 40 chickens (from 22 to 42 d).

[2]Production index = {[daily weight gain × (100 − mortality)]/(10 × feed conversion)}.

[3]PC = positive control; NC1 = −65 kcal/kg, −1.5% CP and digestible amino acids (CP-dAA), −0.15 percent point available P (avP), −0.12 percent point Ca; NC2 = −85 kcal/kg, −3.0% CP-dAA, −0.15 percent point avP, −0.12 percent point Ca; NC3 = −65 kcal/kg, −1.5% CP-dAA, −0.20 percent point avP, −0.16 percent point Ca; NC4 = −85 kcal/kg, −3.0% CP-dAA, −0.20 percent point avP, −0.16 percent point Ca.

[4]Using negative control treatments with or without enzyme supplementation.

[5]EPA = AME, CP, and digestible amino acid reduction.

[6]PCA = available P and Ca reduction.

†$P < 0.1$; *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$, NS: $P > 0.1$.

level, or both avP and Ca levels, were the first limiting nutrient, rather than energy or protein. It is well documented that P deficiency results in feed intake reduction (Kornegay, 1996; Sebastian et al., 1997; Viveros et al., 2002; Cowieson et al., 2006a), but in the present experiment, the reduction on feed intake was also accompanied by a general impairment of the feed:gain, by 3.4% on average. This impairment could be attributed to the dietary energy and CP-dAA dilution applied or to the negative effect of the phytate P on AME, on apparent ileal protein and amino acid digestibility, and on endogenous amino acid flow (Ravindran et al., 2006; Cowieson and Ravindran, 2007).

From 22 to 42 d, NC1 and NC2 diets, containing 0.24% of avP and 0.82% of Ca, with reduced energy (65 to 85 kcal/kg) and CP-dAA (1.5 to 3%) gave similar ADG, lower ADFI, and better feed:gain than the PC diet, suggesting that 0.24% of avP combined with 0.82% of Ca was not limiting for growth, neither the AME nor CP-dAA contents, and also suggesting that birds were able to compensate for the early feed intake restriction due to the avP deficiency during the first 3 wk of the growth period. Further reduction to 0.19% of avP and 0.78% of Ca resulted in lower ADG and reduced ADFI, as well as in an increase of mortality rate. Dhandu and Angel (2003) reported that the nonphytate P (**nPP**) requirement for broilers, based on tibia ash measurements, was 0.20% from 1.5 to 2.2 kg of BW and 0.16% from 2.4 up to 3.0 kg. Yan et al. (2001) estimated that nPP requirement from 21 to 42 d to optimize growth rate was 0.19%. In the present experiment, 0.19% of avP (corresponding to 0.24% of nPP) from 22 to 42 d compromised growth. The values reported in the mentioned studies were derived from birds fed starter diets that met or exceeded the P requirements of the NRC (1994), allowing a good reservoir of P in bones.

At both starter and grower periods, no significant differences were detected between the 2 levels of energy and CP-dAA reduction tested; only significant differences between the 2 levels of avP and Ca could be detected. This might indicate that without enzyme supplementation, avP and Ca reduction was more critical on performance than the energy and CP-dAA reduction.

Over the entire trial period, birds fed NC diets supplemented with the multi-enzyme complex had similar growth rate and better feed:gain compared with birds fed PC diet. Diets NC1 and NC2 were formulated to

provide 65 to 85 kcal/kg, 1.5 to 3% CP-dAA, 0.15% avP, and 0.12% Ca less than the PC. Enzyme complex increased ADFI and ADG and improved feed:gain, and the strongest effects (23 and 27% increased ADFI and ADG, respectively) were observed with the lowest avP and Ca levels (0.23 to 0.19% avP and 0.85 to 0.78% Ca from 0 to 21 d and 22 to 42d, respectively). This might indicate that the first limiting nutrient was P and once the P deficiency was overcome by phytase and feed intake was restored, the carbohydrases could increase the nutritive value of diet compensating for the reduction in AME (by 2.8%) and CP-dAA (by 1.5 to 3.0%). Some reports also suggested that carbohydrases, which are able to break down the cell wall NSP matrix, can facilitate the access of phytase to phytate molecule (Olukosi et al., 2007), supporting the hypothesis that the use of a combination of carbohydrases and phytase can fully strengthen their effects. The beneficial effect of the use of carbohydrases in C-SBM diets is less documented in the literature than the use of enzymes in viscous cereals. Zanella et al. (1999) found that a combination of xylanase, protease, and α-amylase allowed restoring a reduction of 3.4% in dietary AME in broilers fed C-SBM diet from 0 to 45 d. Yu and Chung (2004) found similar results from 0 to 39 d. Recently, Cowieson and Ravindran (2008) reported that a combination of xylanase, amylase, and protease was able to increase the availability of energy by 3%, the apparent N retention by 11.7%, and the apparent ileal digestibility of amino acids, from 0.44% for methionine to 9.1% for cystine, irrespective of the dietary nutrient density. The mode of action of the supplemental enzymes in C-SBM diets has been linked to the disruption of the cell wall matrix, facilitating the release of encapsulated nutrients and the digestive enzyme access, and also to the modification of the intestinal microbiota communities (Bedford, 1996). Moreover, phytase alone might improve dietary AME, apparent amino acid digestibility, apparent CP digestibility, and mineral absorption of reduced nutrient C-SBM diets, according to Santos et al. (2008). Similar responses to those obtained in the present experiment were recorded by Cowieson et al. (2006b) by using a combination of xylanase, amylase, protease and phytase to marginal diets in terms of energy (49 to 155 kcal/kg,), amino acids (1 to 2%), Ca (−0.12%), and avP (−0.13%). Enzyme addition restored performance

Table 6. Effect of dietary nutrient reduction and enzyme supplementation on the bone mineralization of broilers[1]

		Day 21			Day 43		
Item	Enzyme (g/t)	Ash (%)	P (% of DM)	Ca (% of DM)	Ash (%)	P (% of DM)	Ca (% of DM)
Basal diet[2]							
PC	—	49.2	8.33	17.4	44.2	7.49	15.8
NC1	—	45.6	7.39	16.1	42.0	7.18	14.9
NC1	220	48.7	8.19	17.2	43.4	7.32	15.4
NC2	—	46.0	7.50	16.2	42.2	7.08	15.0
NC2	220	48.0	8.04	16.9	42.4	7.17	15.1
NC3	—	43.0	6.83	15.2	38.5	6.47	13.7
NC3	220	47.1	7.90	16.6	44.1	7.38	15.7
NC4	—	42.8	6.89	15.1	40.4	6.78	14.3
NC4	220	47.3	7.96	16.7	42.1	7.14	15.0
Pooled SEM		0.53	0.11	0.20	0.61	0.14	0.22
P-value of contrast							
PC vs. NC1 no enzyme		***	***	***	*	†	**
PC vs. NC2 no enzyme		***	***	***	*	*	*
PC vs. NC3 no enzyme		***	***	***	***	***	***
PC vs. NC4 no enzyme		***	***	***	***	***	***
PC vs. NC1 enzyme		NS	NS	NS	NS	NS	NS
PC vs. NC2 enzyme		NS	†	NS	*	†	*
PC vs. NC3 enzyme		**	**	**	NS	NS	NS
PC vs. NC4 enzyme		*	*	*	*	*	*
P-value of factorial analysis[3]							
EPA reduction[4]		NS	NS	NS	NS	NS	NS
PCA reduction[5]		***	***	***	**	**	**
Enzyme		***	***	***	***	***	***
EPA × PCA		NS	NS	NS	NS	NS	NS
EPA × enzyme		NS	NS	NS	**	†	**
PCA × enzyme		*	**	*	**	**	**
EPA × PCA × enzyme		NS	NS	NS	†	NS	NS

[1]Data are least squares means of 12 birds per treatment (2 birds per pen).

[2]PC = positive control; NC1 = −65 kcal/kg, −1.5% CP and digestible amino acids (CP-dAA), −0.15 percent point available P (avP), −0.12 percent point Ca; NC2 = −85 kcal/kg, −3.0% CP-dAA, −0.15 percent point avP, −0.12 percent point Ca; NC3 = −65 kcal/kg, −1.5% CP-dAA, −0.20 percent point avP, −0.16 percent point Ca; NC4 = −85 kcal/kg, −3.0% CP-dAA, −0.20 percent point avP, −0.16 percent point Ca.

[3]Using negative control treatments with or without enzyme supplementation.

[4]EPA = AME, CP, and digestible amino acid reduction.

[5]PCA = available P and Ca reduction.

†$P < 0.1$; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$, NS: $P > 0.1$.

of birds fed NC diets to that of the PC. Considering growth performance, the first limiting nutrient appears to be the avP through its main effect on feed intake. Conversely, energy and amino acids appeared secondary and even a decrease by 85 kcal of AME and 3% digestible AA did not affect growth rate beyond the avP and Ca effect.

The dietary nutrient reduction resulted in decreased bone mineralization, in terms of tibia ash, P, and Ca contents, compared with the adequate nutrient diet. This reduction was related to the level of avP and Ca, as well as to the level of energy and protein, and age of birds.

At 21 d, enzyme supplementation increased bone mineralization of birds fed all reduced nutrient diets, in a greater extent with the most reduced avP and Ca content diets. When the reductions of avP and Ca were by −0.15 percent point and −0.12 percent point, respectively, enzyme supplementation resulted in a similar bone mineralization to that of the PC. With further reduction on avP and Ca contents (−0.20 percent point avP, −0.16 percent point Ca), supplemental enzyme restored performance, but it was not enough to restore bone mineralization to that of the PC. This might indicate that the dose of phytase used (500 FTU/kg) or the phytate P content in the diet could be insufficient to release the required quantity of P to compensate the −0.20 percent point of avP reduction for a complete bone mineralization. It has been suggested that the nPP requirement for obtaining a maximum mineral deposition in tibia ash is greater than the requirement for obtaining an optimal growth (Yan et al., 2001; Viveros et al., 2002; Persia and Saylor, 2006). Some authors have also reported that the P equivalency of phytase was lower when tibia ash was taken as a response criteria relative to weight gain, both in chickens (Adedokun et al., 2004) and turkeys (Esteve et al., 2005). Moreover, Selle and Ravindran (2007) reported that the collective P equivalency value for phytase was 840 FTU/kg ≡ 1.0 g/kg of inorganic P, depending on dietary concentration of phytate P, in particular, and Ca and nPP and phytase inclusion. In addition, as in the present experiment, Onyango et al. (2005) found that phytase supplementation to low-P diets restored growth rate to that of an adequate P diet, but tibia ash or toe ash remained lower.

Bone mineralization at 21 and 43 d was affected differently by dietary nutrient reduction and enzyme supplementation. Whereas at 21 d, energy and CP-dAA did not affect bone mineralization and enzyme addition restored it to that of PC with the lower reduction on avP and Ca contents, at 43 d, enzyme supplementation increased bone mineralization to the level of PC in birds fed diets reduced in −65 kcal/kg and −1.5% CP-dAA, irrespective of the avP and Ca reduction. Birds fed the most reduced energy and CP-dAA diets (−85 kcal/kg and −3%, respectively), supplemented with enzyme, did not reach the bone mineralization of the PC. It could be hypothesized that not only avP and Ca levels affected bone mineralization, but also the amino acid supply may be through the protein matrix required to sustain optimum growth of the medullary bone matrix.

In summary, results of the present experiment indicate that the use of a multi-enzyme complex containing xylanase, β-glucanase, and phytase as main activities allows the reduction of the AME, CP-dAA, avP, and Ca contents of a C-SBM diet without penalizing performance, but not always restoring fully the bone mineralization at a very low level of avP formulation. The need for an optimum amino acid supply under limited avP and Ca feeding requires further understanding to be fully accounted for in feed formulation. The antagonistic type of response between dietary supplementation with phytase, which restores feed intake reduced by low avP and Ca levels, and the reduced amount of feed consumed with carbohydrases needs to be completely understood to fully benefit from simultaneous enzyme supplementation. The potential synergy between carbohydrases and phytase as well as the modes of action of both types of enzymes need also to be further investigated. How carbohydrases can help the action of phytase and conversely will indeed depend on the sites of action of both enzymes.

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