Contribution of protein, starch, and fat to the apparent ileal digestible energy of corn- and wheat-based broiler diets in response to exogenous xylanase and amylase without or with protease

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ABSTRACT The ileal energy contribution of protein, starch, and fat in response to 2 exogenous enzyme combinations was studied in 2 digestibility assays with 21- (experiment 1; 432 birds) and 42-d-old (experiment 2; 288 birds) Ross 308 broiler chickens. A 2 × 2 × 3 factorial arrangement of treatments with 2 base grains (corn or wheat), without or with high fiber ingredients (corn distillers dried grains with solubles and canola meal), and 3 enzyme treatments was implemented. Enzyme treatments, fed from 12 to 21 d or 32 to 42 d, were 1) without enzymes, 2) with xylanase from *Trichoderma ressei* (2,000 U/kg) and amylase from *Bacillus licheniformis* (200 U/kg; XA), or 3) with XA plus protease from *Bacillus subtilis* (4,000 U/kg; XAP). All diets contained *Escherichia coli* phytase (500 FTU/kg). Apparent ileal digestibility (AID) of protein, starch, and fat, as well as the apparent ileal digestible energy, were determined using titanium dioxide as inert marker. A generalized mixed model was used to test main effects and 2-way interactions at $P < 0.05$. An enzyme × grain interaction was detected for AID of starch at 21 and 42 d, and AID of fat at 21 d, with greater effects of enzymes in wheat-based compared with corn-based diets, but significant increments due to enzymes compared with controls in both diet types. Apparent ileal digestibility of fat at 42 d increased with enzyme supplementation compared with the control treatments. The XA and XAP treatments gradually ($P < 0.05$) increased AID of protein at 21 d, but only XAP increased AID of protein compared with the control at 42 d. Compared with the controls, XA increased AID energy by 52 or 87 kcal, and XAP by 104 or 152 kcal/kg of DM at 21 or 42 d, respectively. The caloric contribution of starch, fat, and protein were affected differentially by base grain and the presence of fibrous ingredients at 21 and 42 d of age.

Key words: enzyme, broiler chicken, starch, fat, protein

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INTRODUCTION

Increasing prices and price volatility of ingredients typically used in poultry feeds have intensified the need to formulate diets with substitute ingredients. More high-fiber ingredients (**HFI**) such as corn distillers dried grains with solubles (**DDGS**), a by-product from the ethanol industry, and other cereal grains, milling by-products, and oilseed meals are being incorporated into broiler diets in regions that traditionally used corn or wheat and soybean meal as the major vegetable ingredients. As a result, broiler diets are now increasingly complex and variable in nutrient content compared with cereal grain and oilseed meal-based broiler diets.

Dietary xylanase, alone and in combination with other exogenous enzymes such as protease, have reportedly improved growth performance, enhanced flock uniformity, improved energy and nutrient availability, and reduced nutrient excretion in broiler chickens (Zanella et al., 1999; Cowieson and Ravindran, 2008; Adeola and Cowieson, 2011). Exogenous xylanases were originally used as an aid to reduce the antinutritional effects of viscosity induced by nonstarch polysaccharides (**NSP**), particularly arabinoxylans, in viscous cereal grains (Bedford and Classen, 1992). However, the use of these exogenous enzymes in corn-based diets is becoming more prevalent due to variable ingredient quality and increased use of HFI. The resulting higher dietary concentrations of insoluble NSP present additional chal-

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lenges for broiler nutrition, such as a reduced digestibility of proteins and lower energy density.

An accurate estimation of improvements on energy digestibility due to exogenous enzymes is relevant for the industry to better account for the effects of enzymes in diet formulations and to calculate the value of these additives in different diet compositions. That is particularly important in diets that contain increased amounts of NSP because the energy efficiency of digestion or fermentation of NSP sugars cannot be directly compared with digestion and absorption of starch, fat, or protein (Chwalibog, 2002). In fact, the energy efficiency of xylose is significantly lower compared with glucose in chickens (Savory, 1992) and other monogastric animals such as pigs (Noblet and Le Goff, 2001). Therefore, calculation of the energy contribution of enzymes using AME or even apparent ileal digestible energy (**AIDE**) may not be enough to understand the net energy that enzymes can deliver to the animal metabolism, particularly in diets with increased levels of NSP.

The objective of the current study was to evaluate changes in apparent ileal digestibility (**AID**) coefficients and ileal energy contribution of protein, starch, and fat in response to exogenous xylanase and amylase combinations without or with protease in broiler chickens fed diets with corn or wheat as the base grain, without or with the inclusion of HFI, at 2 different ages.

MATERIALS AND METHODS

Exogenous Enzymes

A 6-phytase (EC 3.1.3.26) from *Escherichia coli* (Phyzyme XP, Danisco Animal Nutrition, DuPont Industrial Biosciences, Marlborough, UK) was applied in the background of all diets. The XA treatment was an enzyme preparation containing endo-1,4-β-xylanase (EC 3.2.1.8) and α-amylase (EC 3.2.1.1). The XAP treatment (Axtra XAP; Danisco Animal Nutrition, DuPont Industrial Biosciences) was a commercial enzyme preparation containing endo-1,4-β-xylanase (EC 3.2.1.8), α-amylase (EC 3.2.1.1), and a serine protease (EC 3.4.21.62). The xylanase originated from *Trichoderma reesei*; the amylase originated from *Bacillus licheniformis*; and the protease originated from *Bacillus subtilis*. In the XA and XAP treatments, the xylanase and amylase corresponded to the same molecule at the same target concentrations.

Enzymes activities in feed samples (200 g) were measured at the DuPont Nutrition Biosciences Innovation Laboratories (Brabrand, Denmark; Table 1) in duplicate. One FTU was defined as the quantity of enzyme that releases 1 μmol of inorganic P/min from 5.0 m*M* sodium phytate at pH 5.5 at 37°C. One xylanase unit was defined as the amount of enzyme that releases 0.48 μmol of reducing sugar as xylose from wheat arabino xylan per minute at pH 4.2 and 50°C. One unit of *Bacillus licheniformis* α-amylase was the amount of enzyme required to release, in the presence of excess α-glucosidase, 0.20 μmol of glucosidic linkages expressed as *p*-nitrophenol equivalents from a maltoheptaoside substrate per minute at pH 8.0 and 40°C. One protease unit was defined as the amount of enzyme that releases 1.0 μg of phenolic compound, expressed as tyrosine equivalents, from a casein substrate per minute at pH 7.5 and 40°C.

Bird Trials

The experimental procedures employed in these studies were approved by the Animal Ethics Committee of Massey University, New Zealand. Two digestibility assays with 432 (experiment 1, starter phase) or 288 (experiment 2, finisher phase) male broilers (Ross-308) were performed. Experiment 1 was conducted from d 12 to 21. Prior to the introduction of cages, the birds were reared in floor pens and fed the control starter diets. Experiment 2 was conducted from d 32 to 42. Prior to the introduction of cages, the birds were reared in floor pens and fed the control starter and finisher diets. The cages were housed in an environmentally controlled room where the temperature was maintained at 32°C during the first week and then gradually reduced to 24°C by d 21. Birds received 20-h fluorescent illumination and were allowed free access to the diets and water.

Compositions of the basal starter (21 d, experiment 1) and finisher (42 d; experiment 2) diets are presented in Tables 2 and 3, respectively. Diets were provided in mash form and contained a basal level of 500 FTU/kg of *E. coli* phytase and TiO_2 (0.3%) as an indigestible marker. Diets were manufactured in one batch for each diet formulation and phase (starter or finisher), and each of those diets was subdivided in 3 experimental diets, 2 of them containing enzymes. Concentrates of the test enzymes were sprayed into a wheat carrier, activity was measured and standardized, and such enzyme preparations were added to the respective diets in dry form at 0.5 g/kg, after being premixed with 5 g of maize or wheat/kg from the diets.

Experimental Design

In both experiments, a 2 × 2 × 3 factorial arrangement of dietary treatments was used with 2 base grains (corn or wheat), 2 levels of HFI (with or without 10% corn-DDGS and 5% canola meal), and 3 enzyme treatments. The 3 enzyme treatments were: negative control (**NC**); NC with xylanase from *T. ressei* (2,000 U/kg) and amylase from *B. licheniformis* (200 U/kg; XA); or NC with XA plus protease from *B. subtilis* (4,000 U/kg).

In experiment 1, birds were individually weighed on d 12 and assigned to 6 blocks on the basis of BW for a total of 72 cages (6 birds/cage). The 12 dietary treatments were then randomly assigned to 6 cages each. Experimental diets were administered from d 12 until d 21, when all birds were euthanized by intracardial

Table 1. Expected and measured enzyme activities in feed samples from experiments 1 and 2

	Xylanase[3] (XU/kg of feed)		Amylase[3,4] (AU/kg of feed)		Protease[3] (PU/kg of feed)		Phytase[5] (FTU/kg of feed)	
Dietary treatment[1,2]	Expected	Measured (starter/finisher)	Expected	Measured (starter/finisher)	Expected	Measured (starter/finisher)	Expected	Measured (starter/finisher)
Corn-based	0	ND/ND	0	ND/ND	0	ND/ND	500	785/624
Corn-based + XA	2,000	2,970/2,850	200	221/192	0	ND/ND	500	532/609
Corn-based + XAP	2,000	2,620/2,570	200	261/306	4,000	4,820/6,390	500	559/487
Corn-based/HFI	0	ND/ND	0	ND/ND	0	ND/ND	500	530/540
Corn-based/HFI + XA	2,000	2,660/2,630	200	152/182	0	ND/ND	500	771/531
Corn-based/HFI + XAP	2,000	2,761/2,350	200	240/221	4,000	5,030/5,920	500	645/496
Wheat-based	0	ND/ND	0	NA/NA	0	ND/ND	500	1,310/910
Wheat-based + XA	2,000	2,560/2,707	200	NA/NA	0	ND/ND	500	1,200/811
Wheat-based + XAP	2,000	2,420/2,270	200	NA/NA	4,000	4,040/4,410	500	1,150/1,080
Wheat-based/HFI	0	ND/ND	0	NA/NA	0	ND/ND	500	965/837
Wheat-based/HFI + XA	2,000	2,170/2,300	200	NA/NA	0	ND/ND	500	919/791
Wheat-based/HFI + XAP	2,000	2,630/2,480	200	NA/NA	4,000	6,190/4,520	500	1,180/723

[1]In experiment 2, starter diets were used, and experimental treatments were fed from 12 to 21 d of age. In experiment 3, finisher diets were used, and experimental treatments were fed from 35 to 42 d of age.

[2]X = xylanase from *Trichoderma ressei* (2,000 U/kg); A = amylase from *Bacillus licheniformis* (200 U/kg); P = protease from *Bacillus subtilis* (4,000 U/kg). HFI = high-fiber ingredients.

[3]ND = not detectable. XU: xylanase units defined as the amount of enzyme that releases 0.48 μmol of reducing sugar as xylose from wheat arabino xylan per minute at pH 4.2 and 50°C. AU: amylase units defined as the amount of enzyme required to release, in the presence of excess α-glucosidase, 0.20 μmol of glucosidic linkages expressed as *p*-nitrophenol equivalents from a maltoheptaoside substrate per minute at pH 8.0 and 40°C. PU: protease units defined as the amount of enzyme that releases 1.0 μg of phenolic compound, expressed as tyrosine equivalents, from a casein substrate per minute at pH 7.5 and 40°C.

[4]NA = not analyzed. High levels of background amylase in wheat-based diets impaired measuring exogenous amylase with this assay.

[5]All diets contained 500 FTU of *Escherichia coli* phytase/kg of feed in the background. FTU: phytase units defined as the quantity of enzyme that releases 1 μmol of inorganic P/min from 5.0 m*M* sodium phytate at pH 5.5 at 37°C.

Table 2. Ingredient composition (%, as fed) of basal starter diets in experiment 1

Item	Corn	Corn/HFI[1]	Wheat	Wheat/HFI
Ingredient (%)				
Corn	57.77	51.68	0.00	0.00
Wheat (12.5%CP)	0.00	0.00	63.10	56.45
Corn-DDGS[2]	0.00	10.00	0.00	10.00
Wheat bran	1.00	0.00	1.00	0.00
Soybean meal, 48% CP	35.84	27.44	28.95	21.28
Canola meal	0.00	5.00	0.00	5.00
Tallow/soy oil blend	1.18	1.65	2.63	2.95
L-Lys HCl	0.19	0.36	0.33	0.48
DL-Met	0.28	0.28	0.26	0.27
L-Thr	0.08	0.13	0.13	0.17
Titanium dioxide	0.30	0.30	0.30	0.30
Salt	0.38	0.33	0.35	0.30
Limestone	1.21	1.35	1.30	1.43
Dicalcium phosphate	1.46	1.16	1.34	1.06
Trace mineral/vitamin premix[3]	0.31	0.31	0.31	0.31
Calculated analysis				
CP,[4] %	22.50 (22.52)	22.50 (22.50)	22.50 (23.60)	22.50 (23.30)
Starch,[4] %	(35.25)	(33.14)	(36.50)	(30.60)
Ether extract,[4] %	(3.94)	(6.01)	(4.47)	(6.30)
Gross energy,[4] kcal/kg	(3,928)	(4,072)	(3986)	(4,124)
AME, kcal/kg	2,925	2,925	2,925	2,925
Calcium, %	0.95	0.95	0.95	0.95
Available P, %	0.38	0.38	0.38	0.38
Sodium, %	0.18	0.18	0.18	0.18
Digestible Lys, %	1.20	1.20	1.20	1.20
Digestible Met, %	0.59	0.61	0.54	0.56
Digestible Met + Cys, %	0.85	0.85	0.85	0.85
Digestible Thr, %	0.75	0.75	0.75	0.75

[1]HFI = high-fiber ingredients.

[2]DDGS = distillers dried grains with solubles.

[3]The premix supplied per kilogram of diet: antioxidant, 100 mg; biotin, 0.2 mg; calcium pantothenate, 12.8 mg; cholecalciferol, 60 μg; cyanocobalamin, 0.017 mg; folic acid, 5.2 mg; menadione, 4 mg; niacin, 35 mg; pyridoxine, 10 mg; *trans*-retinol, 3.33 mg; riboflavin, 12 mg; thiamine, 3.0 mg; DL-α-tocopheryl acetate, 60 mg; choline chloride, 638 mg; Co, 0.3 mg; Cu, 3 mg; Fe, 25 mg; I, 1 mg; Mn, 125 mg; Mo, 0.5 mg; Se, 200 μg; Zn, 60 mg.

[4]Values in parentheses correspond to measured concentrations in feed.

injection of sodium pentobarbitone. In experiment 2, birds were individually weighed on d 32 and assigned to 6 blocks on the basis of BW for a total of 72 cages (4 birds/cage). The 12 dietary treatments were then randomly assigned to 6 cages each. Experimental diets were administered from d 35 until d 42, when all birds were euthanized by intracardial injection of sodium pentobarbitone.

After euthanasia, contents of the lower half of the ileum were obtained by gentle flushing with distilled water. The ileum was defined as the portion of the small intestine extending from the Meckel's diverticulum to a point approximately 40 mm proximal to the ileo-cecal junction (Ravindran et al., 2005). Digesta from birds within a cage were pooled, resulting in 6 samples per dietary treatment in each experiment. The digesta samples were frozen immediately after collection, lyophilized, and processed.

Chemical Analyses

Samples of digesta and diets were analyzed for Ti, DM, starch, fat, GE, and N. Dry matter content was determined using standard procedures (AOAC International, 2005; method 930.15). Gross energy (**GE**) was determined using an adiabatic bomb calorimeter (Gallenkamp Autobomb, London, UK) standardized with benzoic acid. Nitrogen content was determined by the combustion method (AOAC International, 2005; method 968.06) using CNS-2000 carbon, nitrogen, and sulfur analyzers. Crude fat was determined using Soxhlet extraction procedure (AOAC International, 2005; method 991.36). Starch was determined using the Megazyme Total Starch Assay Procedure (Megazyme International Ireland Ltd., Wicklow, Ireland) based on thermostable α-amylase and amyloglucosidase. Titanium content was measured on a UV spectrophotometer following the method of Short et al. (1996).

AID and Energy Contribution Calculations

The AID was calculated for energy, starch, fat, and N based on the concentration of titanium in diet and digesta, as reported by Ravindran et al. (2005). Apparent ileal digestible energy was calculated by multiplying the diet GE content by the apparent ileal energy digestibility coefficient. Apparent ileal digestible energy contributions from protein, starch, and fat in response to exogenous enzymes were calculated for each experimental unit in the XA and XAP treatments according to the following equation:

$$AIDE_{psf} = (AID_{EU} - AID_{NC}) \times GE_{psf},$$

Table 3. Ingredient composition (%, as fed) of basal finisher diets in experiment 2

Item	Corn	Corn/HFI[1]	Wheat	Wheat/HFI
Ingredient (%)				
Corn	59.00	49.75	0.00	0.00
Wheat (12.5%CP)	0.00	0.00	64.45	54.31
Corn-DDGS[2]	0.00	13.94	0.00	14.00
Soybean meal 48%CP	33.04	22.50	26.00	16.54
Canola meal	0.00	5.00	0.00	5.00
Tallow/soy oil blend	3.81	4.61	5.29	5.86
L-Lys HCl	0.13	0.35	0.27	0.47
DL-Met	0.24	0.26	0.22	0.24
L-Thr	0.06	0.12	0.10	0.16
Titanium dioxide	0.30	0.30	0.30	0.30
Salt	0.40	0.34	0.37	0.32
Limestone	1.20	1.43	1.29	1.51
Dicalcium phosphate	1.50	1.09	1.38	0.99
Trace mineral/vitamin premix[3]	0.31	0.31	0.31	0.31
Calculated analysis				
CP,[4] %	21.00 (19.95)	21.00 (20.25)	21.00 (21.02)	21.00 (21.06)
Starch,[4] %	(39.68)	(32.36)	(36.63)	(30.99)
Ether extract,[4] %	(6.80)	(9.06)	(6.99)	(9.14)
Gross energy,[4] kcal/kg	(4,069)	(4,229)	(4,116)	(4,290)
AME, kcal/kg	3,100	3,100	3,100	3,100
Calcium, %	0.95	0.95	0.95	0.95
Available P, %	0.38	0.38	0.38	0.38
Sodium, %	0.19	0.19	0.19	0.19
Digestible Lys, %	1.07	1.07	1.07	1.07
Digestible Met, %	0.54	0.57	0.49	0.53
Digestible Met + Cys, %	0.78	0.78	0.78	0.78
Digestible Thr, %	0.68	0.68	0.68	0.68

[1]HFI = high-fiber ingredients.

[2]DDGS = distillers dried grains with solubles.

[3]The premix supplied per kilogram of diet: antioxidant, 100 mg; biotin, 0.2 mg; calcium pantothenate, 12.8 mg; cholecalciferol, 60 μg; cyanocobalamin, 0.017 mg; folic acid, 5.2 mg; menadione, 4 mg; niacin, 35 mg; pyridoxine, 10 mg; *trans*-retinol, 3.33 mg; riboflavin, 12 mg; thiamine, 3.0 mg; DL-α-tocopheryl acetate, 60 mg; choline chloride, 638 mg; Co, 0.3 mg; Cu, 3 mg; Fe, 25 mg; I, 1 mg; Mn, 125 mg; Mo, 0.5 mg; Se, 200 μg; Zn, 60 mg.

[4]Values in parentheses correspond to measured concentrations in feed.

where $AIDE_{psf}$ was the apparent digestible energy contribution of each substrate (protein, starch, or fat; kcal/kg of feed DM); AID_{EU} was the apparent ileal digestible substrate of each experimental unit (g/kg of feed DM); AID_{NC} was the arithmetic mean of the apparent ileal digestible substrate of the respective negative control (grain × HFI diet; g/kg of feed DM); and GE_{psf} was the GE density of each substrate (kcal/g). The GE density of protein was assumed to be 5.5 kcal/g; starch was assumed to contain 4.2 kcal/g; and fat was assumed to contain 9.1 kcal/g (Leeson and Summers, 2001).

Data Analysis

Data from the 2 experiments were analyzed independently with a randomized block design and a 2 × 2 × 3 factorial arrangement of treatments, using the General Mixed Procedure of SAS (2002, SAS Institute Inc., Cary, NC). The model included the main effects of grain type, HFI, and enzyme, as well as all possible interactions, and the random effect of block. Nonsignificant 3-way interactions were removed from the model. Significant differences were considered at $P < 0.05$. A group of chickens in a cage were used as the experimental unit.

Data of the energy contribution of substrates were analyzed accordingly, with the difference that the negative control treatments were excluded because data were expressed in relative terms versus the control treatments.

RESULTS

Feed Composition and Enzyme Recoveries

Enzyme recoveries in feed are presented in Table 1. Generally, all samples, including the negative controls, contained phytase activities over 500 FTU/kg, which are typically used in broilers diets. Wheat-based diets presented phytase recoveries that were above those for corn-based diets, presumably due to the presence of endogenous phytase in wheat, given the fact that diets were not pelleted, which would have deactivated endogenous phytase. All dietary treatments with enzymes presented levels of xylanase above the target level of 2,000 xylanase U/kg, and the negative controls had nondetectable levels, which confirmed that enzyme treatments were correctly applied.

Acceptable levels of amylase were measured in corn-based diets treated with enzymes. However, this amylase assay could not be performed in the wheat-based diets used in the study, in both experiments 1 and 2, due to the presence of a high background level of amylase in the feed, which likely came from the batch of wheat used because it was the only ingredient that was different from corn-based diets. It is possible that

endogenous wheat amylase was responsible for this interference with this assay. It is also possible that fungal contamination of the wheat may have occurred or that fungal growth may have occurred in wheat-based diet samples during transportation and storage. Even though mycotoxins were not measured in this study, there was no evidence of reduced feed intake or the presence of clinical signs of mycotoxin contamination in either wheat- or corn-based diets. Given the fact that amylase levels were measured in the enzyme premix, amylase was present in corn-based diets, and xylanase levels were on target, it was assumed that the exogenous amylase was present in all diets with exogenous xylanase. Significant levels protease activity (>4,000 protease U/kg) were only measured in diets with added protease. Overall, enzyme activities corresponded to the experimental design and the activity measurements in enzyme premixes despite the presence of background levels of phytase and amylase in wheat-based diets.

Analysis of the diets for protein (Tables 2 and 3) showed that diets reflected the formulated values, although wheat-based diets were slightly above corn-based diets. In general, diets with inclusion on HFI contained less starch and more fat compared with diets without HFI to reach the same level of AME in the diet formulation. Feed intake was normal during the experimental period of each age of birds and low mortality (<5%) was present.

Nutrient Utilization

The influence of diet-type and enzyme combinations on the AID of nutrients and AIDE at 21 and 42 d of age is summarized in Tables 4 and 5, respectively. At d 21, a significant interaction between grain and HFI was evident for the AID coefficient of energy and the AIDE, with a reduced coefficient of AID due to the inclusion of HFI in corn-based diets, but not in wheat-based diets. Nonetheless, the actual AIDE (kcal/kg) was greater for wheat-based diets with inclusion of HFI compared with wheat-based diets without HFI. An interaction between grain and enzymes was present for the AID of starch and fat. In the case of starch, the effect of both enzyme combinations was greater in wheat-based compared with corn-based diets. In the case of fat, the starting point of fat digestibility was lower for wheat-based diets versus corn-based diets, with both enzyme combinations increasing fat digestibility in wheat-based diets, but only XAP having an effect in corn-based diets. No interactions between HFI and enzymes were detected at 21 d.

At d 21, grain affected the AID of protein as a main effect, with corn-based diets exhibiting higher AID of protein compared with wheat-based diets. The HFI increased the AID of starch and reduced the AID of fat across grain types. There was a significant effect of enzymes in the AID of energy, protein, and in AIDE across the different diets used. The XA combination increased the AID of energy and AIDE (+52 kcal/kg) compared with the control treatment, and the addition of protease in the XAP combination further increased energy digestibility compared with the control treatment (+104 kcal/kg) and XA.

At d 42, interactions between grain and HFI were detected for the AID of energy and fat, and for the AIDE. Similar to the results at 21 d, addition of HFI reduced the AID of energy in corn-based diets, but not in wheat-based. The AID of starch exhibited an interaction between grain and enzymes in which wheat-based diets without enzymes presented a reduced digestibility compared with corn-based diets without enzymes, and both enzyme combinations increased AID of starch to similar levels in both diet types. No interactions between HFI and enzymes were detected.

At d 42, HFI affected the AID of energy and protein across grains and enzymes, with the inclusion of HFI producing a reduced digestibility. There was a significant effect of enzymes across diet types affecting the AID of energy, protein, and fat, as well as the AIDE. Both XA and XAP increased the AID of energy and the AIDE, with no significant differences between enzymes.

Caloric Contributions of Starch, Protein, and Fat to Changes in AIDE

The apparent ileal energy contribution of the exogenous enzymes due to increments in the digestion of protein, starch, and fat versus the relevant control treatments, the sum of these contributions, as well as the change in the measured AIDE due the enzyme treatments versus the respective controls at 21 and 42 d are presented in Table 6. In this analysis, the main effect of enzyme compared the 2 enzyme treatments (XA and XAP), as opposed to evaluating the effect versus the negative controls. Similarly, the interactions between enzyme and grain or HFI evaluated differences in the energy contribution of XA and XAP in response to dietary changes.

No interactions were present for the energy contribution of analyzed nutrients in response to enzymes with the exception of an interaction between grain and HFI in the AID of protein at 21 d, where the inclusion of HFI increased the contribution of XA and XAP enzymes in corn-based diets, but not in wheat-based diets. Grain significantly affected the energy contribution of protein at 42 d, the contribution of starch and fat, and the AIDE response to enzymes at 21 and 42 d. Generally, energy contributions of these enzymes were greater in wheat-based than in corn-based diets for all substrates as it was for AIDE. The HFI influenced the energy contribution of starch and fat in response to enzymes at 42 d, without evident effects in the contribution of these substrates at 21 d. However, HFI affected the combined energy contribution of protein, starch, and fat due to enzymes at 21 and 42 d, where greater effects of enzymes were found in diets with HFI. The

Table 4. Influence of grain type, high-fiber ingredients (HFI), and enzyme combinations on the apparent ileal digestibility of nutrients and apparent ileal digestible energy (AIDE) of broilers at 21 d of age[1]

Item	Ileal digestibility (%)				AIDE (kcal/kg of DM)
	Energy	Protein	Starch	Fat	
Treatment[2]					
Grain					
Corn-based	73.2	84.7[a]	96.9	84.3	3,293
Wheat-based	70.7	83.9[b]	97.8	80.7	3,208
SEM	0.3	0.2	0.1	0.5	13
HFI[3]					
No	72.3	84.9[a]	97.4	81.8	3,213
Yes	71.6	83.7[b]	97.3	83.2	3,288
SEM	0.3	0.2	0.1	0.5	13
Enzyme[4]					
Phytase	70.8[c]	82.7[c]	96.3	80.2	3,199[c]
Phytase + XA	71.9[b]	84.4[b]	97.8	83.3	3,250[b]
Phytase + XAP	73.1[a]	85.8[a]	97.9	84.0	3,303[a]
SEM	0.4	0.3	0.2	0.6	17
Grain × HFI					
Corn-based	74.2[a]	85.5	97.1	84.3	3,284[a]
Corn-based/HFI	72.2[b]	83.9	96.7	84.4	3,303[a]
Wheat-based	70.4[c]	84.2	97.8	79.4	3,143[b]
Wheat-based/HFI	71.0[c]	83.6	97.8	82.0	3,272[a]
SEM	0.4	0.3	0.2	0.7	19
Grain × enzyme					
Corn-based	72.4	83.5	96.2[c]	83.6[ab]	3,257
Corn-based + XA	73.0	84.6	97.2[b]	83.8[ab]	3,284
Corn-based + XAP	74.2	86.0	97.3[b]	85.7[a]	3,339
Wheat-based	69.2	81.8	96.4[c]	76.9[c]	3,140
Wheat-based + XA	70.9	84.3	98.5[a]	82.9[b]	3,217
Wheat-based + XAP	72.0	85.6	98.5[a]	82.4[b]	3,266
SEM	0.5	0.4	0.2	0.9	23
HFI × enzyme					
No HFI	71.2	83.4	96.5	79.5	3,167
No HFI + XA	71.9	84.8	97.8	82.7	3,196
No HFI + XAP	73.7	86.4	97.9	83.3	3,277
HFI	70.4	81.9	96.1	81.0	3,231
HFI + XA	72.0	84.0	97.8	83.9	3,304
HFI + XAP	72.5	85.2	97.9	84.8	3,328
SEM	0.5	0.4	0.2	0.9	23
Source of variation (*P*-value)					
Grain	<0.001	0.017	<0.001	<0.001	<0.001
HFI	0.13	0.001	0.39	0.06	<0.001
Enzyme	<0.001	<0.001	<0.001	<0.001	<0.001
Grain × HFI	0.002	0.13	0.33	0.08	0.005
Grain × enzyme	0.51	0.16	0.042	0.007	0.50
HFI × enzyme	0.42	0.71	0.62	0.98	0.42

[a–c]Means with different superscripts differ at $P < 0.05$.

[1]Each mean represents values from 6 replicate cages.

[2]All diets contained 500 FTU of *Escherichia coli* phytase/kg of feed in the background. FTU: phytase units defined as the quantity of enzyme that releases 1 μmol of inorganic P/min from 5.0 m*M* sodium phytate at pH 5.5 at 37°C.

[3]HFI refers to the inclusion of 10% corn distillers dried grains with solubles and 5% canola meal.

[4]X = xylanase from *Trichoderma ressei* (2,000 U/kg); A = amylase from *Bacillus licheniformis* (200 U/kg); P = protease from *Bacillus subtilis* (4,000 U/kg).

addition of protease on top of XA had an effect in the energy contribution from protein at 21 and 42 d, and fat at 42 d, which were enough to produce a difference in the total ileal energy contribution from protein, starch, and fat, as well as AIDE compared with XA, both at 21 and 42 d.

DISCUSSION

The current study assessed the AID of protein, starch, and fat without or with enzymes to calculate the ileal caloric contribution of these fractions in response to enzymes in different diet formulations. The interactive effects of cereal grains (corn and wheat), inclusion of corn-DDGS and canola meal (HFI), and supplemental enzyme combinations on ileal digestible of energy and nutrients were explored. The responses were tested at 2 different ages, namely 21 and 42 d, under the assumption that the relative response to different enzymes and different diets would be affected by age, due to differences in the maturity of the gastrointestinal tract (Jin et al., 1998), and the intestinal microbiome (Gong et al., 2008).

Table 5. Influence of grain type, high-fiber ingredients (HFI) and enzyme combinations on the apparent ileal digestibility of nutrients and apparent ileal digestible energy (AIDE) of broilers at 42 d of age[1]

Item	Ileal digestibility (%)				AIDE (kcal/kg of DM)
	Energy	Protein	Starch	Fat	
Treatment[2]					
Grain					
Corn-based	75.1	83.3	96.1	87.3	3,482
Wheat-based	75.0	83.9	95.5	88.6	3,512
SEM	0.4	0.4	0.2	0.3	19
HFI[3]					
No	75.6	84.5^{a}	95.8	87.8	3,460
Yes	74.4	82.7^{b}	95.8	88.1	3,534
SEM	0.4	0.4	0.2	0.3	19
Enzyme[4]					
Phytase	73.3^{b}	82.4^{b}	93.9	86.6^{c}	3,417^{b}
Phytase + XA	75.2^{a}	83.2^{b}	96.6	87.9^{b}	3,505^{a}
Phytase + XAP	76.5^{a}	85.1^{a}	97.0	89.4^{a}	3,569^{a}
SEM	0.5	0.5	0.3	0.4	24
Grain × HFI					
Corn-based	76.4^{a}	84.3	96.2	86.7^{b}	3,478^{b}
Corn-based/HFI	73.7^{b}	82.2	96.0	87.9^{ab}	3,486^{b}
Wheat-based	74.9^{ab}	84.6	95.4	88.9^{a}	3,443^{b}
Wheat-based/HFI	75.0^{ab}	83.2	95.6	88.3^{a}	3,582^{a}
SEM	0.6	0.5	0.3	0.4	27
Grain × enzyme					
Corn-based	74.1	82.5	94.8^{b}	86.3	3,440
Corn-based + XA	74.9	82.6	96.7^{a}	87.0	3,473
Corn-based + XAP	76.2	84.8	96.8^{a}	88.6	3,533
Wheat-based	72.5	82.3	93.0^{c}	86.9	3,395
Wheat-based + XA	75.5	83.9	96.4^{a}	88.9	3,536
Wheat-based + XAP	76.9	85.5	97.2^{a}	90.1	3,606
SEM	0.7	0.7	0.4	0.5	33
HFI × enzyme					
No HFI	74.2	83.3	94.5	86.5	3,393
No HFI + XA	76.0	84.2	96.2	87.6	3,477
No HFI + XAP	76.7	85.9	96.8	89.3	3,511
HFI	72.4	81.5	93.4	86.7	3,442
HFI + XA	74.3	82.3	96.9	88.3	3,532
HFI + XAP	76.3	84.4	97.2	89.4	3,627
SEM	0.7	0.7	0.4	0.5	33
Source of variation (P-value)					
Grain	0.86	0.24	0.08	0.004	0.27
HFI	0.035	0.002	0.95	0.47	0.009
Enzyme	<0.001	<0.001	<0.001	<0.001	<0.001
Grain × HFI	0.022	0.47	0.53	0.042	0.020
Grain × enzyme	0.16	0.51	0.023	0.49	0.16
HFI × enzyme	0.59	0.95	0.06	0.79	0.54

[a–c]Means with different superscripts differ at $P < 0.05$.

[1]Each mean represents values from 6 replicate cages.

[2]All diets contained 500 FTU of *Escherichia coli* phytase/kg of feed in the background. FTU: phytase units defined as the quantity of enzyme that releases 1 μmol of inorganic P/min from 5.0 m*M* sodium phytate at pH 5.5 at 37°C.

[3]HFI refers to the inclusion of 10% corn distillers dried grains with solubles and 5% canola meal.

[4]X = xylanase from *Trichoderma ressei* (2,000 U/kg); A = amylase from *Bacillus licheniformis* (200 U/kg); P = protease from *Bacillus subtilis* (4,000 U/kg).

Energy from wheat-based diets has been reported to be less readily available than from corn-based diets in broilers due largely to the presence of soluble NSP, particularly arabinoxylans, which are only marginally degraded in the gastrointestinal tract of broilers and can increase the viscosity of the digesta (Annison and Choct, 1991). In the current work, wheat-based diets showed lower AID of energy compared with corn-based diets at 21 d. Given the fact that ingredients used did not differ between 21 and 42 d, this finding suggests that 42-d-old birds were more capable of extracting energy from the wheat-based diet at the ileal level compared with 21-d-old birds. Palander et al. (2010) reported that 6-wk-old broilers fed wheat-based diets had lower digesta viscosity and higher AME compared with 3-wk-old broilers. Although there were no significant interactions between enzymes and grain on AIDE at either age, when the caloric contributions of enzymes were calculated (Table 6), a significant effect of grain type on AIDE contributions of enzymes was found at 21 and 42 d. The higher response to enzymes in 21-d and 42-d chickens fed wheat-based diets compared with

Table 6. Influence of grain type, high-fiber ingredients (HFI) and enzyme combinations on the apparent ileal digestible energy (AIDE) contribution of protein, starch, and fat due exogenous enzymes versus the respective control treatments in broiler chickens at 21 and 42 d of age[1]

	AIDE contribution from protein[2] (kcal/kg of DM)		AIDE contribution from starch[2] (kcal/kg of DM)		AIDE contribution from fat[2] (kcal/kg of DM)		$AIDE_{psf}$[3] contribution (kcal/kg of DM)		AIDE contribution (kcal/kg of DM)	
Item	21 d	42 d	21 d	42 d	21 d	42 d	21 d	42 d	21 d	42 d
Treatment										
Grain										
Corn-based	24.7	14.0^{b}	16.3^{b}	31.5^{b}	5.7^{b}	12.2^{b}	46.7^{b}	57.6^{b}	54.5^{b}	63.2^{b}
Wheat-based	45.2	30.4^{a}	31.8^{a}	58.1^{a}	31.7^{a}	21.6^{a}	108.8^{a}	110.2^{a}	101.4^{a}	176.2^{a}
SEM	2.7	5.4	2.5	4.1	2.2	2.3	5.0	8.4	15.0	22.1
HFI[4]										
No	31.5	21.4	22.6	35.5^{b}	15.8	13.6^{b}	70.0^{b}	70.5^{b}	70.2	101.5
Yes	38.4	23.0	25.4	54.1^{a}	21.7	20.3^{a}	85.5^{a}	97.3^{a}	85.7	137.9
SEM	2.7	5.4	2.5	4.1	2.2	2.3	5.0	8.4	15.0	22.1
Enzyme[5]										
XA	25.3^{b}	10.0^{b}	23.0	41.6	16.9	11.4^{b}	65.3^{b}	63.0^{b}	51.7^{b}	87.5^{b}
XAP	44.6^{a}	34.3^{a}	25.1	48.0	20.5	22.5^{a}	90.2^{a}	104.8^{a}	104.2^{a}	151.9^{a}
SEM	2.7	5.4	2.5	4.1	2.2	2.3	5.0	8.4	15.0	22.1
Grain × HFI										
Corn-based	17.0^{c}	12.3	14.0	26.9	5.8	11.9	36.7	51.0	44.5	62.3
Corn-based/HFI	32.3^{b}	15.6	18.6	36.0	5.7	12.5	56.6	64.2	64.5	64.1
Wheat-based	46.1^{a}	30.5	31.3	44.2	25.8	15.3	103.2	89.9	96.0	140.8
Wheat-based/HFI	44.4^{a}	30.3	32.3	72.1	37.7	28.0	114.3	130.4	106.8	211.7
SEM	3.9	7.7	2.5	5.8	3.2	3.3	7.0	11.8	21.3	31.3
Grain × enzyme										
Corn-based + XA	15.3	0.3	14.9	30.9	0.9	5.9	31.1	37.1	26.9	33.4
Corn-based + XAP	34.0	27.6	17.7	32.0	10.5	18.5	62.2	78.1	82.1	93.0
Wheat-based + XA	35.4	19.7	31.2	52.3	32.9	16.9	99.5	88.9	76.6	141.6
Wheat-based + XAP	55.1	41.1	32.4	64.0	30.6	26.4	118.1	131.5	126.2	210.9
SEM	3.9	7.7	3.5	5.8	3.2	3.3	7.0	11.8	21.3	31.3
HFI × enzyme										
No HFI + XA	20.8	10.5	21.4	30.7	14.7	7.4	57.0	48.7	29.7	84.6
No HFI + XAP	42.2	32.2	23.9	40.3	16.9	19.7	83.0	92.2	110.8	118.5
HFI + XA	29.8	9.6	24.6	52.4	19.1	15.3	73.6	77.3	73.8	90.3
HFI + XAP	46.9	36.4	26.2	55.7	24.2	25.2	97.3	117.3	97.5	185.4
SEM	3.9	7.7	3.5	5.8	3.2	3.3	7.0	11.8	21.3	31.3
Source of variation (P-value)										
Grain	<0.001	0.038	<0.001	<0.001	<0.001	0.006	<0.001	<0.001	0.033	0.001
HFI	0.08	0.83	0.43	0.003	0.07	0.047	0.033	0.029	0.47	0.25
Enzyme	<0.001	0.003	0.57	0.27	0.26	0.001	0.001	0.001	0.018	0.046
Grain × HFI	0.032	0.82	0.61	0.11	0.07	0.07	0.54	0.26	0.83	0.28
Grain × enzyme	0.89	0.71	0.82	0.37	0.07	0.63	0.38	0.95	0.90	0.88
HFI × enzyme	0.58	0.74	0.90	0.59	0.64	0.72	0.88	0.88	0.18	0.33

[a–c]Means with different superscripts differ at $P < 0.05$.

[1]Statistical analyses excluded the negative control treatments. The energy contribution of different substrates and AIDE were calculated as the difference of the observation in each experimental unit in the treatments fed enzymes versus the mean values of the control treatment.

[2]The calculated AIDE contribution from substrates was based on 1) the difference on the coefficient of ileal digestibility of the substrate versus that of the control treatments, 2) the nutrient content on the basal diets, and 3) a theoretical gross energy content of the substrates. The gross energy content of starch was assumed to be 4.2 kcal/g, fat was assumed to contain 9.1 kcal/g, and protein was assumed to contain 5.5 kcal/g.

[3]Sum of calculated AIDE contributions from protein, starch, and fat.

[4]HFI refers to the inclusion of 10% corn distillers dried grains with solubles and 5% canola meal.

[5]X = xylanase from *Trichoderma ressei* (2,000 U/kg); A = amylase from *Bacillus licheniformis* (200 U/kg); P = protease from *Bacillus subtilis* (4,000 U/kg).

corn-based diets clearly indicates a greater potential of these enzymes to increase the AIDE of wheat- compared with corn-based diets.

The inclusion of HFI such as corn-DDGS and canola meal in grain-soybean meal-based broiler diets reportedly reduces the ileal and total tract energy digestibility of broiler diets (Bell, 1993; Salim et al., 2010). Conventional wisdom would suggest that exogenous xylanase will have a better efficacy in diets with high concentrations of NSP. Nonetheless, the structure of the NSP from different ingredients, and whether they are soluble or insoluble, may determine the response to specific enzymes targeting dietary fiber (Choct, 1997; Choct et al., 2004). Corn-DDGS and canola meal contain high amounts of insoluble arabinoxylans (11.7 and 5.0%, respectively; L. F. Romero, 2012, unpublished data) compared with corn, wheat, and soybean meals typically used in broiler diets. Using the same xylanase enzyme that was used in the current study, Liu et al. (2011) previously reported an interaction between xylanase and corn-DDGS inclusion on digestibility of energy in 21- and 42-d-old broilers, where greater increments in energy digestibility with xylanase were evident in diets with increased corn-DDGS inclusion. In the current study, however, no interactions between enzymes and HFI on the AID of energy or any of the analyzed nutrients were detected at 21 and 42 d.

At 21 d, apparent ileal protein digestibility was increased by XA and additionally increased by XAP across diets, whereas at 42 d, the digestibility of CP was increased by XAP without a significant effect of XA, equally across diets. These results with XA at 21 d are supported by those of Rutherfurd et al. (2007), who reported improvements in AID of protein and amino acids in broilers fed corn-soybean meal-based diets with supplemented carbohydrase enzymes. The diets in Rutherfurd et al. (2007) contained approximately 8% HFI in the form of wheat bran and canola meal and the enzymes tested did not contain protease. However, other studies have not found responses of xylanase and amylase on ileal protein and amino acid digestibility in broilers fed corn-based diets without and with the inclusion of HFI such as corn-DDGS (Romero et al., 2013). The lack of response to xylanase and amylase on protein digestibility at 42 d in the current study, and to similar enzymes in other studies with broiler chickens at 21 d raises the question of what factors determine the response of xylanases and amylases on protein digestibility when it is observed. It is possible that factors influencing endogenous secretions, including changes on microbial populations and intestinal health affect the response to carbohydrases on protein digestibility. For instance, it has been previously reported that arabinooligosaccharides, which can be produced by xylanase activity, elicit probiotic effects (Van Craeyveld et al., 2008) and can reduce endogenous nitrogen secretions (Dänicke et al., 2000). Similarly, amylase supplementation can reduce the secretion of pancreatic α-amylase (Jiang et al., 2008).

In the current study, irrespective of diet type, protease activity increased the AID of protein, presumably due to direct hydrolysis of protein. The additive protein digestibility effect of protease on top of xylanase and amylase had been recently demonstrated in corn-based diets (Romero et al., 2013), but had not been widely examined in wheat-based diets, where xylanase has been the main focus of feed enzyme research. Because phytase was present in all diets including the controls, increments in protein digestibility of other enzymes measured in the current study were additional to effects of phytase, which has, on its own, been reported to increase ileal amino acid digestibility in chickens (Selle and Ravindran, 2007). This is an important feature of the current experimental design because a high proportion of commercial diets contain supplemental phytase and the effects of protein digestibility of phytase and other enzymes are known to be subadditive (Olukosi et al., 2007).

Interactions between grain type and enzymes on starch digestibility were present both at 21 and 42 d, with a greater response to enzymes in wheat- compared with corn-based diets. Similar findings were reported by Almirall et al. (1995) when boilers were fed diets based on cereals with significant levels of soluble NSP such as barley. In their study, increased starch digestibility was observed due to supplemental xylanase and β-glucanase in a barley-based diet, but not in a corn-based diet. Choct et al. (1999) tested a combination of xylanase and protease in broilers fed wheat-based diets and reported a significant increase on starch digestibility. The amylase used in the current study may have directly targeted starch digestion, whereas xylanase may have reduced digesta viscosity caused by soluble arabinoxylans from wheat and enhanced the accessibility of amylases to the cell contents of the grain. Because cereal starch is encased in a protein matrix, protease has also been ascribed to have indirect effects on starch digestibility by increasing accessibility to digestive enzymes (Svihus et al., 2005). However, in the current study, no additional increment on starch digestibility was provided by the addition of protease on top of XA.

Wheat-based diets without enzymes exhibited a reduced AID of fat compared with nonsupplemented corn-based diets at 21 of age. In contrast to the 21 d results when there was not an interaction between grain and HFI, at 42 d, fat digestibility of corn-based diets without HFI was lower than that of wheat-based diets. It has been previously reported that the digestibility of fat is greatly reduced in the presence of soluble pentosans (Dänicke et al., 1999). Again, these data suggest that fat digestibility was less affected by wheat arabinoxylans in older birds. Nonetheless, fat digestibility was increased due to the addition of XA in the wheat-based diets both at 21 and 42 d, which was not evident in the corn-based diets. A reduction in digesta viscosity due to the activity of xylanase may have been the main mechanism to increase fat digestibility in wheat-based diets (Almirall et al., 1995; Mathlouthi et al., 2002;

Rodriguez et al., 2012). Although supplementation of XAP did not increase the AID of fat compared with XA at 21 d, at 42 d, XAP improved fat digestibility regardless of grain type or the presence of HFI. A mode of action of exogenous protease to increase fat digestibility is unknown. However, Kalmendal and Tauson (2012) also reported an increase in the digestibility of fat in response to an exogenous protease in broilers fed wheat-based diets. Contrary to the present study, the addition of protease and a xylanase in combination in their study did not increase the digestibility of fat compared with the use of xylanase alone.

To our knowledge, this is the first study that has directly related the energy digestibility effects to exogenous enzymes and the energy contribution from the main energy yielding substrates (i.e., starch, protein, and fat) at the ileal level in broiler chickens. Wheat-based diets generally presented a greater response to exogenous enzymes in the digestibility of protein, starch, and fat compared with corn-based diets, which was reflected in a greater AIDE response both at 21 and 42 d. Although the inclusion of HFI did not exhibit a significant effect on the AIDE contribution of enzymes, it did affect the sum of the caloric contribution of protein, starch, and fat at 21 and 42 d, with significant effects on the caloric contribution of starch and fat at 42 d only. These data support the original hypothesis that diets with inclusion of HFI may have a greater potential for digestibility improvements due to exogenous enzymes, but that does not appear to be true for all nutrients. Additionally, age, and possibly the maturity of the gastrointestinal tract, may be factors influencing the energy contribution of starch and fat in response to exogenous enzymes in diets with HFI.

At 21 d, the largest contributor to the AIDE improvement with XAP was the protein fraction. Evidently, fat digestibility had a more prominent role in the total energy contribution in response to enzymes in wheat than in corn-based diets at 21 d, with only a marginal contribution of fat digestibility in corn-based diets. The energy contribution from starch at 21 d was also more important in wheat- than in corn-based diets. In general, the measured changes on AIDE due to supplemental enzymes closely resembled the sum of calculated contributions from starch, fat, and protein at 21 d, with some minor differences that may have been caused by the sum of experimental errors from the measurement of substrates in diet, digesta, and the inert marker.

The ileal energy contribution of protein in response to enzymes was relatively lower at 42 d compared with 21 d. The energy contribution of enzymes due to increments in fat digestibility in wheat-based diets was also lower at 42 d compared with 21 d. However, the opposite was observed for corn-based diets, where higher contributions of fat were observed at 42 d compared with 21 d. A relevant finding of the current study was that the relative energy contribution of starch in response to enzymes at 42 d was generally greater compared with 21 d, constituting the main source of the energy due to added enzymes at this age, both in wheat- and corn-based diets. Contrary to the common assumption that starch digestibility in older birds is almost complete following lower digestibilities during the first week of age (Gracia et al., 2003), it is possible that it could be reduced again at the end of the growth cycle if the ratio between ingested starch and endogenous α-amylase production is limiting.

Notably, the measured energy improvement from enzymes in corn-based diets closely resembled the sum of the contributions from protein, starch, and fat at 42 d. However, there was a consistent difference between the energy improvement from enzymes in wheat-based diets and the calculated contributions from protein, starch, and fat, which suggests that the digestibility of other components in the diet may have increased the AIDE response due to enzymes. The obvious contributing substrates to this difference are NSP, but the digestibility of this fraction was not measured in the current study. Enzyme supplementation may have caused either an increased fermentation of NSP or the absorption of pentose sugars in the small intestine. The fact that this difference was minor at 21 d (−7.4 kcal/kg), but was biologically noteworthy in 42-d-old chickens (66 kcal/kg), suggests that an interaction with the microbial populations of older birds may have occurred. Choct et al. (1999) reported that a combination of xylanase and protease reduced the production of short-chain fatty acids (**SCFA**) in the ileum, but increased it in the cecum of 29-d-old broilers fed wheat-based diets, which suggests a reduction on fermentation in the small intestine and an increase in the cecum in response to enzymes. In contrast, Wang et al. (2005) reported that a carbohydrase combination increased SCFA production in the ileum of broilers fed wheat-based diets at 21 d, but did not affect it at 42 d, whereas the production of SCFA in the cecum was increased by carbohydrases at both 21 and 42 d. The current results suggest an increase of digestion of fiber in the small intestine of 42-d-old broilers, which may be related to increased fermentation.

Even though some authors have suggested a direct link between increased fermentation in the small intestine and antinutritive effects of soluble NSP (Choct et al., 1996), it is also likely that the sugars released from the degradation of NSP by enzymes in the small intestine are fermented by the intestinal microbiota and the end products (i.e., SCFA) are subsequently used as energy yielding substrates by broilers. Nonetheless, if NSP digestibility is actually contributing to the measured AIDE or AME in response to enzymes in some diets or ages of broiler chickens, the efficacy of utilization of the energy from NSP is likely to be lower than the efficiency of using energy from fat, starch, or protein for growth (Savory, 1992; Chwalibog, 2002). If that is the case, current recommendations of enzymes that are solely based on AME systems, in particular for enzymes targeting the digestion of fiber such as xyla-

nase and β-glucanase, may be overestimating the net energy contribution of enzymes that animals can use for growth. The metabolic use of energy substrates in response to the addition of exogenous enzymes, as well as the interaction of exogenous enzymes, dietary ingredients, and the composition of microbial populations in chickens requires further study.

The authors acknowledge that results from digestibility studies are not always an indication of animal growth or feed efficiency, and that performance studies are required to reach general conclusions of the value of exogenous enzymes in different diet types. Nonetheless, this study provides information to allow further understanding of the mechanism of action of carbohydrases and proteases in different broiler chicken diets.

In conclusion, the caloric contributions of starch, fat, and protein in response to exogenous enzymes were affected differentially by the base grain (corn or wheat) and the presence of fibrous ingredients at 21 and 42 d of age. Evidently, there is an opportunity for greater accuracy in the development of energy matrices for exogenous enzymes, reflecting the diet composition and physiologic status of the birds, which can allow producers to maximize the value of these additives in their systems.

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