

Wheat-barley-rye- or corn-fed growing pigs respond differently to dietary supplementation with a carbohydrase complex

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ABSTRACT: Thirty-six pigs (22 kg of BW) were used to evaluate a carbohydrase preparation, with xylanase and β -glucanase as main activities, added to either wheat-barley-rye- (WBR) or corn-based diets on performance, intestinal environment, and nutrient digestibility. Pigs were offered 1 of 4 different dietary treatments for 27 d according to a factorial arrangement of treatments (a 2×2) with 2 cereal types (WBR or corn) and 2 levels of supplemental carbohydrase (0 or 0.01%). Pig growth and feed intake were individually measured every week until the end of the experiment when pigs were slaughtered to obtain samples of digesta and tissues. Cereal type affected performance only during wk 1, in which WBR improved ADG (590 vs. 440 g/d; $P = 0.008$) and G:F (0.61 vs. 0.43; $P = 0.045$) compared with corn. The WBR also increased the viscosity of the digestive contents in stomach (1.95 vs. 1.23 mPa·s; $P = 0.001$) and ileum (6.53 vs. 2.80 mPa·s; $P = 0.001$) and resulted in greater cecal starch digestibility (95.7 vs. 93.9%; $P = 0.012$). However, trends for a reduction in digestibility were observed for glucose in the nonstarch polysaccharide (NSP) fraction in the ileum

(64.4 vs. 75.8%; $P = 0.074$) and galactose in the NSP fraction in the cecum (1.4 vs. 1.8%; $P = 0.055$). The use of the enzyme preparation increased ADFI during wk 2 (1,328 vs. 1,215 g/d; $P = 0.028$), and increased villus height (423 vs. 390 μ m; $P = 0.045$) and tended to reduce relative pancreas weight (0.16 vs. 0.17% BW; $P = 0.079$) at d 27. The enzyme also improved cecal starch digestibility (95.5 vs. 94.1%; $P = 0.043$) and tended to improve ileal energy digestibility (61.3 vs. 53.7%; $P = 0.090$) and cecal glucose digestibility in the NSP fraction (76.0 vs. 54.5%; $P = 0.055$). However, it reduced the cecal digestibility of mannose in the NSP fraction (27.0 vs. 50.5%; $P = 0.016$). Interactions ($P < 0.05$) between cereal type and enzyme supplementation were observed for ADG and G:F during wk 2, BW and ADG during wk 3, and BW and ADFI over the whole trial; and also for villus-height-to-crypt-depth ratio and for cecal DM digestibility. In all instances, whereas the added enzyme had no effect in the case of the corn diet, improvements were observed with WBR. In conclusion, the multi-enzyme tested had different effects depending on the type of cereal present in the diet.

Key words: digestibility, β -glucanase, nonstarch polysaccharide, swine, villus height, xylanase

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INTRODUCTION

Swine diets are mainly composed of cereals with variable compositions in nonstarch polysaccharides (NSP). Those may interfere with the digestion of other nutrients (Simon, 1998). The NSP content ranges from 7 to 9% in corn, to 11% in wheat and rye, and 16% in barley (Chesson, 1993; Dierick and Decuyper, 1996). In addition, whereas NSP in corn mainly consist of insoluble arabinoxylans (Summers, 2001), wheat and rye contain

arabinoxylans (both soluble and insoluble) and insoluble β -glucans (Henry, 1987; Evers et al., 1999) and barley contains arabinoxylans (mainly insoluble) and β -glucans (mainly soluble; Chesson, 1993; Partridge, 2001). Although the use of NSP-degrading carbohydrases has been widely and successfully implemented in poultry fed NSP-rich diets (Francesch and Geraert, 2009), the results are not as clear in swine. The efficacy of carbohydrase in pigs might not be due to improvements in nutrient digestibility alone (Omogbenigun et al., 2004; Ji et al., 2008) but also to changes in digestive content characteristics, which can indirectly affect gut mucosa integrity (Jakob et al., 2005b; Nofrarias et al., 2006). Although some authors report improvements in

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pig performance with enzymes (Omogbenigun et al., 2004; Jakob et al., 2005a; Kiarie et al., 2007), others do not (Mavromichalis et al., 2000). The differences in the efficacy observed may be explained by the differences in the NSP fraction of the diets or in tested carbohydrase activities.

Carbohydrase efficacy in growing pigs is hypothesized to be dependent on enzyme activities, as well as the nature (and concentration) of the dietary NSP fraction and how well they match each other. The aim of this study was to test the efficacy of a multi-enzyme preparation (mainly xylanase and β -glucanase) added to either corn- (low-NSP content, mostly insoluble) or wheat-rye-barley- (WBR; high-NSP content, mostly soluble) based diets on growth performance, intestinal environment, and energy and nutrient digestibility in growing pigs.

MATERIALS AND METHODS

This experiment was conducted at the Experimental Farm of the Institut de Recerca i Tecnologia Agroalimentària (IRTA) following approval by IRTA's Ethical Committee on Animal Experimentation. The management, housing, husbandry, and slaughtering conditions were conducted according to the European Union Guidelines (VICH-GL9, 2000).

Animals, Housing, and Dietary Treatments

Thirty-six growing pigs (Landrace \times Pietrain; mixed entire males and females; BW 22.0 ± 0.4 kg) from 9 different litters (4 pigs per litter) of the IRTA farm herd were used. At weaning, piglets were identified by ear tags and maintained on a common pre-experimental diet formulated to meet or exceed the nutrient requirements for weaning pigs until the start of the experiment (NRC, 1998; Table 1). Pigs were kept individually in 36 pens (2×1 m). The facilities were provided with forced ventilation for thermal regulation, and each pen had 1 feeder and 1 water nipple to allow for ad libitum access to feed and water.

At the start of the experiment, pigs from the same litter were randomly assigned to the 4 experimental treatments, providing a total of 9 replicates per treatment. Treatments were designed as a 2×2 factorial arrangement using 2 sources of cereal (corn or WBR), and with or without supplementation with NSP-degrading enzymes (0 or 0.01%). All experimental diets were formulated to be isonutritive (i.e., 3,125 kcal of ME/kg, 16% CP, 10 g/kg of Lys, 9 g/kg of Ca, and 6 g/kg of P) and to meet or exceed the nutrient requirements for growing pigs (NRC, 1998; Table 2), and were provided in mash form. The enzyme preparation tested was a multi-enzyme complex obtained from *Penicillium funiculosum* (Rovabio Excel AP, Adisseo France SAS, Antony, France). This product is guaranteed to provide 22,000 U/g of endo- β -1,4-xylanase and 2,000 U/g of endo- β -1,3(4)-glucanase. Units are defined as

the amount of enzyme, which hydrolyzes wheat arabinoxylans, reducing the viscosity of the solution to give a change in relative fluidity of 1 arbitrary unit·min⁻¹ under the assay conditions (pH 5.5 and 30°C) for xylanase activity, and the quantity of enzyme, which hydrolyzes barley β -glucan (bound to a chromophore), releasing ethanol-soluble oligomers to give an absorbance of 0.820 units at 590 nm for endo- β -1,3(4)-glucanase activity. In addition to endo- β -1,4-xylanase and endo- β -1,3(4)-glucanase activities, other enzymes (cellulases, pectinases, mannanase, and others) are also active in the preparation (Karboune et al., 2008, 2009). The presence of the enzyme preparation in the supplemented diets was confirmed by analyzing the corresponding β -glucanase and xylanase activities (Cosson et al., 1999). Titanium dioxide was added to all the experimental diets as an indigestible marker.

Experimental Procedures and Sampling

The animals were fed the experimental diets for 27 d. Individual BW and feed intake were recorded weekly at d 0, 7, 14, 21, and 27 of the trial. At the end of the experiment, all 36 pigs were euthanized by exsanguination under isoflurane anesthesia. The abdominal cavity was immediately opened, and the whole gastrointestinal tract (GIT) from cardia to rectum was removed. The GIT was tied to retain the digestive contents in the different sections (stomach, small intestine, and cecum). The pH of the digesta in stomach, ileum (100 cm cranial to the ileocecal valve), and cecum were measured with a unipolar electrode pH meter (HI83141 pH meter, Hanna Instruments, Eibar, Spain). Samples of digestive contents, GIT tissues, and mucosa were collected immediately to perform the different analyses. Finally, the weights of the dissected pancreas and the emptied stomach were also recorded.

Table 1. Ingredient composition of the common pre-experimental diet, as-fed basis

| Ingredient, % | Content |
|-------------------------------------|---------|
| Barley | 28.94 |
| Whey, acid, dehydrated | 16.67 |
| Corn | 15.46 |
| Soybean, whole extruded | 14.64 |
| Soybean meal, 48% CP | 11.42 |
| Fish meal | 8.00 |
| Soybean oil | 4.03 |
| Vitamin-mineral premix ¹ | 0.40 |
| Calcium carbonate | 0.17 |
| L-Lys-HCl | 0.13 |
| DL-Met | 0.11 |
| L-Thr | 0.04 |

¹Providing per kilogram of diet: vitamin A, 1.5 mg; vitamin D₃, 0.025 mg; vitamin E, 15 mg; thiamine, 1.3 mg; riboflavin, 3.5 mg; vitamin B₁₂, 0.025 mg; vitamin B₆, 1.5 mg; calcium pantothenate, 10 mg; nicotinic acid, 15 mg; biotin, 0.1 mg; folic acid, 0.6 mg; vitamin K₃, 2 mg; Fe, 80 mg as iron sulfate; Cu, 6 mg as copper sulfate; Co, 0.75 mg as cobalt sulfate; Zn, 60 mg as zinc oxide; Mn, 30 mg as manganese sulfate; I, 0.75 mg as potassium iodate; Se, 0.10 mg as sodium selenite; and ethoxyquin, 150 mg.

Table 2. Ingredient composition and chemical analysis of the experimental diets, as-fed basis

| Item | Corn | | Wheat-barley-rye | |
|-------------------------------------|------------------------|--------------|------------------|--------------|
| | 0% enzyme ¹ | 0.01% enzyme | 0% enzyme | 0.01% enzyme |
| Ingredient, % | | | | |
| Corn | 71.11 | 71.11 | 0 | 0 |
| Wheat | 0 | 0 | 24.58 | 24.58 |
| Barley | 0 | 0 | 24.58 | 24.58 |
| Rye | 0 | 0 | 22.72 | 22.72 |
| Soybean meal, 44% CP | 17.83 | 17.83 | 13.55 | 13.55 |
| Sunflower meal | 6.88 | 6.88 | 6.88 | 6.88 |
| Lard | 0.03 | 0.03 | 3.47 | 3.47 |
| Calcium carbonate | 1.15 | 1.15 | 1.19 | 1.19 |
| Dicalcium phosphate | 1.28 | 1.28 | 1.17 | 1.17 |
| Sodium chloride | 0.37 | 0.37 | 0.50 | 0.50 |
| Vitamin/mineral premix ² | 0.40 | 0.40 | 0.40 | 0.40 |
| L-Lys-HCl | 0.34 | 0.34 | 0.36 | 0.36 |
| L-Thr | 0.04 | 0.04 | 0.07 | 0.07 |
| DL-Met | 0.03 | 0.03 | 0.04 | 0.04 |
| L-Trp | 0.04 | 0.04 | 0.01 | 0.01 |
| Titanium dioxide | 0.50 | 0.50 | 0.50 | 0.50 |
| Analyzed composition | | | | |
| DM, % | 86.82 | 86.89 | 88.78 | 88.96 |
| Ash, % | 5.32 | 5.46 | 5.62 | 5.55 |
| GE, kcal/kg | 3,888 | 3,858 | 4,031 | 4,045 |
| Ether extract, % | 3.23 | 3.13 | 4.56 | 4.7 |
| CP, % | 15.17 | 15.23 | 16.33 | 15.88 |
| Crude fiber, % | 4.21 | 3.72 | 4.76 | 4.63 |
| NSP, ³ % | 9.25 | 9.27 | 13.68 | 14.05 |
| Arabinose, % | 3.22 | 3.29 | 5.37 | 5.18 |
| Xylose, % | 1.38 | 1.31 | 1.86 | 1.29 |
| Mannose, % | 0.32 | 0.34 | 0.51 | 0.26 |
| Glucose, % | 3.08 | 3.04 | 4.86 | 6.29 |
| Galactose, % | 1.25 | 1.29 | 1.08 | 1.04 |
| Enzyme recovery, % | | | | |
| β-Glucanase | — | 92 | — | 92 |
| Xylanase | — | 106 | — | 103 |

¹Rovabio Excel AP (Adisseo France SAS, Antony, France).

²Providing per kilogram of diet: vitamin A, 1.5 mg; vitamin D₃, 0.025 mg; vitamin E, 15 mg; thiamine, 1.3 mg; riboflavin, 3.5 mg; vitamin B₁₂, 0.025 mg; vitamin B₆, 1.5 mg; calcium pantothenate, 10 mg; nicotinic acid, 15 mg; biotin, 0.1 mg; folic acid, 0.6 mg; vitamin K₃, 2 mg; Fe, 80 mg as iron sulfate; Cu, 6 mg as copper sulfate; Co, 0.75 mg as cobalt sulfate; Zn, 60 mg as zinc oxide; Mn, 30 mg as manganese sulfate; I, 0.75 mg as potassium iodate; Se, 0.10 mg as sodium selenite; and ethoxyquin, 150 mg.

³Nonstarch polysaccharides; total neutral sugars.

Viscosity, Enzyme Activity, and Histology Analysis

Fresh samples (approximately 10 mL) of digesta from stomach, ileum (20 cm cranial from the ileocecal valve), and cecum were immediately refrigerated on ice. The liquid fraction of digesta was obtained by centrifugation at $3,500 \times g$ for 10 min at 10°C, and viscosity of the supernatant (0.5 mL) was immediately measured at a shear rate of 12 s^{-1} and 30°C (Digital DV-II cone/plate viscometer, Brookfield Engineering Laboratories, Stoughton, MA) as described by Steinfeldt et al. (1998).

A section of the ileum (10 cm) was opened lengthwise along the mesenteric attachment, rinsed carefully with ice-cold 0.9% NaCl, and blot dried. Then the mucosa layer was scraped with a sterile cell scraper, placed in capped tubes (2 to 3 g), and immediately snap-frozen in liquid N and stored at -80°C until enzyme activities were determined. To analyze saccharase and maltase

activities of the ileal mucosa, the samples were homogenized and diluted in ice-cold 0.9% NaCl (100 mg/mL for saccharase activity and 5 mg/mL for maltase activity). The samples were then incubated with a solution of either saccharose or maltose in maleate buffer 0.2 M (pH 6.5) at 2% for 60 min at 37°C, followed by 10 min at 95°C in a thermocycler. The glucose concentration was then measured (in duplicate) with an assay kit (Amplex Red Glucose/Glucose Oxidase Assay Kit, Invitrogen, Eugene, OR) and read by a spectrophotometer (SpectraMax Plus 384, MDS Analytical Technologies Inc., Sunnyvale, CA) with a software (SoftMax Pro 5.3 Ink Software, MDS Analytical Technologies Inc.). The enzyme activities were adjusted for protein content, which was measured with an assay kit and fluorometer (Quant-iT Protein Assay Kit and Qubit fluorometer, Invitrogen, Eugene, OR).

Another 5 cm section of the ileum (20 cm cranial to the ileocecal valve) was obtained for the histological study of ileal mucosa. Samples were partially opened

(about 3/4 of length) along the mesenteric attachment and fixed by immersion in 10% (vol/vol) formaldehyde (3.7%, pH 7.0, stabilized with methanol). Tissue samples were dehydrated and embedded in paraffin, sectioned at 3 μm , and stained with hematoxylin and eosin. Morphometric measurements were performed with a light microscope (BHS, Olympus, Barcelona, Spain) using a linear ocular micrometer (Olympus, Ref. 209-35040, Microplanet, Barcelona, Spain). Villus height (VH) and crypt depth (CD) were measured on 10 well-oriented villi and crypts per animal, and the VH:CD ratio was calculated.

Digestibility Determination

The remaining ileal and cecal digestive contents were stored at -20°C in hermetic plastic bags until digestibility was determined using TiO_2 as an indigestible marker. Feed and digesta were analyzed for DM, NSP, starch, CP, and energy according to standard procedures (AOAC, 1995). Crude protein ($\text{N} \times 6.25$) was determined by the Dumas method (model TruSpec N, Leco, St. Joseph, MI). Gross energy was determined by combustion under high pressure of oxygen in an adiabatic bomb (model C4000 adiabatic calorimeter, IKA Werke GmbH & Co. KG, Staufen, Germany). Total starch was determined colorimetrically, as glucose liberated after enzymatic incubation of 0.2 g of sample with 0.1 mL of thermostable α -amylase (Ref. A-4551, Sigma, Madrid, Spain) diluted 1/10 with distilled water for 1 h at 100°C , and amyloglucosidase (Ref. A-3514, Sigma) for 6 h at 60°C , according to the method of Theander (1991). Nonstarch polysaccharides were analyzed by gas chromatography according to the method described by Englyst and Cummings (1984). The concentrations of TiO_2 were measured as described by Short et al. (1996). The digestibility coefficients were calculated using the index method (Adeola, 2001).

Statistical Analyses

Data were analyzed using the SAS MIXED procedure (SAS Inst. Inc., Cary, NC) using the animal as the experimental unit. The model included cereal type (corn or WBR), enzyme (0 or 0.01%), and their interaction as fixed effects, and litter as a random effect. In addition, for the analysis of the performance data (Table 3), BW at the start of the corresponding experimental period was used as the covariable. Results are presented as least squares means. The level of significance was set at $P < 0.05$, and trends were discussed at $P < 0.1$.

RESULTS

The presence of the enzyme preparation in the supplemented diets was confirmed (Table 2). Recovery rates were 92 and 92% (β -glucanase activity) and 106 and 103% (xylanase activity) for the supplemented corn and WBR diets, respectively.

Growth Performance

The growth performance of the growing pigs over the whole experimental period is presented in Table 3. Pigs fed the WBR-based diets had greater BW (26.3 vs. 24.9 kg; $P = 0.008$), ADG (590 vs. 440 g/d; $P = 0.008$), and G:F (0.61 vs. 0.43; $P = 0.045$) than the corn diets during the first week of trial and also BW during the second week (31.1 vs. 29.3 kg; $P = 0.006$). No effects of cereal type were observed during wk 3 and 4, or over the whole experimental period. Diet supplementation with the enzyme preparation resulted in greater ADFI during the second week of trial (1,328 vs. 1,215 g/d; $P = 0.028$). Interactions between cereal type and enzyme supplementation were observed for ADG ($P = 0.045$) and G:F ($P = 0.037$) during the second week of trial. Whereas the addition of enzyme improved ADG for the WBR-based diet, it had no effect for the corn diet. Similarly, there were no differences in G:F between the 2 nonsupplemented diets, but adding the enzyme preparation to the WBR diet resulted in greater G:F than the corn diet. During the third week, interactions were observed for BW ($P = 0.031$), ADG ($P = 0.031$), ADFI ($P = 0.081$), and G:F ($P = 0.086$). Similar interactions were observed for ADG ($P = 0.056$) and G:F ($P = 0.075$) during the last week of trial and for BW ($P = 0.013$) and ADFI ($P = 0.035$) over the whole trial.

Characteristics of Digesta and Gut Morphology

Digestive organ weight, digestive content properties, and ileal mucosa enzymatic activity and histology are shown in Table 4. Viscosities of the digestive contents were greater for the WBR than the corn diets in the stomach (1.95 vs. 1.23 mPa·s; $P = 0.001$) and the ileum (6.53 vs. 2.80 mPa·s; $P = 0.001$), but no difference was observed in the cecum. Supplementation of diets with the enzyme preparation increased villus height (423 vs. 390 μm ; $P = 0.045$) and tended to reduce relative pancreas weight (0.16 vs. 0.17%; $P = 0.079$) at the end of the trial. An interaction between cereal nature and enzyme supplementation was observed for the VH:CD ratio ($P = 0.031$) so that the ratio increased with the enzyme for the WBR diet, but there was no difference for the corn diet.

Nutrient Digestibility

Nutrient digestibilities measured in the ileum and cecum are presented in Table 5. Cecal starch digestibility was greater for the WBR-based than for the C diets (95.7 vs. 93.9%; $P = 0.012$). However, the digestibility of the corn-based diets tended to be greater for glucose in the NSP fraction in the ileum (75.8 vs. 64.4%; $P = 0.074$) and for galactose in the NSP fraction in the cecum (1.8 vs. 1.4%; $P = 0.055$). The enzyme preparation improved starch digestibility in the cecum (95.5 vs. 94.1%; $P = 0.043$), and tended to improve energy di-

Table 3. Effect of carbohydrase supplementation to corn- or wheat-barley-rye-based diets on the performance of growing pigs

| Item | Corn | | Wheat-barley-rye | | SED | <i>P</i> -value | | |
|-------------------------|------------------------|--------------|------------------|--------------|-------|-----------------|--------|-----------------|
| | 0% enzyme ¹ | 0.01% enzyme | 0% enzyme | 0.01% enzyme | | Cereal | Enzyme | Cereal × enzyme |
| BW, kg | | | | | | | | |
| d 0 | 21.7 | 22.0 | 22.3 | 22.1 | 0.69 | 0.466 | 0.973 | 0.644 |
| d 7 ² | 25.2 | 25.0 | 26.1 | 26.2 | 0.51 | 0.008 | 0.831 | 0.692 |
| d 14 ² | 29.6 | 29.4 | 30.3 | 31.5 | 0.66 | 0.006 | 0.315 | 0.133 |
| d 21 ² | 35.4 | 34.0 | 34.8 | 36.9 | 1.09 | 0.145 | 0.626 | 0.031 |
| d 27 ² | 41.4 | 38.5 | 39.3 | 42.3 | 1.58 | 0.475 | 0.998 | 0.013 |
| ADG, kg/d | | | | | | | | |
| 0 to 7 d ² | 0.46 | 0.42 | 0.59 | 0.60 | 0.073 | 0.008 | 0.832 | 0.694 |
| 8 to 14 d ³ | 0.63 | 0.63 | 0.59 | 0.75 | 0.055 | 0.303 | 0.048 | 0.045 |
| 15 to 21 d ⁴ | 0.84 | 0.68 | 0.64 | 0.75 | 0.083 | 0.295 | 0.699 | 0.031 |
| 22 to 27 d ⁵ | 1.01 | 0.78 | 0.76 | 0.84 | 0.106 | 0.213 | 0.342 | 0.056 |
| 0 to 27 d ² | 0.72 | 0.61 | 0.64 | 0.75 | 0.058 | 0.475 | 0.998 | 0.013 |
| ADFI, kg/d | | | | | | | | |
| 0 to 7 d ² | 1.02 | 1.04 | 0.96 | 1.15 | 0.087 | 0.661 | 0.104 | 0.179 |
| 8 to 14 d ³ | 1.25 | 1.32 | 1.18 | 1.34 | 0.068 | 0.628 | 0.028 | 0.314 |
| 15 to 21 d ⁴ | 1.65 | 1.58 | 1.35 | 1.45 | 0.068 | 0.001 | 0.700 | 0.081 |
| 22 to 27 d ⁵ | 1.89 | 1.69 | 1.64 | 1.65 | 0.122 | 0.114 | 0.277 | 0.273 |
| 0 to 27 d ² | 1.43 | 1.38 | 1.27 | 1.42 | 0.062 | 0.201 | 0.265 | 0.035 |
| G:F | | | | | | | | |
| 0 to 7 d ² | 0.44 | 0.42 | 0.70 | 0.52 | 0.117 | 0.045 | 0.278 | 0.349 |
| 8 to 14 d ³ | 0.50 | 0.48 | 0.50 | 0.56 | 0.029 | 0.081 | 0.337 | 0.037 |
| 15 to 21 d ⁴ | 0.51 | 0.46 | 0.46 | 0.52 | 0.041 | 0.761 | 0.866 | 0.086 |
| 22 to 27 d ⁵ | 0.54 | 0.43 | 0.47 | 0.51 | 0.056 | 0.944 | 0.442 | 0.075 |
| 0 to 27 d ² | 0.50 | 0.46 | 0.51 | 0.53 | 0.034 | 0.152 | 0.668 | 0.179 |

¹Rovabio Excel AP (Adisseo France SAS, Antony, France).

²BW at d 0 used as covariable.

³BW at d 7 used as covariable.

⁴BW at d 14 used as covariable.

⁵BW at d 21 used as covariable.

Table 4. Effect of carbohydrase supplementation to corn- or wheat-barley-rye-based diets on the weight of digestive organs, the characteristics of the digestive contents, and the enzymatic activity and histology of ileal mucosa of growing pigs

| Item | Corn | | Wheat-barley-rye | | SED | <i>P</i> -value | | |
|---|------------------------|--------------|------------------|--------------|-------|-----------------|--------|-----------------|
| | 0% enzyme ¹ | 0.01% enzyme | 0% enzyme | 0.01% enzyme | | Cereal | Enzyme | Cereal × enzyme |
| Organ weight, % of BW | | | | | | | | |
| Stomach | 0.71 | 0.67 | 0.70 | 0.66 | 0.033 | 0.664 | 0.101 | 0.861 |
| Pancreas | 0.17 | 0.16 | 0.17 | 0.16 | 0.009 | 0.386 | 0.079 | 0.932 |
| Digesta pH | | | | | | | | |
| Stomach | 4.05 | 3.81 | 3.31 | 3.37 | 0.538 | 0.133 | 0.803 | 0.698 |
| Ileum | 6.61 | 6.50 | 6.52 | 6.48 | 0.149 | 0.617 | 0.467 | 0.738 |
| Cecum | 6.08 | 5.91 | 5.95 | 5.96 | 0.111 | 0.599 | 0.319 | 0.258 |
| Digesta viscosity, mPa·s | | | | | | | | |
| Stomach | 1.20 | 1.26 | 1.90 | 2.01 | 0.236 | 0.001 | 0.894 | 0.659 |
| Ileum | 2.07 | 3.53 | 5.52 | 6.08 | 1.177 | 0.001 | 0.243 | 0.600 |
| Cecum | 2.44 | 2.08 | 2.40 | 2.58 | 0.541 | 0.560 | 0.826 | 0.494 |
| Enzyme activity ileal mucosa, U/mg of protein | | | | | | | | |
| Sucrase | 15 | 15 | 12 | 14 | 2.1 | 0.221 | 0.513 | 0.468 |
| Maltase | 204 | 185 | 170 | 188 | 21.3 | 0.319 | 0.959 | 0.224 |
| Morphometry ileal mucosa, μm | | | | | | | | |
| VH ² | 410 | 417 | 370 | 430 | 22.7 | 0.396 | 0.045 | 0.115 |
| CD ³ | 318 | 316 | 328 | 297 | 18.7 | 0.733 | 0.245 | 0.283 |
| VH:CD ratio | 1.29 | 1.32 | 1.16 | 1.46 | 0.085 | 0.949 | 0.008 | 0.031 |

¹Rovabio Excel AP (Adisseo France SAS, Antony, France).

²Villus height.

³Crypt depth.

Table 5. Effect of carbohydrase supplementation to corn- or wheat-barley-rye-based diets on the ileal and cecal digestibility of nutrients in growing pigs

| Item | Corn | | Wheat-barley-rye | | SED | <i>P</i> -value | | |
|------------------------|------------------------|--------------|------------------|--------------|-------|-----------------|--------|-----------------|
| | 0% enzyme ¹ | 0.01% enzyme | 0% enzyme | 0.01% enzyme | | Cereal | Enzyme | Cereal × enzyme |
| Ileal digestibility, % | | | | | | | | |
| DM | 60.1 | 53.8 | 46.8 | 60.9 | 7.29 | 0.557 | 0.463 | 0.060 |
| Starch | 90.1 | 88.2 | 89.9 | 93.5 | 2.94 | 0.242 | 0.690 | 0.211 |
| Energy | 60.4 | 60.6 | 46.9 | 62.0 | 5.85 | 0.176 | 0.090 | 0.100 |
| CP | 67.5 | 63.2 | 63.2 | 70.9 | 5.14 | 0.649 | 0.646 | 0.118 |
| NSP ² | 38.1 | 35.3 | 13.5 | 42.4 | 17.78 | 0.517 | 0.337 | 0.243 |
| Arabinose | 23.2 | -0.8 | -9.4 | 28.6 | 27.52 | 0.939 | 0.736 | 0.144 |
| Xylose | 12.8 | 5.4 | -7.5 | 32.0 | 26.21 | 0.873 | 0.420 | 0.242 |
| Mannose | 7.3 | -3.6 | -9.7 | -51.0 | 30.11 | 0.165 | 0.257 | 0.505 |
| Glucose | 77.5 | 74.0 | 56.9 | 71.9 | 8.18 | 0.074 | 0.356 | 0.142 |
| Galactose | 15.3 | 17.3 | -20.9 | 2.2 | 20.63 | 0.108 | 0.425 | 0.500 |
| Cecal digestibility, % | | | | | | | | |
| DM | 70.2 | 68.3 | 61.4 | 71.3 | 3.69 | 0.287 | 0.143 | 0.037 |
| Starch | 93.4 | 94.4 | 94.8 | 96.6 | 0.94 | 0.012 | 0.043 | 0.548 |
| Energy | 68.9 | 66.6 | 64.0 | 70.3 | 2.98 | 0.785 | 0.370 | 0.066 |
| NSP ² | 21.8 | 21.4 | 22.7 | 22.4 | 3.12 | 0.691 | 0.863 | 0.999 |
| Arabinose | 22.6 | 8.9 | 11.7 | 45.8 | 18.73 | 0.362 | 0.475 | 0.127 |
| Xylose | 29.9 | -7.8 | 43.0 | 45.4 | 26.58 | 0.116 | 0.392 | 0.360 |
| Mannose | 39.5 | 31.8 | 61.6 | 22.1 | 11.57 | 0.480 | 0.016 | 0.105 |
| Glucose | 61.6 | 69.8 | 47.4 | 82.2 | 13.87 | 0.932 | 0.055 | 0.239 |
| Galactose | 2.0 | 1.7 | 1.4 | 1.4 | 0.31 | 0.055 | 0.373 | 0.499 |

¹Rovabio Excel AP (Adisseo France SAS, Antony, France).

²Nonstarch polysaccharides; total neutral sugars.

gestibility in the ileum (61.3 vs. 53.7%; $P = 0.090$) and glucose digestibility in the NSP fraction in the cecum (76.0 vs. 54.5%; $P = 0.055$). On the other hand, the digestibility of mannose in the NSP fraction in the cecum was reduced with the enzyme (27.0 vs. 50.5%; $P = 0.016$). An interaction between cereal and enzyme was found for DM digestibility in the cecum ($P = 0.037$), whereas trends were observed for DM in the ileum ($P = 0.060$) and energy in the cecum ($P = 0.066$).

DISCUSSION

The type and composition of dietary fiber influence the digestion process in the GIT (Cummings et al., 1986), the physicochemical characteristics of digesta (Anguita et al., 2007), the gut morphology (Jørgensen et al., 1996; Nofrarías et al., 2007), and the intestinal function (Correa-Matos et al., 2003). Dietary fiber includes any polysaccharides reaching the hindgut such as resistant starch, some oligosaccharides, and soluble and insoluble NSP (Englyst and Wiggins, 1979). Nonstarch polysaccharides are structural components of the endosperm cell walls in cereals, and they interfere with the access of the digestive enzymes to the nutrients inside the endosperm (Simon, 1998). Exogenous enzymes that are able to hydrolyze the plant cell wall matrix may facilitate the access of the digestive enzymes inside the endosperm and help release nutrients encapsulated in the cell walls or incorporated into the cell wall itself (Bedford and Schulze, 1998). The 2 cereal compositions

used in the present study are representative of diets commonly used in the pig industry and vary substantially in their NSP content and nature. The corn diet was chosen for its low NSP content (mainly insoluble), whereas the WBR-based diet was selected for its high NSP content (mainly soluble). The aim of this experiment was to study possible interactions between enzyme supplementation and the type of cereals in the diet.

In our study, the pigs had a better growth and feed efficiency during the first week of trial for the WBR than the corn-based diets. In subsequent weeks, however, no differences in performance were observed between the 2 cereal compositions, indicating that the 2 types of diet were nutritionally equivalent, as intended. It is possible that the differences observed for the first week may be due to a better adaptation of the pigs to the WBR diet. The use of 3 different cereals at reduced inclusion, instead of a large proportion of corn as the only cereal source, may have facilitated the acceptance of the new diet at the start of the trial (Solà-Oriol et al., 2009). The viscosity of the stomach and ileum digestive contents for the WBR diets was greater than that of the corn diets. This may have impaired nutrient digestibility (Langhout et al., 2000) and, therefore, would not explain the aforementioned differences in performance during the first week. However, it can also be speculated that the greater proportion of soluble NSP in the digestive contents of pigs fed WBR may have delayed gastric emptying and digesta flow rate (Solà-Oriol et al., 2010), favoring nutrient digestibility.

Regarding the enzyme preparation tested, our data support the initial hypothesis for an interaction between the supplementation with carbohydrases and the nature of the dietary NSP fraction. We observed no responses to enzymes with corn-based diets, but positive effects with the WBR-based diets. The carbohydrase supplementation of the corn diet (based on corn and soybean meal) did not improve pig performance. Although it has been proposed that carbohydrase complexes can improve the energy digestibility of corn-soybean meal diets (Kim et al., 2003) in young pigs, older pigs were used in the current trial (20 to 40 kg of BW) and may have had a more mature digestive system. This may explain the different results. Nevertheless, the effects of enzyme supplementation on performance or nutrient digestibility of pigs fed corn-based diets, as reported in literature, are not consistent. Some studies show positive responses to enzyme supplementation (Jakob et al., 2005a; Fang et al., 2007; Ji et al., 2008), but others fail to show any effects (Li et al., 1999; Kim et al., 2004). Corn contains approximately 7 to 9% NSP, mainly insoluble arabinoxylans (Dierick and Decuyper, 1996; Summers, 2001), whereas soybean meal contains approximately 3% soluble NSP and 16% insoluble NSP (Irish and Balnave, 1993). It could be hypothesized that the enzyme complex modified the characteristics of the mainly insoluble NSP fraction of the corn diet, increasing the solubility of its NSP fraction and the viscosity of the digestive contents. This could impair nutrient digestion and absorption, counteracting possible positive effects of the enzyme and resulting in the absence of an overall response. Alternatively, changes in the intestinal bacterial population in response to the modification of fermentable carbohydrates may have also interfered. In this study, the intestinal microbiota was also studied, and it has been reported that enzymes dramatically change the composition of the intestinal microflora for both cereals (Willamil et al., 2009).

The NSP contents in wheat, rye, and barley are greater than corn, with around 11% in wheat and rye and 16% in barley (Chesson, 1993; Slominski et al., 2004). In addition, NSP in wheat and rye are present as a mixture of soluble and insoluble arabinoxylans, and insoluble β -glucans (Henry, 1987; Steinfeldt et al., 1995; Evers et al., 1999), and as a mixture of arabinoxylans (mainly insoluble) and β -glucans (mainly soluble; Chesson, 1993; Partridge, 2001) in barley. Therefore, in our study, a greater efficacy of the carbohydrases was expected for the WBR diet. Indeed, when WBR diets were supplemented with the enzyme complex, improvements in growth (17%) and feed efficiency (6%) were observed. This agrees with trials in which the use of enzymes improved the performance of piglets (Omogbenigun et al., 2004) and growing-finishing pigs (Schulze et al., 1996; Schulze and Campbell, 1998; Jakob et al., 2005a) fed similar cereal-based diets. The improved performance with the enzyme complex for the WBR diets in the present study agrees well with the observed improvements in nutrient digestibilities in the ileum

and cecum. This is in line with the digestibility improvements reported for the enzyme supplementation of wheat-based diets in growing-finishing pigs (Barrera et al., 2004; Woyengo et al., 2008). Our digestibility results indicate that the multicarbohydrase cocktail may have exerted its beneficial effects on nutrient utilization, and thus growth performance, by eliminating the nutrient-encapsulating effect of plant cell wall structural polysaccharides (Bedford and Schulze, 1998). However, it must be considered that, to compensate for the decreased energy content, the WBR diet contained a greater proportion of added fat than the corn diet. This may have favored the efficacy of the enzyme in improving energy digestibility in the WBR diet because the NSP-degrading enzymes have consistently been reported to improve fat digestibility to a greater extent than that of any other nutrient (Bedford and Schulze, 1998). Carbohydrase supplementation of NSP-rich diets promotes a more proximal digestion of the polysaccharides (Omogbenigun et al., 2004; Ji et al., 2008) and changes the nutrients available for microbial growth and fermentation (Summers, 2001; Kiarie et al., 2007; Hanczakowska and Koczywas, 2008). In our study, the composition of the microbiota was also studied and has been reported previously (Willamil et al., 2009).

Saccharase and maltase are intestinal brush border glycoside hydrolases responsible for the final steps of carbohydrate digestion and release of absorbable monosaccharides (Hertel et al., 2000). The digestion and absorption of nutrients in the small intestine determines the amount of fermentable material reaching the hindgut and, therefore, may modulate the carbohydrase activity at the ileal brush border, as suggested by Hedemann et al. (2006). The addition of β -glucanase and xylanase, likely to increase small intestine digestibility, enhances maltase and sucrose activities in the mucosa of jejunum and ileum of piglets (Fan et al., 2009). However, in our study, there was no difference between any of the treatments on the terminal ileum brush border enzyme activities. Similarly, Li et al. (2004) did not find any differences in maltase, saccharase, and lactase activities in the jejunum of piglets fed a barley-based diet supplemented with or without xylanase and β -glucanase. Brush border enzyme activities, however, not only depend on diet composition, but can also be influenced by pig age (Fan et al., 2002), section of the small intestine (Hedemann et al., 2006), and gut microbiota (Willing and Kessel, 2009). These factors might explain the differences among studies.

The viscosity of digesta was not reduced by enzyme supplementation in either corn or WBR diets, indicating that this may not be a key factor for the efficacy of carbohydrases in swine. Similarly, Kiarie et al. (2007) and Mavromichalis et al. (2000) did not find any effect of enzyme supplementation on the ileal digesta viscosity of pigs fed wheat-based diets. As stated by Bedford and Schulze (1998), the viscosity of digesta in pigs is considerably less than in poultry because of the greater concentration of water in pig digesta, and therefore, the

negative effects associated with digesta viscosity in pigs may be less relevant than in poultry.

The NSP amount and composition of the diet influence the epithelial morphology and cell turnover of the gut mucosa (Montagne et al., 2003). For example, pectin (soluble fiber) reduces VH and CD in piglets, whereas barley hulls (insoluble fiber) had no effect or even resulted in increased VH (Hedemann et al., 2006). In our study, enzyme supplementation did not have any effect on ileal morphometry in pigs fed the corn diet, in contrast to what has been reported previously in piglets (Kim et al., 2003; Jakob et al., 2005b). However, in pigs fed the WBR diet, we observed an increase in VH and VH:CD, and a reduction in CD in the ileum with the addition of carbohydrase. This agrees with previous observations in piglets fed enzyme-supplemented wheat-barley diets (Mori et al., 2007). The increased VH and VH:CD indicate improved absorption capacity, which, in turn, may contribute to the increased nutrient digestibilities observed.

In conclusion, substantially different effects of the multi-enzyme preparation (mainly xylanase and β -glucanase) were observed depending on the type of cereal in the diet. For the corn-based diet (low concentrations of primarily insoluble NSP), no effects were observed. The supplementation of the WBR-based diet (high NSP content and soluble), however, improved nutrient utilization and growth performance. Thus, the efficacy of exogenous NSP-degrading enzymes depends on the NSP composition of the diet.

LITERATURE CITED

- Adeola, O. 2001. Digestion and balance techniques in pigs. Pages 903–916 in Swine Nutrition. 2nd ed. A. J. Lewis and L. L. Southern, ed. CRC Press, Boca Raton, FL.
- Anguita, M., J. Gasa, M. Nofrarias, S. M. Martín-Orúe, and J. F. Pérez. 2007. Effect of coarse ground corn, sugar beet pulp and wheat bran on the voluntary intake and physicochemical characteristics of digesta of growing pigs. *Livest. Sci.* 107:182–191.
- AOAC. 1995. Official Methods of Analysis. 16th ed. Assoc. Off. Anal. Chem., Arlington, VA.
- Barrera, M., M. Cervantes, W. C. Sauer, A. B. Araiza, and N. Torrentera. 2004. Ileal amino acid digestibility and performance of growing pigs fed wheat-based diets supplemented with xylanase. *J. Anim. Sci.* 82:1997–2003.
- Bedford, M. R., and H. Schulze. 1998. Exogenous enzymes for pigs and poultry. *Nutr. Res. Rev.* 11:91–114.
- Chesson, A. 1993. Feed enzymes. *Anim. Feed Sci. Technol.* 45:65–79.
- Correa-Matos, N. J., S. M. Donovan, R. E. Isaacson, H. R. Gaskins, B. A. White, and K. A. Tappenden. 2003. Fermentable fiber reduces recovery time and improves intestinal function in piglets following *Salmonella typhimurium* infection. *J. Nutr.* 133:1845–1852.
- Cosson, T., A. M. Pérez Vendrell, B. González Teresa, D. Reñé, P. Taillade, and J. Brufau. 1999. Enzymatic assays for xylanase and β -glucanase feed enzymes. *Anim. Feed Sci. Technol.* 77:345–353.
- Cummings, J. H., H. N. Englyst, and H. S. Wiggins. 1986. The role of carbohydrates in lower gut function. *Nutr. Rev.* 44:50–54.
- Dierick, N., and J. Decuyper. 1996. Mode of action of exogenous enzymes in growing pig nutrition. *Pig News Inf.* 17:41–48.
- Englyst, H., and J. H. Cummings. 1984. Simplified method for the measurement of total non-starch polysaccharides by gas-liquid chromatography of constituent sugars as alditol acetates. *Analyst (Lond.)* 109:937–942.
- Englyst, H., and H. Wiggins. 1979. Measurement of the carbohydrate component of dietary fiber (non-starch polysaccharides). *Gut* 20:A935.
- Evers, A. D., A. B. Blakeney, and L. O'Brien. 1999. Cereal structure and composition. *Aust. J. Agric. Res.* 50:629–650.
- Fan, C. L., X. Y. Han, Z. R. Xu, L. J. Wang, and L. R. Shi. 2009. Effects of β -glucanase and xylanase supplementation on gastrointestinal digestive enzyme activities of weaned piglets fed a barley-based diet. *J. Anim. Physiol. Anim. Nutr. (Berl.)* 93:271–276.
- Fan, M. Z., O. Adeola, E. K. Asem, and D. King. 2002. Postnatal ontogeny of kinetics of porcine jejunal brush border membrane-bound alkaline phosphatase, aminopeptidase N and sucrase activities. *Comp. Biochem. Physiol. A Mol. Integr. Physiol.* 132:599–607.
- Fang, Z. F., J. Peng, Z. L. Liu, and Y. G. Liu. 2007. Responses of non-starch polysaccharide-degrading enzymes on digestibility and performance of growing pigs fed a diet based on corn, soya bean meal and Chinese double-low rapeseed meal. *J. Anim. Physiol. Anim. Nutr. (Berl.)* 91:361–368.
- Francesch, M., and P. A. Geraert. 2009. Enzyme complex containing carbohydrases and phytase improves growth performance and bone mineralization of broilers fed reduced nutrient corn-soybean-based diets. *Poult. Sci.* 88:1915–1924.
- Hanczakowska, E., and E. Koczywas. 2008. Application of non-starch polysaccharide degrading enzymes in pig nutrition. *Wiad. Zootechniczne* 46:9–16.
- Hedemann, M. S., M. Eskildsen, H. N. Lærke, C. Pedersen, J. E. Lindberg, P. Laurinen, and K. E. Bach Knudsen. 2006. Intestinal morphology and enzymatic activity in newly weaned pigs fed contrasting fiber concentrations and fiber properties. *J. Anim. Sci.* 84:1375–1386.
- Henry, R. J. 1987. Pentosan and (1–3),(1–4)-beta-glucan concentrations in endosperm and wholegrain of wheat, barley, oats and rye. *J. Cereal Sci.* 6:253–258.
- Hertel, S., F. Heinz, and M. Vogel. 2000. Hydrolysis of low-molecular-weight oligosaccharides and oligosaccharide alditols by pig intestinal sucrase/isomaltase and glucosidase/maltase. *Carbohydr. Res.* 326:264–276.
- Irish, G. G., and D. Balnave. 1993. Nonstarch polysaccharides and broiler performance on diets containing soybean-meal as the sole protein-concentrate. *Aust. J. Agric. Res.* 44:1483–1499.
- Jakob, S., S. Maisonnier-Grenier, P. Dalibard, and F. X. Roth. 2005a. Interest for utilizing a single multi-enzyme preparation on different types of diets, from post-weaning to slaughter. *J. Rech. Porcine* 37:239–244.
- Jakob, S., J. Wolinski, R. Zabielski, and D. Laubitz. 2005b. Effect of a multi-enzyme preparation on the gut morphology of weaning piglets. *J. Anim. Sci.* 83(Suppl. 1):391. (Abstr.)
- Ji, F., D. P. Casper, P. K. Brown, D. A. Spangler, K. D. Haydon, and J. E. Pettigrew. 2008. Effects of dietary supplementation of an enzyme blend on the ileal and fecal digestibility of nutrients in growing pigs. *J. Anim. Sci.* 86:1533–1543.
- Jørgensen, H., X. Q. Zhao, and B. O. Eggum. 1996. The influence of dietary fibre and environmental temperature on the development of the gastrointestinal tract, digestibility, degree of fermentation in the hind-gut and energy metabolism in pigs. *Br. J. Nutr.* 75:365–378.
- Karboune, S., P. A. Geraert, and S. Kermashat. 2008. Characterization of selected cellulolytic activities of multi-enzymatic complex system from *Penicillium funiculosum*. *J. Agric. Food Chem.* 56:903–909.
- Karboune, S., L. L'Hocine, J. Anthoni, P. A. Geraert, and S. Kermasha. 2009. Properties of selected hemicellulases of a multi-enzymatic system from *Penicillium funiculosum*. *Biosci. Biotechnol. Biochem.* 73:1286–1292.

- Kiarie, E., C. M. Nyachoti, B. A. Slominski, and G. Blank. 2007. Growth performance, gastrointestinal microbial activity, and nutrient digestibility in early-weaned pigs fed diets containing flaxseed and carbohydrase enzyme. *J. Anim. Sci.* 85:2982–2993.
- Kim, B. G., J. Z. Tian, J. S. Lim, D. Y. Kil, H. Y. Jeon, Y. K. Chung, and Y. Y. Kim. 2004. Influences of enzyme complex supplementation on growth, ileal and apparent fecal digestibility and morphology of small intestine in pigs. *Asian-australas. J. Anim. Sci.* 17:1729–1735.
- Kim, S. W., D. A. Knabe, K. J. Hong, and R. A. Easter. 2003. Use of carbohydrases in corn-soybean meal-based nursery diets. *J. Anim. Sci.* 81:2496–2504.
- Langhout, D. J., J. B. Schutte, J. de Jong, H. Sloetjes, M. W. A. Verstegen, and S. Tamminga. 2000. Effect of viscosity on digestion of nutrients in conventional and germ-free chicks. *Br. J. Nutr.* 83:533–540.
- Li, D. F., S. D. Liu, S. Y. Qiao, G. F. Yi, C. Liang, and P. Thacker. 1999. Effect of feeding organic acid with or without enzyme on intestinal microflora, intestinal enzyme activity and performance of weaned pigs. *Asian-australas. J. Anim. Sci.* 12:411–416.
- Li, W.-F., J. Feng, Z.-R. Xu, and C.-M. Yang. 2004. Effects of non-starch polysaccharides enzymes on pancreatic and small intestinal digestive enzyme activities in piglet fed diets containing high amounts of barley. *World J. Gastroenterol.* 10:856–859.
- Mavromichalis, I., J. D. Hancock, B. W. Senne, T. L. Gugle, G. A. Kennedy, R. H. Hines, and C. L. Wyatt. 2000. Enzyme supplementation and particle size of wheat in diets for nursery and finishing pigs. *J. Anim. Sci.* 78:3086–3095.
- Montagne, L., J. R. Pluske, and D. J. Hampson. 2003. A review of interactions between dietary fibre and the intestinal mucosa, and their consequences on digestive health in young non-ruminant animals. *Anim. Feed Sci. Technol.* 108:95–117.
- Mori, A. V., J. Kluess, R. Zabielski, D. Laubitz, J. Wolinski, and P. A. Geraert. 2007. Exogenous NSP-enzymes change the intestinal physiology in piglets. *J. Rech. Porcine* 39:139–142.
- Nofrarias, M., M. Anguita, M. Roca, J. F. Pérez, and N. Majó. 2007. Effect of coarse ground corn, sugar beet pulp and wheat bran on the colonic morphology in growing pigs. *J. Anim. Sci.* 85(Suppl. 1):311. (Abstr.)
- Nofrarias, M., E. G. Manzanilla, J. Pujols, X. Gibert, N. Majó, J. Segalés, and J. Gasa. 2006. Effects of spray-dried porcine plasma and plant extracts on intestinal morphology and on leukocyte cell subsets of weaned pigs. *J. Anim. Sci.* 84:2735–2742.
- NRC. 1998. *Nutrient Requirements of Swine*. 10th rev. ed. Natl. Acad. Press, Washington, DC.
- Omogbenigun, F. O., C. M. Nyachoti, and B. A. Slominski. 2004. Dietary supplementation with multienzyme preparations improves nutrient utilization and growth performance in weaned pigs. *J. Anim. Sci.* 82:1053–1061.
- Partridge, G. C. 2001. The role and efficacy of carbohydrase enzymes in pig nutrition. Pages 161–198 in *Enzymes in Farm Animal Nutrition*. M. B. G. Partridge, ed. CABI Publ., Wallingford, UK.
- Schulze, H., and R. G. Campbell. 1998. Effect of exogenous xylanase on performance of pigs fed corn/soya based diets. *J. Anim. Sci.* 76(Suppl. 1):179. (Abstr.)
- Schulze, H., G. G. Partridge, and D. Creswell. 1996. The effect of feed enzyme supplementation to corn/soya based diets on performance of finisher pigs from 46 to 92 kg. *J. Anim. Sci.* 74(Suppl. 1):191. (Abstr.)
- Short, F. J., P. Gorton, J. Wiseman, and K. N. Boorman. 1996. Determination of titanium dioxide added as an inert marker in chicken digestibility studies. *Anim. Feed Sci. Technol.* 59:215–221.
- Simon, O. 1998. The mode of action of NSP hydrolysing enzymes in the gastrointestinal tract. *J. Anim. Feed Sci.* 7:115–123.
- Slominski, B. A., D. Boros, L. D. Campbell, W. Guenter, and O. Jones. 2004. Wheat by-products in poultry nutrition. Part I. Chemical and nutritive composition of wheat screenings, bakery by-products and wheat mill run. *Can. J. Anim. Sci.* 84:421–428.
- Solà-Oriol, D., E. Roura, and D. Torrallardona. 2009. Feed preference in pigs: Effect of cereal sources at different inclusion rates. *J. Anim. Sci.* 87:562–570.
- Solà-Oriol, D., D. Torrallardona, and J. Gasa. 2010. Role of dietary fibre source and meal size on the ileal transit of digesta in growing pigs. *Livest. Sci.* 133:67–69.
- Steenfeldt, S., K. E. B. Knudsen, C. F. Borsting, and B. O. Eggum. 1995. The nutritive-value of decorticated mill fractions of wheat. 2. Evaluation with raw and enzyme-treated fractions using adult cockerels. *Anim. Feed Sci. Technol.* 54:249–265.
- Steenfeldt, S., A. Mullertz, and J. Jensen. 1998. Enzyme supplementation of wheat-based diets for broilers. 1. Effect on growth performance and intestinal viscosity. *Anim. Feed Sci. Technol.* 75:27–43.
- Summers, J. D. 2001. Maize: Factors affecting its digestibility and variability in feeding value. Pages 109–124 in *Enzymes in Farm Animal Nutrition*. M. B. G. Partridge, ed. CABI Publishing, Wallingford, UK.
- Theander, O. 1991. Chemical analysis of lignocellulose materials. *Anim. Feed Sci. Technol.* 32:35–44.
- VICH-GL9. 2000. Good clinical practice. VICH International cooperation on harmonisation of technical requirements for registration of veterinary medicinal products. Accessed Jul. 18, 2011. http://www.vichsec.org/pdf/2000/Gl09_st7.pdf.
- Willamil, J., I. Badiola, D. Torrallardona, P. A. Geraert, and E. Devillard. 2009. Effect of carbohydrase supplementation on ileal morphology and gut microbiota in growing pigs, fed different cereal-based diets. *Microb. Ecol.* 57:567. (Abstr.)
- Willing, B. P., and A. G. Kessel. 2009. Intestinal microbiota differentially affect brush border enzyme activity and gene expression in the neonatal gnotobiotic pig. *J. Anim. Physiol. Anim. Nutr. (Berl.)* 93:586–595.
- Woyengo, T. A., J. S. Sands, W. Guenter, and C. M. Nyachoti. 2008. Nutrient digestibility and performance responses of growing pigs fed phytase- and xylanase-supplemented wheat-based diets. *J. Anim. Sci.* 86:848–857.