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Non Ruminant Nutrition

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Effect of a carbohydrase admixture in growing pigs fed wheat-based diets in thermoneutral and heat stress conditions

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Abstract

The efficacy of exogenous carbohydrases in pig diets has been suggested to depend on enzyme activity and dietary fiber composition, but recent evidence suggests other factors such as ambient temperature might be important as well. Therefore, we investigated the effect of heat stress (**HS**) on the efficacy of a multienzyme carbohydrase blend in growing pigs. Ninety-six (barrows: gilts; 1:1) growing pigs with initial body weight (**BW**) of 20.15 ± 0.18 kg were randomly assigned to six treatments, with eight replicates of two pigs per pen in a 3 × 2 factorial arrangement: three levels of carbohydrase (0, 1X, or 2X) at two environmental temperatures (20 °C or cyclical 28 °C nighttime and 35 °C day time). The 1X dose (50 g/tonne) provided 1,250 viscosimetry unit (visco-units) endo-β-1,4-xylanase, 4,600 units α-L-arabinofuranosidase and 860 visco-units endo-1,3(4)-β-glucanase per kilogram of feed. Pigs were fed ad libitum for 28 d and 1 pig per pen was sacrificed on day 28. There was no enzyme × temperature interaction on any response criteria; thus, only main effects are reported. Enzyme treatment quadratically increased ($P < 0.05$) BW on day 28, average daily gain (**ADG**) ($P < 0.05$), and average daily feed intake (**ADFI**) ($P < 0.05$) with the 1X level being highest. HS reduced the BW at day 14 ($P < 0.01$) and day 28 ($P < 0.01$), ADG ($P < 0.01$), and ADFI ($P<0.001$). There was a trend of increased feed efficiency (**G:F**) ($P < 0.1$) in the HS pigs. HS increased apparent jejunal digestibility of energy ($P < 0.05$) and apparent ileal digestibility of calcium ($P < 0.01$). At day 1, HS reduced serum glucose ($P < 0.001$) but increased nonesterified fatty acid ($P < 0.01$). In the jejunum, there was a trend of increased villi height by carbohydrases ($P < 0.1$), whereas HS reduced villi height ($P < 0.05$). HS increased the jejunal mRNA abundance of *IL1β* in the jejunum ($P < 0.001$). There was a trend for a reduction in ileal *MUC2* ($P < 0.1$) and *occludin* ($P < 0.1$) by HS, and a trend for increased *PEPT1* ($P < 0.1$). There was no effect of HS on alpha diversity and beta diversity of the fecal microbiome, but there was an increase in the abundance of pathogenic bacteria in the HS group. In conclusion, HS did not alter the efficacy of carbohydrases. This suggests that carbohydrases and HS modulate pig performance independently.

Key words: carbohydrases, growing pig, growth performance, gut microbiome, heat stress

Abbreviations

ADFI	average daily feed intake
ADG	average daily gain
AID	apparent ileal digestibility
AJD	apparent jejunal digestibility
BUN	blood urea nitrogen
BW	body weight
DM	dry matter
EDTA	ethylenediaminetetraacetic acid
G:F	feed efficiency
GE	gross energy
GIT	gastrointestinal tract
HS	heat stress
LEfSe	linear discriminant analysis effect size
NEFA	nonesterified fatty acid
OUT	operational taxonomic units
SID	standardized ileal digestible
STTD	standardized total tract digestibility
TAG	triacylglyceride
TN	thermoneutral

Introduction

Inclusion of a high level of fiber in pig diets may cause reduced growth and nutrient digestibility (Noblet and Goff, 2001). This is partly because most dietary fiber is fermented in the hindgut, a process that is less efficient due to energy loss in heat and gas production (Bach Knudsen, 2001). Additionally, although fiber fermentation results in production of short-chain fatty acids, these have lower energy content compared with glucose. Fiber may also trap and decrease digestibility of other nutrients such as amino acids and minerals which are then fermented in the hindgut where they are poorly available to the animal (Lenis et al., 1996; Gutierrez et al., 2013). For this reason, exogenous fiber degrading carbohydrases (e.g., xylanase, β-glucanase, and arabinofuranosidase) have been used to improve digestibility of energy and protein in pigs (Zijlstra et al., 2010). These carbohydrases hydrolyze non-starch polysaccharides (**NSP**s) in the small intestine, releasing energy and other nutrients within the matrix of the polysaccharides. The released nutrients can then be absorbed in the foregut before they are fermented by the microbes in the hindgut (Bedford, 1995). Despite the potential of exogenous carbohydrases, their effect in pigs has been inconsistent (Adeola and Cowieson, 2011; Torres-Pitarch et al. (2019). Feeding a high fiber diet may also affect growth composition in pigs. As demonstrated by Agyekum et al. (2012), high fiber inclusion reduced empty body weight (**BW**) by increasing the mass of colon, rectum, and portal drained viscera which was ameliorated with multienzyme supplementation. Fiber utilization may also increase metabolic heat load in animals due to heat of fermentation. For example, ruminants are known to be susceptible to HS because of the large amount of heat produced during feed fermentation and digestion (Coppock, 1985; Goetsch and Johnson, 1999; Roy and Collier, 2012). However, the extent to which fermentative heat production affects pigs that are exposed to environmental HS is relatively unknown. Nevertheless, by degrading fiber in the foregut, carbohydrases may cause reduction of fermentative heat load by degrading NSP into components that are absorbed foregut rather than fermented in the hindgut (Mayorga et al., 2019). The action of carbohydrases may also alter the structure and size of materials passing through the gut, leading to a reduction in their abrasive effect on the gut lining and to improvement in intestinal barrier structure and inflammation. The oligomers, which are the products of carbohydrase action on fiber, can alter microbial fermentation by gut microbes. However, the impact of exogenous carbohydrases in pigs fed NSP-rich diets in different ambient temperatures is still unknown. Therefore, because of their potential to increase digestibility of NSPs and decrease hindgut fermentation, we hypothesize that exogenous carbohydrases will improve performance and nutrient digestibility, affect intestinal barrier structure, and reduce intestinal inflammation. We also hypothesize that carbohydrases may alter the gut microbiome characteristics in growing pigs housed under different ambient temperature conditions.

Materials and Methods

Animals

All animal procedures were approved by the Purdue University Animal Care and Use Committee. All pigs used in this study were obtained from the Purdue University Swine Research Unit. A total of 96 growing pigs (Hampshire× Duroc× Yorkshire × Landrace, barrows: gilts = 1:1) with an average initial BW of 20.15 ± 0.18 kg were used in a randomized complete block design. The animals were blocked by initial BW and randomly allocated to one of the six treatments on day 0. The study lasted 28 d. Pigs were housed in floor pens and had ad libitum access to feed and water. Pigs were fed experimental diets in two phases. Diets were without titanium dioxide (TiO_2) from day 0 to 14 and with TiO_2, which was included in the diet at 0.5%, from day 14 to 28.

Dietary treatments

All diets were fed as mash. The experiment was arranged in 3 × 2 factorial arrangement with three levels of xylanase-arabinofuranosidase and β-glucanase admixture (Rovabio Advance at **0, 1X, or 2X**). The three dietary treatments were duplicated in two environmental temperature conditions: thermoneutral (**TN**; constant 20 °C) and heat stress (**HS**; cyclical 28 °C from 2000 to 0600 hours and 35 °C from 0600 to 2000 hours (28/35 °C). The 96 pigs were assigned to the six treatments stated above. There were eight replicate pens per treatment at two pigs per pen (four pens of gilts and four pens of barrows).

The 1X dose is the commercial dose inclusion (50 g/tonne) and provides 1,250 visco-units of endo-β-1,4-xylanase, 4,600 units of α-L-arabinofuranosidase, and 860 visco-units of endo-1,3(4)-β-glucanase per kilogram of feed, whereas the 2X dose (100 g/tonne) is twice the 1X dose (Table 1).

Experimental procedure and sample collection

BW and feed intake were recorded on days 14 and 28, and feed efficiency (**G:F**) was calculated. On days 1, 14, and 28, blood samples were collected from the jugular vein from one pig per pen at about 0800 hours in the morning into a vacutainer tube containing ethylenediaminetetraacetic acid (EDTA). On days 14 and 28, feces were collected through rectal palpation from one pig per pen to determine gut microbial diversity. On day 28, one pig per pen was euthanized by injecting 0.5 mL of Telazol (Fort Dodge Laboratories, Inc., Fort Dodge, IA, USA; 100 mg/mL in 5 mL of xylazine [Veterinary Healthcare Solutions, Inc., Windsor, ON, Canada]) and asphyxiation with CO_2. Digesta from the mid-jejunum and distal ileum were also collected from one pig from each pen to determine apparent ileal digestibility (**AID**) of nutrients. Mucosal samples were collected from the

Table 1. Ingredient composition of experimental diets[1]

Ingredients[1]	CON	1X	2X
Wheat	198	188	178
Rapeseed meal	56	56	56
Soybean meal	150	150	150
Rye	260	260	260
Wheat bran	100	100	100
Wheat middlings	100	100	100
Soy oil	80	80	80
L-Lysine	4	4	4
L-Threonine	1	1	1
DL-Methionine	1	1	1
Limestone	11	11	11
Monocalcium phosphate	6	6	6
Salt	4	4	4
Vitamin premix[2]	2.5	2.5	2.5
Mineral premix[2]	1.5	1.5	1.5
Carbohydrase premix[3]	0	10	20
TiO_2 premix[4]	25	25	25
Total	1,000	1,000	1,000
Calculated composition			
Metabolizable energy, kcal/kg	3,433	3,433	3,433
Ca	0.67	0.67	0.67
Total P	0.66	0.65	0.65
STTD P[5]	0.38	0.38	0.38
SID AA[6]			
Methionine	0.34	0.34	0.34
Histidine	0.40	0.40	0.40
Threonine	0.62	0.62	0.62
Lysine	1.04	1.04	1.04
Tryptophan	0.18	0.18	0.18
CP[1]	19.04	19.03	19.03
Neutral detergent fiber	14.72	14.72	14.72
Acid detergent fiber	5.32	5.32	5.32
Analyzed composition			
GE[1]	4,734	4,763	4,757
CP[1]	21.25	21.5	21.19
Ca	0.7	0.74	0.68
P	0.72	0.73	0.71

[1]CON, control; 1X, 50 g/tonne carbohydrase admixture; 2X, 100 g/tonne of carbohydrase admixture; AA, amino acid; GE, gross energy; CP, crude protein.
[2]Premix composition per kilogram of diet: Vit. A, 7,500 IU; Vit. D_3, 1,500 IU; Vit E, 20 IU; Vit B1, 0.5 mg; Riboflavin, 3.0 mg; D-pantothenic acid, 10.0 mg; Vit B_6, 1.0 mg; Vit B_{12}, 15 ug; Vit PP, 15.0 mg; Vit K3 1.0 mg; Fe, 80 mg; I, 0.50 mg; Cu, 15 mg; Mn, 40 mg; Zn, 80 mg; and Se, 0.25 mg.
[3]Carbohydrase premix made to 0.05 g carbohydrase/g premix added at 10 and 20 g/kg to provide 0.5 and 1.0 g/kg.
[4]TiO_2 premix made at 5 g TiO_2/g premix added at 25 g/kg in each diet to provide 75 g/kg.
[5]STTD, standardized total tract digestibility.
[6]SID, standardized ileal digestible.

mid-jejunum and terminal ileum and stored at −80 °C until processed.

Determination of nutrient digestibility

Jejunal and ileal digesta were freeze-dried and ground. The ground samples were then dried at 105 °C in a drying oven (Precision Scientific Co., Chicago, IL, USA) for 24 h to determine the dry matter (**DM**) content (AOAC International, 2000). Gross energy (**GE**) was determined with a bomb calorimeter (Parr 1261 bomb calorimeter, Parr Instruments Co., Moline, IL, USA). Titanium concentration was determined after samples were ashed and digested in sulfuric acid. Titanium concentration was determined by reading absorbance at 440 nm on a spectrophotometer (Spectronic 21D, Milton Roy Co., Rochester, NY; Fenton and Fenton, 1979). Similarly, for measurement of calcium (Ca) and phosphorus (P), samples were first digested in nitric and hydrochloric acid. Phosphorus concentration was measured by reading absorbance in a spectrophotometer at 630 nm using the method described by Onyango et al. (2004). Concentration of Ca was determined using flame atomic absorption spectrometry (Varian FS240 AA Varian Inc., Palo Alto, CA, USA). Nitrogen content was determined with the combustion method on a TruMac nitrogen analyzer (Leco Corp., St. Joseph, MI, USA). AID or apparent jejunal digestibility (**AJD**) was calculated using the following equation:

$$\text{AID (AJD)}, \% = [1 - (\text{Ti}_i/\text{Ti}_o) \times (\text{Y}_o/\text{Y}_i)] \times 100$$

where Ti_i and Ti_o are the titanium concentrations of the diet and jejunal or ileal output, respectively (mg/kg of DM), and Y_o and Y_i are the concentrations of nutrients in the jejunal or ileal output and diet, respectively (mg/kg of DM).

RNA isolation and quantitative real-time PCR

Total RNA was extracted from jejunal and ileal mucosa with the Trizol reagent (Invitrogen, Grand Island, NY, USA). The concentration of RNA was quantified with a spectrophotometer (ND-1000; NanoDrop Technologies, Inc., Rockland, DE). The quality of the RNA was determined by agarose gel electrophoresis. Isolated RNA was reverse transcribed into complimentary DNA (**cDNA**) using Moloney murine leukemia virus reverse transcriptase (Promega, Madison, WI). Real time polymerase chain reaction (**PCR**) was conducted with a Bio Rad CFX Connect machine (Bio Rad, Hercules, CA) with an SYBR Green Master Mix (Qiagen, Valencia, CA) in a total reaction volume of 20 µL. At the end of each PCR run, a melt curve analysis was performed, and the expression level of each was calculated using the ΔΔCt method with *GAPDH* as the normalizing gene. Gene expression level of heat shock protein 70 and 90 (*HSP70* and *HSP90*), interleukin 1 β (***IL1β***), tumor necrosis factor-alpha (***TNFα***), Mucin gene 2 (***MUC2***), peptide transporter 1 (***PEPT1***), sodium-dependent glucose cotransporter 1 (***SGLT1***), galactoside 2-alpha-L-fucosyltransferase gene 2 (***FUT2***), *Claudin* 4, and *occludin* were measured. Sequences of the primers used for RT-PCR are provided in Table 2.

Analysis of serum

Serum glucose and nonesterified fatty acid (**NEFA**) concentrations were determined with the Autokit kit Glucose and NEFA kit, respectively (Wako Pure Chemical Industries, Chuo-Ku Osaka, Japan). Serum insulin concentration was determined using the porcine insulin ELISA kit (Mercodia, Uppsala, Sweden). Serum triacylglyceride (**TAG**) concentration was determined with the triglyceride determination kit (Sigma Aldrich, St Louis, MO). Blood urea nitrogen (**BUN**) concentration in the serum was determined with the BUN colorimetric detection kit (Arbor Assays, Ann Arbor, MI) according to the manufacturer's instructions.

Histomorphology analysis

The mid-jejunal and proximal ileal tissues were collected and fixed in 10% neutral-buffered formalin (VWR International,

Table 2. Sequences of real-time PCR primers

Gene	Primer sequence (5'-3')	Annealing temperature
HSP 70	F—TTCGTGGACAGAAGCCACAG	55
	R—TTGCTAGGATCTCCACCCGA	
HSP 90	F—GTCGAAAAGGTGGTTGTGTCG	55
	R—TTTGCTGTCCAGCCGTATGT	
IL 1 β	F—CCAAAGAGGGACATGGAGAA	55.7
	R—GGGCTTTTGTTCTGCTTGAG	
TNF α	F—CGTCGCCCACGTTGTAGCCAAT	55.7
	R—GCCCATCTGTCGGCACCACC	
MUC 2	F—AACCAGAAGCTGGTCCTGAA	55
	R—TGTCAGCCATCGTAGGAAAT	
PEPT 1	F—CAGACTTCGACCACAACGGA	55
	R—TTATCCCGCCAGTACCCAGA	
SGLT 1	F—GTGCAGTCAGCACAAAGTGG	55
	R—CCCGGTTCCATAGGCAAACT	
FUT 2	F—CGAGTGGATTGGGATCGAGG	55
	R—AAGAAGAATGGGGAGCCGAG	
Claudin 4	F—CTCTCGGACACCTTCCCAAG	55
	R—GCAGTGGGGAAGGTCAAAGG	
Occludin	F—CTACTCGTCCAACGGGAAAG	61
	R—ACGCCTCCAAGTTACCACTG	
GAPDH	F—GTTTGTGATGGGCGTGAAC	55
	R—ATGGACCGTGGTCATGAGT	

Radnor, PA). Samples were then dehydrated with ethanol (VWR International, Radnor, PA), and then cleared with Sub-X (Polysciences, Inc., Warrington, PA) and fixed in paraffin (Polyfin paraffin, Sigma Polysciences, St. Louis, MO). The segments (5 μm) were stained with hematoxylin and eosin at the Purdue Histology and Phenotyping Laboratory (Purdue University, West Lafayette, IN). Villus height and crypt depth were measured from three complete vertically oriented villi per slide. Villus height was taken from the apical portion of villus to the base, and crypt depth was taken from the base of the villus to the basolateral membrane. Subsequently, the villus height to crypt depth ratio was calculated. All measurements were performed under a binocular light microscope (National Optical and Scientific Instruments, Inc., Schertz, TX).

Fecal DNA extraction, sequencing, and analysis

Bacterial DNA was extracted from the fecal samples (approx. 200 mg) with the FastDNA SPIN Kit for Soil (MP Biomedicals, Irvine, CA) according to the manufacturer's instructions. The 16S rRNA gene in extracted fecal DNA was amplified at the V3-V4 region using specific primers (343-forward TACGGRAGGCAGCAG and 802-reverse CTACCRGGGTATCTAATCC primers). Dual index tags were used to identify each sample according to the manufacturer's instruction (Illumina, San Diego, CA). After amplification and purification, equimolar quantity of each sample was polled and sent to the Purdue Genomics Core Facility for MiSeq Illumina 2 × 250 paired-end sequencing. Sequences were analyzed based using the QIIME pipeline version 1.9.1 (Caporaso et al., 2010). The alpha diversity indices (Chao 1, Observed operational taxonomic units [**OTU**s], Evenness, Shannon and Faith phylogenetic diversity, and Simpson and Simpson evenness) were calculated to compare diversity within a sample. Beta diversity measures were calculated for comparison among communities using non-phylogenic distance (Bray Curtis and Jaccard) and phylogenetic distance (unweighted and weighted Unifrac) (Hamady et al., 2010). Kruskal–Wallis analysis (nonparametric equivalent to analysis of variance [ANOVA]) was used for an overall comparison of the proportions of the treatments at different taxa levels (phyla, class, order, family, and genera) in fecal samples collected on days 14 and 28. The linear discriminant analysis effect size (**LEfSe**) method (Galaxy v1.0; Segata et al., 2011) was used to identify differentially abundant phyla, class, order, family, and genera between groups under different conditions. Significant differences in beta diversity were determined using 999 permutations of PERMANOVA and PERMDISP to ensure that observed differences were not due to differences in dispersion.

Statistical analysis

Data were arranged in a 2 × 3 factorial arrangement, with two environmental temperatures and three levels of the enzyme blend. Data were analyzed using the PROC MIXED procedure of SAS (SAS Inst. Inc., Cary NC) for randomized complete block design with diet, temperature, and sex as the fixed effects, and block (replicate) as a random effect. However, because sex effect was not significant for any of the variables measured, sex was removed from the model to increase the power of the statistical tests. To estimate the linear and quadratic effects of both enzyme inclusion, orthogonal polynomial contrasts were conducted. PROC CORR procedure of SAS was used to estimate the correlation between performance metrics. Results are reported as least square means and SEM. Means were considered significantly different at $P \leq 0.05$. Means were separated using Tukey multiple comparison test. Significant mean differences were indicated with superscript letters.

Results

Growth performance and nutrient digestibility

There was no enzyme × temperature interaction on any growth performance and nutrient digestibility parameters; therefore, only main effects are presented. Pigs in HS had lower BW on day 14 ($P < 0.01$) and day 28 ($P < 0.01$) compared with TN pigs (Table 3). Supplementation with 1X enzyme significantly increased BW at day 28 by 7.7% ($P < 0.05$), whereas the improvement reduced in the 2X treatment to 2.6% ($P > 0.05$) compared with the control (**CON**). As presented in Table 3, there were no effects of enzyme on average daily gain (**ADG**), average daily feed intake (**AFI**), and G:F but pigs in the HS group had significantly lower ADG ($P < 0.01$) and average daily feed intake (**ADFI**; $P < 0.001$) in phase 1 (day 0 to 14). In phase 2 (day 15 to 28), ADFI was higher in the 1X carbohydrase treatment by 10.7% compared with the CON and 2X ($P < 0.05$). There was a quadratic effect of enzyme on ADFI for days 15 to 28 (period 2) ($P < 0.05$; Table 3). There was a trend ($P < 0.1$) of increased ADG by 10.8% with 1X treatment. There was no effect of enzyme on G:F. There was a temperature effect on ADFI ($P < 0.001$), with the HS group having lower feed intake. A trend ($P < 0.1$) was also observed for a higher G:F compared with the TN.

From day 0 to 28, the 1X enzyme treatment had higher ADFI (+7.0%) compared with the CON and 2X ($P < 0.05$) and had higher ADG (+10.4%; $P < 0.05$) without any effect on G:F. The HS group had lower ADG ($P < 0.01$) and ADFI ($P < 0.001$) compared with the TN, and there was also a trend of increased G:F in HS ($P < 0.1$). There was no carbohydrase effect on rectal and skin temperature and respiratory rate. Pigs in HS had higher skin temperature ($P < 0.001$) and respiratory rate ($P < 0.001$). There was a quadratic effect of enzyme on ADG ($P < 0.05$) and ADFI ($P < 0.05$) in the overall period (day 0 to 28). As presented in

Table 4, there were also no effects of enzyme on both jejunal and ileal digestibility. HS led to significantly higher ileal energy, nitrogen, and calcium digestibility ($P < 0.01$; Table 4) and jejunal energy and nitrogen digestibility ($P < 0.05$) compared with the TN.

There was a trend of positive correlation between AID of energy and ADG ($P < 0.1$) and G:F ($P < 0.1$), but there was no correlation between AID and AJD of nitrogen, calcium, and phosphorus digestibility and ADG and G:F (data not shown).

mRNA expression of cytokines, tight junction proteins, and nutrient transporters

There were no enzyme × temperature interactions and treatment effects on ileal gene expression (Table 5). There were trends of decrease in the ileal gene expression of *MUC2* ($P < 0.1$; Table 5) and *occludin* ($P < 0.1$) in the HS compared with the TN, while HS tended ($P < 0.1$) to increase *PEPT1* expression. There were no effects of enzyme on the jejunal gene expression, but HS significantly increased the expression of *IL1β* ($P < 0.001$; Table 6).

Serum metabolites

There was no enzyme × temperature interaction effect on all serum parameters. Both enzyme-supplemented groups (1X and 2X) showed a trend for reduced TAG at day 14 ($P < 0.1$; Table 7). Carbohydrase treatment did not have a significant effect on other serum parameters. HS caused elevated glucose concentration at day 1 ($P < 0.001$). HS increased NEFA concentrations at day 1 ($P < 0.01$; Table 7), and this was reversed on day 28 with the serum NEFA significantly lower compared with the TN ($P < 0.05$). There was a trend for an increase in the serum concentration of

Table 3. Effect of carbohydrase supplementation and environmental temperature on performance of pigs fed wheat-based diets[1]

	Carbohydrase				Temperature			*P*-value			
	CON	1X	2X	SEM	TN	HS	SEM	Enz	Temp	Enz Linear	Enz Quadratic
BW day 0, kg	20.2	20.1	20.1	0.66	20.2	20.2	0.66	0.958	1.000	0.801	0.884
BW day 14, kg	28.4	29.2	29.0	0.82	29.5	28.3	0.80	0.162	0.002	0.185	0.166
BW day 28, kg	39.1[a]	41.0[b]	40.0[a]	1.11	40.8	39.2	1.07	0.025	0.006	0.193	0.016
Period 1, 0 to 14 d											
ADG, g	589	649	632	21.34	664	582	17.45	0.141	0.002	0.159	0.147
ADFI, g	1,177	1,212	1,185	26.87	1,265	1,117	23.37	0.542	0.0001	0.791	0.286
G/F	505	539	532	15.21	527	524	12.42	0.347	0.699	0.204	0.442
Period 2, 15 to 28 d											
ADG, g	760	842	784	43.93	813	777	41.16	0.097	0.261	0.536	0.039
ADFI, g	1.294[a]	1.433[b]	1.290[a]	56.81	1.424	1,253	51.30	0.033	0.001	0.949	0.010
G/F	588	610	622	39.52	572	641	35.36	0.719	0.058	0.428	0.885
Period total, 0 to 28 d											
ADG, g	675[a]	745[b]	708[a]	25.79	738	679	23.84	0.022	0.005	0.179	0.014
ADFI, g	1,235[a]	1,322[b]	1,238[a]	37.10	1,344	1,185	34.05	0.032	0.0001	0.948	0.009
G/F	548	570	575	20.72	549	579	18.89	0.395	0.093	0.205	0.620
Rectal temp	40	40	40	0.05	40	40	0.04	0.564	0.272	0.746	0.304
Skin temp	37.5	37	37	0.16	36.3	38.0	0.13	0.252	0.0001	0.106	0.677
Resp rate	34.5	35	35.5	0.50	31.3	38.7	0.41	0.418	0.0001	0.192	0.879

[1]Data are means of 16 replicates per treatment in carbohydrase level and 24 replicates per treatment among temperature. Means with different superscript are different ($P < 0.05$). CON, Control; 1X, 50 g/tonne carbohydrase admixture; 2X, 100 g/tonne of carbohydrase admixture; TN, thermoneutral; HS, heat stress; Temp, temperature; Enz, carbohydrase treatment. Linear contrast between enzyme groups, Enz Quadratic, Quadratic contrast between enzyme groups.

Table 4. Effect of carbohydrase supplementation and environmental temperature on ileal and jejunal digestibility coefficient of pigs fed wheat-based diets[1]

	Carbohydrase level				Temperature			*P*-value	
	CON	1X	2X	SEM	TN	HS	SEM	Enz	Temp
Ileum									
Gross energy	0.69	0.71	0.71	0.02	0.67	0.75	0.02	0.566	0.002
Nitrogen	0.72	0.76	0.75	0.05	0.69	0.79	0.05	0.581	0.017
Phosphorus	0.51	0.51	0.48	0.04	0.46	0.54	0.03	0.794	0.052
Calcium	0.60	0.63	0.59	0.05	0.54	0.68	0.06	0.832	0.272
Jejunum									
Energy	0.68	0.72	0.68	0.05	0.67	0.72	0.05	0.551	0.039
Nitrogen	0.69	0.74	0.71	0.04	0.69	0.74	0.06	0.684	0.026
Phosphorus	0.55	0.50	0.53	0.03	0.52	0.53	0.02	0.421	0.531
Calcium	0.65	0.68	0.67	0.05	0.66	0.67	0.07	0.427	0.757

[1]Data are means of 16 replicates per treatment in carbohydrase level and 24 replicates per treatment among temperature. Means with different superscript are different ($P < 0.05$). CON, control; 1X, 50 g/tonne carbohydrase admixture; 2X, 100 g/tonne of carbohydrase admixture; TN, thermoneutral; HS, heat stress; Temp, temperature; Enz, Carbohydrase treatment.

Table 5. Effect of carbohydrase supplementation and environmental temperature on ileal gene expression of pigs fed wheat-based diets[1]

	Carbohydrase level			Temperature		P-value	
	CON	1X	2X	TN	HS	Enz	Temp
HSP70	1.3 ± 0.35	1.1 ± 0.37	1.0 ± 0.38	0.9 ± 0.34	1.5 ± 0.33	0.874	0.121
HSP90	0.9 ± 0.13	0.8 ± 0.12	0.7 ± 0.12	0.8 ± 0.11	0.8 ± 0.11	0.378	0.710
MUC2	1.0 ± 0.17	1.1 ± 0.16	1.0 ± 0.16	1.2 ± 0.13	0.9 ± 0.13	0.662	0.096
IL1β	1.4 ± 0.47	1.4 ± 0.46	1.4 ± 0.46	1.4 ± 0.46	1.4 ± 0.46	0.972	0.558
TNFα	1.2 ± 0.28	1.2 ± 0.27	1.10 ± 0.27	1.1 ± 0.27	1.2 ± 0.26	0.524	0.391
PEPT1	0.8 ± 0.21	1.3 ± 0.21	0.9 ± 0.21	0.8 ± 0.19	1.2 ± 0.18	0.134	0.059
SGLT1	1.0 ± 0.19	1.1 ± 0.19	1.0 ± 0.18	1.0 ± 0.17	1.1 ± 0.17	0.880	0.255
FUT2	1.9 ± 1.04	3.6 ± 1.04	3.2 ± 0.91	2.8 ± 0.82	3.0 ± 0.81	0.539	0.879
Claudin 4	2.5 ± 0.42	2.7 ± 0.46	2.4 ± 0.43	2.6 ± 0.38	2.4 ± 0.36	0.860	0.763
Occludin	0.7 ± 0.10	0.8 ± 0.11	0.7 ± 0.10	0.9 ± 0.09	0.6 ± 0.08	0.647	0.074

[1]Data are means of 16 replicates per treatment ± standard error in carbohydrase level and 24 replicates per treatment ± standard error among temperature. Means with different superscript are different ($P < 0.05$). CON, control; 1X, 50 g/tonne carbohydrase admixture; 2X, 100 g/tonne of carbohydrase admixture. *HSP70*, heat shock protein 70; *HSP90*, heat shock protein 90; *MUC2*, mucin 2; *IL1β*, interleukin 1β; *TNFα*, tumor necrosis factor α; *PEPT1*, peptide transporter type 1; *SGLT1*, sodium dependent glucose cotransporter type 1; *FUT2*, fucosyltransferase type 2. TN, thermoneutral; HS, heat stress; Temp, temperature; Enz, carbohydrase treatment.

Table 6. Effect of carbohydrase supplementation and environmental temperature on jejunal gene expression of pigs fed wheat-based diets[1]

	Carbohydrase level			Temperature		P-value	
	CON	1X	2X	TN	HS	Enz	Temp
HSP70	1.7 ± 0.34	1.0 ± 0.36	1.4 ± 0.36	1.3 ± 0.31	1.4 ± 0.31	0.154	0.616
HSP90	1.4 ± 0.21	1.0 ± 0.21	1.2 ± 0.21	1.1 ± 0.19	1.3 ± 0.19	0.400	0.294
MUC2	1.2 ± 0.14	0.9 ± 0.17	1.2 ± 0.16	1.2 ± 0.12	1.0 ± 0.13	0.604	0.479
IL1β	1.3 ± 0.14	1.3 ± 0.16	1.4 ± 0.16	1.0 ± 0.13	1.6 ± 0.14	0.818	0.0003
TNFα	1.4 ± 0.19	1.4 ± 0.2	1.4 ± 0.2	1.2 ± 0.16	1.5 ± 0.15	0.981	0.189
PEPT1	0.7 ± 0.15	0.8 ± 0.13	0.7 ± 0.13	0.6 ± 0.11	0.8 ± 0.12	0.585	0.157
SGLT1	0.7 ± 0.09	0.6 ± 0.09	0.6 ± 0.09	0.6 ± 0.08	0.6 ± 0.08	0.641	0.833
FUT2	1.7 ± 0.5	1.9 ± 0.52	1.7 ± 0.5	1.7 ± 0.44	1.9 ± 0.41	0.924	0.661
Claudin 4	1.5 ± 0.24	1.6 ± 0.22	1.4 ± 0.26	1.6 ± 0.2	1.4 ± 0.2	0.831	0.713
Occludin	1.6 ± 0.18	1.4 ± 0.18	1.8 ± 0.18	1.6 ± 0.15	1.6 ± 0.14	0.441	0.972

[1]Data are means of 16 replicates per treatment ± standard error in carbohydrase level and 24 replicates per treatment ± standard error among temperature. Means with different superscript are different ($P < 0.05$). CON, control; 1X, 50 g/tonne carbohydrase admixture; 2X, 100 g/tonne of carbohydrase admixture. *HSP70*, heat shock protein 70; *HSP90*, heat shock protein 90; *MUC2*, mucin 2; *IL1β*, interleukin 1β; *TNFα*, tumor necrosis factor α; *PEPT1*, peptide transporter type 1; *SGLT1*, sodium dependent glucose cotransporter type 1; *FUT2*, fucosyltransferase type 2. TN, thermoneutral; HS, heat stress; Temp, temperature; Enz, carbohydrase treatment.

TAG in HS at day 1 ($P < 0.01$), with further significant increase at day 14 ($P < 0.05$). HS resulted in a trend for increased serum BUN at day 1 ($P < 0.1$).

Intestinal histology

There was a trend for enzyme × temperature interaction effect in the jejunal villus height ($P < 0.1$; Table 8). In addition, HS reduced jejunal and ileal villi height compared with the TN ($P < 0.05$; Table 9), while there was a trend of increased villi height in the 1X group compared with the CON and the 2X only in the TN environment ($P < 0.1$; Table 9).

Gut microbiome

There was no significant effect of treatments on alpha diversity indices (Chao1, richness, Shannon, Evenness, Faith diversity indices and Observed OTUs, and Simpson and Simpson evenness). There was also no significant effect of both enzyme and temperature on beta diversity indices (Bray Curtis, Jaccard, and Unweighted and weighted UniFrac). However, there was a significant effect of sampling day (PERMANOVA) on Bray Curtis ($P < 0.001$), Jaccard ($P < 0.001$), and Unweighted UniFrac ($P < 0.01$). Additionally, PERMDISP was also significant for Bray Curtis ($P < 0.05$), Jaccard ($P < 0.01$), and Unweighted UniFrac ($P < 0.01$). LEfSe analysis showed that 21 bacterial taxa (5 in TN and 16 in HS) were differentially enriched due to temperature treatment (Supplementary Figure 1), 81 bacteria taxa by experiment day (Supplementary Figure 2), and 7 bacteria taxa by enzyme × temperature in the two sampling days (Supplementary Figures 3–6).

Discussion

Effect on growth performance

A quadratic effect of enzyme observed for BW at day 28 indicated that the optimum concentration of enzyme was at the 1X dose. This quadratic effect of enzyme was also observed with ADG and ADFI from day 0 to 28 which were higher in the 1X enzyme level than CON and 2X. Although there was a 10.8% difference in the ADG between the CON and 1X treatment, the high variability in the data did not allow this to be statistically significant. The modest effect of enzyme observed on performance parameters

Table 7. Effect of carbohydrase supplementation and environmental temperature on serum parameters of pigs fed wheat-based diets[1]

	Carbohydrase level			Temperature		*P*-value	
	CON	1X	2X	TN	HS	Enz	Temp
Glucose, mg/dL							
Day 1	81.8 ± 8.73	85.4 ± 7.10	85.6 ± 6.87	93.9 ± 6.79	74.6 ± 6.25	0.432	0.012
Day 14	66.9 ± 8.31	67.6 ± 5.81	65.4 ± 6.61	68.8 ± 5.41	64.5 ± 5.68	0.861	0.473
Day 28	71.7 ± 11.63	82.8 ± 9.52	79.7 ± 9.79	76.8 ± 8.91	79.3 ± 8.64	0.305	0.889
Insulin, µg/L							
Day 1	0.2 ± 0.05	0.2 ± 0.05	0.2 ± 0.05	0.2 ± 0.04	0.2 ± 0.05	0.721	0.865
Day 14	0.2 ± 0.05	0.2 ± 0.05	0.3 ± 0.04	0.3 ± 0.05	0.2 ± 0.05	0.932	0.311
Day 28	0.2 ± 0.04	0.2 ± 0.04	0.2 ± 0.05	0.2 ± 0.05	0.2 ± 0.04	0.897	0.204
NEFA, mmol/L							
Day 1	0.2 ± 0.05	0.2 ± 0.04	0.2 ± 0.5	0.1 ± 0.02	0.3 ± 0.04	0.631	0.011
Day 14	0.1 ± 0.02	0.1 ± 0.01	0.1 ± 0.01	0.1 ± 0.01	0.1 ± 0.01	0.495	0.872
Day 28	0.1 ± 0.06	0.2 ± 0.07	0.2 ± 0.07	0.2 ± 0.06	0.1 ± 0.07	0.771	0.012
TAG, mmol/L							
Day 1	0.5 ± 0.13	0.6 ± 0.12	0.8 ± 0.13	0.5 ± 0.10	0.7 ± 0.11	0.179	0.013
Day 14	0.5 ± 0.07	0.4 ± 0.08	0.4 ± 0.07	0.4 ± 0.02	0.5 ± 0.03	0.054	0.011
Day 28	0.4 ± 0.08	0.3 ± 0.08	0.3 ± 0.09	0.4 ± 0.03	0.3 ± 0.04	0.225	0.122
BUN, mg/dL							
Day 1	12.4 ± 2.56	14.5 ± 2.31	16.1 ± 2.34	12.3 ± 1.76	16.4 ± 1.70	0.462	0.083
Day 14	8.0 ± 1.67	7.2 ± 1.82	7.2 ± 1.93	7.0 ± 1.56	8.0 ± 1.72	0.559	0.551
Day 28	10.4 ± 1.85	11.0 ± 1.79	12.5 ± 1.72	11.7 ± 2.11	10.8 ± 2.24	0.637	0.612

[1]Data are means of 16 replicates per treatment ± standard error and 24 replicates per treatment ± standard error among temperature. Means with different superscripts are different ($P < 0.05$). 1X, 50 g/tonne carbohydrase admixture; 2X, 100 g/tonne of carbohydrase admixture; BUN, blood urea nitrogen; CON, control; Enz, carbohydrase treatment; HS, heat stress; NEFA, nonesterified fatty acid; TAG, triacyl glycerol; Temp, temperature; TN, thermoneutral.

Table 8. Interaction effect of temperature and carbohydrase supplementation on intestinal histological characteristics in pigs fed wheat-based diets[1]

	Thermoneutral			Heat stress				*P*-value
	CON	1X	2X	CON	1X	2X	SEM	
Jejunum								
Villus height, µm	585.0^{ab}	669.0^{a}	564.2^{b}	551.9^{b}	560.9^{b}	564.2^{b}	23.90	0.080
Crypt depth, µm	234.7	258.5	232.7	216.2	224.8	245.0	16.45	0.327
Villus height/ Crypt depth ratio	2.6	2.7	2.5	2.7	2.5	2.4	0.16	0.672
Ileum								
Villus height, µm	584.3	603.0	585.6	527.6	571.1	535.1	21.29	0.832
Crypt depth, µm	216.6	224.8	242.0	202.1	236.5	215.6	14.91	0.433
Villus height/ Crypt depth ratio	2.8	2.8	2.5	2.7	2.5	2.5	0.11	0.347

[1]Data are means of eight replicates per treatment. Means with different superscripts are different ($P < 0.05$). 1X, 50 g/tonne carbohydrase admixture; 2X, 100 g/tonne of carbohydrase admixture; CON, control.

is similar to reports by Zijlstra et al. (2004) in weanling pigs fed wheat-based diet supplemented with graded level of xylanase and β-glucanase enzyme blend who observed a lack of enzyme effect on BW at day 7 and ADG from day 0 to 7. Although the reason for the quadratic effect of enzyme is unclear, the 2X dose of the enzyme might have increased the breaking of dietary insoluble fiber to soluble fiber which is expected to increase digesta viscosity. Increased digesta viscosity may reduce digesta transit time and reduce access of digestive enzyme to digesta, causing impairment of digestion and growth. The bulking effect of enzyme may also trigger the release of peptide tyrosine tyrosine (**PYY**), a gut satiety peptide induced by xylanase supplementation in broilers (Singh et al., 2012). An increase in PYY is also expected to cause reduced voluntary feed intake. Lu (2018) reported a similar anorexic effect of xylanase in weanling pigs. Another possibility of the reduction ADG by the 2X dose of the enzyme could be the increased release of xylose in the foregut which has been suggested to impair performance. Agyekum et al. (2018) reported that inclusion of D-xylose beyond 15% reduced BW, ADG, and G:F, suggesting that high concentration of xylose in the foregut can have a negative effect on performance. Additional investigations are needed to clarify the effects of exogenous carbohydrases on digesta viscosity, release of pentose sugar in the upper gut, their impact on voluntary feed intake, and the optimal level of carbohydrases required in pig diets.

Table 9. Effect of carbohydrase supplementation and environmental temperature on intestinal histological characteristics in pigs fed wheat-based diets[1]

	Carbohydrase level				Temperature			P-value	
	CON	1X	2X	SEM	TN	HS	SEM	Enz	Temp
Jejunum									
Villus height, µm	568.4	614.9	564.2	16.90	606.0	559.0	13.80	0.074	0.020
Crypt depth, µm	225.4	241.6	239.9	11.86	242.0	229.3	9.86	0.551	0.343
Villus height/ Crypt depth ratio	2.6	2.6	2.4	0.11	2.6	2.5	0.09	0.408	0.662
Ileum									
Villus height, µm	555.9	587.0	560.3	15.08	590.9	544.6	12.33	0.298	0.011
Crypt depth, µm	209.3	230.6	228.8	10.5	227.8	218.0	8.61	0.298	0.427
Villus height/ Crypt depth ratio	2.7	2.6	2.5	0.08	2.7	2.5	0.07	0.150	0.136

[1]Data are means of 16 replicates per treatment and 24 replicates per treatment ± standard error among temperature. Means with different superscripts are different ($P < 0.05$). 1X, 50 g/tonne carbohydrase admixture; 2X, 100 g/tonne of carbohydrase admixture; CON, control; Enz, carbohydrase treatment; HS, heat stress; Temp, temperature; TN, thermoneutral.

As expected, HS significantly reduced the BW at days 14 and 28 with a corresponding decrease in ADG and ADFI. Under high thermal load, animals reduce metabolic heat production, a high proportion of which comes from digestion. Hence, pigs typically reduce ADFI under HS. However, pigs in the HS group had a trend for increased feed efficiency. The observed increased in feed efficiency suggests that the pigs might have been under a mild HS. Increased efficiency of protein deposition observed in pigs under HS depends may depend on the severity of the HS and size of the pigs (Baumgard and Rhoads, 2013). Mild HS leads to increased efficiency compared with severe HS (Baumgard and Rhoads, 2013). HS also reduces basal metabolic rate in pigs which subsequently leads to higher ADG and G:F (Pearce et al., 2013).

Effect on nutrient digestibility

Carbohydrase supplementation did not affect nutrient digestibility both in the jejunum and in the ileum. Although carbohydrases are expected to hydrolyze dietary arabinoxylans and β-glucans in the small intestine, the primary products of hydrolysis by carbohydrases are oligosaccharides and pentose sugars which are not well absorbed in the small intestine but serve as substrates for microbial fermentation in the hindgut. This may explain the lack of significant enzyme effect on AJD and AID in this study. The lack of enzyme effect on nutrient digestibility may also be due to differences in ADFI of the pigs. Studies in pigs with equalized feed intake showed improvement in AID and ATTD of nutrients (Baidoo et al., 1998), whereas, when pigs were allowed voluntary feed intake, there was no effect of carbohydrases on nutrient digestibility (Baas and Thacker 1996). The observed increase in AID of energy, nitrogen, and calcium and AJD of energy and nitrogen in the study by Baas and Thacker (1996) suggests that HS increases nutrient digestibility. This could be due to the mild HS of the HS group. Kellner et al. (2016) also reported an increase in ATTD of energy in pigs under HS.

Effect on mRNA expression of cytokine, tight junction proteins, and nutrient transporters

There was no enzyme × temperature interaction, enzyme, and temperature effect on both SGLT1 and PEPT1 in the ileum and jejunum. Although carbohydrases have been hypothesized to increase release of nutrients in the small intestine, the primary product of carbohydrase hydrolysis is oligosaccharides which cannot be absorbed in the small intestine, leading to minimal effect on nutrient transporter expression. However, there was a trend of increased ileal expression of *PEPT1* in HS pigs that might have been a compensation for the reduced ADFI in this group. HS reduced the mRNA expression of both ileal *occludin* and *MUC2*. The effect of HS on the protein abundance of these genes is unclear since the mRNA level may not correlate to protein. Implication of abundance of these transcripts on gut permeability is also unclear. For instance, Peace et al. (2013) observed that HS upregulated the abundance of both *claudin 3* and *occludin* at the protein level. However, they also observed increased intestinal permeability in pigs under HS, suggesting that the increase in protein abundance of tight junction proteins might have been a compensatory response. Experiments are needed to elaborate on effect of HS on transcript and protein abundance of tight junction proteins, the mechanism of their regulation, and effect of HS on intestinal permeability and function. In the jejunum, HS increased the expression of *IL1β*. This might be due to increased intestinal inflammation at this location in HS. HS has been shown to cause local and systemic inflammation in the intestine in both humans and animals. (Leon, 2007).

Regulation of serum metabolites

Effect of carbohydrases on serum concentrations of glucose and insulin has been inconsistent. In broiler chickens, Gao (2001) reported a lack of effect of xylanase supplementation on plasma glucose concentration despite significant increase in glucose concentration in the digesta. Similarly, Lu et al. (2019) reported that phytase did not affect serum glucose and insulin concentrations in young pigs. However, the reduction in glucose concentration after acute HS (day 1) observed in this study might have been due to utilization of glucose for energy secondary to a reduction in feed intake within the first 24 h of HS. However, there appears to be adaptation to prolonged HS such that pigs maintained tight homeostatic regulation of serum concentrations of glucose and insulin when fed diets with or without carbohydrases. The observed increase in

serum NEFA concentration after acute stress, but a decline at day 28, is similar to the pattern observed in pigs after chronic and systemic exposure to HS by Pearce et al. (2013) and Qu and Ajuwon (2018). Acute HS often leads to a negative energy balance characterized by reduction in feed intake, serum glucose concentration, and a mobilization of NEFA from adipose tissue for energy (Victoria Sanz Fernandez et al., 2015). However, prolonged HS may cause metabolic adaptation that increases lipogenesis in adipose tissue, causing a decline in blood NEFA.

Effect on intestinal histology

HS often causes profound damage to the intestinal epithelium. The decrease in the villi height from HS pigs in both the ileum and jejunum may indicate damage to the villi under HS. Similar reduction of intestinal villi height was reported by Yu et al. (2010) in pigs under HS. Yu et al. (2010) also showed that microvilli height was shorter, mitochondrial were swollen, number of lysosomes was increased, and enterocyte tight junction was altered in HS.

Impact on gut microbiome

Effect of carbohydrases on microbial composition may be dependent on the microbial source of the carbohydrase and the feed material (Zhang et al., 2018). The lack of a significant effect of carbohydrase on the microbiome composition is similar to the findings of Zhang et al. (2018) and Lu (2018) who reported that xylanase did not affect alpha and beta intestinal microbial diversity in weanling pigs. Pigs generally develop a stable bacteria community structure 3 wk after weaning (Frese et al., 2015), and it was possible that a stable microbial community was already established when treatments were started at 10 wk old. In addition, effects of the carbohydrases may not have been strong because the animals were fed diets with similar base ingredients. Although there was no difference in the overall community structure, abundance of specific bacterial groups was differentially regulated, suggesting that specific organism might have been impacted by treatments. The increased abundance of five taxa in the TN pigs compared with HS pigs (Supplementary Figure 1) indicates effects due to temperature difference. Abundance of 16 genera was also increased in the HS pig (Supplementary Figure 1), an example being the pathogenic Clostridiaceae_1 (Grzeskowiak et al., 2019; Zhang et al., 2019). The abundance of Erysipelotrichaceae (Supplementary Figure 5) was significantly reduced in the enzyme group under HS at day 28. This is particularly important because this bacteria taxon is associated with intestinal inflammation and related gastrointestinal disease (Kaakoush, 2015).

In conclusion, results from this study suggest that HS does not affect the efficacy multienzyme carbohydrases, indicating that both HS and carbohydrases impact animal performance independently.

Supplementary Data

Supplementary data are available at *Journal of Animal Science* online.

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Conflict of interest statement

P.C. is an employee of Adisseo France SAS, which provided funds for this study. All other authors disclose that there is no conflict of interest.

Literature Cited

Adeola, O., and A. J. Cowieson. 2011. Board-Invited Review: Opportunities and challenges in using exogenous enzymes to improve nonruminant animal production. *J. Anim. Sci.* **89**:3189–3218. doi:10.2527/jas.2010-3715

Agyekum, A. K., B. A. Slominski, and C. M. Nyachoti. 2012. Organ weight, intestinal morphology and fasting whole-body oxygen consumption in growing pigs fed diets containing distillers dried grains with solubles alone or in combination with a multienzyme supplement. *J. Anim. Sci.* **90**:3032-040. doi:10.2527/jas.2011-4380

Agyekum, A. K., M. C. Walsh, E. Kiarie, J. S. Sands, and C. M. Nyachoti. 2018. Dietary D-xylose effects on growth performance, portal nutrient fluxes, and energy expenditure in growing pigs. *J. Anim. Sci.* **96**:2310–2319. doi:10.1093/jas/sky142

AOAC International. 2000. *Official methods of analysis*. 17th ed. Arlington (DC): Association of Official Analytical Chemists.

Baas, T. C., and P. A. Thacker. 1996. Impact of gastric pH on dietary enzyme activity and survivability in swine fed-glucanase supplemented diets. *Can. J. Anim. Sci.* **76**:245–252. doi:10.4141/cjas96-036

Bach Knudsen, K. E. 2001. The nutritional significance of "dietary fibre" analysis. *Anim. Feed Sci. Technol.* **90**:3–20. doi:10.1016/S0377-8401(01)00193-6

Baidoo, S. K., Y. G. Liu, and D. Yungblut. 1998. Effect of microbial enzyme supplementation on energy, amino acid digestibility and performance of pigs fed hulless barley for swine. *Can. J. Anim. Sci.* **78**:202–210. doi:10.4141/A96-110

Baumgard, L. H., and R. P. Rhoads. 2013. Effects of heat stress on post absorptive metabolism and energetics. *Annu. Rev. Anim. Biosci.* **1**:311–337. doi:10.1146/annurev-animal-031412-103644

Bedford, M. R. 1995. Mechanism of action and potential environmental benefits from the use of feed enzymes. *Anim. Feed Sci. and Tech.* **53**:145–155. doi:10.1016/0377-8401(95)02018-U

Caporaso, J. G., J. Kuczynski, J. Stombaugh, K. Bittinger, F. D. Bushman, E. K. Costello, N. Fierer, A. G. Pena, J. K. Goodrich, J. I. Gordon, *et al.* 2010. QIIME allows analysis of high-throughput community sequencing data. *Nat. Methods* **7**:335–336. doi:10.1038/nmeth.f.303

Coppock, C. E. 1985. Energy nutrition and metabolism of the lactating dairy cow. *J. Dairy Sci.* **68**:3403–3410. doi:10.3168/jds.S0022-0302(85)81253-4

Fenton, T. W., and M. Fenton. 1979. An improved procedure for the determination of chromic oxide in feed and faeces. *Can. J. Anim. Sci.* **59**:631–634. doi:10.4141/cjas79-081

Frese, S. A., K. Parker, C. C. Calvert, and D. A. Mills. 2015. Diet shapes the gut microbiome of pigs during nursing and weaning. *Microbiome* **3**:28. doi:10.1186/s40168-015-0091-8

Gao, F. 2001. *Effects of non-starch polysaccharides enzyme preparations supplemented to diets on the performances in chicken and pig and the mechanisms involved* [PhD dissertation]. Nanjing: Nanjing Agricultural University.

Goetsch, A. L., and Z. B. Johnson. 1999. Feed intake and digestion in the summer and fall by different breeds of ewes consuming forages differing in quality. *Small Rumin. Res.* **31**:109–116. doi:10.1016/S0921-4488(98)00127-8

Grzeskowiak, Ł., T. H. Dadi, J. Zentek, and W. Vahjin. 2019. Developing gut microbiota exerts colonization resistance to

Clostridium (Syn. *Clostridioides*) *deficile* in piglets. *Microorganisms* **7**(8): 218. doi:10.3390/microorganisms7080218

Gutierrez, N. A., B. J. Kerr, and J. F. Patience. 2013. Effect of insoluble-low fermentable fiber from corn-ethanol distillation origin on energy, fiber, and amino acid digestibility, hindgut degradability of fiber, and growth performance of pigs. *J. Anim. Sci.* **91**:5314–5325. doi:10.2527/jas.2013-6328

Hamady, M., C. Lozupone, and R. Knight. 2010. Fast UniFrac: facilitating high-throughput phylogenetic analyses of microbial communities including analysis of pyrosequencing and PhyloChip data. *ISME J*. **4**:17–27. doi:10.1038/ismej.2009.97

Kaakoush, N. O. 2015. Insights into the role of Erysipelotrichaceae in the human host. *Front. Cell. Infect. Microbiol.* **5**:84. doi:10.3389/fcimb.2015.00084

Kellner, T. A., L. H. Baumgard, K. J. Prusa, N. K. Gabler, and J. F. Patience. 2016. Does heat stress alter the pig's response to dietary fat? *J. Anim. Sci.* **94**:4688–4703. doi:10.2527/jas.2016-0756

Lenis, N. P., P. Bikker, J. van der Meulen, J. Th. M. van Diepen, J. G. M. Bakker, and A. W. Jongbloed. 1996. Effect of neutral detergent fiber on ileal digestibility and portal flux of nitrogen and amino acids and on nitrogen utilization in growing pigs. *J. Anim. Sci.* **74**:2687–2699. doi:10.2527/2001.7992418x

Leon, L. R. 2007. Heat stroke and cytokines. *Prog. Brain Res.* **162**:481–524. doi:10.1016/S0079-6123(06)62024-4

Lu, H. 2018. *Effects of exogenous enzymes and live yeast on growth performance, nutrient digestibility and gut microbiome diversity in pigs* [PhD dissertation]. West Lafayette, Indiana: Department of Animal Science, Purdue University.

Lu, H., I. Kühn, M. R. Bedford, H. Whitfield, C. Brearley, O. Adeola, and K. M. Ajuwon. 2019. Effect of phytase on intestinal phytate breakdown, plasma inositol concentrations, and glucose transporter type 4 abundance in muscle membranes of weanling pigs. *J. Anim. Sci.* **97**:3907–3919. doi:10.1093/jas/skz234

Mayorga, E. J., D. Renaudeau, B. C. Ramirez, J. W. Ross, and L. H. Baumgard. 2019. Heat stress adaptations in pigs. *Anim. Front.* **9**:54–61. doi:10.1093/af/vfy035

Noblet, J., and G. L. Goff. 2001. Effect of dietary fiber on the energy value of feeds for pigs. *Anim. Feed Sci. Tech.* **90**:35–52. doi:10.1016/S0377-8401(01)00195-X

Onyango, E. M., M. R. Bedford, and O. Adeola. 2004. The yeast production system in which Escherichia coli phytase is expressed may affect growth performance, bone ash, and nutrient use in broiler chicks. *Poult. Sci.* **83**:421–427. doi:10.1093/ps/83.3.421

Pearce, S. C., N. K. Gabler, J. W. Ross, J. Escobar, J. F. Patience, R. P. Rhoads, and L. H. Baumgard. 2013. The effects of heat stress and plane of nutrition on metabolism in growing pigs. *J. Anim. Sci.* **91**:2108–2118. doi:10.2527/jas.2012-5738

Qu, H., and K. M. Ajuwon. 2018. Adipose tissue specific responses reveal an important role of lipogenesis during heat stress adaptation in pigs. *J. Anim. Sci.* **96**:975–989. doi:10.1093/jas/sky022

Roy, K. S., and R. J. Collier. 2012. Regulation of acclimation to environmental stress. In: Collier, R. J., and J. L. Collier, editors. *Environmental physiology of livestock*. West Sussex (UK): John Wiley & Sons, Inc; p. 49–63.

Segata, N., J. Izard, L. Waldron, D. Gevers, L. Miropolsky, W. S. Garrett, and C. Huttenhower. 2011. Metagenomic biomarker discovery and explanation. *Genome Biol.* **12**:R60. doi:10.1186/gb-2011-12-6-r60

Singh, A., H. V. M. O'Neill, T. K. Ghosh, M. R. Bedford, and S. Haldar. 2012. Effects of xylanase supplementation on performance, total volatile fatty acids and selected bacterial population in caeca, metabolic indices and peptide YY concentrations in serum of broiler chickens fed energy restricted maize–soybean-based diets. *Anim. Feed Sci. Tech.* **177**:194–203. doi:10.1016/j.anifeedsci.2012.08.005

Torres-Pitarch, A., E. G. Manzanilla, G. E. Gardiner, J. V. O'Doherty, and P. G. Lawlor. 2019. Systematic review and meta-analysis of the effect of feed and enzymes on growth and nutrient digestibility in grow-finisher pigs: effect of enzyme type and cereal source. *Anim. Feed Sci. and Tech.* **251**:153–165. doi:10.1016/j.anifeedsci.2018.12.007

Victoria Sanz Fernandez, M., J. S. Johnson, M. Abuajamieh, S. K. Stoakes, J. T. Seibert, L. Cox, S. Kahl, T. H. Elsasser, J. W. Ross, S. C. Isom, *et al.* 2015. Effects of heat stress on carbohydrate and lipid metabolism in growing pigs. *Physiol. Rep.* **3**(2). doi:10.14814/phy2.12315

Yu, J., P. Yin, F. Liu, G. Cheng, K. Guo, A. Lu, X. W. Luan, and J. Xu. 2010. Effect of heat stress on porcine small intestine: a morphological and gene expression study. *Com. Biochem. And Physiol.* **156**:119–128. doi:10.1016/j.cbpa.2010.01.008

Zhang, J., J. Bi, G. Guo, L. Yang, B. Zhu, G. Zhan, S. Li, N. Huang, K. Hashimoto, C. Yang, *et al.* 2019. Abnormal composition of gut microbiota contributes to delirium-like behaviors after abdominal surgery in mice. *CNS Neurosci. Ther.* **25**:685–696. doi:10.1111/cns.13103

Zhang, Z., H. M. Tun, R. Li, B. J. M. Gonzalez, H. Keenes, C. M. Nyachoti, E. Kiarie, and E. Khafipour. 2018. Impact of xylanases on gut microbiota of growing pigs fed corn or wheat-based diets. *Anim. Nutri.* **4**:339–3950. doi:10.1016/j.aninu.2018.06.007

Zijlstra, R. T., A. Owusu-Asiedu, and P. H. Simmins. 2010. Future of NSP-degrading enzymes to improve nutrient utilization of co-products and gut health in pigs. *Livest. Sci.* **134**: 255–257. doi:10.1016/j.livsci.2010.07.017

Zijlstra, R. T., A. Owusu-Asiedu, P. H. Simmins, and J. F. Patience. 2004. Effect of carbohydrase supplementation of wheat and canola meal-based diets on growth performance and nutrient digestibility in group-housed weaned pigs. *Can. J. Anim. Sci.* **84**:689–695. doi:10.4141/A03-127