RESEARCH REPORT
ANIMAL SCIENCE

Title: Gut physiology and metabolomics profile of pigs fed diets with carbohydrases enzymes and distillers dried grains with solubles – **NPB #14-045**

Investigator: Pedro E. Urriola

Co-Investigators: Zhikai Zeng, Gerald C. Shurson, Milena Saqui-Salces, and Chi Chen

Institution: University of Minnesota, St. Paul

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Industry summary

Feeding high fiber feed ingredients (e.g. wheat middlings and corn distillers dried grains with solubles) significantly decreases feed efficiency of pigs. However, the addition of carbohydrase enzymes to feed offer the potential to partially overcome these reductions in feed efficiency. However, results from feeding experiments of high fiber diets supplemented with carbohydrase enzymes (including NPB projects #13-158, 13-191, and 14-234) are have been inconsistent with generally poor effectiveness. One potential reason for the ineffectiveness of carbohydrases, is that there are multiple types of these products, produced by multiple suppliers, and multiple target substrates where upon specific enzymes work. There are no systematic reviews or experiments that allow for comparing products and enzyme activity of the commercial carbohydrases. Likewise, the relative effectiveness of carbohydrase enzymes on growth performance, nutrient digestibility, and gut physiology effects are unknown. We speculate that the overall impact of carbohydrase enzymes on feed efficiency of growing pigs can be described by 1) an improvement of energy and nutrient digestibility or 'nutrient uplift'or by 2) an improvement in energy and nutrient utilization, or a 'non-nutrient' effect. Results from previous UMN projects (NPB 13-014) have shown that use of an *in vitro* digestibility and gas production system can provide a reasonable estimates of DM and GE digestibility of high fiber feed ingredients. This *in vitro* system allowed us to compare digestibility values of 11 different carbohydrase enzymes in fiber from wheat middlings and corn DDGS. Results from these studies suggest that of the 11 of the most common carbohydrase enzymes available in the U.S. market, none of them have a measurable effect on digestibility of DM and GE when added to corn DDGS, and minimal improvements when added to wheat middlings. However, despite of the limited ability of these enzymes to improve digestibility of DM and GE, pigs fed wheat middlings and carbohydrase enzymes had greater feed intake than pigs fed the same diets not supplemented with enzymes. The reason for this improvement in feed intake is not clear, but it may be related to the fact that feeding carbohydrase enzymes modifies the composition and physico-chemical characteristics of digesta in the gastrointestinal tract. Specifically, enzymes increased the release of xylose monosaccharides into the liquid portion of the jejunum and ileum contents. These modifications of fiber in the presence of these carbohydrases also decreased the force required to stir jejunal and ileal contents. In addition, adding carbohydrase enzymes to these diets also modified the immune response of pigs, which varied along different parts of the gastrointestinal tract and between the types of enzyme substrates in the diets (wheat

middlings vs. corn DDGS). These observations are in agreement with our previous NPB reports (13-014), where we observed that fiber has a significant impact on gut cell proliferation, differentiation, and immune function. However, the implications of these observations on other factors such as resistance to infections and other stressors are yet to be determined. In conclusion, feeding enzymes may modify the gastrointestinal mileu and improve feed efficiency of pigs fed fibrous feed ingredients, but not by improving nutrient digestibility, but rather by modifications of gut function. The practical application and consistency of effects need to be further evaluated by conducting studies that help us understand how the physical structure of fiber in DDGS is related to effectiveness of these enzymes. Knowing this, it may be possible to design enzymes that will have greater effectiveness for increasing energy and nutrient digestibility of DDGS and other high fiber ingredients.

Key findings:

1. ***In vitro activity of enzymes.***
    a. None of the 11 commercially available carbohydrase enzymes were effective for improving *in vitro* dry matter or gross energy disappearance in the simulated small intestine digestion or simulated large intestine fermentation when enzymes were added to corn DDGS.
    b. Carbohydrase enzymes only increased *in vitro* dry matter and gross energy dissapearance in the simulated small intestine digestion without affecting the *in vitro* total tract digestibility of DM when applied to wheat middlings.

2. ***In vivo effect of enzymes.*** Feeding diets with high inclusion rates of high fiber ingredients (40% corn DDGS or 30% wheat middlings) decreased feed intake of 25-40 kg pigs.
    a. Addition of carbohydrase enzymes increased feed intake and improved growth rate without changes in feed efficiency in pigs fed wheat middlings.
        i. Effect of enzymes on feed intake may be the result of changes in gut physiological conditions.
            1. ***Site of nutrient digestion.*** Similarly as observed *in vitro,* feeding wheat middlings and corn DDGS with carbohydrase enzymes increased the apparent ileal digestibility of DM, OM, GE, and CP, but it did not affect the apparent total tract digestibility of DM, GE, OM, or CP.
            2. ***Characteristics of digestive content.***
                a. There were no differences in pH of gut contents of pigs fed diets containing enzymes vs. the control diets.
                b. Adding corn DDGS and wheat midds increased the peak shear stress for stirring jejunum and ileum content. Addition of carbohydrase enzymes decreased this peak shear stress.
            3. ***Immune response.*** Feeding wheat midds or corn DDGS to growing pigs excerts a different immune response in the small and large intestine. While pigs fed corn DDGS had greater expression of TNFα in colon, pigs fed wheat midds had greater expression of IL4 and IL25 in the ileum.
            4. ***LC-MS metabolome***. There were differences in metabolite composition of pigs fed wheat middlings and corn DDGS, and adding carbohydrases had no effect on the metabolome profile of pigs at any of the sections of the gastrointestinal tract.

Contact

Pedro E. Urriola, PhD
Department of Animal Science and Department of Veterinary Population Medicine
University of Minnesota
Urrio001@umn.edu

Scientific abstract: This study evaluated the effects of carbohydrase supplementation to diets with wheat middlings (**WM**) or corn distillers dried grains with solubles (**DDGS**) on growth performance, digestibility of nutrients, cytokine profile and characteristics of intestinal mileu of growing pigs. In Exp. 1, The efficacy of 11 commercially available carbohydrases was evaluated measuring *in vitro* digestibility of nutrients and GE. After incubation of WM and DDGS pretreated with carbohydrases in pepsin and pancreatin, the residues were analyzed for DM and GE. A subset of hydrolysis residues were incubated with fecal inocula and allowed to ferment for 72 h. Compared with no enzyme controls carbohydrases increased ($P < 0.05$) *in vitro* ileal digestibility of DM (3.2%) and GE (4.2%) in WM, but not in DDGS. The concentrations of glucose (73.2 vs. 54.1 mg/dL) and soluble protein (1.27 vs. 1.10 mg/mL) released during hydrolysis were increased ($P < 0.05$) by the addition of carbohydrases compared with the non-enzyme treated WM control, but there was no effect in DDGS. During *in vitro* fermentation, gas and VFA production was less ($P < 0.05$) and took longer ($P < 0.05$) time (6.3 vs. 4.8 h) to reach half assyntote $T/2^{-1}$ in the hydrolysis residue from WM treated with carbohydrases. For DDGS, the total gas production (358 vs. 416 mL/g DM) was less ($P < 0.05$) and $T/2^{-1}$ (13.9 vs. 17.6 h) was increased ($P < 0.05$) in several carbohydrase sources compared with the no enzyme control. The WM control had greater ($P < 0.05$) disappearance of DM (45.1 vs. 49.8%) during fermentation than WM supplemented with 5 out the 11 carbohydrases tested, whereas there were no differences observed among DDGS with or without carbohydrases. In Exp. 2, fifty-four individually housed pigs (25.33 ± 0.41 kg) were blocked by BW and sex and fed 1 of 6 dietary treatments (n = 9) in a 2 × 3 factorial design with 2 levels of carbohydrases (0 vs. 100 mg/kg;1,500 U/g xylanase, 1,100 U/g beta-glucanase, 110 U/g mannanase, 35 U/g galactosidase) and 3 basal diets (corn-soybean control, CSB; CSB + 40% DDGS; or CSB + 30% WM). Titanium dioxide (0.5%) and phytase (1,000 FTU/kg) were added to all diets. Pig BW and feed intake were determined weekly. On d 28, pigs were euthanized and intestinal contents were collected to determine digestibility, pH, and rheology. Pigs fed diets that contained DDGS or WM had less ($P < 0.05$) ADG (755 and 751 g/d, respectively) and ADFI (1,474 and 1,435 g/d, respectively) compared with pigs fed CSB diets (803 g/d and 1,582 g/d, respectively). Carbohydrases tended to improve ($P < 0.10$) ADG (787 vs. 752 g/d) and ADFI (1,529 vs. 1,465 g/d). Pigs fed CSB diets had greater ($P < 0.05$) AID and ATTD of DM, OM, and GE compared with pigs fed DDGS and WM diets. Carbohydrases supplementation improved ($P < 0.05$) AID of DM, GE and CP and ATTD of ash in WM diets, but not in DDGS or CSB diets. The addition of carbohydrases increased ($P < 0.05$) viscosity of cecal digesta supernatant only in CSB diet, but decreased peak shear stress (14.2 vs. 28.2 Pa, $P < 0.05$) and K (16.6 vs. 20.9 Pa, $P < 0.05$) in jejunum digesta regardless of basal diets. The metabolome of intestinal content was affected by WM or DDGS, but not by carbohydrases. In conclusion, adding DDGS and WM to CSB diets decreased GE and nutrient digestibility and impaired growth performance. Carbohydrases supplementation improved growth performance of growing pigs by modifying rheology characteristics of intestinal contents and modifications to the epithelial immune system.

Key Words: carbohydrases, cytokines, digestibility, pig, rheology

INTRODUCTION

Use of alternative feed ingredients in swine feeding programs has become more common due to increasing costs of conventional feedstuffs and wheat middling (**WM**) and corn distillers' dried grains with soluble (**DDGS**) are common cereals co-products that can be used as alternative low-cost feed resources (Stein and Shurson, 2009; Kerr and Shurson, 2013). However, the presence of high antinutritional factors (e.g., dietary fiber) in these co-products decrease feed efficiency by decreasing nutrient digestibility and absorption and thus compromising growth performance of pigs (Urriola et al., 2013).

Application of exogenous feed enzymes is a common strategy to cope with antinutritional factors in cereals co-products (Adeola and Cowieson, 2011; Kerr and Shurson, 2013). However, due to the heterogeneity and complex nature of dietary fiber, complete hydrolysis requires a series of cooperatively acting enzymes working in a highly organized way (Harris and Ramalingam, 2010; Larsbrink et al., 2014; Cuskin et al., 2015). Likewise, dietary fiber in cereal co-products vary in composition (Jaworski et al., 2015) making the necessity of matching enzyme type with the substrate. The most common fiber degrading enzymes are mainly endo-glycosidases, which cleave the backbone of arabinxylans. Other enzyme types include glucanases and cellulases (Adeola and Cowieson, 2011). It is assumed that a partial hydrolysis of fiber is sufficient to generate the nutritive benefits of enzymes (Bedford and Schulze, 1998) and carbohydrases improve nutritive value of feedstuffs in swine and poultry by increasing feed efficiency (Li et al., 2004; Fang et al., 2007; Leslie et al., 2007; Pedersen et al., 2015a). However, there are a multitude of enzyme type and suppliers available to US pork producers and there are no reliable methods to measure the effectiveness of these products. Therefore, the first objective of this project was to compare the activity and effectiveness of multiple products using an *in vitro* digestibility and gas production assay previously tested to accurately represent *in vivo* digestibility of fiber and nutrients in DDGS fed to growing pigs (NPB projects #13-014 and #14-045).

Other modes of action of carbohydrase enzymes may include release of fiber degradation products into the gastrointestinal milieu with direct or indirect impact on gut physiology. A summary of multiple carbohydrase feeding experiments, suggest that there is a reduction in mortality of pigs fed carbohydrase enzymes by modification of arabinoxylans contained in the diet (Boyd and Rush, 2015; Zier-Rush et al., 2016). Fiber degradation products or microbial metabolites produced after fiber fermentation may have a direct impact on the immune system, thereby modifying nutrient metabolism and morbidity and mortality. However, the variety of fiber sources (wheat vs. corn), fiber components (arabinoxylans, cellulose, etc), and variety of microbial degradation products (VFA, secondary bile salts, etc) require the use of Systems Biology tools to investigate the mode of action of enzymes. Therefore, a second objective of this project was to characterize the chemical and physical characteristics of the gut contents of pigs fed carbohydrase enzymes, measure cytokine production, and measure metabolome composition.

MATERIALS AND METHODS

Experiment 1. In vitro screening of carbohydrase enzymes

Commercial Carbohydrases, Phytase, and Substrate

A total of 11 commercially available carbohydrases were collected from 7 suppliers in 2015 (Table 1). All enzymes were obtained at the supplier concentration ready for feed application. Carbohydrases were mixed with phosphate buffer solution (100 mL, 0.1 M, pH 6.0) on a vortex mixer for 30 min. The concentration of carbohydrases solution was calculated according to the recommended dose from each enzyme supplier. For each 1 g of WM or cDDGS substrate, the prepared enzyme solution provided 20 times higher amount of enzyme activity than the commercial recommended dose. In addiiton, phytase (Quantum Blue, 5000 FTU/g) was provided by AB Vista (Marlborough, UK). The preapred phytase solution provided 10,000 FTU/kg of WM or cDDGS substrate.

We selected wheat and corn fiber as the most common sources of arabinoxylans in diets for growing pigs in the US. Wheat middlings and corn DDGS were collected from a supplier at the West Central Research and Outreach Center in Morris, MN. These 2 fibrous feed ingredients were selected on the basis of their availability

in diets for growing pigs in the US and their high concentration of arabinoxylans potentially substrates for the carbohydrase enzymes (Table 2).

Enzymatic Hydrolysis

Samples of WM and cDDGS were ground to pass 1 mm-mesh screen before undergoing an *in vitro* pepsin and pancreatin hydrolysis according with the first steps of the method of Boisen and Fernández (1997). Briefly, about 2 g (± 0.01) samples were weighed in 500 mL conical flasks. A phosphate buffer solution (100 mL, 0.1 M, pH 6.0) and an HCl solution (40 mL, 0.2 M) were added into the flasks. The pH was adjusted to 2.0 with 1 *M* HCl or 1 *M* NaOH. 2 ml of a chloramphenicol (Sigma C-0378, Sheboygan Falls, WI) solution (0.5 g 100 mL/L ethanol) was added to inhibit microbial activity. 1 mL of prepared commercial carbohydrases or blank phosphate buffer solution were pipette into the flask. Prepared phytase solution (1 mL, 20 FTU) were transferred into the flask across all of treatments. Fresh porcine pepsin solution (4 mL, 25 g /L, Sigma P-7000) was added and flasks were closed with a rubber stopper and placed for 2 h under gentle agitation in a water-bath at 39 ± 0.5 ˚C.

Afterwards, 40 ml phosphate buffer (0.2 *M*, pH 6.8) and 20 ml of 0.6 *M* NaOH were added into the solution. The pH was adjusted to 6.8 with 1 *M* HCl or 1 *M* NaOH. Fresh pancreatin solution (2 ml, 100 g/l pancreatin, Sigma P-1750) was added and hydrolysis was continued for 4 h under the same conditions. After hydrolysis, the residues were collected by filtration on a nylon bag (42 µm; Ankom Technologies, Macedon, NY), washed with ethanol (2 × 25 ml 95% ethanol) and acetone (2 × 25 ml 99.5% acetone), dried for 48 h at 60 ˚C and weighed. Part of the filtrates were collected (before washing with ethanol and acetone) and centrifuged at 10,000 × g for 10 min. The supernant were stored at -80 ˚C for later analysis. The enzymatic hydrolysis was repeated 12 to 16 times to obtain suficient residue for multiple analyses. Hydrolyzed residues from the different replicates and batches of same treatments (n = 6) were pooled for subsequent *in vitro* fermentation. The rest 6 replicates were individually stored for GE determination in a Isoperibol bomb calorimetry.

In vitro Fermentation

The rate of fermentation of the hydrolyzed substrates was assessed *in vitro*, using a cumulative gas-production technique (Bindelle et al., 2007). Briefly, 200 mg samples were inoculated at 39 °C in a 125 ml-glass bottle with 30 ml buffer solution containing macro- and micro-minerals (Menke and Steingass, 1988) and a fecal inoculum. Feces were donated by 6 finishing pigs (approx. 90 kg of BW) from the the University of Minnesota Southern Research and Outreach Center Swine Research Facility (Waseca, MN). The pigs were fed a corn soybeam meal basal diet voided of antibiotics. Fecal samples were collected directly from the rectum and immediately placed in air tight plastic syringes and kept in a water-bath at 39 °C until incubation (less than 2 h). The inoculum was diluted to 0.05 g feces per mL of the buffer solution. The prepard inoculum was filtered through a 250 µm-screen and transferred into the bottle with fermentation substrates. Bottles were sealed with a rubber stopper and placed for incubation. An anaerobic environment was maintained throughout the experiment, from the inoculum preparation until the incubation step by flushing bags and bottles with CO_2 gas. The gas generated by the fermentation process was manually recorded at 0, 2, 5, 8, 12, 18, 24, 36, 48 and 72 h. The bottles were vented after every measurement. Fermentation was stopped at 48 h of incubation by quenching the bottles in iced water. At the end of the fermentation period, the supernatant from each bottle was collected in frozen until analysis for VFA (e.i., acetic, proprionic, butyric, and branched chain short chain fatty acids). Samples of the inoculum prior to fermentation were also analyzed for VFA.

Chemical Analyses

All samples and the ingredients were ground with a laboratory mill to pass through 1 mm mesh screen. Chemical analyses were performed according to the Association of Official Analytical Chemists standard procedures (AOAC, 2007) with specific methods as follows: DM (135 ◦C for 2 h, AOAC 930.15), N (AOAC 968.06; using an elemental analyzer LECO FP528, St Joseph MI, USA; CP = N×6.25), ether extract using Soxhlet apparatus and petroleum ether (AOAC 920.39), and ash (AOAC 942.05). Gross energy was determined by an adiabatic bomb calorimeter (Parr 6400; Parr Instrument Company, Moline, IL) and benzoic acid was used as standard.

Soluble protein and glucose concentration in enzymatic hydrolysis filtrates were measured by Pierce BCA Protein Assay Kit (CAT# 23225, Thermo. Scientific) and a Glucose Colorimetric Assay Kit (Cayman Chemical, Ann Arbor, MI). The VFA were determined via gas chromatography (Agilent 6890 system, Germany) after extraction with diethyl ether (Greenberg et al., 1992). Briefly, 2 mL of the samples were transferred into the

centrifuge tube and then 0.5 mL of sulfuric acid (1/1), 0.4 g sodium chloride, 0.4 mL internal standard and finally 2 mL of diethyl ether were added. The samples were mixed for 2 min and centrifuged at 3000 rpm for 3 min. After that, the etheric layer was transferred to the vial tube and finally loaded on gas chromatography (Agilent 6890 system, Germany). Branched-chain fatty acids (**BCFA**) content was calculated as the sum of the iso-butyric and iso-valeric acids.

Calculations and Statistical Analyses

In vitro degradability of DM (IVDMD) and GE (IVDGE) during the pepsin and pancreatin hydrolysis were calculated as follows:

IVDMD (GE) = (dry weight (GE) of the sample before hydrolysis − dry weight (GE) of the residue) /dry weight (GE) of the sample before hydrolysis

In vitro fermentability of DM (IVFMD) during feces fermentation was calculated as follows:

IVFDM = (dry weight of the hydrolyzed residue − dry weight of the residue after fermentaion) / dry weight of the hydrolyzed residue

Gas accumulation curves recorded during the 72 h of fermentation were modified according to France et al. (1993):

G (mL g/DM) = 0, if $0 < t < L$

G (mL g/DM) = $G_f(1\text{-}\exp(-[b(t-L) + c(\sqrt{t} - \sqrt{L})]))$, if $t \geq L$

where G denotes the gas accumulation at a specific time (t), G_f (ml g−1 DM) the maximum gas volume for $t = \infty$ and L (h) the lag time before the fermentation starts. In the present study, gas accumulation rapidly reached one fourth of the maximum accumulation in 2 hours, the parameters L was very close to 0, which resulted the model fail to convege. Therefore, L(h) were removed from the final model. The constants b (h^{-1}) and c ($h^{-1/2}$) determine the fractional rate of degradation of the substrate μ (h−1), which is postulated to vary with time as follows:

$\mu = b + c/(2\sqrt{t})$, if $t \geq L$

Kinetics parameters (Gf, L, t=T/2 and μ) were compared in the statistical analysis. T/2 is the time to half-asymptote when $G = G_{f/2}$.

The VFA (acetic [A], propionic [P], butyric [B], and valeric [V] acids) production per kilogram of enzymatic digestion residues DM was estimated by the following equation:$[(A+ P + B+ V)]_{72h} - (A+ P + B+ V)]_{0h}$/g of residue DM. The equivalent energy for each VFA in kilocalories per mole was assumed to be 208, 364, 520, and 676 kcal/mol for acetic, propionic, butyric, and valeric acids, respectively (Weast, 1977). The energy derived from VFA per kg of feed DM was then calculated by multipling IVDMD.

All data were analyzed using the MIXED procedure of SAS version 9.4 (SAS Inst. Inc., Cary, NC) with individual bottles as the experimental unit. The model included treatment as fixed effect and bathes of samples as random effect. The least square means of individual treatments was separated by adjusted Tukey. Outliers were identified and removed if the obsoluted studentized residues exceeded 3. Results were considered significant at $P \leq 0.05$ and a trend at $P \leq 0.10$.

Experiment 2. In vivo effects of carbohydrase enzymes

All the procedures of this study were approved by the University of Minnesota Institutional Animal Care and Use Committee, protocol 1604-33628A.

Preparation of Feed Enzyme

The commercial feed enzyme was supplied by Archer Daniels Midland Company (ADM, Decatur, IL) in this study. The cocktail enzyme contains 1,500, 1,100 and 110 U/g xylanase, glucanase and mannanase, respectively.

Animals, Housing and Experimental Design

This experiment was conducted at the University of Minnesota Southern Research and Outreach Center Swine Research Facility in Waseca. The selected pigs were terminal offspring of Yorkshire × Landrace sows (TOPIGS USA, Des Moines, IA) sired by Duroc boars (Compart Boar Store, Nicollet, MN). Fifty-four pigs (initial BW 25 ± 0.41 kg) were assigned to 1 of 6 experimental diets on the basis of weight in a completely randomized block design. Pigs were blocked by initial BW and sex with 5 barrows and 4 gilts per treatments. Three basal diets were mixed to contain 1) corn-soybean meal (**CSB**), 2) as 1 + 40% corn DDGS, and 3) as 1 + 30% wheat

middling (**WM**). All diets were formulated to meet or exceed the NRC (2012) nutrient requirements for growing pigs (20-50kg BW). Another 3 diets were similar to diets 1-3, but with the addition of the test enzyme (100 mg/kg). Titanium oxide (0.25%) were added as a digestibility marker. All diets were fed in mash form and contain supplemental phytase with an activity of 1000 FTU/kg (Quantum, ABvista). The 1000 FTU phytase/kg supply 0.1% Ca and 0.12% digestible P (Table 3). Pigs had ad libitum access to their assigned dietary treatments and water throughout the experiment. Feeders were located at the front of each pen, and a nipple waterer was located at the side of the pen. Room temperature were maintained at 25 ± 1°C to meet the comfort needs of the pigs.

Growth Performance and Sample Collection

Pigs were weighed individually every week to calculate ADG. On each weigh day, feed disappearance was measured to calculate ADFI of pigs. The individual ADG and ADFI were used to calculate G:F.

From d 24 to 28 of the experiment, approximately 200 g of feces were collected from each pig by using the grab sample technique. Briefly, rectal palpation was performed to ensure a fresh fecal sample was collected from each pig. Fecal samples were pooled by animal and immediately frozen at -20°C until subsequent chemical analysis was conducted.

On the morning of d29, an 8 ml sample of blood was obtained by vena cava puncture from each pig using a 10 ml tube with anticoagulant (Becton, Dickinson, Franklin Lakes, NJ). According to Pagano et al. (2007), the pigs were not allowed to consume feed for 8 h and subsequently given *ad libitum* access to feed for 12 h before slaughter to normalize the presence of digesta throughout the gastrointestinal tract. The small intestine was divided into three parts by cutting from the Ligament of Treitz to the ileocecal valve. The contents of the jejunum, ileum, cecum, and colon were aseptically collected, pooled within pigs. A 1.5 mL digesta was then transferred to 2 mL EP tubes and immediately immersed in liquid nitrogen and preserved at -80 ^{o}C for later LC-MS analysis. The rest of digesta were stored at -20 ^{o}C for viscosity and chemical analysis. Tissue samples of duodenum, jejunum, ileum, and colon were collected. Each intestinal segment was divided into 4 parts. Three out of 4 samples were frozen in liquid nitrogen and stored at -80°C and 1 sample were fixed with 10% formaldehyde-phosphate buffer, and kept at 4 ^{o}C for histological evaluation.

Chemical Analyses

Feces were thawed and dried in a drying at 65°C for 72 h and ground through a 1-mm screen. Digesta samples were thawed and lyophilized in a Vacuum-Freeze Dryer, and then ground through a 1-mm screen.

Analysis of DM (AOAC Method 930.15), CP (AOAC Method 984.13), ash (AOAC Method 923.03) and acid hydrolysis fat (AOAC Method 948.15) were conducted according to the methods of AOAC (2007). Organic matters (OM) were calculated by the difference of DM and ash. GE was determined by an Automatic Energy Analyzer (Parr 6400, Parr Instrument Company, Moline, IL). Titanium concentration in feed, digesta, and feces was determined as described by Myers et al. (2004).

Rheology and pH

The pH value of digesta (duodenum, jejunum, ileum, cecum, and colon) were directly measured on the harvest day by using portable pH meters.

An Rheometric Expansion System (ARES G2, TA Instruments, New Castle, DE, USA) that is strain controlled was used to perform all rheological measurements. Frozen digesta samples were thawed at 4 °C for 3 h and then loaded into a temperature controlled cup (39 °C) and pre-warmed for 5 min before measurements. The peak hold test was then performed at 0.1 s^{-1} shear rate for 2 min. Steady shear flow measurements were subsequently performed on the same samples. A cone and vane geometry was applied to ensure a homogeneous shear field, as suggested by Lentle and Janssen (2008). The gap to bottom was maintained at 3 mm for all measures to avoid any slip or artefact due to the larger particulates in the digesta samples. The steady shear measurements were performed for shear rates ranging from 0.1 to 100 s^{-1}. The obtained data were used to calculate the peak shear stress (Pa), consistency constant K and power index n.

The digesta was recovered and immediately transferred into 50 mL polypropylene centrifuge tubes (Easy Reader^{TM}) incubated with ice after the rheological measures. Samples were then centrifuged at 3,500 ×g (Heraeus Biofuge 22R Centrifuge, Hanau, Germany) for 10 min. The liquid and solid volume of digesta were estimated based on stratification and the liquid fraction ratio of digesta were calculated. About 15 mL supernatant of the digesta were re-loaded in to the rheometric machine to determine the viscosity of the supernatant portion of

digesta. Steady shear flow measurements were performed with a standardized cone geometry. The shear rates ranged from 0.1 to 100 s^{-1} and the gap to the bottom cup was controlled at 3 mm.

Metabolomic Analysis

The following chemicals were used: LC-MS-grade water and acetonitrile (ACN) (Fisher Scientific, Houston, TX); 2-hydrazinoquinoline (HQ) and triphenylphosphine (TPP) (Alfa Aesar, Ward Hill, MA); 2,20-dipyridyl disulfide(DPDS) (MP Biomedicals, Santa Ana, CA); and3-amino-9-ethylcarbazole (AEC), sodium cyanoborohydride ($NaCNBH_3$), dansyl chloride (DC) and n-butanol (Sigma-Aldrich, St. Louis, MO). The metabolite standards used for structural confirmation were from Sigma-Aldrich, Fisher Scientific, Alfa Aesar, Ark Pharm (Libertyville, IL), Frontier Scientific (Logan, UT), and Steraloids (Newport, RI), respectively.

Untargeted metabolites analysis of ileal and cecal digesta collected from pigs fed the 6 experimental dies were conducted at the Department of Food Science and Nutrition of the University of Minnesota. A liquid chromatography-mass spectroscopy (LC-MS) based metabolomic analysis comprised of the steps 1) sample preparation, 2) chemical derivatization, 3) LC-MS analysis, 4) data deconvolution and processing, 5) multivariate data analysis (MDA), and 6) marker characterization and quantification (Chen et al., 2007).

For LC-MS analysis, ileal and cecal samples were mixed with 50% aqueous ACN in 1:9 (w/v) ratio and then centrifuged at 18,000 ×g for 10 min to obtain extract supernatants. For detecting carboxylic acids, aldehydes and ketones, the samples were derivatized with HQ prior to the LC-MS analysis (Lu et al., 2013). Briefly, 2 μL of sample was added into a 100 μL of freshly prepared ACN solution containing 1 mmol/L DPDS, 1 mmol/L TPP, and 1 mmol/L HQ. The reaction mixture was incubated at 60°C for 30 min, chilled on ice, and then mixed with 100 μL of ice-cold H_2O. After centrifugation at 18,000 ×g for 10 min, the supernatant was transferred into a HPLC vial for LC-MS analysis as described by Wang et al. (2016). For detecting mono-saccharides, the digesta supernatant (18,000 ×g for 10) were derivatized with AEC and $NaCNBH_3$ prior to LC-MS analysis. Briefly, 50 μL of sample were added into 100 μL of 25mM AEC (in MeOH), 50 μL of 50 mM $NaCNBH_3$ (in H_2O), 50 μL of 0.1 mg/mL d2-glucose, and 20 μL acetic acid. The reaction mixture was incubated at 60°C for 80 min, chilled on ice, and then mixed with 300 μL of ice-cold H_2O and CH_2Cl_2:Hexane (2:1 v/v). After centrifugation at 18,000 ×g for 10 min, the supernatant was transferred into a HPLC vial for LC-MS analysis.

A 5 μl of sample aliquot will be injected into an Acquity ultra-performance liquid chromatography (UPLC) system (Waters, Milford, MA) and separated in a BEH C18 column (Waters). The mobile phase for HQ-derivatized samples contains A: H_2O containing 0.05 % acetic acid (v/v) and 2 mM ammonium acetate; B: H_2O:ACN = 5:95 (v/v) containing 0.05 % acetic acid (v/v) and 2 mM ammonium acetate. The mobile phase gradient ranged from 0.5% B to 100% B over a 10-min run. LC eluant will be introduced into a Xevo-G2-S quadrupole time-of-flight mass spectrometer (QTOFMS, Waters) for accurate mass measurement and ion counting. Capillary voltage and cone voltage for electrospray ionization will be maintained at 3 kV and 30 V for positive-mode detection, or at -3 kV and -35 V for negative-mode detection, respectively. Source temperature and desolvation temperature will be set at 120°C and 350°C, respectively. Nitrogen will be used as both cone gas (50 L/h) and desolvation gas (600 L/h), and argon as collision gas. For accurate mass measurement, the mass spectrometer will be calibrated with sodium formate solution with mass-to-charge ratio (*m/z*) of 50-1,000 and monitored by the intermittent injection of the lock mass leucine enkephalin ($[M+H]^+$ = *m/z* 556.2771 and ($[M-H]^-$ = *m/z* 554.2615) in real time. Mass chromatograms and mass spectral data will be acquired and processed by MassLynx™ software (Waters) in centroided format. Additional structural information will be obtained using tandem MS (MSMS) fragmentation with collision energies ranging from 15 to 40 eV.

After data acquisition in the UPLC-QTOFMS system, chromatographic and spectral data of samples were deconvoluted by MarkerLynx™ software (Waters). A multivariate data matrix containing information on sample identity, ion identity (retention time and *m/z*), and ion abundance was generated through centroiding, deisotoping, filtering, peak recognition, and integration. The intensity of each ion was calculated by normalizing the single ion counts (SIC) versus the total ion counts (TIC) in the whole chromatogram. The processed data matrix was exported into SIMCA-P+™ software (Umetrics, Kinnelon, NJ), transformed by *pareto* scaling, and then analyzed by unsupervised principal components analysis (PCA) and supervised partial least squares-discriminant analysis (OPLS-DA).

Cytokine Profile of Ileal and Colonic Tissues

Total RNA from ileal and colon tissues was isolated and 500 ng used for cDNA synthesis. Gene expression was determined by quantitative real time PCR (qPCR) and the cycle time (ΔCt) of INFγ, TNFα, IL1β, IL2, IL4, IL6, IL8, IL10, IL11, IL12p40, IL17A, IL23A and IL25 was calculated in reference to housekeeping genes such as hypothanthine phosphoribosyltransferase (HPRT), glyceraldehyde-3-phosphate dehydrogenase (GAPDH), and the 18s. Data were corrected for primer efficiency using the Pfaffl method (Pfaffl, 2001).

Calculations

The calculation of apparent total tract and ileal digestibility (ATTD and AID) of energy or nutrients followed the method of Stein et al. (2007)

AID or ATTD = [1- (Ns/Nf) (Tif/Tis)] ×100%

In which Ns is the concentration of nutrients or other component in the ileal digesta or feces [g/kg of DM], Nf is the concentration of nutrients or other component in the diets [g/kg of DM], Tif represents titanium concentration in the diet [g/kg of DM], and Tis represents titanium concentration in the ileal digesta or feces [g/kg of DM]. The hindgut fermentation was calculated by subtracting AID from ATTD for nutrients or other component.

The peak shear stress was extracted as the maximum shear stress value that recorded at 0.1 s^{-1} shear rate for 2 continuous minutes. The viscosity of whole digesta was recorded at different shear rate (from 1 to 100 s^{-1}, Figure 1A) and data were fitted to a power law model according to Holdsworth (1971).

$\eta=K\times\gamma^{n-1}$

where η = apparent viscosity, K = consistency constant, γ = shear rate and n = power law index. The lower the value of n, the greater the viscosity decreases with increasing shear rate; when n = 1 corresponds to a newtonian fluid which has a constant viscosity. And K represents the viscosity at a shear rate of 1 s^{-1}.

The viscosity of digesta supernatant were stable when shear rate was above 10 s^{-1} (Figure 1B). Therefore, the values measured above 10 s^{-1} shear rate were averaged to represent the viscosity of digesta supernatant.

Statistical Analysis

All statistical analyses were performed using SAS 9.4 (SAS Inst. Inc., NC) with pigs as the experimental unit. Performance data were submitted to repeated measures by using linear mixed model followed 2 × 3 factorial design. The main effects of carbohydrases, basal diet types, their interaction and sex served as fixed effects, block served as random effect and experimental period was the repeated term in the model. The digestibility data were submitted to the similar mixed model without the effect of experimental period. For rheological characteristics data, the intestine site effects (fixed) were examined in the previous mixed model. Subsequently, the rheological data were re-analyzed in the same mixed model with liquid to solid ratio (centrifuged at 3500 × g for 10 min) as covariaence and witout intestine site effects. The least square means of main effects were seperated by adjust tukey methods and the leat square means of simple effects (carbohydrases × basal diets) were seperated by planned t test when the interaction was significant ($P < 0.05$). Pearson correlation factors between the rheological parameters were also investigated. Differences were considered significant at $P < 0.05$ and trends ($0.05 > P < 0.10$) were discussed.

RESULTS

Experiment 1

Degree of Enzymatic Hydrolysis of WM and cDDGS

Addition of carbohydrase enzymes effectively increased ($P < 0.01$) IVDMD, IVDGE, and solubilized glucose and protein in the supernatant when incubated with WM, but not with cDDGS (Table 4). There were no differneces in IVDMD between Enzyme 11 and WM control, whereas the other carbohydrases increased ($P < 0.05$) IVDMD compared with the WD control. All collected carbohydrases ($P < 0.05$) improved IVDGE. Enzyme5 had greater IVDMD of WM than Enzyme 7, 9, and 11and showed the highest value among all treatments. Greater IVDGE was observed in Enzyme 1, 2, 5, and 8 compared with Enzyme 10 and 11.

Kinetics of Gas Production

In general, carbohydrases supplemented into WM slowed down fermentation speed and reduced total gas production compared with the control (Figure 2).The WM with carbohydrases consumed more ($P < 0.05$) time to achieve half gas accumulation (T/2) and yeilded less ($P < 0.05$) total gas production (G_f) compared with the no enzyme WM control (Table 5). The IVFDM during fermentation was the greatest for the WM control (49.8%),

which was greater ($P < 0.05$) than WM supplemented with Enzyme 1, 3, 5, 8, and 9. The fractional rates of degradation at T/2 ($\mu_{T/2}$) were greater ($P < 0.05$) in the control compared with WM with enzyme 8 and 10. No differences were observed among groups of WM with different commercial carbohydrases. Samples of WM supplemented with Enzyme 11 showed the second highest disappearance of DM (48.9%) and greater then WM with Enzyme 1, 3, and 8.

In cDDGS, gas accumulation curves was close among all treatments. No differences were observed in IVFDM during fermenation for all treatments. However, the control had greater ($P < 0.05$) G_f than DDGS with Enzyme 1, 3, 5, 6, 9 and 11, and greater ($P < 0.05$) T/2 than DDGS with Enzyme 9. There was no difference in T/2 and G_f for the control and DDGS with enzyme 4, 7, 8, and 10. The fractional rates of degradation at T/2 ($\mu_{T/2}$) were less ($P < 0.05$) in the control compared with DDGS supplemented with Enzyme 6, 8, 9 and 11.

Profile of End-Products After In vitro Fermentation

Acetic acid was the most abundant VFA during *in vitro* fermentation regardless of cDDGS or WM (Table 6). Carbohydrases decreased ($P < 0.05$) the *in vitro* acetic acid, total VFA production and the amount of energy derived from VFA compared to the WM control, as well as the fermented energy to GE ratio. There was a decrease tendecy ($P = 0.08$) in branched-chain fatty acids production for WM treated with carbohydrases compared to the WM control. However, the VFA and the derived energy were not affected by carbohydrases supplementing in cDDGS.

Experiment 2

Growth Performance

No interactions among period and dietary treatments were observed in growth performance data. Pigs fed diets that contained DDGS or WM had less ($P < 0.05$) ADG (755 and 751 g/d, respectively) and ADFI (1,474 and 1,435 g/d, respectively) compared with pigs fed CSB diets (803 g/d and 1,582 g/d, respectively). Carbohydrases supplementation tended to improve ($P < 0.10$) ADG (787 vs. 752 g/d) and ADFI (1,529 vs. 1,465 g/d) regardless of basal diets (Table 7).

Nutrients Digestibility and Hindgut Fermentation

Pigs fed CSB diets had greater ($P < 0.05$) AID and ATTD of DM, OM, and GE compared with pigs fed DDGS and WM diets (Table 8). The inclusion of DDGS resulted in an increase AID of acid hydrolyzed fat and decreases in ATTD of DM, GE, OM, CP, and acid hydrolyzed fat compared with pigs fed WM diets. As indicated by the interactions ($P < 0.05$) between enzyme and basal diets, Carbohydrases supplementation improved ($P < 0.05$) ATTD of ash and AID of DM, GE and CP in WM diets, but not in DDGS or CSB diets. Pigs fed diets with carbohydrases tended to have greater ($P < 0.05$) AID of ash compared to pigs fed diets without carbohydrases. Pigs fed CSB diets had a greater ($P < 0.05$) DE intake compared to WM and DDGS diets and tended to had a greated energy:gain efficiency ($P = 0.093$) compared to WM diets. The inclusion of WM increased hindgut fermentation of DM, GE, OM, and acid hydrolyzed fat compared to pigs fed CSB and DDGS diets. The hindgut fermentation of CP was also stimulated by inclusion of WM compared to pigs fed CSB diets. There were no effects of carbohydrases addition on ATTD of DM, OM, CP, or EE and hindgut fermentaion of nutrients in any of the 3 diets.

Rheology Characteristics and pH of Duodenum, Jejunum, Ileum, Cecum, and Colon Content

There were no differences in pH of duodenum, jejunum, ileum, cecum, and colon content in pigs fed CSB, DDGS, or WM nor there were differences when carbohydrase enzymes were added (Table 9).

The liquid to solid ratio of whole digesta at ileum was associated with a larger coefficent of variarance (CV, 22 % vs. 15 and 9%), but the value were less ($P < 0.05$) than jejunum and cecum (Figure 3A). The cecal viscosity of digesta supernatant were greater ($P < 0.05$) than ileum and jejunum (Figure 3B). The ileal digesta had a greater ($P < 0.05$) consitency constant than jejunal digesta and a greater peak shear stress ($P < 0.05$) than jejunal and cecal digesta (Figure 3 C and D). The CV of whole digesta viscosity characteristics (> 85%) were much larger than the digesta supernatant (33-52%) from small intestine to large intestine.

The liquid to solid ratio of whole digesta negatively associated with ($P < 0.01$) peak shear stress (maximum value measured at 0.1 s^{-1} shear rate for 2 min) and consistency constant K of jejunum, ileum, and cecum digesta, as well as ileal viscosity of digesta supernatant (Table 10). The consistency constant (K) had strong positive correlationship ($P < 0.01$, $r > 0.87$) with peak shear stress at all measured intestinal site. The power index negtively

associated (P <0.01) with peak shear stress and K at ileum and cecum. The positive correlation beween viscosity of digesta supernatant and power index at ileum was significant with $P = 0.035$.

Carbohydrases supplementation decreased ($P < 0.05$) the peak shear stress and consistency constant K of jejunum digesta and tended to decrease ($P = 0.053$) the power index of cecal digesta (Table 11). The inclusion of DDGS induced a lower ($P < 0.05$) the peak shear stress and consistency constant K of cecal digesta, while inclusion of WM resulted in a greater ($P < 0.05$) peak shear stress at ileum and lower power index at jejunum compared to the CSB diets.

Pigs fed CSB diets had greater ($P < 0.05$) jejunal digesta viscosity than pigs fed WM diets and greater ($P < 0.05$) viscosity of digesta supernatant at jejunum and ileum than pigs fed DDGS diets (Table 12). The addition of carbohydrases increased ($P < 0.05$) viscosity of cecal digesta supernatant only in CSB diet, but not WM and DDGS diets, as indicated by the interaction ($P < 0.05$) between basal diets and carbohydrases addition. There was also a tendency interaction ($P = 0.06$) in viscosity of ileal digesta supernatant between basal diets and carbohydrases addition.

Jejunal, Ileal and Cecal Monosaccharides and Metabolomes

The metabolites in intestinal digesta, including short-chain fatty acids, bile acids, lipids and mono-saccharides were analyzed by LC-MS following their respective derivatization procedures. The LC-MS data were analyzed by the PCA and PLS-DA modeling. The PLS-DA model clearly separated cecal metabolomes of CSB, DDGS and WM diets (positive and negative detection mode), but not with or without carbohydrases (Figure 4A-B) Dietary treatments differences were not be separated based on ileal metabolome.

However, the mono-saccharides concentrations of liquid phase of digesta (different sections) were altered by basal diets and carbohydrases supplementation (Table 13). The addition of carbohydrases increased ($P < 0.05$) xylose concentration of jejunal and ileal digesta supernatant only in WM diet, but not in CSB and DDGS diets, as indicated by the interaction ($P < 0.05$) between basal diets and carbohydrases addition. The interaction ($P < 0.05$) was also observed for galactose concentration of ileal fluid, which was increased ($P < 0.05$) by carbohydrases addition into CSB diet, but not into WM and DDGS diets. The glucose concentration of ileal fluid was improved ($P < 0.05$) by carbohydrases addition regardless of basal diets. Pigs fed CSB diets had greater ($P < 0.05$) glucose and mannose concentration at ileum and galactose concentration at cecum compared to pigs fed WM and DDGS diets. The inclusion of DDGS resulted decreases in glucose concentration of jejunal fluid and galactose concentration of ileal fluid compared to CSB diets. Pigs fed WM diets had greater mannose concentration at jejunum and galactose concentration at ileum compared to DDGS diets. The addition of WM also resulted increases in xylose concentration of ileal and cecal fluids compared to CSB and DDGS diets.

Gene Expression of Cytokines in Ileal and Colonic Tissue

The addition of carbohydrases decreased ($P < 0.05$) expression of ileal IL11 and colonic IFNγ only in WM and DDGS diets, but not in CSB diets, as indicated by the interaction ($P < 0.01$) between basal diets and carbohydrases addition (Table 14). The interaction ($P < 0.05$) was also observed for ileal IL4 expression, which was increased ($P < 0.05$) by carbohydrases addition into WM diet, but not into WM and DDGS diets. The addition of carbohydrases decreased ($P < 0.05$) expression of colonic TNFα expression in WM and DDGS diets, but increased it in CSB diets. Without the presence of carbohydrases, pigs fed WM diet had a greater ($P < 0.05$) expression of IL25 in the ileum compared to pigs fed CSB and DDGS diets. Carbohydrases supplementation increased ($P < 0.05$) expression of ileal IL1 β and colonic IL10, IL23A, and IL25, and decreased ($P < 0.05$) expression of ileal IL17A and colonic IL11 and IL17A. The addition of carbohydrases also resulted in a decreased tendency in expression of IL2, IL8, and IL10 in the ileum and an increased tendency in expression of colonic IL4. Pigs fed WM diets had a greater ($P < 0.05$) expression of IL1β in the ileum compared to pigs fed CSB diets.

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Table 1. Characteristics of exogenous carbohydrases

Supplier	Trade name	Enzymes	Stated activity	Inclusion, mg/kg
Novus	Xylanase	Xylanase	11820 U/g	100
Danisco	Xylanase 4000 G	Xylanase	40,000 U/g	25-100
Danisco	-	Xylanase	40,000 U/g	25-100
AB Vista	Econase XT25	Xylanase	160,000 BXU/g	60-150
	Econase GT 200 P	Glucanase	200,000 U/g	60-150
ADISSEO	Rovabio-Excel	Xylanase Glucanase	2,200 U/g 200 U/g	454
ADM	Easyzyme A	Xylanase cellulose	100,000 U/g 125,000 U/g	24
	Easyzyme B	Xylanase β-lucanase β-mannanase	1,500 U/g 1,100U/g 110U/g	100
DSM	Ronoztme @G2G	Xylanase Glucanase Cellulase	2700 U/g 700 U/g 800 U/g	50-200
Sunhy	Sunhy	Xylanase Glucanase	20,000 U/g 1,800 U/g	500
Huvepharma	Hostazyme	Xylanase Glucanase Cellulase	1,000 U/g 7,000 U/g 5,000 U/g	100

Table 2. Chemical composition of wheat middlings (WM) and corn distillers dried grains with solubles (DDGS) used as substrate for *in vitro* digestibility and fermentation procedures

Items	**WM**	**DDGS**
Dry matter, %	88.62	91.83
Gross energy, kcal/kg	4,065	4,683
Crude protein, %	17.67	28.21
Acid hydrolysis fat, %	3.66	8.25
Total monosaccharide content, % of DM		
Arabinose	5.60	5.56
Xylose	6.19	4.61
Mannose	0.35	1.48
Galactose	1.42	1.70
Glucose	17.02	6.40

Table 3. Ingredient and calculated nutrient composition of the corn and soybean meal diet (CSB), corn distillers dried grains with solubles (DDGS), and wheat middling (WM)

Item	CSB	DDGS	WM
Ingredient composition, %			
Yellow dent corn	72.00	42.02	46.66
Soybean meal	25.00	15.00	18.00
Corn distillers dried grains with solubles	-	40.00	-
Wheat middling	-	-	30.00
Soybean oil	-		2.23
Dicalcium phosphate	0.30	-	-
Limestone	1.36	1.66	1.56
Salt	0.25	0.25	0.25
L-Lys HCl, 78%	0.27	0.35	0.38
DL-Met, 98%	0.06	-	0.09
L-Thr, 98%	0.05	-	0.12
L-Trp, 99%	-	0.01	-
Phytase, 10,000 FTU/g	0.01	0.01	0.01
Vitamin premix[1]	0.25	0.25	0.25
Mineral premix[2]	0.15	0.15	0.15
Titanium dioxide, 40% Ti	0.30	0.30	0.30
Total	100	100	100
Calculated nutrient composition			
ME, Kcal/kg	3,285	3,295	3,285
NE, Kcal/kg	2,446	2,373	2,425
CP, %	18.18	22.76	17.56
Ether extract, %	2.89	5.10	5.14
NDF, %	8.61	17.30	17.22
ADF, %	3.39	6.15	5.78
Total Ca, %	0.66	0.66	0.66
Total P	0.42	0.59	0.56
Standardized total tract digestible P	0.31	0.45	0.40
Standardized ileal digestible AA, %			
Lys	1.00	1.00	1.00
Met + Cys	0.57	0.71	0.57
Thr	0.60	0.67	0.60
Trp	0.18	0.18	0.18
Val	0.71	0.86	0.65

[1,2] The premix provided the following per kilogram of complete diet: vitamin A, 12,000 IU; vitamin D3, 2,500 IU; vitamin E, 30 IU; vitamin K3, 3 mg; vitamin B12, 0.012 mg; riboflavin, 4 mg; niacin, 40 mg; pantothenic acid, 15 mg; choline chloride, 400 mg; folic acid, 0.7 mg; thiamin, 1.5 mg; pyridoxine, 3 mg; biotin, 0.1 mg; Zn, 105 mg; Mn, 22 mg; Fe, 84 mg; Cu, 10 mg; I, 0.50 mg; Se, 0.35 mg.

Table 4. Effects of different commercial carbohydrases on *in vitro* dry matter (IVDMD) and gross ennery (IVGED) digestibility and nutrients releases in filtrate for wheat middlings (WM) and corn distillers dried grains with solubles (DDGS).

Treatments	In vitro digestibility, %				Nutrients release in filtrate			
	WM		Corn DDGS		WM		Corn DDGS	
	DM	GE	DM	GE	Glucose ug/mL	AA mg/mL	Glucose Ug/mL	AA mg/mL
Control	54.7^{e}	52.8^{d}	52.7	51.3	54.1^{b}	1.10^{b}	22.1	1.58
Enzyme1	58.5^{abc}	57.5^{a}	52.8	52.6	71.4^{a}	1.27^{a}	21.6	1.62
Enzyme2	57.6^{abc}	57.9^{a}	53.5	52.6	67.1^{ab}	1.14^{ab}	22.3	1.62
Enzyme3	58.9^{ab}	57.2^{ab}	53.1	52.2	66.9^{ab}	1.16^{ab}	21.7	1.61
Enzyme4	57.9^{abc}	57.2^{ab}	52.8	52.5	69.2^{ab}	1.21^{ab}	24.2	1.61
Enzyme5	59.2^{a}	57.7^{a}	53.3	52.4	73.9^{a}	1.27^{a}	24.2	1.68
Enzyme6	58.8^{ab}	57.4^{ab}	53.0	52.7	75.8^{a}	1.18^{ab}	22.0	1.68
Enzyme7	57.5^{bcd}	56.9^{ab}	53.1	51.8	72.1^{a}	1.17^{ab}	22.9	1.63
Enzyme8	58.2^{abc}	57.5^{a}	52.6	51.5	67.3^{ab}	1.19^{ab}	25.1	1.68
Enzyme9	57.1^{cd}	56.8^{ab}	53.0	52.9	75.3^{a}	1.20^{ab}	22.1	1.63
Enzyme10	57.2^{abc}	55.6^{bc}	52.6	51.3	70.5^{a}	1.22^{ab}	22.2	1.60
Enzyme11	55.5^{de}	54.9^{c}	53.6	52.0	63.3^{ab}	1.14^{ab}	26.3	1.69
SEM	0.51	0.88	0.42	0.71	4.53	0.045	3.25	0.048
P-value	<0.01	<0.01	0.302	0.279	<0.01	<0.01	0.93	0.32

Table 5. Fitted kinetics parameters (means) of gas accumulation recorded for wheat middlings (WM) and corn distillers dried grains with solubles (DDGS) supplemented with different commerical carbohydrases inoculated with a fecal inoculum from pigs

	WM				DDGS			
Item	T/2[1], h	μ[2]	Gf[3] mL/g	IVFDM[4], %	T/2[1], h	μ[2]	Gf[3] mL/g	IVFDM[4], %
Control	4.8^{b}	0.107^{ab}	365^{a}	49.8^{a}	17.6^{abc}	0.024^{de}	416^{a}	65.3
Enzymes								
1	5.9^{a}	0.110^{a}	246^{b}	44.7^{cd}	16.5^{abcd}	0.028^{abcde}	349^{cd}	66.5
2	6.3^{a}	0.110^{a}	252^{b}	47.8^{abc}	16.4^{abcd}	0.028^{abcde}	382^{abc}	65.0
3	6.4^{a}	0.109^{ab}	257^{b}	45.2^{cd}	14.4^{bcd}	0.032^{abcd}	365^{bcd}	65.4
4	6.7^{a}	0.104^{abc}	261^{b}	47.9^{abc}	15.7^{bcd}	0.029^{abcde}	388^{ab}	63.9
5	7.0^{a}	0.099^{bc}	248^{b}	45.9^{bcd}	14.4^{bcd}	0.031^{abcde}	363^{bcd}	64.3
6	6.9^{a}	0.099^{bc}	262^{b}	46.8^{abcd}	13.3^{cd}	0.033^{abc}	350^{cd}	63.5
7	6.7^{a}	0.102^{abc}	275^{b}	47.7^{abc}	18.4^{ab}	0.025^{cde}	409^{a}	66.1
8	6.8^{a}	0.095^{c}	268^{b}	43.9^{d}	20.2^{a}	0.023^{e}	408^{a}	62.2
9	6.7^{a}	0.099^{bc}	273^{b}	45.8^{bcd}	12.8^{d}	0.034^{ab}	341^{d}	64.6
10	7.0^{a}	0.097^{c}	257^{b}	47.1^{abc}	16.2^{abcd}	0.026^{bcde}	390^{ab}	64.0
11	6.6^{a}	0.101^{abc}	283^{b}	48.9^{ab}	13.0^{cd}	0.035^{a}	353^{cd}	65.3
SEM	0.34	0.002	9.7	0.72	1.01	0.002	8.7	1.03
P-value	< 0.01	< 0.01	< 0.01	< 0.01	<0.01	<0.01	<0.01	0.322

[1]Half-time to asymptote (h).

[2]Fractional rate of degradation (h−1) at t = T/2.

[3]Maximum gas volume (ml per g DM inoculated).

[4] *In vitro* fermentibility of dry matter.

Table 6. Effects of different commercial carbohydrases on short chain fatty acids concentration in wheat middlings (WM) and corn distillers dried grains with solubles (DDGS) inoculated with a fecal inoculum from pigs.

Item	Mmol/g DM inoculated					Energy from VFA[3] Kcal/kg DM	Fermented GE[4] ratio, %
	Acetic acid	Propionic acid	Butyric acid	BCFA[1]	Total VFA[2]		
WM							
Control	4.19[a]	1.10	0.63	0.24	6.27[a]	0.87[a]	18.87[a]
Enzyme1	3.74[b]	0.99	0.53	0.22	5.58[b]	0.76[b]	16.66[b]
Enzyme2	3.71[b]	0.94	0.56	0.21	5.50[b]	0.75[b]	16.21[b]
Enzyme3	3.76[b]	0.92	0.58	0.19	5.54[b]	0.75[b]	16.36[b]
Enzyme4	3.62[b]	0.94	0.50	0.20	5.34[b]	0.73[b]	15.85[b]
Enzyme5	3.65[b]	0.99	0.60	0.22	5.54[b]	0.76[b]	16.58[b]
Enzyme6	3.75[b]	0.94	0.58	0.22	5.58[b]	0.76[b]	16.64[b]
Enzyme7	3.68[b]	0.94	0.61	0.20	5.52[b]	0.76[b]	16.48[b]
Enzyme8	3.70[b]	1.00	0.59	0.22	5.59[b]	0.77[b]	16.91[b]
Enzyme9	3.69[b]	0.93	0.58	0.23	5.53[b]	0.76[b]	16.53[b]
Enzyme10	3.70[b]	0.96	0.54	0.23	5.51[b]	0.76[b]	16.54[b]
Enzyme11	3.67[b]	1.02	0.59	0.23	5.61[b]	0.76[b]	16.70[b]
SEM	0.10	0.06	0.05	0.01	0.13	0.02	0.44
P Value	< 0.01	0.56	0.74	0.08	<0.01	<0.0.1	<0.01
DDGS							
Control	4.85	1.49	0.55	0.32	7.35	1.01	21.61
Enzyme1	4.87	1.41	0.51	0.31	7.23	0.98	20.55
Enzyme2	5.00	1.51	0.55	0.32	7.52	1.01	21.21
Enzyme3	4.77	1.49	0.58	0.33	7.31	1.01	21.10
Enzyme4	4.79	1.49	0.57	0.32	7.34	1.02	21.30
Enzyme5	4.75	1.52	0.58	0.33	7.33	1.01	21.14
Enzyme6	4.82	1.42	0.56	0.31	7.25	0.99	20.77
Enzyme7	4.82	1.39	0.54	0.31	7.15	0.96	20.19
Enzyme8	4.84	1.44	0.60	0.29	7.30	1.00	21.02
Enzyme9	4.84	1.45	0.56	0.32	7.31	1.00	20.94
Enzyme10	4.95	1.46	0.52	0.28	7.33	0.99	20.76
Enzyme11	4.89	1.39	0.50	0.29	7.22	0.96	20.14
SEM	0.14	0.07	0.04	0.02	0.21	0.03	0.70
P Value	0.99	0.90	0.82	0.54	0.99	0.95	0.90

[1] Branched-chain fatty acids (sum of iso-butyric and iso-valeric acids)
[2] Short chain fatty acid
[3] The energy equivalent of each VFA (kJ/mol) was assumed as follows: acetic acid = 875, propionic acid 1,528, butyric acid = 2,185, and valeric acid = 2,839 (CRC, 1977)
[4] Calculated from energy from VFA and the GE of ingredients.

Table 7. Effects of carbohydrases supplementation on growth performance of growing pigs fed with different fiber sources[1]

Items	CSB		DDGS		WM			*P* - value		
	-	+	-	+	-	+	SEM	Diet	Enzyme	D × E
BW, kg										
Initial	24.85	24.86	24.87	24.59	24.78	24.39				
1wk	29.20	28.92	28.61	28.44	28.23	28.77				
2wk	35.27	35.71	34.31	34.39	34.26	35.03				
3wk	41.13	41.59	39.51	40.38	39.37	40.72				
4wk	47.54	47.64	45.59	45.78	45.13	46.64	0.71	0.089	0.327	0.849
ADG, g										
1wk	617	575	485	546	488	619				
2wk	863	966	883	845	858	888				
3wk	834	836	724	851	725	807				
4wk	912	860	861	768	818	841				
Overall	806^{x}	809^{x}	738^{y}	752^{y}	722^{xy}	789^{xy}	22	0.024	0.051	0.419
ADFI, g										
1wk	1,156	1,056	930	1,004	934	1,082				
2wk	1,624	1,773	1,521	1,572	1,493	1,608				
3wk	1,670	1,696	1,709	1,737	1,455	1,608				
4wk	1,848	1,832	1,638	1,681	1,604	1,697				
Overall	1,575^{x}	1,589^{x}	1,449^{y}	1,498^{y}	1,372^{y}	1,499^{y}	44	<0.01	0.076	0.428
G:F										
1wk	535	544	560	541	521	562				
2wk	529	546	556	536	572	556				
3wk	497	500	442	487	496	510				
4wk	491	470	466	461	508	500				
Overall	513	515	506	506	524	532	13	0.288	0.525	0.999

[x,y] Means within a row without same superscript are different ($P < 0.05$) for main effects

[1] CSB: corn soybean meal basal diets; DDGS: diet included 40% DDGS; WD: diets included 30% wheat middling. +/- represented with or without 100 mg/kg carbohydrases mixture, which contains 1,500 U/g xylanase, 1,100 U/g beta-glucanase, 110 U/g mannanse, 35 U/g galactosidase.

Table 8. Effects of carbohydrases supplementation on apparent ileal digestibility (AID), apparent total tract digestibility (ATTD), and hindgut disappearance of energy and nutrient in growing pigs fed with different fiber sources

Items, %	CSB		DDGS		WM		SEM	*P* - value		
	-	+	-	+	-	+		Diet	Enzyme	D × E
AID										
DM	75.9^{a}	75.4^{a}	64.4^{b}	64.2^{b}	60.8^{c}	64.8^{b}	1.37	< 0.01	0.161	0.049
GE	76.6^{a}	74.8^{a}	65.3^{b}	65.2^{b}	61.0^{c}	65.2^{b}	1.61	< 0.01	0.437	0.041
OM	77.4^{x}	77.0^{x}	66.1^{y}	66.0^{y}	62.9^{y}	66.4^{y}	1.48	< 0.01	0.237	0.138
CP	79.1^{ab}	79.4^{a}	71.1^{c}	71.7^{c}	69.6^{c}	75.8^{b}	1.60	< 0.01	0.017	0.025
EE	63.4^{x}	59.9^{x}	64.9^{x}	58.6^{x}	52.5^{y}	56.5^{y}	3.92	0.020	0.436	0.199
Ash	47.7^{x}	47.6^{x}	34.1^{y}	37.3^{y}	25.9^{y}	39.7^{y}	5.12	< 0.01	0.058	0.138
ATTD										
DM	87.4^{x}	87.5^{x}	78.5^{z}	78.3^{z}	80.0^{y}	82.3^{y}	1.13	< 0.01	0.258	0.240
GE	86.5^{x}	86.1^{x}	77.3^{z}	77.6^{z}	80.7^{y}	81.9^{y}	1.01	< 0.01	0.535	0.504
OM	89.1^{x}	89.1^{x}	80.3^{z}	80.1^{z}	82.0^{y}	84.2^{y}	1.14	< 0.01	0.309	0.274
CP	84.1^{x}	85.0^{x}	79.1^{y}	78.8^{y}	84.1^{x}	85.8^{x}	1.45	< 0.01	0.360	0.621
EE	42.4^{z}	40.0^{z}	47.7^{y}	47.5^{y}	55.0^{x}	54.2^{x}	3.80	< 0.01	0.610	0.914
Ash	55.8^{a}	57.9^{a}	46.6^{c}	45.7^{c}	45.6^{c}	51.9^{b}	1.87	< 0.01	0.025	0.030
DE intake, Mcal/d	5.3^{x}	5.2^{x}	4.6^{y}	4.8^{y}	4.4^{y}	4.9^{y}	0.15	<0.01	0.124	0.175
DE efficiency Mcal/kg gain	6.6	6.4	6.4	6.3	6.1	6.2	0.14	0.093	0.722	0.622
Hindgut disappearance										
DM	11.5^{y}	12.0^{y}	14.1^{y}	14.6^{y}	18.9^{x}	17.4^{x}	1.92	< 0.01	0.864	0.703
GE	9.9^{y}	11.3^{y}	11.9^{y}	12.9^{y}	19.5^{x}	16.5^{x}	2.03	< 0.01	0.871	0.264
OM	11.7^{y}	12.0^{y}	14.2^{y}	14.7^{y}	18.9^{x}	17.7^{x}	2.02	< 0.01	0.903	0.822
CP	4.8^{y}	5.6^{y}	8.0^{xy}	7.9^{xy}	14.1^{x}	9.8^{x}	2.34	< 0.01	0.378	0.282
EE	-20.5^{y}	-21.3^{y}	-18.8^{y}	-10.3^{y}	2.2^{x}	-3.7^{x}	5.60	< 0.01	0.854	0.196
Ash	8.0	9.8	12.9	8.6	19.4	12.3	5.65	0.196	0.323	0.526

$^{x\text{-}z,\ a\text{-}c}$ Means within a row without same superscript are different ($P < 0.05$) for main effects and simple effects (if interaction was significant), respectively.

[1] CSB: corn soybean meal basal diets; DDGS: diet included 40% DDGS; WD: diets included 30% wheat middling. +/- represented with or without 100 mg/kg carbohydrases mixture, which contains 1,500 U/g xylanase, 1,100 U/g beta-glucanase, 110 U/g mannanse, 35 U/g galactosidase.

Table 9. Effects of carbohydrases supplementation on pH in different sections of the gastrointestinal tract of growing pigs fed with different fiber sources

Item	CSB		DDGS		WM			*P* - value		
	-	+	-	+	-	+	SEM	Diet	Enzyme	D × E
Duodenum	5.41	5.39	5.41	5.41	5.35	5.50	0.30	0.990	0.804	0.899
Jejunum	6.48	6.48	6.38	6.33	6.58	6.24	0.17	0.579	0.171	0.317
Ileum	6.04	6.11	6.22	6.26	6.30	6.63	0.30	0.242	0.429	0.790
Cecum	5.23	5.36	5.35	5.34	5.30	5.40	0.09	0.618	0.179	0.532
Colon	5.31	5.20	5.02	5.13	5.18	5.40	0.27	0.484	0.649	0.692

[1]CSB: corn soybean meal basal diets; DDGS: diet included 40% DDGS; WD: diets included 30% wheat middling. +/- represented with or without 100 mg/kg carbohydrases mixture, which contains 1,500 U/g xylanase, 1,100 U/g beta-glucanase, 110 U/g mannanse, 35 U/g galactosidase.

Table 10. Correlation coefficients between liquid ratio and viscous characteristics of digesta in different sections of the gastrointestinal tract of growing pigs

Item	Liquid	K	n	Viscosity	Peak
Jejunum					
Liquid	1.00				
K	-0.70**	1.00			
n	0.10	-0.14	1.00		
Viscosity, mPa.s	0.16	0.20	0.14	1.00	
Peak, Pa	-0.58**	0.87**	-0.15	0.02	1.00
Ileum					
Liquid	1.00				
K	-0.52**	1.00			
n	0.12	-0.42**	1.00		
Viscosity, mPa.s	-0.35*	-0.06	0.29*	1.00	
Peak, Pa	-0.46**	0.88**	-0.41**	-0.05	1.00
Cecum					
Liquid	1.00				
K	-0.33*	1.00			
n	0.27	-0.50**	1.00		
Viscosity, mPa.s	-0.17	-0.09	-0.07	1.00	
Peak, Pa	-0.44**	0.91**	-0.41**	-0.14	1.00

*Criteria are significantly correlated at $P < 0.05$

**Criteria are significantly correlated at $P < 0.01$

Table 11. Effects of carbohydrases supplementation on rheology characteristics of whole digesta in different sections of the gastrointestinal tract of growing pigs fed with different fiber sources

Item	CSB		DDGS		WM			*P* value		
	-	+	-	+	-	+	SEM	Diet	Enzyme	D × E
Peak shear stress[2], Pa										
Jejunum	15.1^{A}	14.5^{B}	23.1^{A}	11.0^{B}	46.2^{A}	17.2^{B}	10.00	0.045	0.018	0.146
Ileum	54.8^{y}	61.8^{y}	85.5^{xy}	74.8^{xy}	131.8^{x}	160.7^{x}	9.64	0.020	0.741	0.821
Cecum	59.0^{x}	55.1^{x}	17.8^{y}	9.2^{y}	97.5^{x}	75.9^{x}	9.40	< 0.01	0.434	0.865
Consistency constant, k[3]										
Jejunum	35.9^{A}	19.6^{B}	26.2^{A}	13.6^{B}	30.7^{A}	15.8^{B}	10.31	0.517	0.015	0.966
Ileum	38.0	48.9	43.7	58.0	57.8	46.6	10.08	0.688	0.609	0.484
Cecum	38.4^{x}	44.7^{x}	14.7^{y}	16.9^{y}	41.9^{x}	46.0^{x}	9.80	< 0.01	0.536	0.965
Power index, n[4]										
Jejunum	0.236^{x}	0.161^{x}	0.01^{xy}	0.078^{xy}	0.047^{y}	-0.015^{y}	0.102	0.030	0.684	0.544
Ileum	0.068	0.101	0.124	0.078	0.028	0.002	0.098	0.130	0.715	0.657
Cecum	0.082	0.021	0.139	-0.040	0.003	-0.091	0.100	0.339	0.053	0.658

$^{x,y;A,B}$Means within a row without same superscript are different ($P < 0.05$) for main effects (Diets or Enzyme), respectively.

[1] CSB: corn soybean meal basal diets; DDGS: diet included 40% DDGS; WD: diets included 30% wheat middling. +/- represented with or without 100 mg/kg carbohydrases mixture, which contains 1,500 U/g xylanase, 1,100 U/g beta-glucanase, 110 U/g mannanse, 35 U/g galactosidase.

[2]Peak shear stress was the maximum shear stress recorded at 0.1 s^{-1} shear rate for 2 min.

[3]Consistency constant, k and K represents the viscosity at a shear rate of 1 s^{-1}.

[4]The lower the value of n, the greater the viscosity decreases with increasing shear rate; when n = 1 corresponds to a newtonian fluid which has a constant viscosity.

Table 12 Effects of carbohydrases supplementation on viscosity of digesta supernatant in different sections of the gastrointestinal tract of growing pigs fed with different fiber sources

	CSB		DDGS		WM			*P* value		
Item	-	+	-	+	-	+	SEM	Diet	Enzyme	D × E
Viscosity, mPa.s										
Jejunum	1.60^{x}	1.99^{x}	1.22^{y}	1.19^{y}	1.43^{y}	1.17^{y}	0.19^{y}	< 0.01	0.724	0.056
Ileum	1.93^{x}	1.73^{x}	1.48^{y}	1.32^{y}	1.51^{xy}	1.73^{xy}	0.18	< 0.01	0.619	0.210
Cecum	2.42^{b}	4.25^{a}	3.54^{ab}	4.01^{a}	2.97^{ab}	1.82^{b}	0.18	0.05	0.397	0.032

$^{x,y;ab}$ Means within a row without same superscript are different ($P < 0.05$) for main effects (Diets or Enzyme) and simple effects (if interaction was significant), respectively.

[1] CSB: corn soybean meal basal diets; DDGS: diet included 40% DDGS; WD: diets included 30% wheat middling. +/- represented with or without 100 mg/kg carbohydrases mixture, which contains 1,500 U/g xylanase, 1,100 U/g beta-glucanase, 110 U/g mannanse, 35 U/g galactosidase.

Table 13. Effects of carbohydrases supplementation on mono-saccharides concentration (mg/mL) in liquid phase of different sections of the gastrointestinal tract of growing pigs fed with different fiber sources.

Items	CSB		DDGS		WM		SEM	*P* value		
	-	+	-	+	-	+		Diet	Enzyme	D × E
Jejunum										
Arabinose	0.425	0.512	0.608	0.628	0.512	0.702	0.15	0.281	0.246	0.713
Xylose	0.001^{b}	0.013^{b}	0.009^{b}	0.016^{b}	0.083^{b}	0.306^{a}	0.05	<0.01	<0.01	<0.01
Glucose	15.6^{x}	16.0^{x}	11.6^{y}	11.5^{y}	13.1^{xy}	13.1^{xy}	1.97	0.019	0.942	0.990
Mannose	1.11^{xy}	0.90^{xy}	0.82^{y}	0.46^{y}	1.93^{x}	1.32^{x}	0.39	<0.01	0.068	0.752
Galactose	0.710	1.022	0.535	0.619	0.927	0.757	0.34	0.394	0.699	0.616
Ileum										
Arabinose	1.30	1.10	0.68	0.82	0.91	1.01	0.34	0.197	0.950	0.738
Xylose	0.005^{c}	0.027^{c}	0.003^{c}	0.017^{c}	0.426^{b}	0.955^{a}	0.14	<0.01	0.029	0.025
Glucose	11.94^{xB}	14.14^{xA}	1.23^{yB}	4.74^{yA}	4.41^{yB}	6.41^{yA}	1.95	<0.01	0.024	0.827
Mannose	6.12^{x}	4.85^{x}	1.04^{y}	1.72^{y}	2.84^{y}	3.97^{y}	0.81	<0.01	0.693	0.085
Galactose	3.92^{a}	2.63^{b}	0.89^{c}	0.99^{c}	1.80^{bc}	2.32^{b}	0.49	<0.01	0.440	0.033
Cecum										
Arabinose	2.37	2.26	2.99	2.46	2.27	1.97	0.47	0.169	0.245	0.818
Xylose	0.62^{y}	0.81^{y}	1.24^{y}	1.05^{y}	2.26^{x}	2.03^{x}	0.31	<0.01	0.671	0.578
Glucose	4.43	3.06	3.66	3.48	3.96	3.05	1.01	0.937	0.146	0.684
Mannose	1.03^{x}	0.66^{x}	0.50^{xy}	0.29^{xy}	0.21^{y}	0.25^{y}	0.36	0.048	0.376	0.694
Galactose	0.911^{x}	0.566^{x}	0.328^{y}	0.241^{y}	0.285^{y}	0.299^{y}	0.19	<0.01	0.180	0.370

$^{x,y;A,B;ab}$ Means within a row without same superscript are different ($P < 0.05$) for main effects (Diets or Enzyme) and simple effects (if interaction was significant), respectively.

[1] CSB: corn soybean meal basal diets; DDGS: diet included 40% DDGS; WD: diets included 30% wheat middling. +/- represented with or without 100 mg/kg carbohydrases mixture, which contains 1,500 U/g xylanase, 1,100 U/g beta-glucanase, 110 U/g mannanse, 35 U/g galactosidase.

Table 14. Gene expression in ileum and colon of pigs fed high-fiber diets with and without carbohydrases enzyme supplementation[1,2]

Item	CSB		DDGS		WM		*P*-value[3]		
	-	+	-	+	-	+	Diet	Enzyme	D × E
Ileum									
IFNγ	0.06	0.07	0.06	0.07	0.07	0.07	0.53	0.67	0.89
TNFα	97.0	86.3	107.7	82.8	95.6	101.5	0.83	0.29	0.40
IL1β	$0.05^{y,B}$	$0.07^{y,A}$	$0.06^{xy,B}$	$0.08^{xy,A}$	$0.06^{x,B}$	$0.09^{x,A}$	0.03	<0.01	0.07
IL2	1.95	1.94	1.99	1.87	1.92	1.91	0.60	0.06	0.14
IL4	$0.03^{a,b}$	$0.03^{a,b}$	0.03^{b}	0.03^{b}	0.04^{a}	0.03^{b}	0.14	0.01	0.01
IL6	0.02	0.02	0.02	0.02	0.02	0.02	0.35	0.11	0.31
IL8	12.3	11.7	12.5	11.5	11.7	11.4	0.40	0.07	0.73
IL10	1.98	1.96	1.99	1.92	1.96	1.95	0.75	0.08	0.48
IL11	455.8^{a}	422.4^{a}	435.8^{a}	196.6^{c}	297.9^{b}	140.6^{c}	<0.01	<0.01	<0.01
IL12p40	0.005	0.005	0.005	0.006	0.005	0.006	0.37	0.31	0.37
IL17A	674.9^{A}	548.3^{B}	692.4^{A}	575.6^{B}	590.3^{A}	486.7^{B}	0.06	<0.01	0.96
IL23A	479.7	521.4	502.2	530.5	513.2	614.9	0.44	0.17	0.74
IL25	0.13^{a}	$0.15^{a,b}$	$0.14^{a,b}$	0.16^{b}	0.17^{b}	$0.15^{a,b}$	0.02	0.29	0.01
Colon									
IFNγ	$0.07^{a,b}$	$0.06^{a,b}$	0.06^{a}	0.07^{b}	0.06^{a}	0.08^{b}	0.59	<0.01	<0.01
TNFα	$119.2^{b,c}$	142.8^{a}	141.5^{a}	$114.5^{b,c}$	$134.2^{a,b}$	108.1^{c}	0.20	0.02	<0.01
IL1β	0.04	0.04	0.04	0.05	0.05	0.04	0.59	0.55	0.19
IL2	1.93	1.97	2	1.99	1.95	1.95	0.06	0.57	0.42
IL4	0.01	0.02	0.02	0.02	0.02	0.02	0.14	0.06	0.30
IL6	0.03	0.03	0.03	0.03	0.03	0.03	0.59	0.33	0.47
IL8	12.2	13.3	13.6	13.4	12.9	13.2	0.11	0.21	0.21
IL10	1.95^{B}	2.01^{A}	2.01^{B}	2.05^{A}	1.96^{B}	2.01^{A}	0.08	0.01	0.98
IL11	480.2^{A}	396.9^{B}	537.5^{A}	342.6^{B}	532.6^{A}	321.8^{B}	0.80	<0.01	0.09
IL12p40	0.004	0.004	0.004	0.005	0.004	0.005	0.07	0.17	0.23
IL17A	1295^{A}	953^{B}	1500^{A}	940^{B}	1318^{A}	741^{B}	0.07	<0.01	0.29
IL23A	786^{B}	1006^{A}	963^{B}	961^{A}	823^{B}	976.9^{A}	0.33	<0.01	0.08
IL25	0.11^{B}	0.11^{A}	0.11^{B}	0.12^{A}	0.11^{B}	0.13^{A}	0.17	0.01	0.08

[x,y;A,B;ab] Means within a row without same superscript are different ($P < 0.05$) for main effects (Diets or Enzyme) and simple effects (if interaction was significant), respectively. Relative expression mean values (n = 9).

[1]Values are expressed as a relative ratio of the amount of target gene copies to the amount of HPRT, Gapdh, and 18s (housekeeping genes) copies.

[2] CSB: corn soybean meal basal diets; DDGS: diet included 40% DDGS; WD: diets included 30% wheat middling. +/- represented with or without 100 mg/kg carbohydrases mixture, which contains 1,500 U/g xylanase, 1,100 U/g beta-glucanase, 110 U/g mannanse, 35 U/g galactosidase.

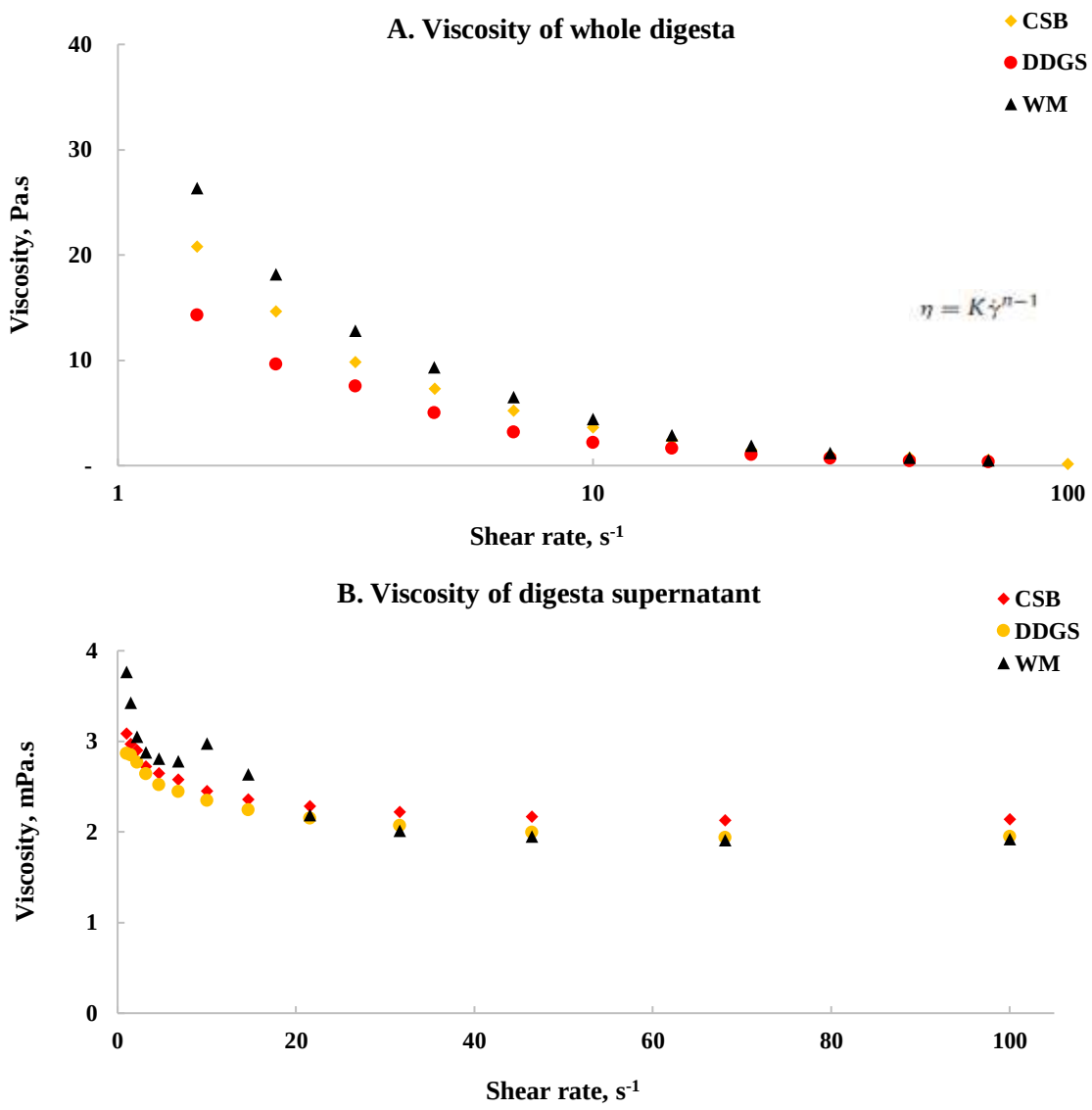

Figure 1. Rheological characteristics of whole digesta (A) and supernatant (B) for pigs fed corn and soybean meal diet (CSB), a CSB with 40% corn distillers dried grains with solubles (DDGS), and a CSB diet with 30% wheat middling (WM).

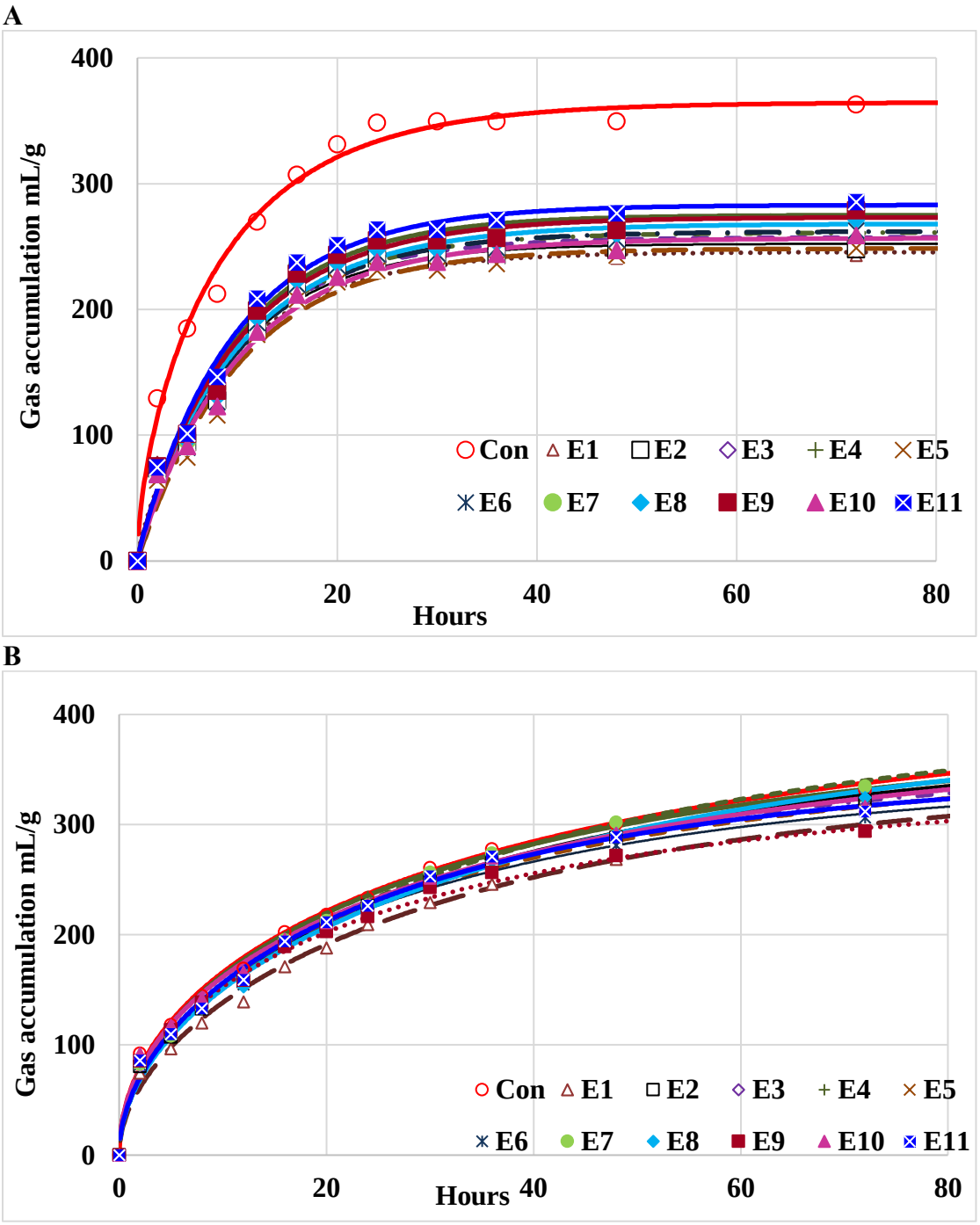

Figure 2. Gas accumulation curves for wheat middlings [A] or corn distillers dried grains with solubles [B] without enzymes (Con) or supplemented with 11 commerical carbohydrases (E1 to E11) inoculated with feces from growing pigs, which were modified according to France et al. (1993)

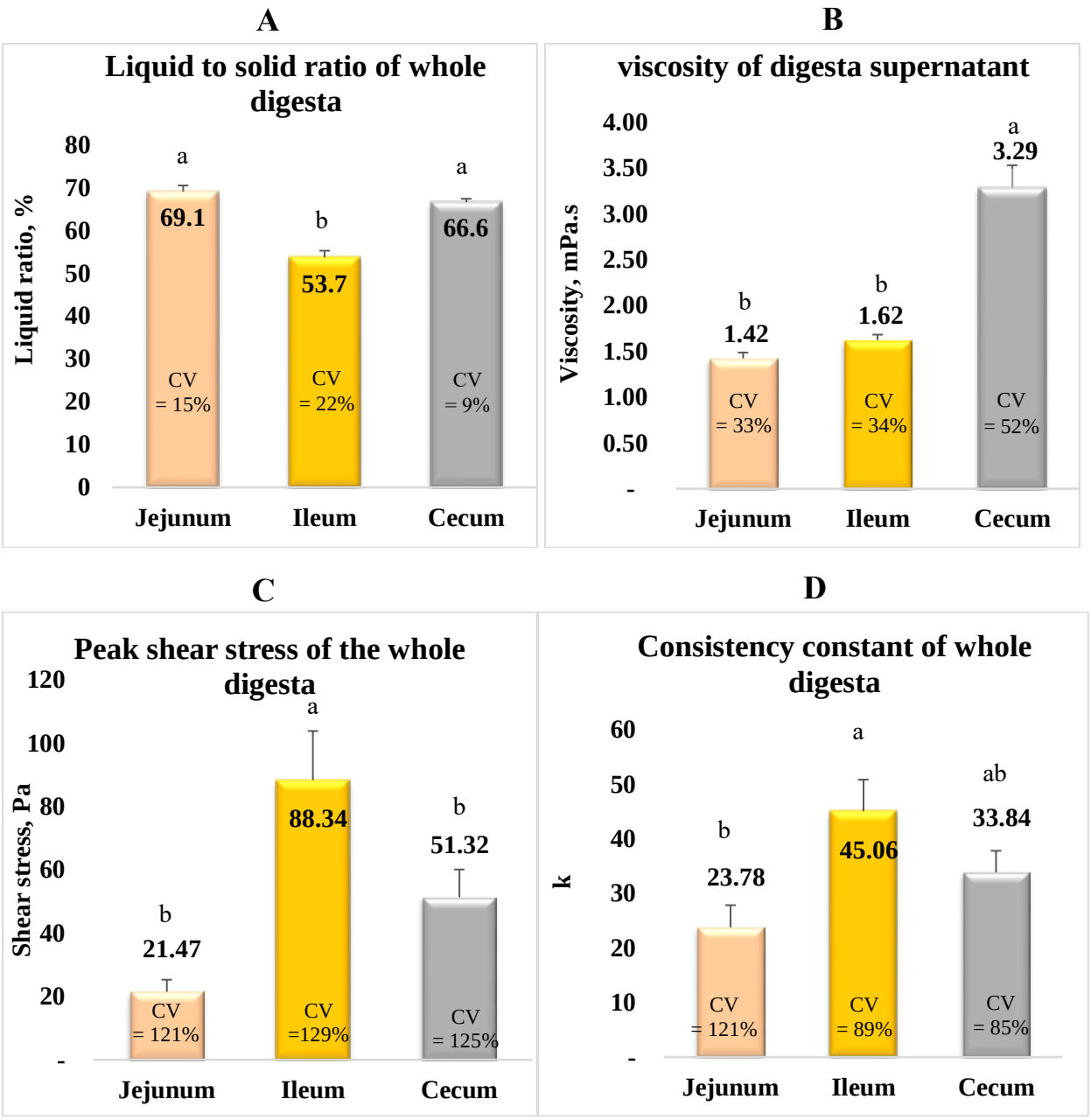

Figure 3. The viscosity characteristics of digesta at different sections of the gastrointestinal tract of growing pigs. (A) liquid phase ratio of whole digesta (centrifuged at 3000 ×g for 10 min); (B) Viscosity of the liquid part of digesta (centrifuged at 3000 ×g for 10 min); (C) The peak shear stress of whole digesta measured at 0.01 s^{-1}shear rate for continuously 2 min; (D) The consistency constant of whole digesta measured at shear rate ranged from 1 to 100 s^{-1}. a,b means within a row without same superscript are different ($P > 0.05$).

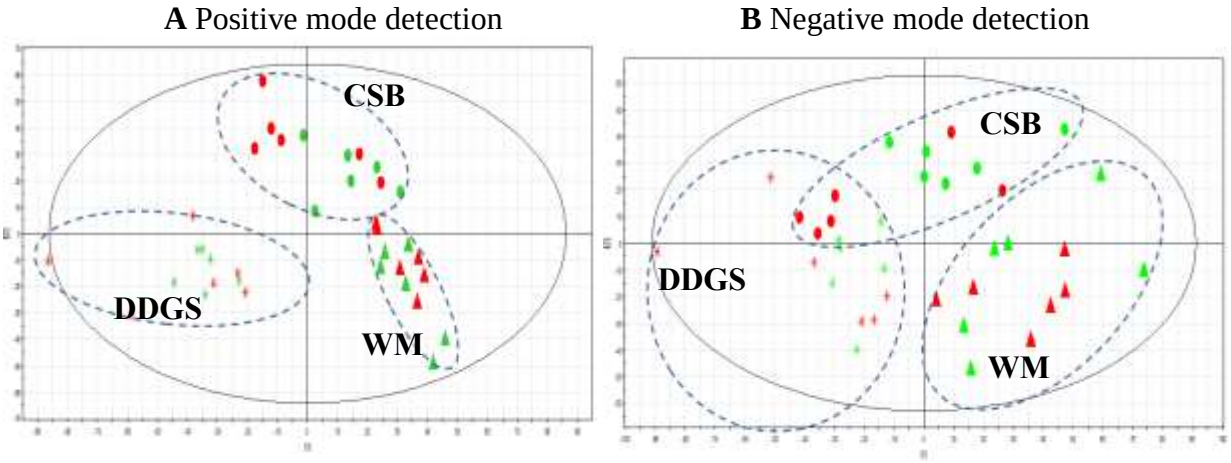

Figure 4. Liquid chromatography mass spectrometry (LC-MS) based metabolomics analysis of cecal digesta from pigs fed a corn and soybean meal diet (CSB), a CSB with 40% corn distillers dried grains with solubles (DDGS), and a CSB diet with 30% wheat middling (WM) with or without carbohydrases. The dots represent CSB diets, stars represent DDGS and triangles represent WM diets. The red figures represent diets with carbohydrases inclusion and green figures represent diets without carbohydrases inclusion.