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Research Article
Impact of Fermented Corn Straw on Growth Performance, Digestibility and Cecal Micro Flora of Grower Pigs

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Abstract

Objective: The aims of the present experiment were to evaluate the impact of fermented corn straws on growth performance, nutrients digestibility and cecal micro flora of grower to finishing pigs. **Methodology:** The total of 72 healthy pigs (Landrace, large white, Duroc breeds) aged 64 days with initial body weight of 30.4±1.53 kg were used for 80 days study period. Pigs were housed in groups of six of the same gender, with each treatment replicated 3 times in a completely randomised block design. Diets containing fermented corn straws were formulated to be isoenergetic and had similar crude protein content, then randomly assigned to: (1) control (no fermented corn straws), (2) 10%, (3) 15% and (4) 20% of fermented corn straws derivatives. Pigs were fed *ad libitum* and had free access to drinking water. Feed intake and body weight were was measured and recorded daily. After slaughtered (on 80th days) the cecal tissues samples were collected for analysis. Nutrients digestibility analysis was performed using the acid insoluble ash as the internal marker, whereby the cecal micro floras were then evaluated using the PCR-DGGE. **Results:** The results demonstrated the significant differences on crude proteins (82.41±1.93, 61.39±2.31), crude fiber (68.03±3.13, 26.49±0.74), apparent detergent fiber (78.20±1.41, 30.19±1.98) and neutral detergent fiber (73.83±2.30, 48.24±2.78) digestibility in pigs fed supplements compared to the control ($p<0.05$). The total of 22747 high-quality sequences were recovered but only average of 21104 OTUs (97%) were determined through high throughput V3 region of 16S rRNA for sequencing ($p>0.05$). **Conclusion:** Although, the fermented corn straws had declined nutrients digestibility in supplemented pigs, there were no differences reported on growth performance, small chain fatty acids and cecal micro flora diversity and this suggest that the use of fermented corn straw in growing to finisher pigs can help reduce feed cost.

Key words: Microbiota, performance, digestibility, fattened pigs, fermented corn straw

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Feed additives including antibiotics as growth promoters have been used in livestock farming as supplements for growth performance improvements and good carcass quality[1,2]. However, with the side effects on bacterial resistance such as *Staphylococcus aureus* and *Escherichia coli* blood stream infections caused by antibiotics on animal products (pork, white and red meat) has caused public outcry due to the health concern and this has resulted in banning the use of antibiotics in many parts of the world such as in Europe and Asian countries such as China, which strictly prohibit the use of antibiotics as additive[3-5]. Conversely, pending to the ever growing human population in the world, there is a prominent need to improve production. Hence, the current banning of antibiotics as supplements to improve performance in pigs is of prominent concern within the research fraternity to find an alternative way to replace the use of antibiotics[6].

In China, most of the pig farmers (particularly the commercial) rely on supplementing diet with fermented corn straws as an alternative to antibiotics growth promoters to improve the growing-finishing pigs[7]. Whereas the most of the smallholder pig farmers uses only the available feeds such as kitchen wastes as feeds for their animals[8]. The Chinese crop farmers produced about 2.7 billion tons of corn stalk with about 33.8% from different types of straws[9,10]. Although most of these farmers experience the challenges on the use of fermented corn straws due to its own characteristics such as higher lignin and lower nutritional content[11].

Study by Van Winsen *et al.*[12] reported fermented corn straws to have positive effects when supplemented in the diet of fattening pigs thereby improving growth and influencing the probiotic bacteria colonization in the gastrointestinal. Although, Chu *et al.*[13] demonstrated that there are few studies reported the use of fermented corn straws as feed additives in animal nutrition with the richness of Lactic Acid Bacteria (LAB) and Volatile Fatty Acids (VFA). The production of lactic acid and hydrogen peroxide is essential in maintaining a healthier micro flora that will prevent the growth of pathogenic bacteria in the gastrointestinal tract[14,15]. The aim of the present study was to evaluate the impact of fermented corn straws on growth performance, digestibility and cecal micro flora of grower to finishing pigs.

MATERIALS AND METHODS

The College of Animal Nutrition and Feed Sciences together with the Committee of Jilin Agricultural University have reviewed and approved the procedure applied in this study.

Study side: The study was conducted at the experimental farm of Jilin Agricultural University, Changchun city in Jilin province of the People's Republic of China. Mean daily temperatures in winter (November-March) ranges between -8.3°C (17°F) to -20.3°C (-4.6°F) and in summer (May-July) between 16°C (61.4°F) and 28°C (81.2°F), respectively. The annual rainfall ranges between 350-1000 mm. Chemical analysis were carried out at the nutrition laboratory of the College of Animal Science, Jilin Agricultural University, Borui Research and Development Laboratory and the Veterinary Hospital Laboratory of the Jilin Agricultural University. The microbiological analysis were performed respectively at Shanghai Rui Yi Biological Company and Yin Yu testing center in Changchun city.

Preparation of the corn stalks: The corn stalks were purchased from the local company in Changchun city of Jilin province, in China. After obtaining the corn stalk, the leaves were removed and then dried at the temperature of 60-70°C until constant weight was achieved. The corn stalks were completely dried and were further cut into smaller pieces (10-30 cm), crushed into fine powder to allow passing through a >3.5 mm mesh mini laboratory sieve to determine the level of dry matter.

Fermentation of the corn stalks: The commercial baker's yeast (Rapid-rise yeast) was purchased from the local company in Changchun city of Jilin province, in China and used as *Saccharomyces cerevisiae* to speed up the fermentation processes. The rapid-rise yeast was then inoculated into the medium having glucose 50 g L^{-1}, peptone 5 g L^{-1}, $MgSO_4$. $7H_2O$ 1 g L^{-1}, K_2HPO_4 and 5 g L^{-1} of yeast. The medium was autoclaved at 121°C (249°F) for 15 min. After autoclaved, the yeast was inoculated on the orbital shaker for a period of 18 h at 30°C, 50 rpm.

Experimental design, animals and housing management

Experiment 1: Growth performance: A total of 72 female growing pigs [(Landrace×Large white)×Duroc] aged 64 days old, with initial live body weight of 30.4±1.53 kg were purchased from Kai De Li animal husbandry Co., Ltd., Jilin province, Changchun city, China. The experimental pigs were randomly assigned to four treatments in a 4×1 (treatment×sex) factorial experiment in a completely randomized design according to their initial body weight. Each experimental unit consisted of 6 pigs per pen and each treatment was replicated three times, making 18 pigs per treatment. The experimental pigs were housed in a traditional

Table 1: Ingredients and chemical compositions of diets fed to pigs

	Treatments			
Ingredients	Control	10%	15%	20%
Corn	70	63	60	56
Soybean meal oil cake	14	11.5	11	11
Wheat bran	13	11.5	11	10
$CaHPO_4$	0.9	0.9	0.9	0.9
Limestone	0.6	0.6	0.6	0.6
NaCl	0.5	0.5	0.5	0.5
Corn stalks	0	10	15	20
Premix	1	1	1	1
Chemical analysis				
DE (MJ kg^{-1})	13.13	12.38	12.02	11.66
Crude protein	13.5	12.42	11.85	11.64
Neutral detergent fiber	27.65	31.99	34.01	37.12
Acid detergent fiber	4.71	8.62	10.52	12.5
Calcium	0.52	0.51	0.51	0.51
Phosphorus	0.45	0.45	0.43	0.43

Premix components: Vitamins A 30 kIU, vitamin D_3 1 kIU, vitamin E ≥24 mg, vitamin K_3 ≥36 mg, vitamin B_2 ≥8.3 mg vitamin B_6 ≥20 mg, pantothenic acid ≥24 mg, Copper: 2.5 mg, Manganese: 22 mg, Iron: 120 mg, Zinc 120 mg, Selenium: 0.12 mg and Iodine: 0.11 mg

small scale type pens (4.5 m length×3.0 m breadth×1.0 m width) whereby the walls and the floors were covered by the solid concrete.

Experiment 2: Nutrients digestibility: Seventy-two crossbred [(Landrace×Large white)×Duroc] female growing pigs from growth performance experiment weighing±65.9 kg live weight were used in this study. The pigs were fed *ad libitum* experimental diets twice a day (at 5 h 30 am and 17 h 30 pm) and had free access to drinking water through drinking nipples. After 80 days of feeding, all pigs were slaughtered to collect cecal tissues to determine the nutrients digestibility and microbial floral analysis. Nutrient digestibility was performed using the acid insoluble ash as the internal marker. The cecal microbioata were evaluated by the PCR-DGGE method.

Experimental diets: The total composition and nutrient level of the feeds and ingredients used in this study are illustrated in Table 1. The pigs were fed on one of four diets which lasted for 80 days. The diets were supplied *ad libitum* and water was made available at all times through drinking nipples. The dietary treatments were: (1) commercial feed (control no FCS), (2) 10%, (3) 15% and (4) 20% FCS. All diets were formulated to be isoenergetic and have similar crude protein content and to meet or exceed the National Research Council[16] recommendations for all nutrients.

Sampling and measurements: During growth performance experiment, the pigs were weighed individually at the start of the trial and continued at weekly intervals until the end of the trial. Daily feed intake was measured by weighing the feed offered and refusals every morning. This data was used to determine the Average Daily Gain (ADG), Average Daily Feed Intake (ADFI) and to calculate the Feed Conversion Ratio (FCR). Mortalities and morbidities were noted and all mortalities were subjected to post-mortem examination. Morbidities were diagnosed and the necessary treatments were done.

In experiment 2, the fecal samples were collected twice a day at 4 h 30 am and 16 h 30 pm, respectively. All feces remaining in the cages were cleared and placed in the plastic bags, stored in a freezer at -200°C temperature for further analysis[17]. On the last day (80th days) fecal samples were thawed and dried in a forced air oven at 63°C PV and 65°C SV for 1 week. Fecal samples were then grinded into powder to pass >3.5 mm mesh mini laboratory sieve for further chemical analysis[18,19].

Genomic DNA extraction: The samples were defrosted at room temperature. Total bacterial DNA was extracted using the DNeasy tissue kit (QIAGEN, Hilden, Germany) following the manufacturer's instructions. The size and quality of the DNA were assessed by the gel electrophoresis on 1% agarose gels which was stained with ethidium bromide.

PCR amplification and high throughput sequencing of the V3 region of 16S rRNA: The V3 region of the 16S rRNA gene was amplified by PCR using the universal bacterial primers GC-338 F (5'-CGC CCG CCG CGC GCG GCG GGC GGG GCG GGG GCA CGG GGG GAC TCC TAC GGG AGG CAG CAG-3') and 518 R (5'-ATT ACC GCG GCT GCT GG-3'). The mixture for PCR system included: 2×Taq master mix 12.5 and 1 µL DNA

Table 2: Effects of fermented corn straw on growth performance in finishing pigs (30-100 kg)

	Treatments (Mean±SD)					
Parameters	Control	10%	15%	20%	SEM	p-value
IW	30.4±1.53	30.4±1.53	30±1.53	30±1.53	0.4±1.53	0.050
FW	95.0±2.91^{a}	90.6±0.30^{b}	91.2±2.90^{b}	92.8.0±1.42^{ab}	92.43±1.06	0.216
ADG (g days^{-1})	824.3±33.0^{a}	756.4±8.84^{a}	756.6±21.3^{a}	787.49±16.1^{a}	37.5676	0.072
ADFI (g day^{-1})	2330.10^{b}	2336.83^{b}	2478.19^{ab}	2640.34^{a}	95.2522	0.072
FCR	2.83±0.035^{c}	3.09±0.010^{b}	3.28±0.078^{a}	3.35±0.023^{a}	0.0773	0.279

IW: Initial weight, FW: Final weight, ADFI: Average daily feed intake, FCR: Data in small letters mean significant difference (p<0.05), the same letter or letter indicates no significant difference (p>0.05)

Table 3: Effects of fermented corn straw on nutrient digestibility in finishing pigs

	Treatments (Mean±SD)					
Parameters	Control	10%	15%	20%	R-MSE	p-value
CP (g kg^{-1})	82.41±1.93^{a}	69.69±2.52^{b}	67.17±0.46^{bc}	61.39±2.31^{c}	3.4347	0.0004
CF (g kg^{-1})	68.03±3.13^{a}	28.50±1.76^{b}	26.49±0.74^{b}	27.20±3.10^{b}	4.2816	0.0001
ADF (g kg^{-1})	78.20±1.41^{a}	38.90±2.71^{b}	33.76±.99^{bc}	30.19±1.98^{c}	3.5451	0.0001
NDF (g kg^{-1})	73.83±2.30^{a}	58.76±2.92^{b}	59.12±1.89^{b}	48.24±2.78^{c}	4.3502	0.0007
GE (MJ kg^{-1})	57.75±2.64^{b}	76.95±1.57^{a}	80.75±0.20^{a}	57.81±4.25^{b}	4.4150	0.0003

Different letters mean significant difference (p<0.05), the same letter indicates no significant difference (p>0.05)

template, forward and reverse primers, each 0.5 µL and ddH_2O was added to make up to 25 µL. The PCR condition were as follows: 95°C for 5 min, 30 cycles of 95°C for 1 min, 60°C for 1 min and 72 for 1 min and 72 for 5 min. The size and quality of the PCR results were assessed by the gel electrophoresis on 1% agarose gels which was stained with ethidium bromide. To assess the composition and diversity of the bacterial abundance in the cecum within the three groups, the PCR products were sent to Sangon Biotech (Shanghai) Co., Ltd. for high throughput sequencing of the gene.

Determination of short chain fatty acids: Volatile fatty acid concentrations in digesta from the cecum, colon and rectum were determined by a gas chromatography method following the procedures of Eggeman and Verser[20]. After digesta samples (1 g) were mixed with 2 mL of distilled water in the screw-capped tube, the suspension liquid was centrifuged (12,000×g) at 4°C for 10 min. Two milliliter of supernatant were mixed with 0.2 mL of metaphosphoric acid and centrifuged for 30 min at 4°C. After centrifugation, 1 mL of supernatant was transferred into an ampoule (1.5 mL) and mixed with 200 µL of 25% metaphosphoric acid (HPO_3) and stored at -20°C until analysis were done. The lower detectable limit for all VFA was 0.1 mM.

Statistical analysis: The data carried out in this study was subjected to General Linear Model (GLM) univariate analysis of variance using the SPSS computer software (SPSS statistics for windows, version 17.0. Chicago: SPSS Inc.) using one-way ANOVA[21]. The results were also presented as Mean±Standard Deviation. Differences between means were assessed by Duncan method test and effects with a probability of p< 0.05.

RESULTS

The effects of feeding different levels of fermented corn straw on the growth performance of grower pigs are portrayed in Table 2. There were no differences (p>0.05) in the initial weight, ADG, ADFI and FCR between treatments. The control had a higher (p<0.05) final weight compared to fermented corn straw diets. However, 20% fermented corn straw diet had higher (p<0.05) final weight compared to 10 and 15% fermented corn straw diets. Also, 20% fermented corn straw diet had higher (p<0.05) ADFI compared to 10 and 15% fermented corn straw diets. However, control had a lower (p<0.05) FCR compared to fermented corn straw diets. Also, 15 and 20% fermented corn straw diets had higher (p<0.05) FCR compared to 10% fermented corn straw diet.

Data on nutrient digestibility in finishing pigs fed different levels of fermented corn straw are presented in Table 3. Control had a higher (p<0.05) CP, CF, ADF and NDF digestibility than fermented corn straw diets. However, 10 and 15% fermented corn straw diet had a higher CP, NDF and ADF digestibility than 20% fermented corn straw diet. There were no difference (p>0.05) between fermented corn straw diets on digestibility of CF. However, 10 and 15% fermented corn straw diets had a higher (p<0.05) GE (MJ) digestibility than control and 20% fermented corn straw diet.

There were no differences (p>0.05) in the pH, acetic acid, methylycwtic, butyric acid and TVFA between treatments in Table 4. However, 10% fermented corn straw diet had a lower (p<0.05) pH than control diet. Also, control and 15% fermented corn straw diets had a higher (p<0.05) butyric acid than 10 and 20% fermented corn straw diets.

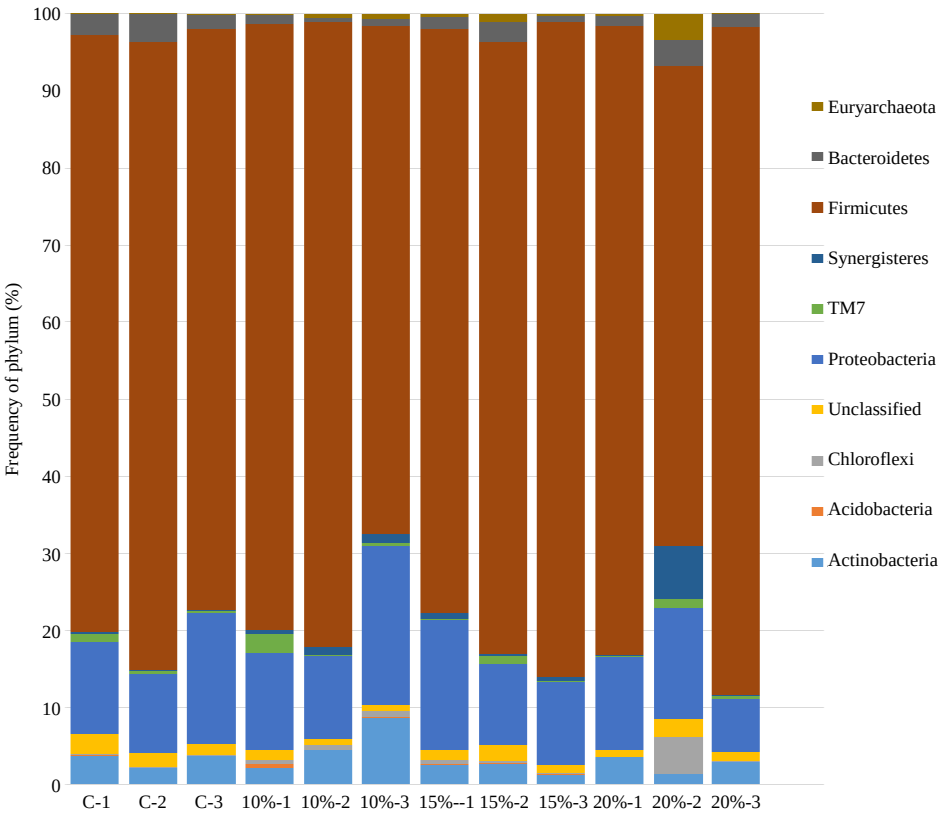

Fig. 1: Taxonomic distribution of bacterial phylum from cecum

Table 4: Effect of fermented corn straw on analysis of cecum pH in finishing pigs

	Treatments (Mean±SD)					
Parameters	Control	10%	15%	20%	R-MSE	p-value
pH	5.92±0.086^{a}	5.86±0.021^{ab}	5.69±0.046^{b}	5.78±0.055^{ab}	0.0992	0.0960
Acetic acid	55.12±5.42	67.76±7.42	67.65±6.41	56.63±2.84	10.0443	0.3131
Methylycetic	25.68±2.81	25.50±0.62	25.68±2.01	24.72±0.31	3.0605	0.9762
Butyric acid	15.02±1.33^{a}	13.57±1.38^{ab}	14.65±1.46^{a}	10.06±0.48^{b}	2.1333	0.0750
TVFA	95.81±6.99	106.83±9.25	107.98±4.68	91.45±3.12	11.1704	0.2637

Data in small letters mean significant difference (p<0.05), the same letter or letter indicates no significant difference (p>0.05)

The results for high throughput sequencing indicating the bacterial abundance in the cecum between the treatments are indicated in Fig. 1 and 2, respectively. Figure 1 indicates the abundance of the phylum in the cecum while, Fig. 2 indicates the abundance within the species. It is of interest that some species (i.e., *Ochrobactrum*) were more abundant in the supplemented group while they were not found in the control group.

DISCUSSION

The fibrous feeds are known to improve the average daily feed intake and the gut tract health by fighting against the enteric pathogens which may cause clinical diseases such as diarrhea and ulcer. These farm diseases are associated with the decline in gastrointestinal pH of fattened pigs[22].

This results showed that the FW, ADG, ADFI and FCR ratio were not affected by inclusion of level of fermented corn straw. However, pigs fed the fermented corn diets diet had higher ADFFI than those fed the control diet. Results from other researchers showed that the effects of fermented grains feed on growth performance are variable[23]. Canibe *et al.*[24] reported that growth performance was not improved by feeding pig's fermented wheat. However, Scholten *et al.*[25] reported that the G/F was improved when pigs were fed liquid feed containing 45% fermented wheat compared with those

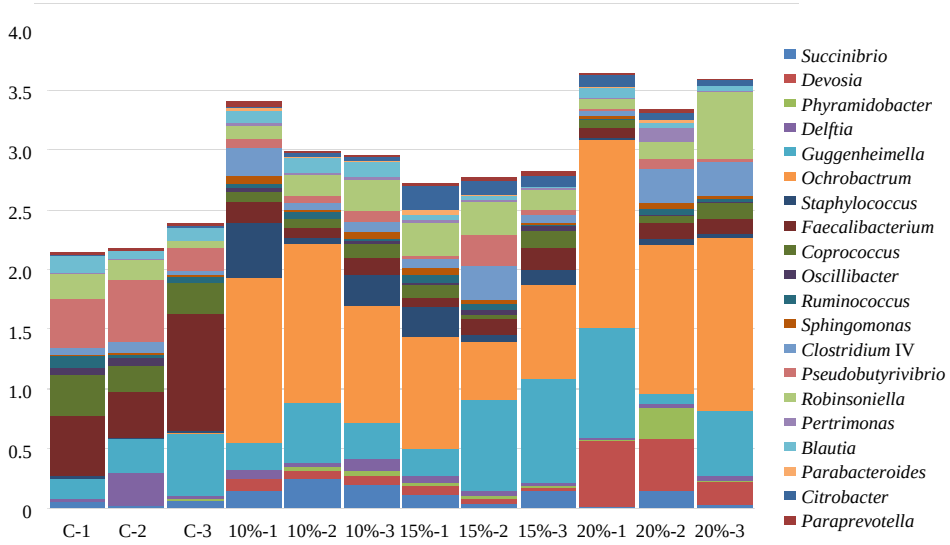

Fig. 2: Taxonomic distribution of bacterial species from cecum

fed non fermented liquid feed. Pedersen[26] also reported that piglets fed diets containing 66% liquid fermented grain of total grain could improve the G/F.

In this study, ADF and NDF digestibility was improved in pigs fed 10% fermented corn straw diet and GE digestibility was improved in pigs fed 10 and 15% fermented corn straw diets, which may be the reason for an improved ADFI compared with pigs fed the control diet. Brooks *et al.*[27] reported that fermented cereal grains could decrease the pH value and improve the palatability of fermented liquid feed. Boesen *et al.*[22] also reported the impacts of fermented liquid diets on decreased gastric pH and on increased gastric lactic acid concentration in pigs.

The relative abundance of gut microbiota results observed in Fig. 1 and 2 are in line with the previous studies by Scholten *et al.*[25]. Thus such abundance, critically suggest to have maintained the balance and prevention of the pathogenic bacterial growth in the cecum[28]. The intestinal micro ecological balance in monogastric animals begin as early as from birth until adults to slaughter. However, little has been reported on the benefits of the gut microbiota such as *Lactobacillus* genus[29], *Bacteroides*, *Fusobacterium* (*Clostridium*) in the large intestine[30]. Under normal circumstances the significance of intestinal micro flora should at least contain the bacteria genera (*Prevotella*), *Streptococcus*, *Lactobacillus* with at least a lower level of bacteria genera (*Mitsoukella*) and giant *Aureus* (*Megasphaera*).

The significant roles of cecal *Fusobacterium* (*Fusobacteria*) and the *Eubacteria* in maintaining a good gut health were observed in the present study and it is in agreement with study by Takahashi *et al.*[31], Krawielitzki *et al.*[32] and previously Kornegay and Risley[33] demostrated that the fibrous feeds act as probiotics and they play a very apex function on microbiota growth and stability repairs in pigs. As such, the hyphothesis on the use of probiotics in pigs was that they could eliminate the environmental pollution impacts from the manure through improving the feed efficiency and nutrient retentions during digestion[34]. This results are also similar to those reported by Giang *et al.*[35].

CONCLUSION

Although, the decline on nutrients digestibility were reported to have a significant difference in pigs fed FCS as compared to the control pigs, there were no negative impacts observed on the growth performance. These observations were similar to the SCFA and cecal micro floras. These suggest that the use of fermented corn straw in growing to finisher pigs can help reduce feed cost. Thus, study that will evaluate the effects of similar dietary inclusion levels of FCS on breeding pigs and on reproductive performance may be warranted. This is because the age of the pigs plays a major role when feeding FCS diets.

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