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Effects of different dietary inclusions of whole-plant corn silage on growth performance, nutrient availability and jejunal development in growing-finishing pigs

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Abstract: This study aimed to evaluate the effects of whole-plant corn silage (WCS) on growth performance, nutrient availability and intestinal development in growing-finishing pigs. A total of 32 barrows (33.1 ± 3.49 kg) were randomly allocated to four treatments. Control was the basal diet, and the low, medium, and high treatments were basal diets substituted with WCS (air-dry basis) at 5.0%, 7.5%, and 10.0% from day 1 to 42, and 10.0%, 12.5%, and 15.0% from day 43 to 98, respectively. The results showed that the average daily feed intake (ADFI), feed/gain (F/G), and crypt depth (CD) increased linearly ($P < 0.05$) with the increasing of WCS. However, the final body weight, average daily gain (ADG), biological value (BV), net protein utilization (NPU), dry matter, crude protein (CP), ether extract, crude fibre (CF), and gross energy decreased linearly ($P < 0.05$). High treatment significantly deteriorated BW, ADG, NPU, and BV compared to control, low, and medium treatments ($P < 0.05$). The low and medium treatments showed significantly greater ADFI, apparent digestibility (CP and CF), villus height (VH), VH/CD, relative mRNA and protein expression of occludin, claudin-1 and sodium-glucose cotransporter (SGLT1) than the high treatment ($P < 0.05$). The highest F/G, CD, relative mRNA and protein expression of cationic amino acid transporter (CAT1) were observed in high treatment ($P < 0.05$). Our results suggested that WCS can replace 7.5% and 12.5% of diet during growing and finishing periods of pigs, respectively. This is of great significance for reducing feeding costs and alleviating food security crises.

Keywords: swine production; dietary fiber; silage feedstuff; intestinal health; nutrient absorption

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The utilization of forages in growing pigs could have a positive impact in reducing gastric mucosal damage as already demonstrated (Mason et al. 2013). Meanwhile, rapid expansion of animal production requires the establishment of an efficient feed industry. Compared with other feed sources, corn silage is considered an efficient and cost-effective feed (Armstrong et al. 2008). However, corn silage is mainly used as feed for ruminants, but it is scarcely used in monogastric animals. The rise in corn grain prices and grain drying cost has stimulated interest in corn silage products in pig feeding (Capraro et al. 2017).

Whole-plant corn silage (WCS) is a valuable source of dietary fibre (DF), organic acids, and abundant beneficial bacteria, which play crucial roles in promoting the intestinal development and maintaining normal functions of the gastrointestinal tract (Liu et al. 2022). Previous studies pointed out that a high-fibre diet would affect nutrient utilization and growth performance of pigs (Leek et al. 2007). Chen et al. (2015) found that in pigs receiving a wheat bran diet containing 5% cellulose, xylan or β-glucan short-chain fatty acid (SCFA) concentrations and beneficial bacteria abundance in caecum could be increased. Consistently, Wu et al. (2018) also found that diets containing 5% xylan or β-glucan significantly increased the mRNA expressions of genes related to intestinal barrier (*ZO-1* and *claudin-1*) and nutrient transporters (*SGLT1* and *GLUT2*). Besides, gastric infusion of SCFAs containing acetic, propionic, and butyric acid improved the jejunal and intestinal barrier function in weaned piglets by increasing the mRNA expressions of tight junction proteins (*occludin* and *claudin-1*) and intestinal development-related genes (*GLP-2R*, *IGF-1*, and *IGF-1R*) (Diao et al. 2019). However, scarce scientific literature reported the appropriate supplementation proportion of WCS in growing-finishing pigs.

Therefore, this study was conducted to evaluate the effects of supplementing different proportions of WCS on growth performance, nutrient availability, and intestinal development in growing-finishing pigs so that we could provide a valuable insight into the applications of WCS in the pig industry.

MATERIAL AND METHODS

The present study was conducted at the Animal Nutrition Research Institute of Shandong Agricultural University (Tai'an, China). All experimental procedures used in this study were approved by the Shandong Agricultural University Animal Care and Use Committee (Approval Number: SDAUA-2019-019).

Preparation of whole-plant corn silage

The fresh corn plants were harvested 30 cm in height above the ground during the milky stage, and they were cut into 5 to 10 mm segments using a straw chopper (1 000–500, Zhengzhou Yiyi Machinery Equipment Co., Ltd, Zhengzhou, Henan, China), followed by being mixed with 0.8 g/kg fermentation strains using a feed mixer (HS-SLC-2, Qufu Hongsheng Machinery Co., Ltd, Qufu, Shandong, China). The strains for silage fermentation were a combination of *Lactobacillus casei* (5.0×10^9 CFU/g), *Saccharomyces cerevisiae* (4.6×10^9 CFU/g), and *Bacillus subtilis* (1.9×10^{10} CFU/g) (KeWeiBo Biotechnology Co., Beijing, China). Then the mixture was compacted and sealed layer by layer into the plastic-lined nylon bag for silage via stretch-film-wrapped silage technology (1 000 ± 5.00 kg/bale). From the 18th day, the pH value of WCS was measured with pH meter (PHS-3C PH, Shanghai, China) every other day. The WCS could be used when the pH value was stable at 3.8–4.0. Samples of WCS were collected for chemical analysis, including moisture, gross energy, crude protein, crude fibre, calcium, phosphorus, and amino acid profile according to AOAC (2012), respectively. Table 1 contains gross energy and chemical composition of WCS.

Table 1. The main ingredient content of whole-plant corn silage (air-dry basis) in %

Items (%)	Laboratory analysis
Gross energy (MJ/kg)	15.3
Crude protein	7.27
Crude fiber	15.3
Calcium	0.02
Phosphorus	0.09
Lysine	0.05
Methionine	0.03
Threonine	0.05
Tryptophan	0.02

https://doi.org/10.17221/153/2023-CJAS

Animals and treatments

A total of 32 Duroc × Landrace × Yorkshire barrows (DLY, 33.1 ± 3.49 kg) were randomly allocated to four treatment groups with eight replicates per group. Control barrows received the basal diet. The low, medium, and high treatments were the test diet in which the basal diet was supplemented with WCS (air-dry basis) at 5.0%, 7.5%, and 10.0% from day 1 to 42, and at 10.0%, 12.5%, and 15.0% from day 43 to 98, respectively. The basal diet (Table 2) was formulated according to the National Research Council (NRC 2012). Barrows were housed individually in the metabolic cages which were placed in a temperature- and humidity-controlled room (26–28 °C, 55–60% RH). Pigs were fed three times a day at 8:00, 14:00 and 18:00. During the period of the experiment, feed and water were offered *ad libitum*. Daily feed intake per barrow was recorded, and barrows were weighed individually on day 42 and day 98 before breakfast.

Table 2. Composition and nutrient levels of basal diet of growing pigs in %

Ingredients	30–60 kg	60–100 kg
Corn	70.1	70.4
Soybean meal, 44.1% CP	17.5	10.5
Wheat bran	5.50	13.0
Soybean oil	3.00	2.50
L-Lysine, 76.8%	0.31	0.24
DL-Methionine, 98.5%	0.05	0.03
L-Threonine, 98.0%	0.10	0.02
Calcium hydrophosphate	1.00	1.00
Limestone, pulverized	1.00	0.90
Sodium chloride	0.30	0.30
Choline	0.10	0.10
Premix[1]	1.00	1.00
Total	100	100
Nutrient levels[2]		
Digestible energy (MJ/kg)	14.6	14.1
Crude fiber	3.00	3.50
Crude protein	14.2	12.4
Ether extract	2.61	2.09
Calcium	0.66	0.61
STTD phosphorus	0.30	0.28
Lysine	0.86	0.75
Methionine	0.26	0.23
Threonine	0.58	0.49

[1]The premix provided the following per kg DM of diets: vitamin A 1 700 IU, vitamin D_3 150 IU, vitamin E 11 IU, vitamin K_3 0.55 mg, vitamin B_1 1.00 mg, vitamin B_2 2.50 mg, vitamin B_6 1.25 mg, vitamin B_{12} 0.01 mg, pantothenic acid 7.00 mg, niacin 22.5 mg, biotin 0.05 mg, folic acid 0.30 mg, Mn ($MnSO_4 \cdot H_2O$) 5.00 mg, Fe ($FeSO_4 \cdot H_2O$) 70 mg, Zn ($ZnSO_4 \cdot H_2O$) 70 mg, Cu ($CuSO_4 \cdot 5H_2O$) 5.00 mg, I (KIO_3) 0.21 mg, and Se (Na_2SeO_3) 0.30 mg

[2]Digestible energy, crude protein, crude fibre, calcium and standardized total tract digestibility (STTD) phosphorus were analysed values, while the other nutrient levels were calculated values

Nutrient availability

From day 70 to 76 of the experiment, faeces and urine excreted by each barrow were collected daily to determine nutrient digestibility and metabolic rate. The daily collected faeces and urine samples of each barrow were preserved by addition of a few drops of 10% sulphuric acid to avoid evaporation of nitrogen in the form of ammonia after being measured and separately homogenized, and then stored at –20 °C and 4 °C, respectively. After 7-day collection, the weekly representative feed and faeces samples of each barrow were pooled for chemical analyses, including dry matter (DM), ether extract (EE), and crude fibre (CF), while the urine samples were pooled for urinary energy (UE) analysis, according to AOAC (2012), respectively. Metabolic energy was determined as the difference between gross energy (GE) and the sum of faecal energy (FE) and urinary energy (UE). Besides, net protein utilization (NPU) and protein biological value (BV) were calculated as indicated below:

$$NPU = [(NI - FN - UN)/NI] \times 100 \quad (1)$$

$$BV = [(NI - FN - UN)/(NI - FN)] \times 100 \quad (2)$$

where:
NPU – net protein utilization;
NI – nitrogen intake;
FN – faecal nitrogen;
UN – urinary nitrogen;
BV – protein biological value.

Sample collection and analysis

In the morning of day 98, all pigs were euthanized by electric shock to the neck after being fasted for 12 h. After then, two segments of jeju-

https://doi.org/10.17221/153/2023-CJAS

nal samples (about 2 cm) were immediately removed under sterile conditions. One segment was stored at −80 °C for subsequent mRNA and protein expression analysis, and the other segment was promptly fixed in Bouin's fluid for morphological examination.

Histological analysis

For morphometric analysis, eight consecutive 5-μm-thick sections of the jejunal segments were fixed, embedded in paraffin, and subjected to haematoxylin-eosin staining. Ten intact, well-oriented crypt-villus units were randomly selected from each section, and both villus height (VH) and crypt depth (CD) were visualized and measured at 40× magnification using an Olympus BX51 microscope equipped with a DP70 digital camera (Olympus, Tokyo, Japan).

qRT-PCR analysis

Total RNA of jejunal samples was extracted using RNAiso Plus (D9108B; Takara, Dalian, China) according to the manufacturer's instructions. Then, the 260/280 nm absorbance ratio of RNA was measured using Eppendorf Biophotometer (RS323C; Eppendorf, Hamburg, Germany) to determine the purity and concentrations. The reverse transcription was reacted as described in the instructions of the Prime Script@ RT Master Mix Perfect Real Time Kit (DDR036A, TaKaRa, Dalian, China). The volume of reagents was added according to PCR kit instructions, and the total reaction system was 20 μl. The qRT-PCR reactions were performed at 95 °C for 30 s, followed by 43 cycles at 95 °C for 5 s, 60 °C for 34 s, 95 °C for 15 s, and 60 °C for 60 s, with a final extension step at 95 °C for 15 s (AB7500; Applied Biosystems, Waltham, MA, USA). The primer sequences and amplicon lengths are shown in Table 3. In this study, *GAPDH* was considered as the internal control, and the relative mRNA expression was calculated by the $2^{-\triangle\triangle CT}$ method (Livak and Schmittgen 2001).

Western blot analysis

The protein samples were extracted from jejunal samples using lysis buffer (Beyotime, Shanghai, China). Then, the concentrations of samples were detected using a BCA protein assay kit (Beyotime, Shanghai, China). Equivalent proteins (50 μg) were separated by electrophoresis on polyacrylamide gels and then transferred to nitrocellulose membranes (Solarbio, Beijing, China). Then, the membranes were incubated in 5% skimmed milk powder for 2 h, washed three times using TBST Tris-buffered saline plus Tween (TBST, pH 7.6), and then incubated with the primary antibodies: rabbit monoclonal anti-actin (1 : 1 500; Beyotime, Shanghai, China), rabbit polyclonal occludin (1 : 500, ab216327; Abcam, Shanghai, China), rabbit polyclonal claudin-1 (1 : 5 000, ab180158; Abcam, Shanghai, China), rabbit polyclonal SGLT1 (1 : 1 000, ab14686; Abcam, Shanghai, China), and rabbit polyclonal CAT1 (1 : 1 000, ab37588; Abcam, Shanghai, China) in an antibody dilution buffer (Beyotime, Shanghai, China) at 4 °C overnight. After washing three times using TBST, the membranes were incubated in di-

Table 3. Primer sequences used for quantitative real-time PCR

Genes	Accession No.	Primer sequences (5'–3')	Production length (bp)
GAPDH	NM_001206359.1	F: ATGGTGAAGGTCGGAGTGAA R: CGTGGGTGGAATCATACTGG	149
Occludin	NM_001163647.1	F: CTCCTCCCCTTTCGGACTAT R: GCCGCTTCTCGTTCACTTT	81
Claudin-1	NM_001161635.1	F: ACCCCAGTCAATGCCAGATA R: GGCGAAGGTTTTGGATAGG	155
SGLT1	M34044.1	F: TCATCATCGTCCTGGTCGTCTC R: CTTCTGGGGCTTCTTGAATGTC	144
CAT1	NM 001012613	F: CATCAAAAACTGGCAGCTCA R: TGGTAGCGATGCAGTCAAAG	185

CAT1 = cationic amino acid transporter; *SGLT1* = sodium-glucose cotransporter

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luted anti-rabbit IgG antibody (1 : 5 000, Beyotime, Shanghai, China) or anti-mouse IgG (1 : 5 000, Beyotime, Shanghai, China) at 37 °C for 2.5 hours. After washing again with TBST, the blots were detected using the ECL chemiluminescence method (BeyoECL plus; Beyotime, Shanghai, China) and analysed using Ipp v6.0 (Image Pro-Plus v6.0; Media Cybernetics, Silver Spring, MD, USA).

Statistical analysis

Individual barrow was considered as an experimental unit. The data were analyzed using the generalized linear model procedure of SAS v9.2 (SAS Institute Inc., Cary, NC, USA) following the normality of the data assessment with the Shapiro-Wilk statistic ($W > 0.05$). Variations among the four treatments were compared with each other by Duncan's multiple range test. Besides, in order to evaluate the overall effect of the treatments, orthogonal polynomial contrasts were used to determine linear responses to the WCS level. The data present as mean ± standard error of the means (SEM), and the differences between treatments were considered significant when $P < 0.05$.

RESULTS

Growth performance

As shown in Figure 1, substitution of WCS at increasing levels had a linear effect ($P < 0.05$) on decreased BWs (day 42 and 98) and ADG (day 1–42 and day 43–98), and increased ADFI and F/G both at day 1–42 and day 43–98. The BWs (day 42 and 98) and ADG (day 1–4 and day 43–98) in control, low and medium treatments were significantly higher than those in high treatment ($P < 0.05$), however, the F/G was exactly the opposite. The ADFI (day 1–42 and day 43–98) in low, medium and high treatments was significantly higher than that in the control ($P < 0.05$), especially, from day 43 to 98, ADFI in medium treatment was the highest, which was significantly higher than that in low and high

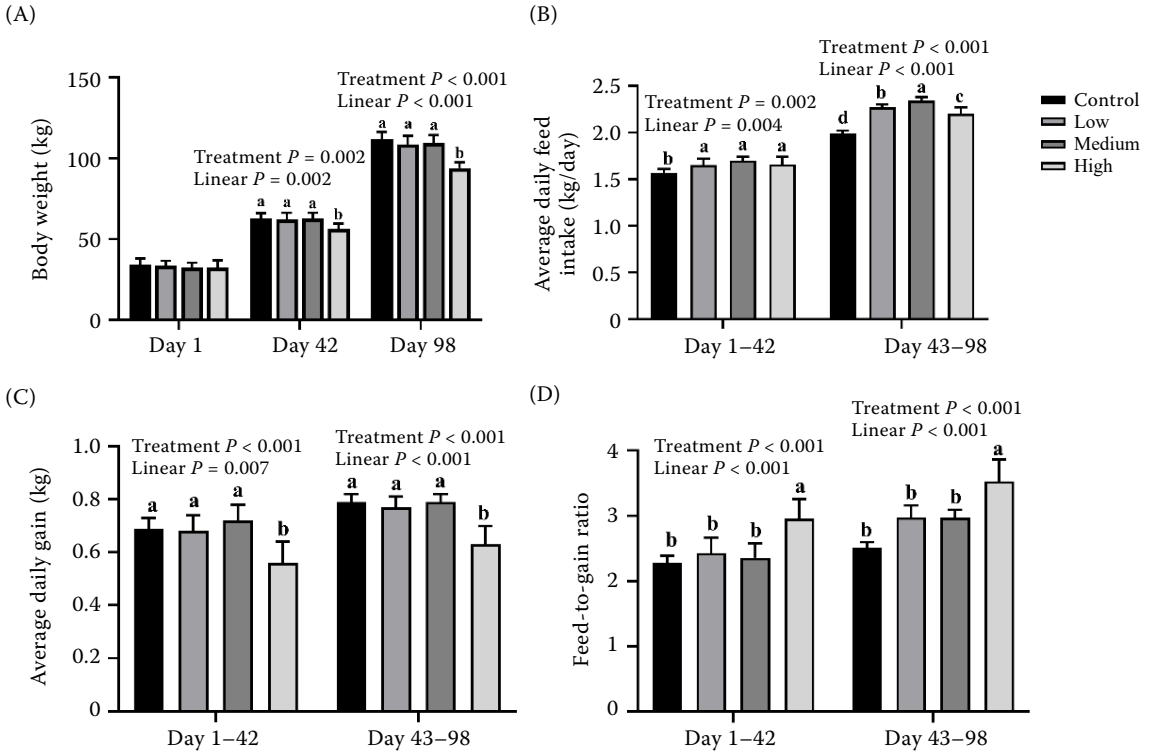

Figure 1. Effect of whole-plant corn silage (WCS) on the growth performance of growing-finishing pigs

Control was basal diet, and low, medium, and high treatments were test diets in which basal diet was substituted with WCS (air-dry basis) at 5.0%, 7.5%, and 10.0% from day 1 to 42, and 10.0%, 12.5%, and 15.0% from day 43 to 98 of the experiment, respectively

[a–d]Different letters on bars differ significantly ($P < 0.05$); values are mean ± standard deviation

https://doi.org/10.17221/153/2023-CJAS

treatments ($P < 0.05$), and pigs in low treatment had significantly higher ADFI than pigs in high treatment ($P < 0.05$).

Apparent nutrient availability

The BV, NPU, and apparent digestibility of DM, CP, EE, CF, and GE in growing-finishing pigs decreased linearly with the increasing WCS substitution ($P < 0.05$; Table 4). Pigs fed WCS had significantly lower apparent digestibility of DM, OM, CP, EE and GE, as well as ME/GE, compared with pigs fed the basal diet ($P < 0.05$). Medium and high level of WCS caused a significant decrease in apparent CF digestibility and BV ($P < 0.05$), and pigs in high treatment had the lowest apparent of digestibility of CP and CF, NPU and BV which were significantly lower than those in the other treatments ($P < 0.05$).

Jejunal morphology

No macroscopic pathological changes were observed in the paraffin-embedded jejunal sections of growing-finishing pigs in the four treatments (Figure 2). However, significant differences caused by the WCS replacement were observed in jejunal development of pigs (Table 5). The VH and VH/CD ratio of the low and medium treatments were significantly higher than those of the control and high treatments ($P < 0.05$), and the VH/CD ratio of the high treatment was significantly lower than those of the other three treatments ($P < 0.05$). Besides, the jejunal CD showed a linear increase ($P < 0.05$) with the increasing WCS replacement.

Relative mRNA expression

As shown in Figure 3, the relative mRNA expression of *occludin* and *claudin-1* in the jejunum of pigs was significantly higher in the low and medium treatments than that in the control ($P < 0.05$), while the control was higher than the high treatment ($P < 0.05$). The relative mRNA expression of *SGLT1* in the jejunum of pigs was significantly higher in control, low and medium treatments than in the high treatment ($P < 0.05$), while the opposite was true for *CAT1*.

Relative protein expression

As shown in Figure 4, the relative protein expressions of occludin, claudin-1, SGLT1, and CAT1 were generally consistent with the observed pat-

Table 4. Effect of whole-plant corn silage (WCS) on nutrient apparent availability of growing-fattening pigs from day 70 to 76 of the experiment

Items	Control	Low	Medium	High	*P*-values	
					treatment	linear
DM	81.7 ± 1.16[a]	77.3 ± 3.04[b]	77.4 ± 2.37[b]	77.0 ± 1.80[b]	0.007	0.025
OM	84.1 ± 1.35[a]	80.5 ± 2.67[b]	80.2 ± 2.00[b]	80.2 ± 2.52[b]	0.022	0.054
CP	74.7 ± 1.90[a]	65.7 ± 4.63[b]	65.4 ± 3.27[b]	57.2 ± 5.86[c]	< 0.001	< 0.001
EE	91.8 ± 1.33[a]	87.6 ± 1.05[b]	87.4 ± 1.32[b]	86.5 ± 1.79[b]	< 0.001	< 0.001
CF	17.0 ± 0.53[a]	17.6 ± 0.85[a]	16.3 ± 0.42[b]	14.6 ± 0. 97[c]	0.039	< 0.001
GE	83.7 ± 1.39[a]	80.4 ± 2.76[b]	80.5 ± 1.96[b]	79.4 ± 0.87[b]	0.014	0.014
NPU (%)	52.1 ± 3.58[a]	51.6 ± 1.73[a]	50.0 ± 3.15[a]	45.1 ± 1.89[b]	< 0.001	0.008
BV (%)	78.8 ± 6.83[a]	79.6 ± 4.50[a]	76.6 ± 2.29[a]	69.1 ± 8.11[b]	0.023	< 0.001
ME/GE	81.7 ± 1.42[a]	78.5 ± 2.34[b]	78.1 ± 2.91[b]	78.0 ± 2.72[b]	0.048	0.056

BV = biological value; CP = crude protein; DM = dry matter; EE = ether extract; GE = gross energy; ME/GE = metabolizable energy/gross energy; NPU = net protein utilization; OM = organic matter

Control was basal diet, and low, medium, and high treatments were test diets in which basal diet was substituted with WCS (air-dry basis) at 5.0%, 7.5%, and 10.0% from day 1 to 42, and 10.0%, 12.5%, and 15.0% from day 43 to 98 of the experiment, respectively

[a–c]Values are mean ± standard deviation. Different letters within a row differ significantly ($P < 0.05$)

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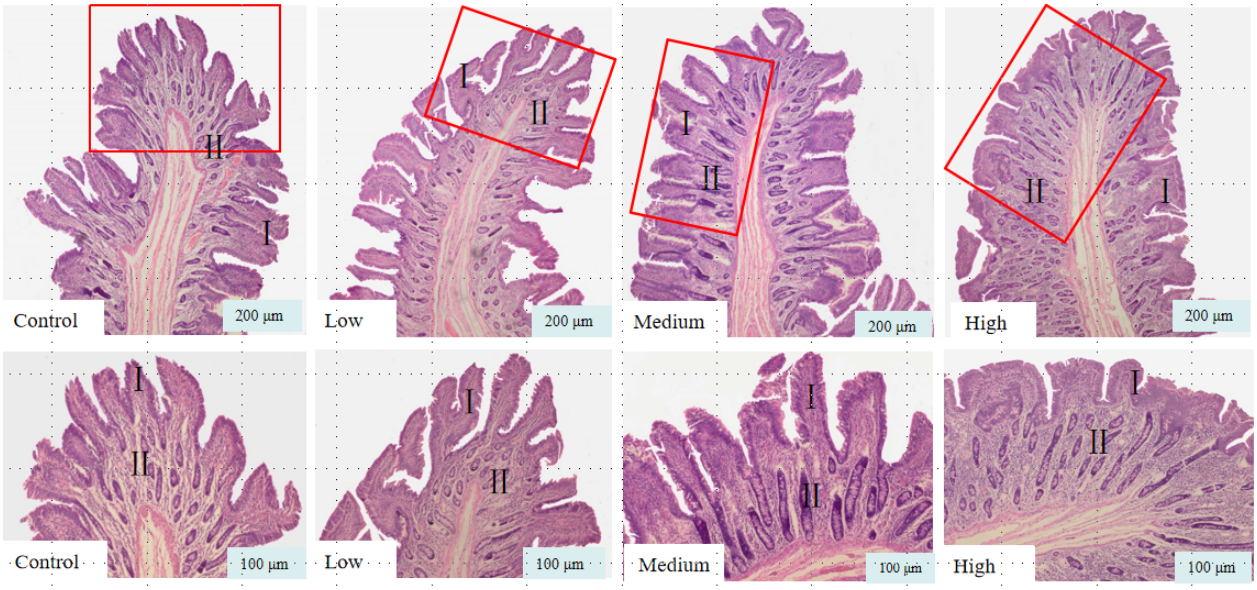

Figure 2. Effect of whole-plant corn silage (WCS) on the jejunal morphology of growing-finishing pigs
(I) Small intestinal villi; (II) small intestinal glands. The red frames in the figure are respectively enlarged and displayed below. Control was basal diet, and low, medium, and high treatments were test diets in which basal diet was substituted with WCS (air-dry basis) at 5.0%, 7.5%, and 10.0% from day 1 to 42, and 10.0%, 12.5%, and 15.0% from day 43 to 98 of the experiment, respectively

Table 5. Effect of whole-plant corn silage (WCS) on the jejunal morphology of growing-fattening pigs

Items	Control	Low	Medium	High	*P*-values	
					treatment	linear
Villus height (μm)	336 ± 23.7^{b}	553 ± 27.8^{a}	511 ± 44.2^{a}	305 ± 25.2^{b}	< 0.001	0.216
Crypt depth (μm)	248 ± 25.2^{c}	295 ± 27.2^{b}	294 ± 28.2^{b}	354 ± 21.5^{a}	< 0.001	0.032
Villus height/crypt depth	1.35 ± 0.10^{b}	1.88 ± 0.07^{a}	1.74 ± 0.13^{a}	0.86 ± 0.06^{c}	< 0.001	0.267

Control was basal diet, and low, medium, and high treatments were test diets in which basal diet was substituted with WCS (air-dry basis) at 5.0%, 7.5%, and 10.0% from day 1 to 42, and 10.0%, 12.5%, and 15.0% from d 43 to 98 of the experiment, respectively

$^{a–c}$Values within a row with different letters mean significantly different ($P < 0.05$)

terns in mRNA expression. The relative protein expressions of jejunal occludin and claudin-1 in pigs were significantly higher in control, low and medium treatments than in high treatment ($P < 0.05$), and in the control they were higher than in high treatment ($P < 0.05$). Pigs in control, low and medium treatments had significantly higher relative protein expression of SGLT1 ($P < 0.05$) and lower relative protein expression of CAT1 ($P < 0.05$) in the jejunum than pigs in high treatment. Besides, there were no significant differences in the protein expressions of SGLT1 and CAT1 between control, low, and medium treatments ($P > 0.05$).

DISCUSSION

Under the action of microorganisms, the fibre in feed is degraded into small molecular substances which are beneficial for absorption and utilization of nutrients (Liu et al. 2023). Data from our study showed that partial replacement (except high treatment) of basal diet with WCS had no negative effects on the growth performance of growing-finishing pigs, indicating the potential values to use WCS diets in pig production. Previous study found that the average daily feed intake on air-dry basis of low, medium, and high treatments was 105%, 108%, and 105% of the control from day 1 to 42, and 118%,

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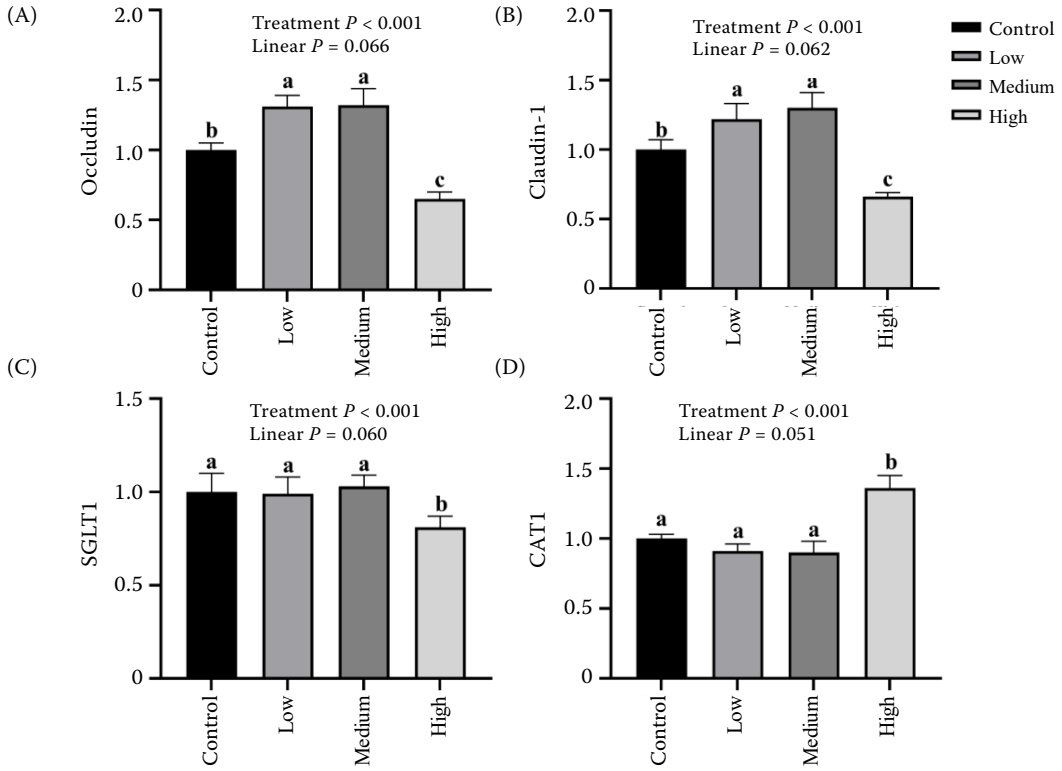

Figure 3. Effect of whole-plant corn silage (WCS) on jejunal gene expressions of growing-finishing pigs

Control was basal diet, and low, medium, and high treatments were test diets in which basal diet was substituted with WCS (air-dry basis) at 5.0%, 7.5%, and 10.0% from day 1 to 42, and 10.0%, 12.5%, and 15.0% from day 43 to 98 of the experiment, respectively

[a–c]Different letters on bars differ significantly ($P < 0.05$). Values are mean ± standard deviation

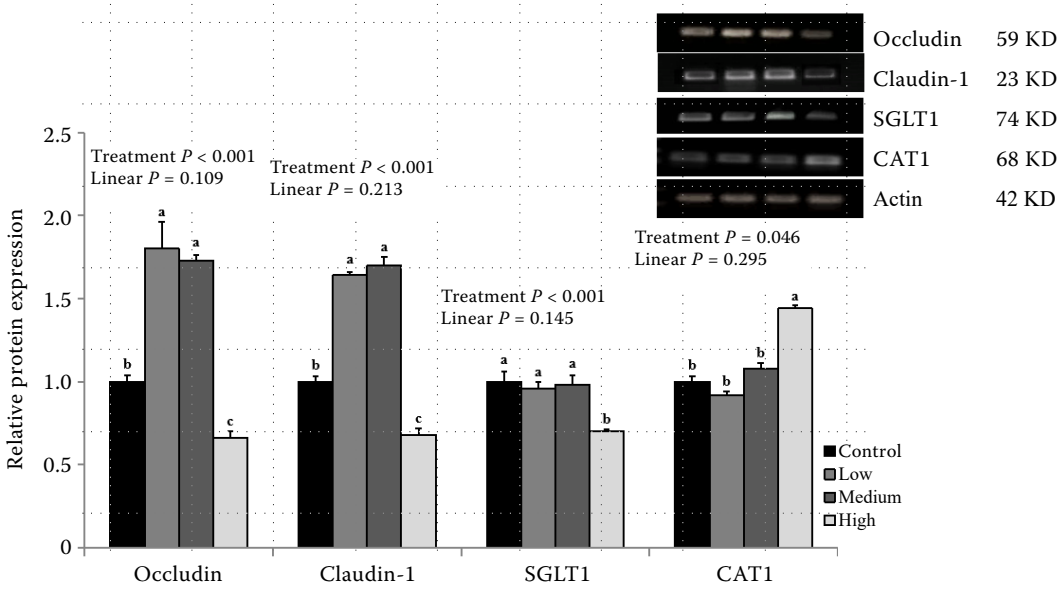

Figure 4. Effect of whole-plant corn silage (WCS) as a fibre source on the relative protein expression of occludin, claudin-1, sodium-glucose cotransporter 1 (SGLT1) and cationic amino acid transporter 1 (CAT1) in the jejunum of growing-finishing pigs

Control was basal diet, and low, medium, and high treatments were test diets in which basal diet was substituted with WCS (air-dry basis) at 5.0%, 7.5%, and 10.0% from day 1 to 42, and 10.0%, 12.5%, and 15.0% from day 43 to 98 of the experiment, respectively

[a–c]Values are mean ± standard deviation. Different letters on bars differ significantly ($P < 0.05$)

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121%, and 112% from day 43 to 98, respectively (Sun et al. 2018). Therefore, the reduction in energy, protein, vitamins, and other nutrients brought about by WCS replacing different levels of basic diet was an effective compensation by increasing feed intake (except high treatment). Similarly, Zanfi et al. (2014) showed that diets containing 15% and 30% WCS (DM basis) did not decrease ADFI and ADG of fattening pigs with an average body weight of 118 kg. However, Galassi et al. (2016a) indicated that dietary supplementation of WCS at 200 g/kg DM significantly reduced the final body weight and ADG, and increased F/G in finishing pigs with the initial BW of 89.5 kg. The reason for differences might be that finishing pigs had the well-developed gut and could digest DF more efficiently compared to growing pigs (Galassi et al. 2016b). The significantly decreased ADG, ADFI, and increased F/G of pigs in high treatment may be related to the insufficient intake of nutrients including energy, protein, and vitamins. Additionally, poor nutrient digestibility and intestinal development may be another mechanism.

In the present study, we found that nutrient (DM, CP, EE, CF, and GE) apparent digestibility, NPU, and BV were all decreased with the increasing WCS replacement. Consistently, Zanfi et al. (2014) reported that pigs fed the diet containing 30% of WCS showed lower CP digestibility compared with pigs fed the control diet and diet containing 15% of WCS. The CF content of WCS used in our study was 15.3% on air-dry basis. Due to the fibre source, type, and inclusion proportion, high fibre diets could impact adversely the nutrient digestibility (Wu et al. 2018). Insoluble fibre could reduce nutrient digestibility through accelerating the evacuation of digesta in the gastrointestinal tract (Choct et al. 2010). While the soluble fibre, such as hemicellulose, has been reported to decrease nutrient digestibility by decelerating the diffusion of substrates and enzymes in the porcine small intestine, which may be attributed to increased digesta viscosity (Hooda et al. 2011). Gao et al. (2015) recently observed that the addition of 5% inulin reduced ileal nutrient digestibility, probably by increasing the ileal nutrient flow. Therefore, the decreased nutrient digestibilities, NPU, and BV in low and medium treatment might be caused by the excessive DF content in the diets.

The intestinal morphology is regarded as an important evidence to assess the overall health of the intestine. Briefly, the VH and the VH/CD denote the absorption efficiency of small intestine indirectly. In the present study, partial replacement of complete formula feed with low and medium levels of WCS increased the jejunal VH and VH/CD ratio. Previous studies demonstrated that 10% wheat straw supplementation in the diets could increase the VH/CD ratio in the jejuna and ilea of growing pigs (Jin et al. 1994). Under the action of microorganisms, dietary fibre fermentation produces abundant SCFAs including acetic, propionic, and butyric acid, which play a vital role in energy supply and intestinal development (Jha and Berrocoso 2015). However, our data showed that the VH/CD of the high treatment was the lowest among the four treatments, which may be due to excessive fibre causing the accelerated digestive fluid flow, causing a large amount of insoluble fibre to scrape the jejunal mucosa or the intestinal mucosa (Mateos et al. 2012). Overall, our present study demonstrated that 7.5–10.0% and 12.5-15.0% (air-dry basis) are the optimal WCS levels for the growing pigs and finishing pigs, respectively.

Expression of the nutrient transporters (*SGLT1* and *CAT1*) provides crucial indices to evaluate the intestinal absorption capacity. Generally speaking, *SGLT1* is responsible for glucose transporting, while *CAT1* is associated with amino acid absorption (Hu et al. 2008). Chen et al. (2014) found that the expression of *SGLT1* significantly increased in growing-finishing pigs fed a 10-30% wheat bran diet, which may be attributed to the abundant butyrate level. Similarly to the observation related to jejunal morphology, we found that the high treatment significantly decreased *SGLT1* expression compared to the other groups, indicating reduced glucose transport efficiency in the small intestine. The lower *SGLT1* expression of the high treatment may be due to timely unabsorbed SCFAs in the intestinal mucosa because of speeding up the flow of digesta induced by excessive fibre. In the present study, *CAT1* expression was significantly increased in the high treatment. A previous study reported that dietary protein affected the *CAT1* expression of growing pigs (Garcia-Villalobos et al. 2012). Therefore, it is likely that a biofeedback signal from the decrease of BV and NPU promoted *CAT1* expression induced by higher fibre in the high treatment of growing-finishing pigs. We presumed that the dietary high fibre-induced decreased protein absorption in jejunum promoted the ex-

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pression of protein transporters in turn. However, the mechanism underlying our presumption should be investigated further.

Tight junction proteins have been recognized to exhibit a vital role in the maintenance of intestinal barrier integrity (Richter et al. 2014). Claudin and occludin were reported to inhibit the colonization of harmful bacteria and other antigens through sealing the paracellular spaces between epithelial cells.(Ma et al. 2022). Plenty of studies illustrated the beneficial effects of dietary fibre related to the intestinal barrier integrity. Wu et al. (2018) proved that dietary supplementation of 5% xylan and 5% β-glucan increased the expressions of *occludin* and *claudin-1* in the duodenum, as well as *claudin-1* in the jejunum. Consistent with previous studies, our study revealed that the expressions of *occludin* and *claudin-1* in the jejunum of the low and medium treatments were significantly greater than in the high and control treatments. The enhancement could potentially be attributed to the colonization of beneficial bacteria in the intestinal tract via fermentation, resulting in the production of SCFAs and ultimately improving intestinal health (Ulluwishewa et al. 2011). However, significantly decreased *occludin* and *claudin-1* expressions were also found in the high treatment. Similarly, Chen et al. (2013) also reported the adverse effect of dietary fibre (10% corn fibre) on the expression of tight junction proteins in the small intestines of weanling pigs. The conflicting results can be explained by the addition proportion, which needs further confirmation in the future. Summarily, our results suggested that 10.0% (from day 1 to 42) and 15.0% (from day 43 to 98) WCS substitution could damage the intestinal barrier integrity.

CONCLUSION

In conclusion, our present study suggested that partially replacing the complete formula feed with WCS up to 7.5% and 12.5% on air-dry basis during growing and finishing stages, respectively, promoted the development of the small intestine without affecting the growth performance of pigs, despite a decrease in the nutrient digestibilities. Further research is needed on the molecular mechanisms of nutrient metabolism and growth promotion of WCS in pigs at different growth stages.

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Conflict of interest

The authors declare no conflict of interest.

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