

Impact of different dietary fibre levels on the roughage resistance of the Dahe black pig

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Abstract: To investigate the effects and molecular mechanism of different levels of dietary fibre (Chinese milk vetch) on Dahe black and Duroc × (Landrace × Yorkshire) (DLY) pigs, 54 of each type of pig were selected and fed diets that contained 3.5%, 5.5% and 7.5% levels of fibre in the growing (30–60 kg) and fattening stages (60–120 kg). The production performance, serum physicochemical indices, nutrient digestibility, the amylase activity in the small intestine, contents of the muscle crude protein and ether extract, and the levels of transcription of the *PRKAG3* and *Fsp27* genes were determined. The production performance of the Dahe black pigs with a 5.5% level of dietary fibre was significantly higher than those with dietary fibre levels of 3.5% and 7.5%. A diet high in fibre also significantly affected the production performance of the DLY pigs. The apparent digestibility of the nutrients decreased with an increase in the dietary fibre level, and the Dahe black pigs appeared to more effectively digest the dietary fibre than the DLY pigs. The serum physicochemical indices, amylase activity, and the expression levels of the *PRKAG3* and *Fsp27* genes from the Dahe black pigs were significantly higher than those of the DLY pigs, and the intramuscular fat content of the Dahe black pigs fed a high fibre diet was significantly higher than that of the DLY pigs. The dietary fibre levels of 5.5% and 7.5% did not affect the production performance of the Dahe black pigs during the 30–120 kg period, but they significantly reduced the production performance of the DLY pigs. The Dahe black pigs were evidently tolerant to high amounts of fibre when fed a high fibre diet.

Keywords: production performance; nutrient digestibility; *PRKAG3*; *Fsp27*; Chinese milk vetch; gene expression

Dahe black pigs in Yunnan Province are primarily concentrated in the area of Dahe and Fuyuan. They are a new transgenic variety bred from Yunnan Wujin and Duroc pigs. The pork produced from Dahe black pigs is popular among consumers ow-

ing to the strong physique, coarse feeding tolerance, strong stress resistance, high intramuscular fat content and good flavour of the meat from these animals, and it is the primary material used to produce Xuanwei ham (Shi et al. 2019). The level

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of dietary fibre is one of the key factors that affects the quality of pork (Han et al. 2020). The water-absorbing quality of crude fibre (CF) can not only change the physical properties of feed, but can also promote the physiological activities of the intestinal tract of the animals. CF is rich in nutrients, such as pectin and polysaccharides. Moisture and polysaccharides combine to produce viscosity after the pigs have eaten dietary fibre, and the exchange of molecules can enhance the viscosity, thereby reducing the digestibility of other nutrients. Chinese milk vetch (*Astragalus sinicus* L.) is a type of green fertiliser. It can not only improve the soil environment (Zhou et al. 2019; Zhang et al. 2020; Zhong et al. 2021), but can also serve as a feed supplement to improve the quality of the pig's bodies. Fresh Chinese milk vetch plants contain a substantial amount of water, but they can be harvested and prepared to serve as a feed additive. The addition of the Chinese milk vetch additive to the feed can ensure that the pigs will perform highly and reduce the cost of their feed.

The retention of water and viscosity of dietary fibre can change the viscosity and rate of flow of the chyme in the digestive tract, dilute the dietary energy, and reduce the digestibility of the nutrients (Taghipoor et al. 2014; Chen et al. 2015; Xu et al. 2020). The digestive system of piglets is not fully developed, and chyme is digested by the pancreatic juice in the small intestine. Pancreatic amylase is the primary enzyme that digests cellulose. Currently, there are few studies on the enzymes that digest dietary fibre. The related studies primarily focus on the activities of the intestinal digestive enzymes during a short period after consuming dietary fibre, and there are few studies on the effects of a longer digestive time after the pigs have been fed dietary fibre. Hedemann et al. (2006) compared the results of a diet supplemented with barley shells and one supplemented with a high level of pectin. The diet supplemented with a high level of pectin significantly reduced the average daily feed intake and average daily gain (ADG) of the pigs. When growing-finishing pigs are fed a suitable amount of dietary fibre, it can improve their production performance, intestinal health, and intestinal environment (Hermes et al. 2009). A high level of dietary fibre absorbs a large amount of water in the digestive tract and increases the flow of the ileal chyme. When the increased ileal chyme passes through the intestine, the in-

testinal mucosa is damaged, and the apparent digestibility of other nutrients in the diet is affected. Wilfart et al. (2007) fed growing pigs with different levels of wheat bran diets, and the apparent digestibility of crude protein (CP), ether extract (EE) and energy decreased as the supplemental level increased. Von Heimendahl et al. (2010) showed that the apparent digestibility of CP and EE decreased in growing pigs fed with beet slag, while the apparent digestibility of the neutral detergent fibre (NDF) and acid detergent fibre (ADF) increased. Dietary fibre helps the pigs feel full and stimulates intestinal tract movements, which is conducive to excretion. The addition of CF to the feed of growing-finishing pigs can improve the intestinal health of pigs, reduce the addition of grain in the feed, and reduce the cost of the feed. Rist et al. (2014) showed that the combination of feed with high levels of protein and low fibre can promote the proliferation of bacteria that ferment protein in the large intestine of piglets. CF can absorb part of the cholesterol and triglycerides in the diet and excrete them with the excreta, effectively reducing the content of the cholesterol and triglycerides in pig's blood and liver and improving the health of the animals. Dietary fibre can also convert cholesterol in the liver to choline, reducing the levels of cholesterol in the animals. In addition, the dietary fibre can also reduce the blood glucose and blood pressure by changing the permeability of the cell membranes (Johansen and Knudsen 1994). Roth-Maier et al. (1993) showed that the contents of urinary nitrogen and blood urea nitrogen increased as sows were fed with decreasing amounts of pectin. *PRKAG3* is a gene related to the pork quality and stress responses (Uimari and Sironen 2014). Changes in the environment can stress the animals, and the activation of adenosine monophosphate kinase in the body enhances the ability of cells to absorb glucose, thus, increasing the amount of glycogen in the skeletal muscle cells (Yang et al. 2015; Weng et al. 2016). Owing to its location on lipid droplets, the *Fsp27* protein can promote the fusion of these droplets, regulate the storage of triglycerides (Price et al. 2020), and inhibit the rate of fat hydrolysis, which leads to the deposition of fat (Xu et al. 2015). The effects of different levels of dietary fibre on the production performance, serum physicochemical indices and the expression of the *PRKAG3* and *Fsp27* genes in Dahe black pigs merit further study.

To explore the different levels of dietary fibre on each index in the fattening growth stage of Dahe black and Duroc × (Landrace × Yorkshire) (DLY) pigs, this study measured the serum biochemical indices, production performance, nutrient digestibility, digestive enzyme activities and the expression of genes related to the meat quality using a variety of modern biological techniques and Chinese milk vetch as a source of fibre. We sought to explore the tolerance of Dahe black pigs to roughage in their diet through these experiments. This study not only has a guiding role in the scientific breeding of Dahe black pigs and the utilisation of their excellent meat quality, but also has important practical significance for the further exploration of the tolerance of Dahe black pigs to consuming roughage and the optimal utilisation of roughage resources.

MATERIAL AND METHODS

Experimental design

Experimental animals. Owing to the different genetic basis of Dahe black pigs and DLY pigs, there are significant differences in their feed intake and growth rate. Therefore, a single factor experimental design was adopted for the two breeds under the same dietary composition, level of nutrients, and feeding and management conditions, where the DLY pigs were used as controls. A total of 54 healthy Dahe black pigs and 54 healthy DLY pigs with a body weight of nearly 30 kg were enrolled and randomly divided into three groups. The pigs used in the experiment were half male and half female.

The added fibre source was Chinese milk vetch, and its nutritional composition was 17.86% CP, 39.89% CF and 0.65% EE. After drying and crushing, the Chinese milk vetch was fully mixed with a premix, corn and soybean meal. The experiment was divided into the growth stage (30–60 kg) and fattening stage (60–120 kg). The pigs were fed experimental diets with a fibre level of 3.5%, 5.5% and 7.5% in each stage. The experiment was initiated after three days of feeding. The digestion test was conducted four days before the end of each stage, and the experiment ended when the weight of the pigs reached approximately 120 kg. The pigs ate and drank freely during the experiment.

Ration preparation. The experimental diet was formulated according to the National Research Council (NRC 2012) pig nutrient requirements, and

the dietary fibre source was supplemented at a level of 3.5%, 5.5% and 7.5% of Chinese milk vetch. The dietary composition and levels of the nutrients are shown in Table 1.

Experimental methods

Collection of test samples. During the preparation of the experimental diets, a 200 g sample was taken from each of the three diets and stored at –20 °C.

Faecal samples were collected from the Haihe pig farm in Xuanwei City, Yunnan Province. Digestibility of the nutrients of the roughage was determined by the total faeces collection method. The samples were collected four days before the end of each stage of the test. Fresh faeces were collected and weighed at 8 a.m. each day, followed by the collection of 200 g of faeces to which 1 ml of sulfuric acid was added for nitrogen fixation. The faeces were quickly dried under an infrared lamp and placed in bags for storage at –20 °C.

Fasting blood was collected from all the pigs at the end of each stage. A volume of 10 ml of blood was collected using anticoagulant tubing at the porcine anterior vena cava and immediately centrifuged at 3 500 rpm for 10 minutes. The serum was prepared and stored at –20 °C for future use.

Two pigs were selected for dissection at the end of the fattening stage in each experiment, and the contents of the duodenum were taken and stored in a tube of 5 ml liquid nitrogen for future use. The longissimus dorsi muscle was sampled from the left carcass of the pigs normally slaughtered and stored in a 5 ml cryopreservation tube at –80 °C for future use, and then 200 g of the longissimus dorsi muscle was sampled and stored in a Ziplock bag at –20 °C for future use.

Determination of the production performance and nutrient digestibility. The amount of feed and the residual amount of feed were recorded daily in each stage of the experiment, and the feed intake was calculated at the end of the stage. The piglets were weighed on an empty stomach before the test. At the end of the growth and fattening stages, the piglets were weighed on an empty stomach and recorded again. The average daily feed intake, average daily gain and feed/gain ratio of pigs in the two stages were calculated.

The contents of the water, CP, CF, ADF, NDF, Ca, P, EE and other nutrients in the faecal samples

Table 1. Composition and nutrient levels of the diets (air dry basis)*

Weight stage (kg)	30–60			60–120		
	3.50	5.50	7.50	3.50	5.50	7.50
Dietary fibre level (%)						
Corn	69.00	63.00	59.00	72.00	66.20	62.40
Soybean meal 43	23.00	21.60	20.00	20.20	18.60	16.00
Chinese milk vetch	3.00	9.00	14.00	3.00	9.00	14.00
Soybean oil	1.00	2.40	3.00	0.80	2.20	3.60
Premix (9240A)	4.00	4.00	4.00	4.00	4.00	4.00
Total	100.00	100.00	100.00	100.00	100.00	100.00
Nutritional level (%)						
Digestive energy (MJ/kg)	13.48	13.43	13.41	13.47	13.44	13.47
Crude protein	16.00	15.91	15.93	15.01	14.89	14.86
Crude fibre	3.51	5.52	7.51	3.52	5.51	7.49
Neutral detergent fibre	12.56	17.41	19.03	12.83	17.34	19.27
Acid detergent fibre	6.62	8.53	10.01	6.54	8.21	10.28
Ca	0.70	0.70	0.70	0.70	0.70	0.70
P	0.50	0.50	0.50	0.45	0.45	0.45
Lys	0.94	0.94	0.93	0.81	0.82	0.82
Met	0.28	0.28	0.27	0.24	0.23	0.23
Thr	0.67	0.69	0.7	0.63	0.65	0.64
Trp	0.18	0.18	0.18	0.17	0.17	0.17

*Provided per kilogram of diet: 12 000 IU vitamin A; 2700 IU vitamin D₃; 35 mg vitamin E; 2.25 mg vitamin K₃; 3 mg vitamin B₁; 8 mg riboflavin; 4.5 mg vitamin B₆; 24 mg vitamin B₁₂; 60 mg niacin; 1.5 mg folic acid; 0.2 mg biotin; 18 mg pantothenic acid; 50 mg Zn (as ZnSO₄); 105 mg Mn (as MnO₂); 60 mg Fe (as FeSO₄·7H₂O); 5 mg Cu (as CuSO₄·5H₂O); 0.68 mg I (as KI); 0.18 mg Se (as Na₂SeO₃·5H₂O)

The digestive energy was a calculated value and the other ones were measured values

were measured, and the apparent digestibility of the dietary nutrients was calculated.

Serum biochemical indices and the assay of the amylase activity. The contents of the total protein (TP), albumin (ALB), total cholesterol (TC), triglycerides (TG) and urea in all the serum samples were measured using kits (Nanjing Jiancheng Company, Nanjing, China).

The activity of amylase activity was assayed using an amylase kit. The amount of hydrolysed starch and amylase activity was calculated by a spectrophotometer (UV-8000; Cany Precision Instruments Co., Ltd, Shanghai, China).

Detection of pork nutritional components. The water content in the longissimus dorsi muscle was measured using the method in GB/T 9595.15-2008 Determination of moisture-direct drying method. The CP content was measured using the Kjeldahl nitrogen determination method, and the EE content was measured by Soxhlet extraction.

Fluorogenic quantitative PCR. The genome sequences of *PRKAG3*, *Fsp27* and *β-actin* were downloaded from the National Center for Biotechnology Information (www.ncbi.nlm.nih.gov/). Primer v5.0 (Premier Biosoft, Palo Alto, CA, USA) was used to design the primers, and the primer sequences (Table 2) were sent to Shanghai Ruijie Biological Co., Ltd (Shanghai, China) for the DNA synthesis. The TRIzol method was used to extract the total RNA from the pork samples, and reverse transcription was performed using a reverse transcription kit (TaKaRa Bio, Inc., Shiga, Japan). The Eva Green I method was adopted. Fluorescence quantitative polymerase chain reaction (PCR) was performed using SsoFast™ EvaGREEN® Supermix 10 µl (Bio-Rad, Hercules, CA, USA) with upstream and downstream primers (100 µM) 1.0 µl of each, cDNA 2.0 µl, and the addition of sterilised deionised water to 20 µl. The reaction conditions were 95 °C for 10 min, 95 °C for 5 s, 60 °C for 20 s, 72 °C for 15 s, and 40 cycles.

Table 2. Primers for the real-time PCR

Gene	Primers	Length (bp)
<i>PRKAG3</i> (AY264345.1)	F: 5'-CTTGGGCTGGTGGAAAGAGAA-3' R: 5'-CCCACGAAGCTCTGCTTCTT-3'	269
<i>Fsp27</i> (JF923469.1)	F: 5'-TCACAGCAGGGCACTAGGTA-3' R: 5'-AGGTTGAAGTTACACGGGC-3'	83
β - <i>actin</i> (AJ312193.1)	F: 5'-GTGCGGGACATCAAGGAGAA-3' R: 5'-ATGTCCACGTGCGACTTCAT-3'	242

Statistical analyses

The levels of the gene expression were expressed as the relative levels of the expression using β -*actin* from the pigs as an internal reference. The fluorescence quantitative results were calculated by the Pfaffl method, and the other test results were analysed using Microsoft Excel 2003 (Redmond, WA, USA). The significance of the statistics were analysed using a one-way analysis of variance (ANOVA) in SPSS v19.0 (IBM, Inc., Armonk, NY, USA). The results were expressed as the mean \pm SD (standard deviation). A *P*-value less than 0.05 was considered statistically significant.

RESULTS

Effects of the level of dietary fibre on the production performance of the pigs

Effects of the different levels of dietary fibre on the production performance of the Dahe black pigs. As shown in Table 3, both the average daily weight gain and average daily feed intake of the Dahe black pigs during the growth stage (30–60 kg) differed significantly between the groups with 3.5% and 5.5% CF and the group with 7.5% CF (all *P* < 0.05), and the feed/gain ratio also differed significantly between the 5.5% group and those at 3.5% or 7.5% (both *P* < 0.05). The average daily feed intake during the fattening stage (60–120 kg) of the Dahe black pigs differed significantly between the 5.5% and 7.5% groups (*P* < 0.01), as well as between the 3.5% and 5.5% groups (*P* < 0.05), and the feed/gain ratio in the 5.5% group was significantly higher than those in the 3.5% and 7.5% groups (both *P* < 0.05).

Effects of the different levels of dietary fibre on the production performance of the DLY pigs. As Table 4 indicates, the average daily gain of the DLY pigs differed significantly between the 3.5%,

5.5% and 7.5% groups (both *P* < 0.05). The average daily feed intake differed significantly between the 5.5% and 3.5% groups, as well as between the 5.5% and 7.5% groups (both *P* < 0.01), and the feed/gain ratio differed significantly between the 5.5% and 7.5% groups (*P* < 0.05). The average daily weight gain of the fattening pigs differed significantly between the 7.5% and 3.5% fibre groups, as well as between the 7.5% and 5.5% groups (both *P* < 0.05). The average daily feed intake differed significantly between the 5.5% and 3.5% fibre groups, as well as between the 5.5% and 7.5% groups (both *P* < 0.01), and the feed/gain ratio also differed significantly between the 3.5% and 5.5% groups (*P* < 0.01), as well as between 3.5% and 7.5% groups (*P* < 0.05). The average daily weight gain of the growing-finishing DLY pigs differed significantly between the 7.5% and 3.5% groups, as well as between the 7.5% and 5.5% groups (both *P* < 0.01). The average daily feed intake differed significantly between the 5.5% and 7.5% fibre groups (*P* < 0.05), and the feed/gain ratio differed significantly between the 7.5% and 3.5% groups, as well as between the 7.5% and 5.5% groups (both *P* < 0.01).

Effects of the level of dietary fibre on the apparent digestibility of the nutrients by the pigs

Effects of the different levels of the dietary fibre on the apparent digestibility of the nutrients by the Dahe black pigs. As shown in Table 5, the apparent digestibility of the CP during the growth stage (30–60 kg) of Dahe black pigs differed significantly between the 3.5% and 5.5% groups, as well as between the 3.5% and 7.5% groups (both *P* < 0.01). The apparent digestibility of the CF differed significantly between the 3.5% and 7.5% groups (*P* < 0.01), as well as between the 5.5% and 7.5% groups (*P* < 0.05). The apparent digestibility of the NDF and ADF

Table 3. Effects of the dietary fibre levels on the growth performance of the Dahe black pig

Weight stage (kg)	30–60			60–120			30–120		
	3.50	5.50	7.50	3.50	5.50	7.50	3.50	5.50	7.50
Dietary fibre level (%)	698.01 ± 15.43 ^a	703.37 ± 10.07 ^a	637.40 ± 28.03 ^b	551.46 ± 21.87	557.19 ± 26.41	503.51 ± 5.26	594.11 ± 13.32	598.88 ± 21.25	542.49 ± 8.49
ADG (g/day)	1.90 ± 0.10 ^a	1.63 ± 0.01 ^b	1.78 ± 0.05 ^b	2.09 ± 0.05 ^a	2.28 ± 0.07 ^{Ab}	1.96 ± 0.02 ^{Ba}	2.04 ± 0.06	2.01 ± 0.05	1.91 ± 0.00
ADFI (kg/day)	2.72 ± 0.17 ^a	2.31 ± 0.02 ^b	2.79 ± 0.07 ^a	3.79 ± 0.06 ^b	4.09 ± 0.23 ^a	3.89 ± 0.04 ^b	3.43 ± 0.03	3.36 ± 0.09	3.50 ± 0.06
F/G									

ADFI = average daily feed intake; ADG = average daily gain; F/G = feed/gain ratio

Lowercase, superscript letters are used to indicate significant differences among the means within a row at the same weight stage

^{a,b}Means lacking a common lowercase superscript were statistically different at $P < 0.05$

^{A,B}Means lacking a common uppercase superscript were statistically different at $P < 0.10$

Table 4. Effects of the dietary fibre levels on the production performance of the finishing DLY pigs

Weight stage (kg)	30–60			60–120			30–120		
	3.50	5.50	7.50	3.50	5.50	7.50	3.50	5.50	7.50
Dietary fibre level (%)	732.06 ± 41.95 ^a	681.05 ± 5.59 ^b	590.01 ± 35.98 ^c	627.88 ± 19.24 ^a	598.65 ± 25.47 ^a	542.03 ± 12.97 ^b	658.52 ± 25.85 ^{Aa}	633.09 ± 13.11 ^a	558.51 ± 4.74 ^{Bb}
ADG (g/day)	1.80 ± 0.07 ^{Bb}	1.61 ± 0.00 ^{Aa}	1.64 ± 0.06 ^{Aa}	2.11 ± 0.04 ^{Bb}	2.39 ± 0.00 ^{Aa}	2.09 ± 0.00 ^{Bb}	2.04 ± 0.04	2.06 ± 0.00	1.98 ± 0.00
ADFI (kg/day)	2.46 ± 0.09 ^{ab}	2.37 ± 0.02 ^a	2.79 ± 0.17 ^b	3.37 ± 0.04 ^{Bb}	3.99 ± 0.18 ^{Aa}	3.86 ± 0.09 ^{Ba}	3.10 ± 0.05 ^{Bb}	3.26 ± 0.07 ^{Bb}	3.54 ± 0.01 ^{Aa}
F/G									

ADFI = average daily feed intake; ADG = average daily gain; F/G = feed/gain ratio

Lowercase, superscript letters are used to indicate significant differences among the means within a row at the same weight stage

^{a–c}Means lacking a common lowercase superscript were statistically different at $P < 0.05$

^{A,B}Means lacking a common uppercase superscript were statistically different at $P < 0.10$

Table 5. Effects of the dietary fibre levels on the apparent digestibility of the dietary nutrients of the Dahe black pig

Weight stage (kg)	30–60			60–120			
	Dietary fibre level (%)	3.50	5.50	7.50	3.50	5.50	7.50
Crude protein		75.50 ± 0.76 ^{Aa}	70.67 ± 0.80 ^{Bb}	65.33 ± 1.23 ^{Cc}	78.78 ± 0.49 ^{Aa}	74.89 ± 0.42 ^{Bb}	70.89 ± 0.35 ^{Cc}
Crude fibre		56.83 ± 1.28 ^{Aa}	54.83 ± 1.25 ^{ABa}	51.00 ± 0.73 ^{Bb}	60.56 ± 0.96 ^a	58.44 ± 0.73 ^a	51.33 ± 0.80 ^b
Neutral detergent fibre		46.83 ± 0.60 ^{Aa}	43.50 ± 0.92 ^{Bb}	42.83 ± 0.31 ^{Bb}	53.11 ± 0.94 ^{Aa}	48.00 ± 0.65 ^{Bb}	41.44 ± 0.84 ^{Cc}
Acid detergent fibre		38.17 ± 0.98 ^{Aa}	33.33 ± 0.42 ^{Bb}	32.33 ± 0.67 ^{Bb}	41.22 ± 0.78 ^{Aa}	38.11 ± 0.26 ^{Bb}	34.33 ± 0.60 ^{Cc}
Ether extract		78.17 ± 1.30 ^{Aa}	75.00 ± 1.18 ^{Aa}	70.33 ± 0.67 ^{Bb}	71.67 ± 0.76 ^{Aa}	67.11 ± 0.63 ^{Bb}	62.56 ± 0.85 ^{Cc}
Ca		43.33 ± 2.65 ^{Aa}	36.17 ± 1.92 ^{Bb}	33.00 ± 2.03 ^{Bb}	45.33 ± 0.50 ^{Aa}	41.89 ± 0.48 ^{Bb}	39.00 ± 0.67 ^{Cc}
P		39.33 ± 2.61 ^{Aa}	32.00 ± 1.32 ^{ABb}	25.17 ± 1.33 ^{Bc}	31.22 ± 2.60 ^{ab}	34.78 ± 1.67 ^a	28.00 ± 0.82 ^b

Lowercase, superscript letters are used to indicate significant differences among the means within a row at the same weight stage

^{a-c}Means lacking a common lowercase superscript were statistically different at $P < 0.05$

^{A-C}Means lacking a common uppercase superscript were statistically different at $P < 0.10$

differed significantly between the 3.5% and 5.5% groups, as well as between the 3.5% and 7.5% groups (both $P < 0.01$). The apparent digestibility of the EE differed significantly between the 7.5% and 3.5% groups, as well as between 7.5% and 5.5% groups (both $P < 0.01$). The apparent digestibility of the Ca differed significantly between the 3.5% and 7.5% groups ($P < 0.01$), as well as between the 3.5% and 5.5% groups ($P < 0.05$). The apparent digestibility of the P differed significantly between the 3.5% and 7.5% groups ($P < 0.01$), and it also differed significantly between the 5.5% and 3.5% groups, as well as between the 5.5% and 7.5% groups (both $P < 0.05$). The apparent digestibility of the CP during the fattening stage (60–120 kg) of Dahe black pigs differed significantly among the 3.5%, 5.5% and 7.5% dietary fibre groups (all $P < 0.01$). The apparent digestibility of the CF differed significantly between the 7.5% and 3.5% groups, as well as between the 7.5% and 5.5% groups (both $P < 0.05$). The apparent digestibility of the NDF, ADF, EE and Ca were the same and exhibited significant differences between the 3.5%, 5.5% and 7.5% fibre groups (all $P < 0.01$), and the apparent digestibility of the P differed significantly between the 5.5% and 7.5% fibre groups ($P < 0.05$).

Effects of the levels of the different dietary fibre on the apparent digestibility of the nutrients by the DLY pigs. As shown in Table 6, the apparent digestibility of the CP differed significantly among the 3.5%, 5.5% and 7.5% dietary fibre groups during the growth stage (30–60 kg) of the DLY pigs (all $P < 0.01$). The apparent digestibility of the CF was significant between the 3.5% and 7.5% groups

($P < 0.01$) and the 5.5% and 7.5% groups ($P < 0.01$), as well as between the 3.5% and 5.5% groups ($P < 0.05$). The apparent digestibility of the NDF differed significantly between the 3.5% and 7.5% groups, as well as between the 5.5% and 7.5% groups (both $P < 0.05$). The apparent digestibility of the ADF differed significantly between the 3.5% and 5.5% groups, as well as between the 3.5% and 7.5% groups (both $P < 0.01$). The apparent digestibility of the EE differed significantly between the 7.5% and 3.5% groups, as well as between the 7.5% and 5.5% groups (both $P < 0.01$). The apparent digestibility of the Ca differed significantly between the 3.5% and 5.5% groups ($P < 0.05$), and the apparent digestibility of the P differed significantly between the 3.5% and 7.5% groups ($P < 0.01$), as well as between the 5.5% and 7.5% groups ($P < 0.05$).

The apparent digestibility of the CP during the fattening stage of the DLY pigs differed significantly among the 3.5%, 5.5% and 7.5% groups (all $P < 0.01$). The apparent digestibility of the CF differed significantly between the 7.5% and 3.5% groups as well as between the 7.5% and 5.5% groups (both $P < 0.05$). The apparent digestibility of the NDF differed significantly between the 7.5% and 3.5% groups, as well as between the 7.5% and 5.5% groups (both $P < 0.01$). The apparent digestibility of the ADF differed significantly among the 3.5%, 5.5% and 7.5% fibre groups (all $P < 0.01$). The apparent digestibility of the EE and Ca were the same and differed significantly between the 7.5% and 3.5% groups, as well as between the 7.5% and 5.5% groups (both $P < 0.01$). The apparent digestibility of the P differed significantly between the 3.5% and 7.5% groups ($P < 0.05$).

Table 6. Effects of the dietary fibre levels on the apparent digestibility of the dietary nutrients in finishing DLY pig

Weight stage (kg)	30–60			60–120			
	Dietary fibre level (%)	3.50	5.50	7.50	3.50	5.50	7.50
Crude protein		79.83 ± 0.48 ^{Aa}	71.67 ± 0.56 ^{Bb}	61.83 ± 0.87 ^{Cc}	80.56 ± 0.69 ^{Aa}	72.33 ± 0.44 ^{Bb}	63.89 ± 0.79 ^{Cc}
Crude fibre		54.00 ± 0.82 ^{Aa}	51.17 ± 0.65 ^{Ab}	46.67 ± 1.23 ^{Bc}	57.78 ± 0.76 ^{Aa}	59.11 ± 0.61 ^{Aa}	46.44 ± 0.29 ^{Bb}
Neutral detergent fibre		40.33 ± 0.49 ^a	40.17 ± 0.75 ^a	38.33 ± 0.49 ^b	45.67 ± 0.69 ^{Aa}	44.00 ± 0.44 ^{Aa}	35.78 ± 0.64 ^{Bb}
Acid detergent fibre		34.67 ± 0.61 ^{Aa}	30.67 ± 0.49 ^{Bb}	28.17 ± 1.35 ^{Bb}	37.89 ± 0.54 ^{Aa}	33.00 ± 0.67 ^{Bb}	27.83 ± 0.97 ^{Cc}
Ether extract		76.50 ± 2.57 ^{Aa}	70.83 ± 0.60 ^{Ab}	64.00 ± 0.58 ^{Bc}	72.11 ± 0.92 ^{Aa}	64.56 ± 0.85 ^{Bb}	56.89 ± 0.59 ^{Cc}
Ca		40.67 ± 1.52 ^a	34.33 ± 2.12 ^b	35.50 ± 1.75 ^{ab}	47.78 ± 0.80 ^{Aa}	40.67 ± 0.24 ^{Bb}	34.33 ± 0.75 ^{Cc}
P		37.83 ± 2.81 ^{Aa}	35.00 ± 1.16 ^{ABa}	27.67 ± 2.03 ^{Bb}	32.56 ± 1.14 ^a	29.00 ± 2.25 ^{ab}	26.89 ± 1.24 ^b

Lowercase, superscript letters are used to indicate significant differences among the means within a row at the same weight stage

^{a-c}Means lacking a common lowercase superscript were statistically different at $P < 0.05$

^{A-C}Means lacking a common uppercase superscript were statistically different at $P < 0.10$

Effects of the levels of the dietary fibre on the physicochemical indices of the pig serum

Effects of the levels of the different dietary fibre on the physicochemical indices of the Dahe black pig serum. As shown in Table 7, the content of total protein (TP) in the serum during the growth stage (30–60 kg) of Dahe black pigs differed significantly between the 7.5% and 3.5% groups, as well as between the 7.5% and 5.5% groups (both $P < 0.01$). The albumin (ALB) content differed significantly between the 3.5% and 5.5% groups ($P < 0.05$); and there was also a significant difference in the total cholesterol (TC) content in the serum between the 3.5% and 5.5% groups ($P < 0.01$).

The content of the serum TP during the fattening stage (60–120 kg) of Dahe black pigs differed significantly between the 7.5% and 3.5% groups

($P < 0.01$) and between the 7.5% and 5.5% groups ($P < 0.01$), as well as between the 3.5% and 5.5% groups ($P < 0.05$). The serum ALB content differed significantly between the 7.5% and 3.5% groups, as well as between the 7.5% and 5.5% groups (both $P < 0.01$). The serum GLU content differed significantly between the 3.5% and 7.5% groups ($P < 0.05$). The TC content in the serum differed significantly between the 3.5% and 7.5% groups ($P < 0.01$), as well as between the 3.5% and 5.5% groups ($P < 0.05$). The triglyceride (TG) content in the serum differed significantly between the 3.5% and 5.5% groups ($P < 0.01$), as well as between the 3.5% and 7.5% groups ($P < 0.05$).

Effects of the levels of the different dietary fibre on the physicochemical indices of the DLY pig serum. As shown in Table 8, the serum TP content during the growth stage (30–60 kg) of the DLY pigs differed significantly between 7.5% and 3.5% groups,

Table 7. Effects of the dietary fibre levels on the serum biochemical parameters in the finishing Dahe black pigs

Weight stage (kg)	30–60			60–120			
	Dietary fibre level (%)	3.50	5.50	7.50	3.50	5.50	7.50
Total protein (g/l)		41.4 ± 6.78 ^{Bb}	43.65 ± 4.78 ^{Bb}	73.59 ± 2.55 ^{Aa}	80.75 ± 1.93 ^{Aa}	70.86 ± 3.11 ^{Ab}	55.37 ± 2.52 ^{Bc}
Albumin (g/l)		32.12 ± 6.01 ^b	46.86 ± 3.14 ^a	40.12 ± 3.13 ^{ab}	47.80 ± 1.56 ^{Aa}	44.16 ± 1.45 ^{Aa}	34.70 ± 1.67 ^{Bc}
Urea (mmol/l)		3.11 ± 0.25	4.57 ± 0.90	3.77 ± 0.68	4.32 ± 0.17 ^a	4.02 ± 0.10 ^{ab}	3.79 ± 0.17 ^b
Total cholesterol (mmol/l)		1.85 ± 0.24 ^{Bb}	3.14 ± 0.36 ^{Aa}	2.54 ± 0.28 ^{ABab}	3.00 ± 0.20 ^{Aa}	2.54 ± 0.12 ^{ABb}	2.29 ± 0.10 ^{Bb}
Triglycerides (mmol/l)		0.39 ± 0.10	0.56 ± 0.12	0.47 ± 0.14	0.86 ± 0.13 ^{Aa}	0.43 ± 0.05 ^{Bb}	0.55 ± 0.06 ^{Bb}

Lowercase, superscript letters are used to indicate significant differences among the means within a row at the same weight stage

^{a-c}Means lacking a common lowercase superscript were statistically different at $P < 0.05$

^{A,B}Means lacking a common uppercase superscript were statistically different at $P < 0.10$

Table 8. Effects of the dietary fibre levels on the serum biochemical parameters in the finishing DLY pigs

Weight stage (kg)	30–60			60–120			
	Dietary fibre level (%)	3.50	5.50	7.50	3.50	5.50	7.50
Total protein (g/l)		107.24 ± 4.19 ^{Aa}	99.94 ± 4.31 ^{Aa}	64.16 ± 9.66 ^{Bb}	88.15 ± 5.71	88.83 ± 1.38	86.08 ± 5.58
Albumin (g/l)		58.35 ± 3.63	59.28 ± 3.80	45.36 ± 6.65	56.45 ± 5.68	49.40 ± 1.42	47.83 ± 2.30
Urea (mmol/l)		6.72 ± 0.76 ^a	6.30 ± 0.45 ^a	4.17 ± 0.70 ^b	5.23 ± 0.35	5.08 ± 0.36	4.92 ± 0.22
Total cholesterol (mmol/l)		3.85 ± 0.23 ^a	3.87 ± 0.17 ^a	2.81 ± 0.40 ^b	3.20 ± 0.16	3.11 ± 0.07	3.68 ± 0.46
Triglycerides (mmol/l)		0.78 ± 0.12	0.72 ± 0.14	0.45 ± 0.11	1.11 ± 0.33	0.71 ± 0.06	0.83 ± 0.07

Lowercase, superscript letters are used to indicate significant differences among the means within a row at the same weight stage

^{a,b}Means lacking a common lowercase superscript were statistically different at $P < 0.05$

^{A,B}Means lacking a common uppercase superscript were statistically different at $P < 0.10$

as well as between the 7.5% and 5.5% groups (both $P < 0.01$). The urea content in the serum differed significantly between the 7.5% and 3.5% groups, as well as between the 7.5% and 5.5% groups (both $P < 0.05$). The serum TC content also differed significantly between the 7.5% and 3.5% groups, as well as between the 7.5% and 5.5% groups (both $P < 0.05$). The serum TP, ALB, GLU, TC and TG contents in the DLY fattening pigs did not differ significantly.

Effects of the levels of the dietary fibre on the activity of small intestine amylase in the pigs

As shown in Figure 1, the amylase activity of the Dahe black pigs in the small intestine differed significantly between the 3.5% and 5.5% groups ($P < 0.01$) and the 3.5% and 7.5% groups ($P < 0.01$), as well as between the 5.5% and 7.5% groups ($P < 0.05$). Figure 1 indicates that the amylase activity of the DLY pigs differed significantly between the 3.5% and 7.5% groups ($P < 0.05$).

Effects of the levels of the dietary fibre on the nutritional composition of the pork

As shown in Table 9, there were no significant differences in the moisture and CP contents among the experimental groups in the pork from the Dahe black pigs, but there were significant differences in the EE contents between the 7.5% and 3.5% groups, as well as between the 7.5% and 5.5% groups (both $P < 0.01$). The moisture, CP and EE contents

of the DLY pig pork had no significant differences among the groups.

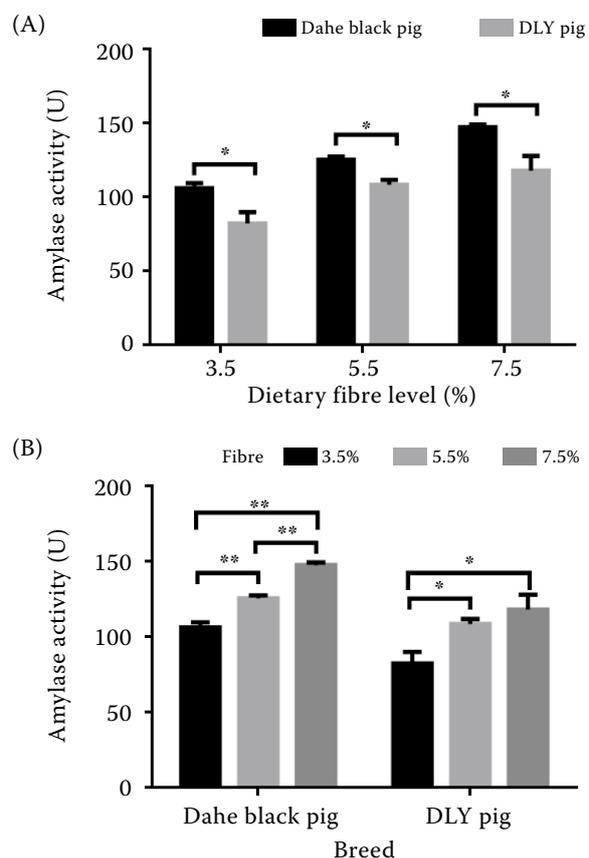


Figure 1. Effects of the levels of the dietary fibre on the amylase activity in the pigs

(A) Comparison of the amylase activity between different pig breeds at the same dietary fibre level; (B) comparison of the amylase activity between the different dietary fibre levels by the same pig breed

*A significant difference between groups ($P < 0.05$)

**A significant difference between groups ($P < 0.01$)

Table 9. Effects of the dietary fibre levels on the meat quality in the finishing Dahe black pigs and the finishing DLY pigs

Breed	Dahe black pig			DLY pig		
	3.50	5.50	7.50	3.50	5.50	7.50
Water content (%)	70.29 ± 0.88	68.42 ± 1.04	66.70 ± 2.34	70.79 ± 0.42	71.00 ± 0.16	70.42 ± 1.26
Crude protein (%)	23.2 ± 0.70	24.66 ± 1.07	24.46 ± 0.80	24.85 ± 0.12	23.75 ± 0.28	23.89 ± 1.29
Ether extract (%)	3.32 ± 0.03 ^{Bb}	3.74 ± 0.06 ^{Bb}	4.88 ± 0.28 ^{Aa}	2.11 ± 0.06	2.43 ± 0.14	2.59 ± 0.22

Lowercase, superscript letters are used to indicate significant differences among the means within a row at the same weight stage

^{a,b}Means lacking a common lowercase superscript were statistically different at $P < 0.05$

^{A,B}Means lacking a common uppercase superscript were statistically different at $P < 0.10$

Effects of the levels of the dietary fibre on the expression of *PRKAG3* and *Fsp27* in the pigs

As shown in Figure 2, the *PRKAG3* expression level in the Dahe black pigs in the 7.5% fibre group

differed significantly from those in the 3.5% and 5.5% groups (both $P < 0.01$), and the *Fsp27* expression level in the 3.5% fibre group differed significantly from those in the 5.5% and 7.5% groups (both $P < 0.01$). The *PRKAG3* expression level differed significantly between the 5.5% and 3.5% groups ($P < 0.05$)

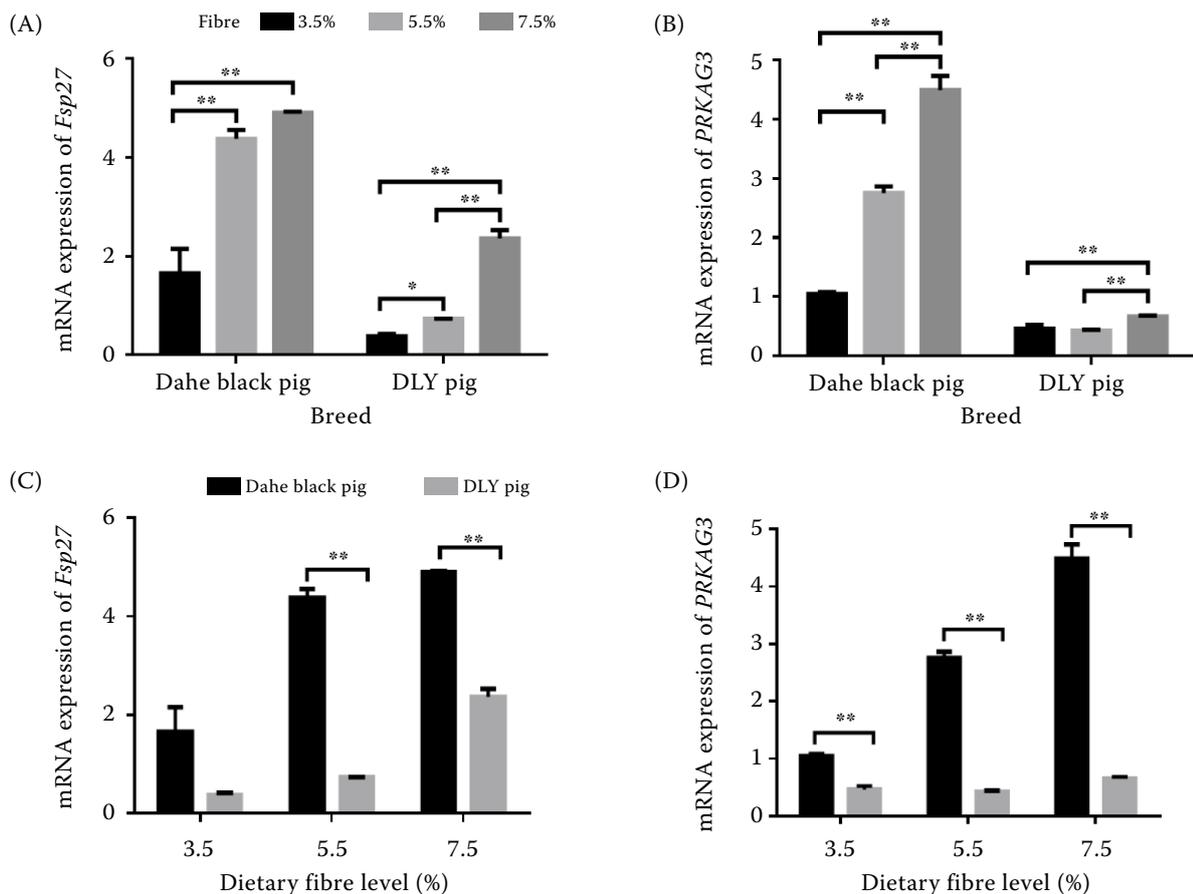


Figure 2. Effects of the dietary fibre levels on the *PRKAG3* and *Fsp27* genes in the finishing Dahe black pigs and the DLY pigs

(A, B) Comparison of the mRNA expression between the different dietary fibre levels by the same pig breed; (C, D) comparison of the mRNA expression between the different pig breeds at the same dietary fibre level

*A significant difference between groups ($P < 0.05$); **a significant difference between groups ($P < 0.01$)

and the 5.5% and 7.5% groups ($P < 0.05$), as well as between the 3.5% and 7.5% groups ($P < 0.01$). The *Fsp27* expression level differed significantly between the 7.5% and 3.5% groups, as well as between the 7.5% and 5.5% groups (both $P < 0.05$).

DISCUSSION

In recent years, the addition of high-quality fibre to a pig's diet has become an important subject of nutritional research in animals. CF feed is a relatively cheap and abundant feed resource. Pigs cannot completely digest dietary fibre, but microbial fermentation can act on the dietary fibre to produce volatile fatty acids in the large intestine, which can be absorbed by the pigs, and the energy produced can provide approximately 30% of the energy used for body growth and development (Urriola and Stein 2010; Zhao et al. 2020). Hermes et al. (2009) reported that piglets fed with dietary fibre had improved growth performance, intestinal development, and intestinal microflora. The results showed that there were significant differences in the digestibility and utilisation of dietary fibre between the Dahe black and DLY pigs, which was related to the characteristics of the pig breeds and the digestibility of the intestinal nutrients. DLY pigs are a breed of pig that performs at a high level in production. They can perform well at a level of 3.5% dietary fibre, but they do not thrive when fed a diet high in fibre. Dahe black pigs have the pedigree of local Wujin pigs. When fed a diet that has a higher level of fibre (CF 5.5% and 7.5%) in the 30–120 kg stage, the Dahe black pigs had a better genetic growth potential and obvious tolerance to being fed roughage. Dahe black pigs fully utilise the nutrients in the feed and develop their genetic growth potential when fed lower amounts. In contrast, DLY pigs require a high level of nutrition to perform at optimal levels.

Studies have shown that pigs that were fed diets supplemented with CF digest other nutrients in their diet more poorly (Wilfart et al. 2007). The results in this study showed that with an increase in the dietary fibre, the digestibility of the CP, CF and EE in Dahe black pigs decreased by 15.56%, 11.43% and 11.14%, respectively, while those in the DLY pigs decreased by 29.11%, 15.71% and 19.13%, respectively. There could be two reasons for this. First, the soluble fibre in the dietary fibre increases the viscosity of the chyme and reduces the contact between the intes-

tinal mucosa and the nutrients, while the water-holding capacity of the dietary fibre accelerates the rate of flow of the chyme in the digestive tract. As a result, the small intestine does not have enough time to absorb nutrients, which leads to a decrease in the ability to digest dietary nutrients. Second, the dietary fibre damages the intestinal mucosa, increasing the burden on the digestive tract and reducing the digestibility of the dietary nutrients. The index of nutrient digestibility decreased with an increase in the level of the dietary fibre in both types of pigs. When the dietary fibre level was 3.5%, the nutrient digestibility of the DLY pigs was higher than that of the Dahe black pigs. However, with an increase in the level of the dietary fibre, the ability of Dahe black pigs to digest nutrients gradually became higher than that of the DLY pigs. The results suggest that Dahe black pigs had more advantages under a high level of dietary fibre. The Dahe black pig was bred by introducing the Duroc pedigree based on the selection and breeding of the Yunnan Wujin pig. Whether the advantages of Dahe black pigs at high levels of fibre are related to genetics merits further study. If it is owing to the genetic differences between the breeds, this would provide a powerful advantage that should be utilised.

Among the results, the ALB content in the growing Dahe black and DLY pigs decreased with an increase in the level of fibre, suggesting that a higher level of dietary fibre may improve the metabolic level and immunity of pigs. Dietary fibre improves hyperglycaemia through its physicochemical properties and possible modulation of the gut hormone secretion, such as glucagon-like peptide 1 (Kim et al. 2016). The blood sugar content of both the Dahe black and DLY pigs tended to continuously decrease as the level of dietary fibre increased. In the fattening stage of the Dahe black pigs and the growth stage of the DLY pigs, the TP values differed significantly between the 7.5% and 3.5% fibre groups, as well as between the 7.5% and 5.5% fibre groups. The TP values tended to increase at first and then decrease during the growth stage of the Dahe black pigs and the fattening stage of the DLY pigs. In contrast, during the fattening stage of the Dahe black pigs and the growth stage of the DLY pigs, the values of TP gradually decreased with an increase in the dietary level, but the value in DLY pigs always outranked that in the Dahe black pigs. The Dahe black pigs could effectively utilise the dietary fibre. The performance of Dahe black pigs in production was not affected at higher levels

of fibre, and the serum indicators showed that the Dahe black pigs were healthier. Pancreatic amylase is the main enzyme that degrades dietary fibre in animals, and the activity of the pancreatic amylase is positively correlated with the content of the dietary fibre. Vahouny and Cassidy (1985) found that dietary fibre can improve the activity of the pancreatic digestive enzymes through *in vitro* experiments. This study found that the amylase activity of both the Dahe black and DLY pigs increased with an increase in the level of the dietary fibre, but the rate of increase in the Dahe black pigs was quicker, which also indicated that the Dahe black pigs could tolerate roughage in their feed. A comparison of amylase and digestibility indicates that the high amylase activity and decreased nutrient digestibility correlated. This could be caused by the fact that an increase in the level of fibre caused the chyme to move faster in the digestive tract and reduced the time that it was in contact with the digestive enzymes.

The intramuscular fat content is the primary factor that affects the pork quality. In this study, the intramuscular fat content of the Dahe black pigs increased by 57.3%, 53.9% and 88.4% than those of DLY pigs, respectively, and the quality of pork significantly improved. In addition, the intramuscular fat content of the pork increased with the level of the dietary fibre. Muscle is the last place in which fat is deposited during the growth process. It is difficult to improve the fat content in the muscle without affecting the lean meat rate. Therefore, improving the quality of the pork from a heredity perspective is an effective method. *PRKAG3* is the main gene that affects the pork quality, and the *Fsp27* gene can accelerate the fusion between the lipid droplets. In this study, the level of *PRKAG3* gene expression in both pig breeds increased as the level of dietary fibre increased, and that in Dahe black pigs was always significantly higher than that in the DLY pigs. This suggests that Dahe black pigs can more effectively utilise nutrients, improve the gene expression, synthesise intramuscular fat, and increase the flavour and taste of meat under a higher level of dietary fibre. To our knowledge, there have been no reports on the expression of *Fsp27* under different levels of dietary fibre. The level of expression of the *Fsp27* gene in the Dahe black pigs was significantly higher than that in the DLY pigs at all the levels of dietary fibre, and the level of expression of the *Fsp27* gene in both pig breeds increased with an increase in the levels of the dietary fibre. There was a positive correlation

between the level of the dietary fibre and the expression of the *Fsp27* gene. Therefore, we hypothesised that the level of the dietary fibre can regulate the transcription and translation of the *Fsp27* gene in pigs and affect the fat metabolism. The specific mechanism by which dietary fibre influences the fat metabolism remains unclear, and further research is merited. With an increase in the level of the dietary fibre, the expression levels of two intramuscular fat-related genes increased significantly, and the expression levels of the Dahe black pigs were much higher than those of the DLY pigs. The intramuscular fat content has always been an important index used in evaluating the pork quality and flavour. A higher intramuscular fat content results in better pork quality. Therefore, an increase in the level of the dietary fibre is highly significant for improving the flavour of both types of pork. In addition, the high *Fsp27* gene expression level in the Dahe black pig lays a theoretical foundation for the development and utilisation of the advantages of local pig breeds and has practical significance in improving the pig breeds and pork quality.

CONCLUSION

The digestibility of Dahe black pigs and DLY pigs showed significant differences under diets with different fibre levels, where the Dahe black pigs obviously tolerated roughage under diets with 5.5% and 7.5% fibre levels.

Feeding the pigs with diets of 5.5% and 7.5% fibre induced the up-regulation of the *PRKAG3* and *Fsp27* gene expression in the muscle and, thus, affected the meat quality from the Dahe black pigs compared with the DLY pigs. Increasing the dietary fibre level could significantly increase the intramuscular fat content of Dahe black pigs.

Conflict of interest

The authors declare no conflict of interest.

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