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Effect of conditioning temperature on pelleting characteristics, nutrient digestibility and gut microbiota of sorghum-based diets for growing pigs

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ABSTRACT

This study was conducted to determine the effect of the conditioning temperature on the pelleting characteristics, nutrient digestibility (*in vitro* and *in vivo*) and gut microbiota of sorghum-based diets for pigs. The experimental design included an evaluation of the effects of 5 conditioning temperatures (65, 70, 75, 80, 85℃) on sorghum-based diet pelleting. Increasing the conditioning temperature led to an increase of resistant starch ($P < 0.05$), decrease of the protein solubility of pellet ($P < 0.05$) and increase of the hardness of pelleted diets ($P < 0.01$) but had a quadratic interaction with gelatinized starch ($P < 0.05$) and the RS (resistant starch) /GS (gelatinized starch) ratio ($P < 0.05$), and the contents of gelatinized starch at 75 and 80℃ were higher than those found in the other groups. There was a quadratic relationship ($P < 0.05$) between dry matter (DM) and crude protein (CP) digestibility with the conditioning temperature *in vitro*. Additionally, increasing the conditioning temperature produced a quadratic relationships between the apparent digestibility and ileum apparent digestibility of both CP and starch *in vivo* ($P < 0.05$), and their greatest digestibility occurred at both 75 and 80℃. As for growth performance, the average daily gain (ADG), feed conversion rate (FCR) and diarrhea ratio reached optimal values at temperatures of 75 and 80℃. Moreover, the increase of the conditioning temperature from 75 to 80℃ significantly increased ($P < 0.05$) the number of beneficial bacteria (*Bifidobacterium* sp. and *Lactobacillus* sp.) in the gut and feces as well as suppressed ($P < 0.05$) bacterial pathogens (*Escherichia coli*), which effectively promoted the diversity of the gut microflora. Overall, these results indicate that 75 and 80℃ are suitable conditioning temperatures of sorghum-based diets for pigs.

1. Introduction

It is generally accepted that the pelleting of feed enhances the economics of production by improving the growth and feed utilization responses in pigs (Rojas et al., 2016; Ruiz et al., 2017). Among all steps of the pelleting process, the conditioning

Abbreviations: DM, dry matter; CP, crud protein; RS, resistant starch; GS, gelatinised starch; GE, gross energy; ADFI, average daily feed intake; ADG, average daily gain; FCR, feed conversion rate; PM, Protein matrix; SG, Starch ganule; BW, body weight; SEM, Standard error of mean

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temperature is one of the most important factors in steam-conditioning, which affects the pellet quality, nutritional quality and feeding value of animals (Selle et al., 2013). In the process of conditioning, the characteristics of the pellets and the structure of the main nutrients, such as starch and protein, will change with the conditioning temperature (Lewis et al., 2015). An appropriate conditioning temperature will increase starch gelatinisation and hardness, which may promote the growth performance and digestibility of animals; otherwise, the conditioning temperature would be counterproductive (Abdollahi et al., 2011, 2012; Abdollahi et al., 2013). On one hand, when the conditioning temperature is excessively high, the hardness of pellet feed may affect animals' digestion. On the other hand, a low conditioning temperature may decrease starch gelatinization and cause insufficient feed viscosity, resulting in low durability and poor pellet quality, accompanied by low protein digestibility and a high diarrhea rate in pigs (Park et al., 2013). Thus, proper heat treatment of feed will achieve the best nutritional utilization and performance.

To identify the optimal conditioning temperature of different grains, much research has been performed on the effect of the conditioning temperature on diet processing characteristics, nutrient digestibility and performance. Abdollahi et al. showed that increasing the conditioning temperature (60, 75 and 90°C) of wheat-based diets decreased the ileal digestibility of starch in broiler chickens (Abdollahi et al., 2012). However, in another of their studies, starch digestibility was unaffected by the conditioning temperature (60, 75 and 90°C) in maize-based diets (Abdollahi et al., 2010). In addition, by comparing the ileal nitrogen digestibility of wheat, sorghum and maize diets, it was found that the conditioned wheat diet had the highest digestibility at 75°C. On the contrary, at 75°C, the conditioned maize and sorghum diet had significant negative effects on the digestibility of nitrogen (Abdollahi et al., 2010) in broiler chickens. These results show that the suitable conditioning temperature of different grain-based diets varies.

As an important cereal, sorghum has broad developmental prospects in swine production; on some level, sorghum is believed to be an economical replacement for maize (Liu et al., 2013; Paulk et al., 2015). Previous studies have shown that sorghum starch normally has a higher gelatinization temperature than both maize and wheat (Taylor and Dewar, 2001). Another study on maize and sorghum diets found that the use of sorghum was superior to that of maize with the addition of fat and had a better ratio of backfat fatty acids and better meat quality, but improper pelleting temperatures may compromise the nutrient value of sorghum (Benz et al., 2011). Remarkably, sorghum is rich in resistant starch, which has been reported to be partly or totally fermented by the colonic microbiota and is beneficial to the colonization of *Bifidobacterium* (Da et al., 2005). However, it is not clear whether the conditioning temperature of sorghum-based diets can be attributed to alterations in the gut microbiota. Considering some adverse effects of an excessively high or low conditioning temperature, it is necessary to identify the optimum conditioning temperature for sorghum-based diets in pigs. To our knowledge, research into conditioning temperatures of sorghum-based diets has concentrated on poultry more than pigs (Loar et al., 2014; Abdollahi et al., 2011). It's worth mentioning that digestive tracts of pigs and poultry are very different so that findings in poultry may or may not apply to pigs. The objectives of this study were to evaluate the effect of the conditioning temperature on the pelleting characteristics, nutrient digestibility and gut microbiota of sorghum-based diets for growing pigs and to comprehensively select the appropriate conditioning temperature for sorghum diet processing.

2. Materials and methods

2.1. Feed processing and dietary treatment

Grain sorghum containing 10.21% crude protein (CP), 2.67% crude fiber, and 12.20% moisture was obtained from a commercial source (New Hope Liuhe Feed Ltd., Shandong, China), as shown in Table 1. The experimental diets were sorghum-based diets with sorghum conditioning at 5 different temperatures (65°C, 70°C, 75°C, 80°C, 85°C), which were achieved using a modulator and screens with 3.0 mm openings. Diets were steam-pelleted through a Palmer PP300 pellet press (Palmer, Milling Engineering, Griffith, NSW, Australia). The diets were also subject to conditioning temperatures of 65°C, 70°C, 75°C, 80°C, 85°C by the controlled introduction of steam into the conditioner with a residence time of 7 s and the conditioning temperatures were continuously recorded by a thermal probe at the exit of the conditioner. Samples of the pelleted diets were collected to determine the contents of gelatinized starch and resistant starch and the solubility of protein.

Individual pellet samples were inserted between a pressure piston and a bar, and by increasing the pressure applied by the pressure piston, the force (Newton) required to break the pellets was determined as a measure of pellet hardness (Svihus et al., 2004). Each sample was measured 15 times. The gelatinized and resistant starch contents of the diets were determined using Megazyme assay kits (Megazyme International Ireland Ltd., Wicklow, Ireland). Protein solubility was determined according to the method of Dale (1987).

The structure of the starch and protein in the sorghum samples (mash) was observed by scanning electron microscopy (JSM-6390/LV). The sorghum samples were freeze-dried, and an adhesive was placed on the surface of the sample after the gold-plated film was examined. Each observation point was magnified by approximately 1500 times, and clear, high-definition structure photos were selected for comparison.

2.2. Nutrient digestibility (in vitro) of sorghum at different conditioning temperatures

The dry matter (DM) and CP digestibility were evaluated according to a previously described *in vitro* digestion method (Gauthier et al., 1986). The enzyme incubation and dialysis procedures consisted of two-step proteolysis at 40°C, a 1 h incubation of the sample with pepsin (P7000; Sigma, St. Louis, MO, US) at pH 2.0, followed by proteolysis of pancreatic enzymes (P1750; Sigma) at pH 7.0 for 6 h in dialysis bags (ET9004; Sigma) with a 12,000–14,000 molecular weight cutoff for the continuous elimination of the digested products into a replaceable buffer. After proteolysis, the buffer was substituted with ice water to terminate the protease digestion. To

Table 1
Ingredient and chemical composition (g/kg as fed) of the basal diets.

Ingredients (%)	Sorghum-based diet
Sorghum	71.84
Extruded soybean	5.00
Soybean meal	14.05
Soybean oil	0.12
Flour	5.00
Limestone	1.06
Dicalcium phosphate	0.97
Salt	0.36
Lysine-H_2SO4	0.75
Methionine	0.17
Threonine	0.15
Tryptophan	0.01
Phytase	0.02
Premix[a]	0.50
Chemical composition	
Gross energy (MJ·kg-1)[b]	16.24
Dry matters (%)[c]	87.84
Crude protein (%)[c]	18.72
Ether extract (%)[c]	4.10
Crude fiber (%)[c]	2.23
Nitrogen free extract (%)[c]	57.03
Ash (%)[c]	6.20
Ca (%)[c]	0.84
P (%)[c]	0.53

[a] Provided per kilogram of diet: vitamin A,11,750 IU; vitamin D3, 50 IU; vitamin E, 50 IU; vitamin K, 1.75 mg; vitamin B1, 1 mg; vitamin B 2, 10 mg; vitamin B 6, 1 mg; vitamin B 12, 27.5 mg; niacin, 38 mg; calcium pantothenate, 35.75 mg; choline chloride, 750 mg; biotin, 100 μg; folic acid, 0.5 mg; Cu, 125 mg as copper sulfate; I, 0.75 mg as potassium iodide; Fe, 152.5 mg as iron sulfate; Mn, 35 mg as manganous oxide; Mg, 125 mg as magnesium sulfate; and Zn, 137.5 mg as zinc sulfate.
[b] Calculated value.
[c] Analyzed value.

remove all of the digested products, the dialysis bags were filtered in a water bath (0°C) that was placed on a magnetic stirrer for 72 h. Finally, the contents of the dialysis bags were freeze-dried and retained for DM and CP determination. The digestibility of DM or CP *in vitro* was calculated based on the different contents of DM or CP before and after digestion in the sample divided by the contents of DM or CP before digestion.

2.3. Digestibility experiment with growing pigs (in vivo)

The animal handling protocol (permit number: HZAUSW2017-0006) followed in the current study was approved by the Animal Care and Use Committee of the College of Animal Sciences and Technology, Huazhong Agricultural University, and was in compliance with the National Research Council's Guide for the Care and Use of Laboratory Animals. The New Hope Liuhe company approved the animal studies. Five Duroc × Landrace × Yorkshire growing castrated boars with an initial body weight (BW) of 21.40 ± 0.39 kg were individually fed in pens that were equipped with a feeder and a nipple drinker. The environment temperature was approximately 21-25°C. Pigs were surgically fitted with a T-cannula at the distal ileum using the procedures described by Stein et al.(1998). After recovering from surgery, five pigs were selected from the seven pigs to be used as experimental animals. Five pigs were allotted to 5 dietary treatments in a 5 × 5 Latin square design with 5 diets (Table 1) that included titanium dioxide (TiO_2); the experiment lasted for 35 d (Table 2). Chyme samples from each pig were collected on days 6 and 7 over a 10 h period each day (between 0800 and 1800 h) (Jacela et al., 2007). A few drops of 4.6 g/L formic acid were added to the collected chyme to prevent degradation by microorganisms and were then stored at -20°C. Feces from each pig were collected between 0830 and 2200 h from the fifth day to the end of the week for each trial period. Once collected, the fecal samples were mixed with HCl at a concentration of 10% to limit microbial growth and reduce the loss of ammonia. Then, the fecal samples were immediately stored at −20°C. At the end of the collection period, fecal samples from each pig were pooled and dried in an oven at 65°C for 72 h. After drying and grinding, subsamples were obtained from the total collected fecal samples for chemical analysis. The gross energy (GE) contents of chyme and feces were determined using a bomb calorimeter (Parr Instrument Company, Moline, IL, US). Detection of DM and CP was performed according to the National standard method [Standard of the People's Republic of China (1994, 2006); GB/T 6432 and 6435]. The starch content of the chyme was determined according to Kamphues et al(2007). TiO_2 in the feed and chyme (or feces) was analyzed by spectrophotometry at 450 nm after ashing (Short et al., 1996).

The calculations of the apparent digestibility of DM, CP, and GE and the ileal digestibility of starch were according to the

Table 2
Design of digestibility experiment for growing pigs[a].

Trial period	Pig number				
	NO.1	NO.2	NO.3	NO.4	NO.5
First week	D	C	E	B	A
Second week	E	D	A	C	B
Third week	A	E	B	D	C
Fourth week	B	A	C	E	D
Fifth week	C	B	D	A	E

[a] A, B, C, D, E means the sorghum-based diets in conditioning temperature of 65℃, 70℃, 75℃, 80, 85℃. Five Duroc × Landrace × Yorkshire growing castrated boars with an initial body weight (BW) of 21.40 ± 0.39 kg were individually fed in pens that were equipped with a feeder and a nipple drinker. The environment temperature was approximately 21–25℃. Pigs were surgically fitted with a T-cannula at the distal ileum.

following formula: 1-the nutrient content of chyme (or feces)/the TiO_2 content of chyme (or feces) × the TiO_2 content of feed/ the nutrient content of feed × 100%

2.4. Feeding experiment in pigs

Three hundred Duroc × Landrace × Yorkshire pigs (initial BW = 45.73 kg) were randomly allocated to 5 treatments, resulting in 12 pigs per pen and 5 pens per treatment. Pigs were fed the sorghum-based diets at different conditioning temperatures (65℃, 70℃, 75℃, 80℃, 85℃). The basal diet was a sorghum-soybean meal-based diet that was formulated according to the nutrient requirements recommended by the NRC (1998). The ingredients and chemical composition of the basal diet are shown in Table 1. The experiment lasted for 25 d, and the average daily gain (ADG), average daily feed intake (ADFI), and feed conversation ratio (FCR) were determined by weighing the pigs and measuring feed disappearance every 5 d. According to Heo et al. (2008), feces were visually examined daily to determine the fecal consistency scores and the incidence of diarrhea. At the end of the experiment, 5 pigs from each treatment (randomly collected 1 pig per pen) were sacrificed by administering a pentobarbital overdose after monitoring was finished. The abdominal cavity of each pig was opened, and the entire small and large intestines were removed. The small intestine and the cecum were carefully dissected from the mesentery. The duodenum, jejunum, ileum, and cecum were classified and secured with ligatures to avoid digesta flow into other parts of the gut (Sauer et al., 2012). Digesta from the duodenum, jejunum, ileum, and cecum for quantification of microbiota were aseptically collected and placed in an ice-water bath until storage at −80℃. Fecal samples were randomly collected from 6 pigs per pen, and fecal samples from each pen were pooled.

2.5. Real-time PCR assay for the quantification of microbiota in the gut and feces of piglets

Digesta from duodenum, jejunum, ileum, cecum, and fecal samples were collected for the Real-time PCR assay. The species-specific PCR primers are listed in Table 3. Real-time PCR was performed on an iCycler IQ real-time detection system using the iCycler optical system interface software version 2.3 (Bio-Rad, Veenen-daal, Netherlands) as previously described (Namkung et al., 2004). The reaction mixture (25 μL) consisted of 12.5 μL of a master mix (IQ SYBR Green Supermix; Bio-Rad), 0.2 μM of each primer set, and 5 μL of template DNA. The amount of DNA in each treatment was determined, and the mean values were calculated. A standard curve was generated by using the serially diluted 16 S rRNA gene amplicons obtained from *Lactobacillus sp*. The species-specific primer LAC1 and the primer Lab0677 (Su et al., 2008) were used for the quantification of *Lactobacillus* sp. with the following conditions: an initial DNA denaturation step at 95 °C for 3 min, followed by 40 cycles of denaturation at 95 °C for 30 s and primer annealing and extension at 60 °C for 1 min. Total *Bifidobacterium* sp. were quantified using the following PCR program: 2 min at 50 °C,10 min at 95 °C, followed by 40 cycles of 15 s at 95 °C and 1 min at 60 °C (Delroisse et al., 2006). Total *E. coli* were quantified using the combination of the forward primer K88AD and reserve primer K88AD (Alexa et al., 2001) and the following cycling program: after the initial denaturation 92 °C for 45 s, 40 cycles were applied at 50 °C for 45, and binding and extension at 75 °C for 45 s.

Table 3
Primers used for several microflora.

Bacteria	Primer	Sequence (5′-3′)	Reference
Lactobacillus sp.	Forward: LAC1	AGCAGTAGGGAATCTTCCA	Su et al. (2008)
	Reverse: Lab0677	CACCGCTACACATGGAG	
Bifidobacterium sp.	Forward: bifido	CGCGTCYGGTGTGAAAG	Delroisse et al. (2006)
	Reverse: bifido	CCCCACATCCAGCATCCA	
Escherichia coli	Forward: K88AD	GGCACTAAAGTTGGTTCA	Alexa et al. (2001)
	Reverse: K88AD	CACCCTTGAGTTCAGAATT	

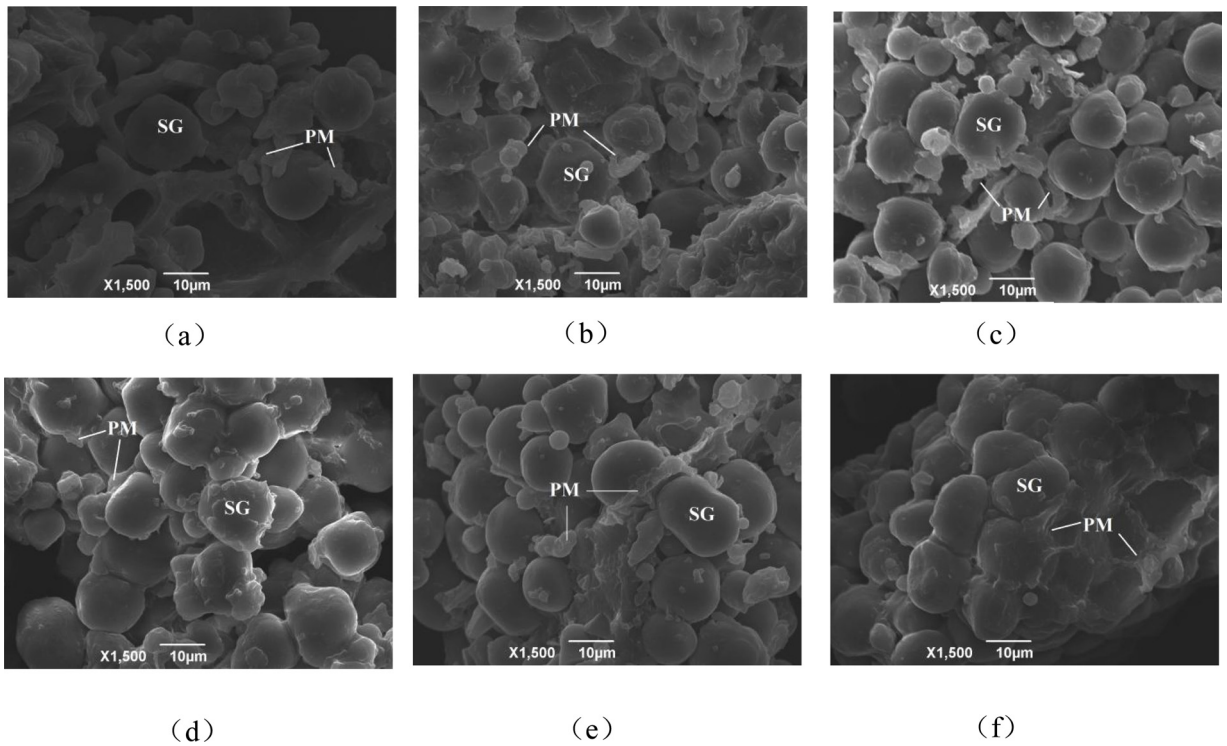

Fig. 1. Effect of conditioning temperature on structure of starch and protein (Scanning electron microscopy).
(a) Crushed sorghum grain without conditioning; (b) the sorghum after steam-conditioning in 65℃; (c) the sorghum after steam-conditioning in 70℃; (d) the sorghum after steam-conditioning in 75℃; (e) the sorghum after steam-conditioning in 80℃; (f) the sorghum after steam-conditioning in 85℃. PM protein matrix (protein bodies), SG starch granule.

2.6. Statistical analysis

Data were analyzed as a randomized complete block design with the pen used as the experimental unit. Pigs were blocked based on weight in all experiments, and ANOVA was performed using the MIXED procedure of SAS (SAS Inst., Inc., Cary, NC, US). Contrasts were used to determine the effect of the conditioning temperature on sorghum-based diets. Linear and quadratic polynomial contrasts were used to determine the effect of the increasing conditioning temperature of sorghum. The relationships between conditioning temperature and the digestibility were determined by iterative nonlinear least-squares regression. Data are presented as the least squares means. Means were considered significantly different at $P < 0.05$.

3. Results

3.1. Effect of the conditioning temperature of sorghum on the processing characteristics

It was found that the results of various related studies on sorghum-diets were different due to the different composition of sorghum diets. Therefore, in this study, sorghum was used as the only research object and scanning electron microscopy was used to study the differences in the protein and starch structures of sorghum under different conditioning temperatures. The scanning electron microscopy results of sorghum samples after conditioning are shown in Fig. 1. Obviously, it was seen that the crosslinking of the PM and SG after conditioning in sorghum were closer than those without conditioning; additionally, with a gradually increasing conditioning temperature, the crosslinking among PM was closer, and SG were more tightly encapsulated by PM in sorghum.

The results of the pelleting characteristics and pellet quality of sorghum-based diets are shown in Table 4. It was found that increasing the conditioning temperature contributed to the increase of resistant starch (linear, $P < 0.05$). Additionally, with the increase of temperature from 65℃ to 85℃, the gelatinized starch content of pellets first increased and then decreased, showing a quadratic relationship ($P < 0.05$) between starch gelatinization and the conditioning temperature. Furthermore, there was an interaction (quadratic, $P < 0.05$) between the conditioning temperature and RS/GS ratio. The RS/GS ratio first decreased, but increasing the conditioning temperature to 85℃ ultimately increased the RS/GS ratio. Moreover, the increasing temperature contributed to the decrease of the protein solubility of the pellet (linear, $P < 0.05$) and increase of the hardness of the pelleted diets (linear, $P < 0.01$).

Table 4
Effect of conditioning temperature on processing characteristics and pellet quality. [a].

Item	Conditioning temperature (°C)					SEM	*P*-value	
	65	70	75	80	85		Linear	Quadratic
Resistant starch (%)	2.32	2.35	2.44	2.49	3.12	0.153	0.002	0.215
Gelatinised Starch (%)	14.51	15.89	16.50	16.95	16.43	0.250	0.001	0.027
RS/GS ratio (%)	15.99	14.79	14.81	14.70	18.99	0.757	0.132	0.023
Protein solubility(%)	73.74	72.40	69.50	65.30	59.71	1.477	0.001	0.110
Hardness(Newton)	13.33	16.07	17.44	18.82	21.66	0.076	0.0003	0.0013

[a] Pellet samples of each group were used to determine processing characteristics. RS, Resistant starch; GS, Gelatinised starch.

3.2. Effect of the conditioning temperature on the nutrient digestibility (in vitro) of sorghum

Table 5 shows the effect of the conditioning temperature on the nutrient digestibility (*in vitro*) of sorghum. Both the DM and CP digestibility first increased and then decreased with the increase of the conditioning temperature from 65 to 85°C, and there was an obvious quadratic relationship ($P < 0.05$) between DM digestibility and the conditioning temperature as well as the CP digestibility ($P < 0.05$). Moreover, the digestibility of both DM and CP, which ranged from 70 to 80 °C, was significantly higher than that of the other groups.

3.3. Effect of the conditioning temperature on the nutrition coefficient (in vivo) of sorghum-based diets

In the present study, fecal samples were collected to analyze DM and CP digestibility *in vivo*. T-cannulas were surgically fitted at the distal ileum for chyme collection and to study the *in vivo* starch digestibility of sorghum-based diets. Table 6 shows the effect of the conditioning temperature on the apparent *in vivo* digestibility coefficient, and no significant difference was observed with regard to DM digestibility($P > 0.05$). Interestingly, both the CP and energy results showed a quadratic relationship ($P < 0.05$) with the increasing conditioning temperature, to a maximum of 75 and 80°C ($P < 0.05$).

Additionally, according to Table 6, it was found that among the five temperature treatments, no significant differences were observed in regard to DM and GE ileal digestibility ($P > 0.05$). However, there was an increase of ileal digestibility of CP and then decreased with increasing conditioning temperature from 65 to 85°C, and reached the greatest ileal digestibility at 75 and 80 °C (quadratic, $P < 0.05$). In addition, the ileal digestibility of starch had a similar variation trend with CP ileal digestibility (quadratic, $P < 0.01$), and the ileal digestibility of CP at 75 and 80 °C was significantly higher than that at the other temperatures. Compared with the other conditioning temperatures, sorghum-based diets at 75 and 80°C were digested and absorbed more effectively.

3.4. Effect of the conditioning temperature of sorghum-based diets on the growth performance of growing pigs

Table 7 shows the effect of the sorghum conditioning temperature on pig growth performance. We found that among the five treatments, no significant difference was observed in regards to the ADFI and diarrhea ratio. However, both ADG and FCR first increased and then decreased with the increase of the conditioning temperature from 65 to 85°C, which showed an obvious quadratic relationship ($P = 0.017$) with the increasing conditioning temperature of sorghum-based diets. Furthermore, both of the largest ADG and FCR values ($P < 0.05$) of the pigs occurred for temperatures of 75 and 80°C. Altogether, it seemed that diets with a conditioning temperature of sorghum that ranged from 75 to 80°C led to a better performance on growing pigs.

3.5. Effect of the conditioning temperature of sorghum-based diets on the gut microbiota in growing pigs

To evaluate the effect of the conditioning temperature on the gut microbiota in growing pigs, real-time PCR was used to quantify *Lactobacillus* sp., *Bifidobacterium* sp., *and E. coli* in duodenum, jejunum, ileum, cecum and feces samples of pigs (Table 8). The assay showed that the gene copy numbers of total *Lactobacillus* sp. 16S rRNA in the duodenum, ileum and feces were greater ($P < 0.05$) in pigs fed with sorghum at conditioning temperatures of 75 and 80°C than the other groups. In addition, there was an increase of the numbers of *Lactobacillus* sp. in the jejunum and cecum and then decreased with increasing conditioning temperature from 65 to 85°C,

Table 5
Effect of conditioning temperature of sorghum on nutrient digestibility (*in vitro*) of DM and CP (%)[a].

Item	Conditioning temperature(°C)					SEM	*P*-value	
	65	70	75	80	85		Linear	Quadratic
DM	30.58	33.15	33.34	34.61	26.01	0.876	0.101	0.030
CP	49.00	54.50	56.02	55.14	44.82	1.209	0.253	0.024

[a] Three samples of each group were used to determine nutrient digestibility(*in vitro*) of DM and CP (%). DM dry matter, CP, crude protein.

Table 6
Effect of conditioning temperature of sorghum-based diets on nutrition apparent digestibility and ileal apparent digestibility in pigs (*in vivo*) (%).

Item	Conditioning temperature (°C)					SEM	*P*-value	
	65	70	75	80	85		Linear	Quadratic
Nutrition apparent digestibility (feces)								
DM	88.90	88.98	89.52	89.39	88.40	0.237	0.720	0.361
CP	84.54	84.98	86.56	86.54	85.06	0.421	0.101	0.025
Energy	87.00	87.02	90.30	89.97	86.50	0.244	0.240	0.046
Nutrition ileal apparent digestibility								
DM	85.95	86.19	86.16	86.07	86.38	0.310	0.577	0.136
CP	85.33	86.72	87.94	87.95	85.01	0.410	0.640	0.042
GE	86.44	86.39	87.97	86.75	85.66	0.289	0.564	0.108
Starch	90.41	91.29	92.26	91.58	89.96	0.240	0.729	0.0011

Table 7
The effect of conditioning temperature on growth performance in pig[a].

Item	Conditioning temperature (°C)					SEM	*P*-value	
	65	70	75	80	85		Linear	Quadratic
ADFI(kg/d)	2.238	2.152	2.269	2.268	2.216	0.0216	0.124	0.693
ADG(kg/d)	0.971	0.974	0.988	0.985	0.899	0.0111	0.086	0.017
FCR(kg/kg)	2.446	2.438	2.317	2.328	2.458	0.0270	0.253	0.025
Diarrhea ratio(%)	2.591	2.602	1.695	1.797	2.335	0.0570	0.086	0.027

[a] A total of 300 Duroc × Landrace × Yorkshire pigs (5 pens per treatment and 12 pigs per pen) with an average initial BW of 45.73 kg and an average final BW of 70.04 kg. Pigs were fed a sorghum-soybean meal-based diet supplemented with sorghum at different conditioning temperatures (65°C, 70°C, 75°C, 80°C, 85°C).

Table 8
Quantitative real-time PCR analysis of total Lactobacillus sp., Bifidobacterium sp., *Escherichia coli* in porcine gut and feces samples [Log 10 (copies/g wet weight)][a].

Item	Conditioning temperature (°C)					SEM	*P*-value	
	65	70	75	80	85		Linear	Quadratic
Lactobacillus sp.								
Duodenum	7.75	7.82	8.15	8.05	7.92	0.32	0.125	0.047
Jejunum	7.25	7.48	7.56	7.49	7.37	0.42	0.113	0.097
Ileum	7.65	7.78	8.11	8.35	8.12	0.23	0.067	0.038
Cecum	8.21	8.26	8.35	8.47	8.42	0.54	0.134	0.087
Feces	8.41	8.34	8.64	8.75	8.69	0.21	0.076	0.043
Bifidobacterium sp.								
Duodenum	6.42	7.25	7.62	7.89	7.72	0.35	0.107	0.036
Jejunum	7.18	7.36	7.27	7.39	7.48	0.44	0.186	0.101
Ileum	7.45	7.49	7.58	7.74	7.48	0.12	0.064	0.021
Cecum	7.24	7.35	7.48	7.72	7.69	0.45	0.043	0.023
Feces	8.42	8.44	8.62	8.57	8.46	0.26	0.095	0.075
Escherichia coli								
Duodenum	7.95	7.96	7.74	7.84	7.98	0.36	0.061	0.047
Jejunum	8.16	8.07	7.55	7.49	7.89	0.65	0.106	0.016
Ileum	8.48	8.42	8.37	8.40	8.49	0.47	0.094	0.048
Cecum	8.98	8.85	8.48	8.78	8.82	0.15	0.086	0.035
Feces	9.24	9.18	8.91	9.03	9.12	0.34	0.133	0.029

[a] A total of 300 growing pigs (5 pens per treatment and 12 pigs per pen) with an average initial BW of 45.73 kg and an average final BW of 70.04 kg. Gut samples were randomly collected from 1 pigs per pen, fecal samples were randomly collected from 6 pigs per pen, and the fecal samples from each pen were pooled.

and the largest numbers of *Lactobacillus* sp. in the jejunum and cecum occurred over the temperature range of 75 and 80°C. For *Bifidobacterium* sp., similarly, the results showed that the bacterial numbers in the duodenum, ileum and cecum were increased ($P < 0.05$) with sorghum at conditioning temperatures of 75 and 80°C compared to other groups in the experiment, and the greatest numbers of *Bifidobacterium* sp. in the jejunum and feces were also occurred over the temperature range of 75 to 80°C. Furthermore, all the *E. coli* results showed an obvious quadratic relationship ($P < 0.05$) with the increasing conditioning temperature of sorghum-based diets, but compared to the other groups, conditioning temperatures of 75 and 80°C led to the smallest numbers of *E. coli*.

4. Discussion

In the present study, our pelleting characteristic results showed that increasing the conditioning temperature reduced the protein solubility of sorghum-based diets, which was expected and consistent with the results of previous research in poultry (Selle et al., 2013). It has been demonstrated that a high processing temperature will affect the noncovalent interactions of proteins and change the structures of proteins, which may expose hydrophobic amino acid residues within the protein molecule and result in a decrease of protein solubility (Goelema and Smits, 1999). Furthermore, as shown in the scanning electron microscopy images, with an increasing conditioning temperature, the binding of the sorghum protein matrix increased. This finding is consistent with the results of Duodu et al. (2003). It has been reported that a high conditioning temperature allows kafirin in sorghum to produce disulfide bonds during hydrothermal processing in the cysteine-rich beta- and gamma-kafirin fractions which are located in the periphery of kafirin protein bodies, thus increasing the protein binding, which could be another explanation for the sorghum protein solubility results (Duodu et al., 2003).

Regarding the characteristics of starch, it is known that starch gelatinization directly influences feed viscosity during the conditioning process and starch digestion in the small intestine (Svihus et al., 2005a). Our results showed that increasing the conditioning temperature promoted starch gelatinization; however, when the temperature increased to 85℃, starch gelatinization was reduced. This result was different from the results of Lewis et al. (2015) in corn-based diets. In general, as the conditioning temperature is increased, at some level, the breakage of the chemical bonds in starch granules is promoted to induce starch gelatinization. Nevertheless, in sorghum-based diets, starch granules are surrounded by kafirin protein bodies, and these interactions between starch and protein may reduce the breakage of chemical bonds in starch granules and affect starch gelatinization (Rooney and Pflugfelder, 1986). As shown in the scanning electron microscopy images, with an increasing conditioning temperature, encapsulation of the starch granule by protein was enhanced, possibly because an excessive conditioning temperature promotes the extension of the protein carbon skeleton, allowing the encapsulation of starch granules by protein (Tudorica et al., 2002). Due to the increase in the interactions between the proteins and starch in sorghum, an excessively high temperature may inhibit the gelatinization of starch. However, the starch-protein interactions in sorghum cannot be over-estimated despite the fact that they are not perfectly understood yet.

Pellet hardness is an important processing indicator of pellet feed. Our results showed that increasing the conditioning temperature enhanced the pellet hardness of sorghum-based diets, which was consistent with the results of a previous study (Abdollahi et al., 2010). In this previous study, it was found that the hardness of maize and sorghum broiler diets increased with increasing temperature (60℃-90℃). In feed processing, low hardness is not conducive to feed storage and transportation, while excessive hardness may cause wear and tear on the ring mold of a granulator. Therefore, according to our results, it seems that the conditioning temperature between 70 and 80℃ is appropriate for the hardness of sorghum-based diet pellets.

Additionally, researchers focused on nutritional implications determined that the conditioning temperature of feed ingredient influenced the digestibility of nutrients on the basis of the results of *in vitro* and *in vivo* trials (Loar et al., 2014; Bryden et al., 2009). To study the effect of the conditioning temperature on the nutrient digestibility of sorghum-based diets, we first performed an *in vitro* digestion trial for sorghum to determine the digestive characteristics of sorghum itself. Our results showed that *in vitro*, the digestibility of both DM and CP in sorghum had a quadratic relationship with the increasing conditioning temperature. There is no doubt that heat processing can change the spatial structure of a protein and stretch its molecular structure, which may increase the contact region of digestive enzymes and proteins, thereby enhancing protein digestibility (Traylor et al., 1998). However, an excessively high temperature will promote the reaction of amino acids and glucose *via* the Maillard reaction, which may reduce protein solubility and lead to the decrease of CP digestibility (Thomas et al., 1998). After the *in vitro* digestion trial, an interesting result was observed. The results showed that increasing the conditioning temperature had no effect on DM digestibility and GE in the ileum of pigs. However, a quadratic relationship between the apparent digestibility and ileum apparent digestibility of CP and the conditioning temperature was observed as the conditioning temperature increased. A previous study have reported that increasing the conditioning temperature results in protein denaturation and increases the digestibility of CP (Portela, 1978). However, as shown in our previous results, an excessively high temperature increases the binding of kafirin in sorghum, thus decreasing the protein solubility, which may decrease the digestibility of CP.

As for the *in vivo* digestibility of starch, in this study, it was observed that increasing the conditioning temperature resulted in increased starch digestibility; as the conditioning temperature increased, there was a quadratic relationship between the apparent digestibility and ileum apparent digestibility of starch and the conditioning temperature. The reduction of starch digestibility at 85℃ could be due to the large increase in the resistant starch content achieved by conditioning at 85℃. The results of the resistant starch and RS/GS ratio showed that increasing the conditioning temperature increased the RS content, although a higher content of GS was also observed in the pellet diet conditioned at 85℃, but the RS/GS ratio was much higher in this diet compared to the diets conditioned at 70, 75 and 80℃. Furthermore, another explanation could be the cross-linking of the protein matrix and starch. Starch granules were embedded in the protein more tightly as the temperature increased, which decreased the digestibility of starch. The highest conditioning temperature (85℃) increased resistant starch and some of the starch cross-link with gliadin in the pellet, which might have reduced the starch utilization of the small intestine. In addition, to our knowledge, increasing the conditioning temperature of the pellet results in greater hardness, which may increase the difficulty of softening the pellet in the stomach and during digestion(Abdollahi et al., 2010).

The results of previous studies have indicated that increasing the conditioning temperature of grains contributes to macronutrient digestion, with the consequences of improved animal growth performance (Selle et al., 2013; Silversides, 1999). Regarding the effect of the conditioning temperature of sorghum-based diets on growth performance, the present indicated that the efficiency of the

digestion and utilization of sorghum-based diets in pigs reached the highest values at temperatures of 75 and 80°C, as the ADG reached a maximum value, and FCR and diarrhea ratio reached minimum values at temperatures from 75 to 80°C. Similarly, Lundblad et al (2011) found that ADG and feed utilization increased as the conditioning temperature increased from 47 to 90°C in wheat-based diets in broiler. However, other researchers failed to observe improvements in ADG or FCR in pigs when increasing the conditioning temperature. These different effects of conditioning temperature increases may be attributed to the difference in the grains used in the experiments. Cowieson et al. (2005) found that in broilers fed wheat-based diets, increasing the pelleting temperature from 70°C to 85°C resulted in increased weight gain, whereas our results showed that feed conditioned at 75°C to 80°C led to better ADG and FCR than the other conditioning temperatures. This finding was consistent with the results of *in vivo* nutrient digestibility due to the greatest digestibility of starch and protein being observed at 75 and 80°C; therefore, ADG and FCR were better in this temperature range.

A Previous study indicated that feed processing can modify the composition of the microbiota, affecting the total bacterial count and the genera and predominant species (Bao et al., 2016). Our quantitative real-time PCR results demonstrated that the different conditioning temperatures of sorghum-based diets resulted in changes in the gut bacteria diversity in pigs. Interestingly, the greatest numbers of beneficial *Lactobacillus* sp. and *Bifidobacterium* sp. occurred at 75 and 80°C. Whereas, conditioning temperatures of sorghum-based diets of 75 and 80°C led the smallest number of *E. coli* in all the intestinal segments and feces. This bacterial community phenomenon may be due to the increasing content of resistant starch. It has been reported that resistant starch could potentialize the bifidogenic effect of leguminous material in rats (Da et al., 2005). Another explanation of the bacterial community phenomenon may be the relationship between resistant starch and protein fermentation. Recent *in vitro* studies have indicated that the addition of corn resistant starch may influence protein fermentation and increase the copies of total bacteria, such as *Bifidobacterium* and *Lactobacillus* (He et al., 2017).These findings are consistent with our results. The rich contents of soluble dietary fiber and resistant starch in sorghum may be the reason for *Bifidobacterium* sp. and *Lactobacillus* sp. colonization (Hughes et al., 2007). These bacteria are seen as beneficial to healthy hosts (Cummings et al., 2004), which is probably why conditioning temperatures of 75 and 80°C resulted in increased ADG in the pig performance experiment. Moreover, as one of the most important causes of diarrhea in pigs (Fairbrother et al., 2005), the reduced *E. coli* numbers may be the reason why the diarrhea ratio was the smallest at 75 and 80°C.

5. Conclusion

Overall, the data from the present study show that the ileal digestibility of starch and protein and growing pigs' performance at 75 and 80°C reach maximum values to maintain healthy intestinal microflora; in addition, the temperatures for starch gelatinization and pellet hardness were also appropriate. This study suggests that 75 and 80°C are the best conditioning temperatures for sorghum-based diets in pigs. Future studies are warranted to investigate the possible ways of maintaining higher nutrient digestibility and energy utilization obtained by different conditioning methods while simultaneously achieving a good pellet quality and higher growth performance of pigs.

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Declaration of Competing Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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