



Effect of particle size of wheat on nutrient digestibility, growth performance, and gut microbiota in growing pigs



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ABSTRACT

This research was conducted to determine the effect of particle size of wheat on digestibility (in vitro and in vivo), growth performance, and gut microbiota in growing pigs. The pig were fed wheat–soybean meal-based diets containing 70.85% wheat ground to mean particle sizes of 330, 430, 450, 470, 580, and 670 μm . Before blended into diet, the ground wheat were used to measure dry matter (DM) and crude protein (CP) in vitro digestibility by the two-step method (pepsin and trypsin). Then, a total of 30 barrows with an average bodyweight (BW) of 21.2 ± 1.2 kg were fed 6 diets containing different particle sizes of wheat (330, 430, 450, 470, 580, and 670 μm) to determine apparent digestibility of DM, gross energy (GE), and N in vivo. Finally, the effect of the particle size of wheat on growth performance and microbiota was assessed by feeding the same diets for 28 d with 6 pens per diet and 10 pigs (initial BW: 10.4 ± 0.9 kg) per pen. Reducing particle size from 670 to 330 μm increased the DM digestibility from 17% to 26% (linear, $P < 0.05$) and CP digestibility from 55% to 66% (linear, $P < 0.05$). Digestibility experiment in growing pigs demonstrated that particle size exerted no effect on DM and GE digestibility in vivo. In contrast with in vitro digestibility, as the particle size of wheat reduced, apparent N in vivo digestibility increased first and then decreased, with the particle size of 430 μm supporting the greatest N digestibility (quadratic, $P = 0.028$). Average daily feed intake (ADFI) and gain/feed were unaffected by particle sizes. However, the diarrhea incidence increased ($P < 0.05$) with the particle size reduced. A quadratic effect ($P = 0.038$) of the particle size and average daily gain (ADG) was also observed, with the greatest ADG occurring with 430–470 μm . Moreover, the increase in the particle size of wheat from 430 to 470 μm increased ($P < 0.05$) the number of beneficial bacteria (*Bifidobacterium* sp. and *Lactobacillus* sp.), but also suppressed ($P < 0.05$) bacterial pathogens (*Escherichia coli*), which effectively promoted ($P = 0.02$) the diversity of gut microflora. The results in the study indicated that the particle size of wheat from 430 to 470 μm was acceptable in diets for growing pig.

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1. Introduction

Most of the feed ingredients are typically processed before being mixed into swine diets. It is widely accepted that grinding increases the surface area for enzyme action and influences gut fermentation and other digestive processes (Lahaye et al., 2008; Mößeler et al., 2010; Valencia et al., 2008). As a consequence, appropriate grinding can effectively improve nutrient digestibility and feed efficiency (Lawrence et al., 2003), resulting in a better animal growth performance (Seerley et al., 1988). However, it has been reported that fine grinding contributes to the development of ulcers in the esophageal region of the pig (Wondra et al., 1995a).

Stomach morphology is also negatively affected by excessive grinding of corn and sorghum. Reducing the particle size of grains increase the pig's susceptibility to harmful bacteria colonization (Hedemann et al., 2005; Mikkelsen et al., 2004). Considering some adverse effects of fine grinding, it would be necessary to identify the optimum particle size for pig diets.

To identify the optimal particle size, a great deal of efforts have been devoted to study nutrient digestibility. Previous investigations have showed that reduction in particle size improves the nutrient digestibility both in vitro and in vivo (Blasel et al., 2006; Al-Rabadi et al., 2009; Wondra et al., 1995a). In general, in vivo digestibility is greater than in vitro digestibility during the early part of incubation (Sun et al., 2006). The environment in the gastrointestinal tract is rather complicated; however, it is a general conclusion that the results getting from in vitro digestion experiments can be extrapolated to the digestion process under the physiological condition. Microbial fermentation in the hindgut of

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pigs is an important factor that can distinguish in vitro and in vivo digestion. The composition of the gut microbiota depends on a variety of exogenous factors such as diet (Leser et al., 2000), and the alteration in the diet can change microbial population in the gastrointestinal tract (Leser et al., 2000) and the distribution of microbiota (Krause et al., 1995). Although it has been demonstrated that difference in particle size influences gut fermentation (Lahaye et al., 2008; Valencia et al., 2008), it is not clear whether the difference of digestibility between in vitro and in vivo can be attributed to the alteration in gut microbiota.

In contrast to mounting research on corn and soybean meal (Fastinger and Mahan, 2003; Healy et al., 1994; Lawrence et al., 2003), little information exists on the optimization of grinding used for wheat. It is important to investigate the optimal particle size of wheat because wheat has become an important alternative feed ingredient for corn in pig diets. The experiment reported herein was to investigate the effect of wheat particle size on nutrient digestibility, growth performance, and gut microbiota in pigs fed wheat-based diets.

2. Materials and methods

2.1. Particle size processing and dietary treatment

Spring wheat (red and soft), containing 12.8% crude protein (CP), 5.2% fat, and 12.3% moisture, was obtained from a commercial source (New Hope Liuhe Feed Ltd., Shandong, China). The experimental diets were wheat–soybean meal-based diet with the wheat ground to 6 different particle size, which was achieved using a hammer mill (Jiangsu Muyang Co., Ltd., Jiangsu, China) and screens with 3.5-, 3.0-, 2.5-, 2.0-, 1.5-, and 1.0-mm openings. Three samples (approximately 500 g) of ground wheat were collected to determine the geometric mean particle size and in vitro nutrient digestibility. Mean particle size was measured using 13 sieves (4.00, 2.26, 1.70, 1.18, 0.85, 0.60, 0.43, 0.30, 0.21, 0.15, 0.11, 0.08, and 0.05 mm) and a pan on a sieve shaker (W. S. Tyler, Mentor, OH) using the ASAE (2001) method (S319.3). Wheat was milled to 6 mean particle sizes: 670, 580, 470, 450, 430, and 330 μm . A constant motor load during milling was maintained to measure the production rates and electrical energy consumption.

2.2. Nutrient digestibility (in vitro) of wheat with different particle size

The dry matter (DM) and CP digestibility were evaluated according to an in vitro digestion method described before (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 digested products into a replaceable buffer. After proteolysis, buffer was substituted by ice water to terminate the digestion of protease. To remove all of the digested products, dialysis bags were filtered in a water bath (0 °C) lying on a magnetic stirrer for 72 h. Finally, the content 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 difference content of DM or CP before and after digestion in sample divided by the content of DM or CP before digestion in sample.

2.3. Digestibility experiment with growing pigs

The protocol of this study was approved by the Animal Care

Table 1
Ingredient and chemical composition of basal diet.

Item	Content
Ingredients (%)	
Wheat	70.85
Soybean oil	1.52
Extruded soybean	3.00
Soybean meal	20.00
Limestone	1.23
Dicalcium phosphate	0.57
Sodium chloride	0.55
Vitamine-trace mineral premix ^a	1.00
Lys	0.84
Met	0.09
Thr	0.15
Chromic oxide	0.20
Chemical composition	
Gross energy (Mcal/kg) ^b	3.90
Dry matters (%) ^c	84.26
Crude protein (%) ^c	19.74
Ether extract (%) ^c	3.63
Crude fiber (%) ^c	2.54
Ash (%) ^c	2.16
Ca (%) ^c	0.81
P (%) ^c	0.54

^a Provided per kilogram of diet: vitamin A, 11,750 IU; vitamin D₃, 1500 IU; vitamin E, 50 IU; vitamin K, 1.75 mg; vitamin B₁, 1 mg; vitamin B₂, 10 mg; vitamin B₆, 1 mg; vitamin B₁₂, 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 manganese oxide; Mg, 125 mg as magnesium sulfate; and Zn, 137.5 mg as zinc sulfate.

^b Calculated value.

^c Analyzed value.

and Use Committee of College of Animal Sciences and Technology (Huazhong Agricultural University, Wuhan, China), and was carried out in accordance with the guidelines (NRC, 2011).

Thirty Duroc $\times$ Landrace $\times$ Yorkshire barrows with an average initial bodyweight (BW) of 21.2 ± 1.2 kg were allotted to 1 of 6 treatments in a randomized complete block design, with their BW as a block, and housed in individual metabolic crates, resulting in 5 replications per treatment. Pigs were fed the basal diet supplemented with different particle sizes (330, 430, 450, 470, 580, and 670 μm) of wheat. The basal diet was wheat–soybean meal-based diet and formulated according to the nutrient requirement recommended by NRC (1998). The ingredient and chemical composition of basal diet are shown in Table 1. Chromic oxide was added to the diets (0.2%) as an indigestible marker. All the diets were fed in a meal form. Piglets were subjected to a 7-d adaptation period followed by a 5-d total collection of feces. Pigs were provided ad libitum access to water and fed respective experimental diets equivalent to 4% of the initial BW, which were equally divided into 2 feedings and provided at 0800 h and 1600 h during both the adaptation and experimental periods. If there was any feed remaining from the previous feeding period, it was removed and subtracted from the amount offered to determine average daily feed consumption. Room temperature was maintained at 20–28 °C.

After the 7-d adaptation period, feces from each pig were collected between 0830 to 2200 h. Once collected, fecal samples were mixed with 15 mL of 6 N HCl to limit the microbial growth and reduce the loss of ammonia. After that, 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

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