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**Feeds**

# Effects of different processing techniques of palm kernel cake on processing quality of pellet feed, nutrient digestibility, and intestinal microbiota of pigs

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## Abstract

The present study was conducted to investigate the effects of extrusion, fermentation, and enzymolysis of palm kernel cake on processing quality of pellet feed, nutrient digestibility, and intestinal microbiota of pigs. First, the pretreatment parameters of extrusion, enzymolysis, and fermentation of palm kernel cake were optimized. Then, PKC after three processing techniques were used to prepare pellet feed. A total of 160 crossbred piglets (Duroc × Landrace × Yorkshire) with an average body weight of 28 ± 0.5 kg were used in an 8-wk feeding experiment. Pigs were randomly assigned to five treatments with four replicates per treatment and eight pigs per replicates. The five experimental groups were as follows: basal diet group (whole corn–soybean meal), 10% PKC group (**PKC**), 10% extrusion PKC group (**PPKC**), 10% enzymolysis PKC group (**EPKC**), and 10% fermented PKC group (**FPKC**), respectively. At the end of the experiment, four pigs from each treatment (randomly collected one pig per pen) were sacrificed by administering a pentobarbital overdose, the gut and blood samples were collected for the quantification analysis of microbiota, hematological parameters, and apparent total tract nutrient digestibility. The results showed that all three processing techniques significantly decreased the contents of crude fiber of PKC ($P < 0.01$), pulverization rate ($P < 0.01$), powder content ($P < 0.01$), and increased the hardness and gelatinization starch of pellet feed ($P < 0.05$) compared to PKC group. In addition, PPKC significantly improved the dry matter, crude protein, and ether extract content, blood indices and average daily feed intake compared to PKC group ($P < 0.01$), while the parameters were similar among FPKC, EPKC, and control group ($P > 0.01$). Furthermore, all three processing techniques significantly increased the *Lactobacillus* and decreased the *Escherichia* levels in feces or gut compared to PKC. Collectively, extrusion, fermentation, and enzymolysis of PKC had positively enhanced the pellet quality, growth performance, nutrient digestibility, and gut microbiota, extrusion exhibited a superior feeding effect compared to fermentation and enzymolysis.

## Lay Summary

Palm kernel cake (**PKC**) has lower nutritional value compared with soybean meal, cottonseed meal, and rapeseed meal, but its cost advantage is great, and it has been gradually used in the ruminant feeding. Due to its high crude fiber content, the processing technique applied to the PKC has a significant impact on its effectiveness. However, the different processing techniques of PKC on pellet quality, and performance of pigs have been poorly reported. The present study was conducted to investigate the effects of extrusion, fermentation, and enzymolysis pretreatment of PKC on processing quality of pellet feed, nutrient digestibility (in vivo), and intestinal microbiota of growing–finishing pigs. This study provides the optimal processing parameters of the three processing techniques, and demonstrated that PKC after processing could significantly improve the pellet quality, performance, and intestinal microbiota of growing–finishing pigs, while extrusion exhibited a superior feeding effect compared to fermentation and enzymolysis.

**Key words:** different processing techniques, growing and finishing pigs, intestinal microbiota, palm kernel cake, pellet feed

**Abbreviations:** ADFI, average daily feed intake; ADG, average daily gain; ALB, albumin; ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate transaminase; DNS, dinitrosalicylic acid; EPKC, enzymatic PKC; F/G, feed/gain ratio; FBW, final body weight; FPKC, fermented PKC; IBW, initial body weight; NSP, nonstarch polysaccharide; PKC, palm kernel cake; PPKC, extrusion palm kernel cake; TP, total protein

## Introduction

Soybean meal is currently the most common source of vegetable protein in livestock and poultry feed. Replacing soybean meal with a cheaper source of vegetable protein will help reduce feed cost (Yun et al., 2005). Palm kernel cake (**PKC**) is the residue obtained after oil extraction from African palm fruits. The annual output of palm cake in the world is more than 10 million tons, and the output growth rate is about 10%. The price of PKC is less than half of that of soybean meal. The crude protein content of PKC ranges from 14.0% to 21.3%, and the variation of CP value is related to the different processing methods (Pasaribu, 2018). Although PKC has lower nutritional value compared with soybean meal, cottonseed meal, and rapeseed meal, its cost advantage is great, and it has been gradually used in the feeding of ruminants.

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Traditionally, PKC has not been widely used in pig and poultry feed, mainly because it contains high levels of non-starch polysaccharide (**NSP**) and poor palatability. Dissolved in water, NSP increases the viscosity of chyme and reduces the digestibility of nutrients. At present, the processing techniques to eliminate anti-nutrition factors in plant feed include extrusion, fermentation, and enzymolysis. The extrusion technique can increase the soluble dietary fiber content in the ingredients, and promote the digestion of proteins, amino acids, and other nutrients in the extrudate in animals (Gajula et al., 2008; Hole et al., 2013). It has been reported that using a single screw extruding machine to treat PKC, when the temperature curve was 90, 100, and 110 °C, the crude fat and ash contents of PKC were not affected, but the crude fiber content was reduced by 21% (Faridah et al., 2020). Meanwhile, apparent metabolizable energy and crude fat digestibility of broilers increased by 6% and 32% respectively. Fermentation is a dynamic process involving microorganisms, substrates, and environmental conditions that transform complex substrates into simpler compounds (Niba et al., 2009). It has been shown that the content of hemicellulose and cellulose in the solid-state fermentation of PKC with *Lactobacillus* was significantly reduced, while the content of reducing sugar was significantly increased (Alshelmani et al., 2021). Enzymolysis refers to the process of degrading biological macromolecules such as protein and cellulose into small molecules such as free amino acids and glucose based on biological enzymolysis technology through the action of various decomposing enzymes such as protease and cellulase (Aguirre and Garro 2008). The contents of neutral detergent fiber, acid detergent fiber, cellulose, and hemicellulose were significantly reduced when PKC was treated with cellulolytic enzymes, but dry matter and ether extract contents were not significantly affected (Saenphoom et al., 2011). Therefore, it is of great significance to study the effects of different processing techniques on PKC antinutritional factors.

Pellet feed has the advantages of convenient transportation, storage, feeding, palatability, and improving the digestibility of animal nutrients (Colovic et al., 2010). However, many factors can affect the quality of pellet feed, among which the composition of raw materials has the greatest influence on the quality characteristics of pellet feed, accounting for about 40% of all factors (Colovic et al., 2010). The contents of protein, starch and cellulose of PKC will be changed after pretreatment or different processing techniques. In addition, PKC has a high content of crude fiber, which ferments into short-chain fatty acids at the end of the intestinal tract. Short-chain fatty acids provide energy for intestinal cells, which may regulate the structure of intestinal microbiota. Therefore, the present study was conducted to investigate the effects of extrusion, fermentation, and enzymolysis of palm kernel cake on processing quality of pellet feed, nutrient digestibility, and intestinal microbiota of pigs.

## Materials and Methods

### Animals and diets

This experiment was approved by the Animal Care and Use Committee of 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. A total of 160 crossbred piglets (Duroc × Landrace × Yorkshire) with an average body weight of 28 ± 0.5 kg were used in an 8-wk feeding experiment. Pigs were randomly assigned to five treatments with four replicates per treatment and eight pigs per replicates. The five experimental groups were as follows: basal diet group (whole corn–soybean meal), 10% PKC group (PKC), 10% extrusion PKC group (PPKC), 10% enzymolysis PKC group (EPKC), and 10% fermented PKC group (FPKC), respectively. The nutrient levels of metabolizable energy and crude protein in each group were balanced. Dietary contents were analyzed according to NRC (2012) recommendations (Table 1). At the end of the experiment, four pigs from each treatment (randomly collected one pig per pen) were sacrificed by administering a pentobarbital overdose, the gut and blood samples were collected for the quantification analysis of microbiota, hematological parameters, and apparent total tract nutrient digestibility.

### Method for determination of reducing sugars in samples

Firstly, the standard curve of reducing sugar was prepared. Nine test tubes with plug scale were numbered and glucose standard solution, distilled water, and dinitrosalicylic acid (**DNS**) reagent were added respectively according to Table 2 to prepare glucose reaction solution with different concentrations. The prepared glucose reaction solution was sealed and boiled in a boiling water bath for 5 min, and then put in a cold water bath for cooling, and then the volume was fixed to 25 mL. With No. 0 tube as the control, the light absorption value was measured at 540 nm. The glucose mass was taken as the y-axis and the light absorption value as the x-axis, and the curve was fitted. Secondly, 3.00 g samples were accurately weighed and transferred into a 50-mL centrifuge tube, 27 mL boiled distilled water was added, and the samples were shaken for 30 min to leach the reducing sugar, and then centrifuged at 4,000 rpm for 5 min. At this time, the supernatant is 10 times diluent, which can be diluted to an appropriate multiple according to the needs of the experiment. Take four test tubes with plugs, numbered 0, 1, 2, 3. Using the same method for making the standard curve, 1 mL of test liquid and 1 mL of distilled water and 1.5 mL of DNS were added to test tubes 1 to 3, while 2 mL of distilled water and 1.5 mL of DNS were added to control tubes 0. Seal in boiling water bath for 5 min, cool in cold water bath, constant volume to 25 mL. Absorbance was measured at 540 nm. The content of reducing sugar in the sample was calculated according to the fitting curve.

### Methods for testing the quality of pellet feed

The determination of hardness: about 20 g representative samples were selected from each group of hard particle feed samples, and 20 feed particles with roughly the same length of about 1.0 cm were selected from each part by quartering method, the hardness and of feed particles were determined by texture analyzer (TA. XT2, Stable Mico Systems Co. Ltd, UK). The maximum and minimum values of the measured values are removed and the final result is expressed as an average value.

Measurement of particle pulverization rate: select two samples of 500 g granule feed with representative, respectively into the pulverization rate tester rotation box, set the speed of 500 rpm, start the machine, and make the box rotation for 10 min. At the end of rotation, the feed samples were taken out and passed through the specified standard screen (in this test, 3.0 mm particles passed through 2.5 mm screen, and 2.0

**Table 1.** Ingredients and chemical composition of experimental diets (air-dry basis)%

| Items | Control group | | PKC[1] | | PPKC[1] | | FPKC[1] | | EPKC[1] | |
|---|---|---|---|---|---|---|---|---|---|---|
| Period | Growing | Finishing | Growing | Finishing | Growing | Finishing | Growing | Finishing | Growing | Finishing |
| Corn | 69 | 73.79 | 62 | 66.76 | 62.5 | 66.86 | 62.9 | 67.06 | 63 | 66.96 |
| Soybean meal | 24 | 20.1 | 21 | 17.1 | 20.5 | 17.0 | 19.9 | 16.8 | 19.9 | 16.9 |
| Soybean oil | 3.2 | 2.57 | 3.2 | 2.6 | 3.2 | 2.60 | 3.4 | 2.6 | 3.3 | 2.6 |
| PKC | 0 | 0 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 |
| Limestone | 0.79 | 0.96 | 0.79 | 0.96 | 0.79 | 0.96 | 0.79 | 0.96 | 0.79 | 0.96 |
| $CaHPO_4$ | 0.99 | 0.8 | 0.99 | 0.8 | 0.99 | 0.8 | 0.99 | 0.8 | 0.99 | 0.8 |
| Premix | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| NaCl | 0.34 | 0.34 | 0.34 | 0.34 | 0.34 | 0.34 | 0.34 | 0.34 | 0.34 | 0.34 |
| lysine | 0.36 | 0.3 | 0.36 | 0.3 | 0.36 | 0.3 | 0.36 | 0.3 | 0.36 | 0.3 |
| Methionine | 0.27 | 0.11 | 0.27 | 0.11 | 0.27 | 0.11 | 0.27 | 0.11 | 0.27 | 0.11 |
| Threonine acid | 0.05 | 0.03 | 0.05 | 0.03 | 0.05 | 0.03 | 0.05 | 0.03 | 0.05 | 0.03 |
| Nutrient levels | | | | | | | | | | |
| DE[2] (MJ/kg) | 14.26 | 14.24 | 14.26 | 14.24 | 14.26 | 14.24 | 14.26 | 14.24 | 14.26 | 14.24 |
| CP[2] | 16.08 | 15.18 | 16.08 | 15.18 | 16.08 | 15.18 | 16.08 | 15.18 | 16.08 | 15.18 |
| Ether extract | 4.10 | 4.15 | 4.10 | 4.15 | 4.10 | 4.15 | 4.10 | 4.15 | 4.10 | 4.15 |
| Ash | 6.20 | 6.44 | 6.20 | 6.44 | 6.20 | 6.44 | 6.20 | 6.44 | 6.20 | 6.44 |
| Ca[2] | 0.6 | 0.5 | 0.6 | 0.5 | 0.6 | 0.5 | 0.6 | 0.5 | 0.6 | 0.5 |
| P[2] | 0.53 | 0.47 | 0.53 | 0.47 | 0.53 | 0.47 | 0.53 | 0.47 | 0.53 | 0.47 |

Note: The premix provided the following per kilogram of diet: Fe, 100 mg as ferrous sulfate; Cu, 15 mg as copper sulfate; Zn, 120 mg as zinc sulfate; Mn, 40 mg as manganese sulfate; Se, 0.3 mg as sodium selenite; I, 0.25 mg as potassium iodide; VA 13500 IU; $VD_3$ 2250 IU; VE 36 IU; $VK_3$ 4 mg;$VB_1$ 5 mg; $VB_2$ 12.5 mg; $VB_3$ 25 mg; $VB_5$ 15 mg; $VB_6$ 7.5 mg; $VB_{12}$ 0.05 mg; folic acid 2.4 mg; niacin 50 mg; biotin 0.15 mg.
[1]PKC, palm kernel cake; PPKC, extrusion palm kernel cake; FPKC, fermented palm kernel cake; EPKC, enzymatic palm kernel cake.
[2]DE, digestive energy; CP, crude protein; Ca, calcium; P, phosphorus.

**Table 2.** Glucose standard curve

| Reagent | Test tube number | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |
| Glucose standard solution, mL | 0 | 0.2 | 0.4 | 0.6 | 0.8 | 1.0 | 1.2 | 1.4 | 1.6 |
| Distilled water, mL | 2 | 1.8 | 1.6 | 1.4 | 1.2 | 1.0 | 0.8 | 0.6 | 0.4 |
| DNS[1], mL | 1.5 | 1.5 | 1.5 | 1.5 | 1.5 | 1.5 | 1.5 | 1.5 | 1.5 |
| Glucose content (mg) | 0 | 0.2 | 0.4 | 0.6 | 0.8 | 1.0 | 1.2 | 1.4 | 1.6 |

[1]Dinitrosalicylic acid.

mm particles passed through 1.7 mm screen). The screening time was 1 min, and then the weight of the objects under the screen was weighed and expressed as the average value of the measured results on both sides.

$$\text{Pulverization rate}\,(\%) = \frac{m2}{m1} \times 100\%.$$

Determination of particle powder content: about 1.0 kg of freshly cooled pellet feed was taken from the feed of each group, and the sample of pellet feed was divided into two parts by quaternization method, each of which was about 500 g (m1). The sample was screened on the vibrating screen for 3 min, and the material under the screen was weighed (m2). The arithmetic mean of the two test results was used as the powder content of the feed sample, and the difference between the two test results should be less than 1%.

$$\text{Powder content}\,(\%) = \frac{m2}{m1} \times 100\%.$$

The method of Xiong (2000) was adopted for the determination of starch activation, which was as follows: two samples were accurately weighed, each 0.2 g (accurate to 0.0002 g), and placed in 25 mL scale test tubes, one of which was for the preparation of all gelatinized samples, and the other was for the determination of samples. Add 15 mL buffer solution to the sample, mix and heat the test tube in the boiling water bath for 1 h (shaking two to three times during the process), that is, the fully gelatinized sample. Cool the test tube with tap water, and add an appropriate amount of distilled water to restore the liquid level to the position before heating. Fifteen milliliters of buffer solution were added to the test sample, and 1 mL enzyme solution was added to both the gelatinized sample and the test sample. Take another test tube and add 15 mL buffer solution and 1 mL enzyme solution as blank. Keep warm in a water bath at 40 °C for 1 h. Shake once at first and once every 15 min thereafter. After holding for 1 h, add 2 mL 10%$ZnSO_4 \cdot 7H_2O$, mix well, and add 1 mL 0.5mol/L

NaOH. Dilute to 25 mL with water, mix well and strain. Accurately absorb 0.1 mL filtrate and 2 mL copper reagent into a 25-mL graduated test tube. Put the test tube in boiling water bath for 6 min, keep boiling, add 2 mL phosphomolybdate reagent, and continue heating for 2 min. Cool the test tube with tap water, dilute it to 25 mL with distilled water, plug the mouth of the test tube (using a gloved thumb or palm), reverse the test tube repeatedly to mix it well, and use a spectrophotometer to read the absorption value at 420 nm.

## Growth performance

Accurate feed intake was recorded every day during the trial, and the pigs were weighed on an empty stomach at the beginning and end of the trial, then average daily gain (**ADG**), average daily feed intake (**ADFI**), and the ratio of consumption to gain (feed:gain rate, F/G).

Initial weight (**IBW**): Pig weight (kg) as measured at the beginning of the test
Final weight (**FBW**): Pig weight (kg) at the end of the trial
ADG (kg/d) = (finishing weight kg – starting weight kg)/number of heads/trial days d
ADFI (kg/d) = Total feed intake kg/head count per column/test days d
F/G = ADFI/ADG

## Nutrient digestibility

Fecal samples from each pig were pooled and dried in an oven at 60 °C for 4 d. The dried fecal samples together with diet samples were ground through a 0.75-mm screen in a centrifugal mill (model ZM200; Retsch GmbH, Haan, Germany). The ground samples were analyzed as follows: Phase 1 diets and fecal samples for dry matter (**DM**), ether extract (**EE**), crude protein (**CP**), and titanium contents; the samples were analyzed for DM by oven drying at 135 °C for 2 h (method 930.15), CP by a combustion procedure (method 990.03) as per AOAC (2005). Titanium dioxide in samples was determined by spectrophotometry (model Spectra MAX 190, Molecular Devices, Sunnyvale, CA) at 408 nm after ashing at 525 °C for 10 h AOAC (2005)(Myers et al., 2004). The EE was analyzed using a Goldfisch fat extraction apparatus (model 35001, Labconco, Kansas City, MO). The samples were not acid-hydrolyzed before ether extraction.

## Blood biochemical index

Blood samples were collected randomly from 4 pigs per treatment (each pig per pen) at the end of the experiment. Pigs were bled via puncture of the jugular vein before the morning feeding. Blood samples were collected into 9 mL vacutainer tubes (Becton–Dickinson Vacutainer Systems, Franklin Lakes, NJ, US). After blood collection, all samples were transferred to centrifuge tubes and centrifuged for 15 min at 8,000 × *g* and 25 °C. The serum was carefully removed and stored at −80 °C for further analysis. The levels of serum total protein (**TP**), albumin (**ALB**), aspartate aminotransferase (**AST**), alanine aminotransferase (**ALT**), and alkaline phosphatase (**ALP**) were determined using commercial kits according to the instructions of the manufacturer (Nanjing Jiancheng Bioengineering company, Jiangsu, China). Plasma urea N was measured with a swine ELISA kit (Huijia Biotech Company, Xiamen, China) following the manufacturer's instructions.

## Real-time PCR assay for the quantification of microbiota in the gut and feces of pigs

Digesta from colon, 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 (25μL) consisted of 12.5μL of a master mix (IQ SYBR Green Supermix; Bio-Rad), 0.2μM of each primer set, and 5μL 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 16S 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.

## Statistical analysis

All the results from experiment were analyzed by using the one-way ANOVA, performed using the MIXED procedure of SAS (SAS Inst., Inc., Cary, NC, US). The sow or litter was the experimental unit. The results in the tables are shown with

**Table 3.** Primers used for several microflora

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

the means + SEM. Means are considered significantly different at $P < 0.05$.

## Results

### Effects of different processing techniques on the release of reducing sugar from palm kernel cake

It was found that β-mannan was reduced to Mannose oligosaccharides after degradation, and accounted for 78% of nonstarch polysaccharides in PKC. Therefore, in this study, the pretreatment and processing parameters of PKC were optimized with reducing sugar release as the evaluation label. The effects of different processing parameters on reducing sugar content of fermented PKC are shown in Table 4. The results showed that fermentation temperature and water content significantly affected the release of reducing sugar from the fermented products ($P < 0.05$). The order of nonstarch polysaccharide degradation of the three factors was water content > fermentation temperature > fermentation time. The optimal fermentation parameters were time 4 d, water content 55%, and temperature 36 °C.

As shown in Table 5, extrusion temperature and water content significantly affected the release of reducing sugar from extrusion products of PKC ($P < 0.05$). The order of nonstarch polysaccharide degradation of three factors was extrusion temperature > water content > feeding rate. The optimal fermentation parameters of PKC were extrusion temperature 100 °C, feeding rate 22.34 kg/min and water content 20%.

The effects of different processing parameters on reducing sugar content of enzymolysis PKC are shown in Table 6. The results showed that pH and enzymolysis temperature had significant effects on the release of reducing sugar from enzymolysis products of PKC ($P < 0.05$). The order of the three factors on the degradation of non-starch polysaccharides was pH > temperature > time. The best enzymolysis parameters of PKC were pH 5, temperature 60 °C and time 7 h.

**Table 4.** Values of experimental parameters in experiment of reducing sugar of fermented PKC

| Number | Factors | | | Reducing sugar |
|---|---|---|---|---|
| | Time, d | Water content, % | Temperature, °C | |
| 1 | 4 | 45 | 34 | 3.88 ± 0.46 |
| 2 | 4 | 50 | 38 | 5.23 ± 0.21 |
| 3 | 4 | 55 | 36 | 6.37 ± 0.21 |
| 4 | 5 | 45 | 36 | 5.37 ± 1.20 |
| 5 | 5 | 50 | 34 | 4.00 ± 0.50 |
| 6 | 5 | 55 | 38 | 5.78 ± 0.54 |
| 7 | 6 | 45 | 38 | 4.06 ± 0.42 |
| 8 | 6 | 50 | 36 | 4.50 ± 0.57 |
| 9 | 6 | 55 | 34 | 5.51 ± 0.52 |
| Optimum level | 4 | 55 | 36 | |
| R-value | 0.46 | 1.45 | 0.95 | |
| F-value | 1.305 | 13.802 | 4.933 | |
| *P*-value | 0.293 | <0.01 | 0.018 | |

**Table 5.** Values of experimental parameters in experiment of reducing sugar of extrusion PKC

| Number | Factors | | | Reducing sugar |
|---|---|---|---|---|
| | Temperature, °C | Feeding rate, kg/min | Water content, % | |
| 1 | 100 | 17.25 | 12 | 1.62 ± 0.55 |
| 2 | 100 | 22.34 | 20 | 2.42 ± 0.29 |
| 3 | 100 | 27.35 | 16 | 2.17 ± 0.36 |
| 4 | 120 | 17.25 | 16 | 1.47 ± 0.38 |
| 5 | 120 | 22.34 | 12 | 1.30 ± 0.39 |
| 6 | 120 | 27.35 | 20 | 1.46 ± 0.17 |
| 7 | 140 | 17.25 | 20 | 1.40 ± 0.01 |
| 8 | 140 | 22.34 | 16 | 1.47 ± 0.40 |
| 9 | 140 | 27.35 | 12 | 1.15 ± 0.19 |
| Optimum level | 100 | 22.34 | 20 | |
| R-value | 0.73 | 0.23 | 0.4 | |
| F-value | 13.313 | 1.104 | 3.883 | |
| *P*-value | <0.01 | 0.351 | 0.038 | |

**Table 6.** Values of experimental parameters in experiment of reducing sugar of enzymatic PKC

| Number | Factors | | | Reducing sugar |
|---|---|---|---|---|
| | pH | Temperature, °C | Time, h | |
| 1 | 5 | 50 | 6 | 8.28 ± 0.53 |
| 2 | 5 | 60 | 8 | 9.00 ± 0.78 |
| 3 | 5 | 70 | 7 | 8.44 ± 0.47 |
| 4 | 6 | 50 | 7 | 6.81 ± 0.61 |
| 5 | 6 | 60 | 6 | 7.09 ± 0.20 |
| 6 | 6 | 70 | 8 | 6.07 ± 0.32 |
| 7 | 7 | 50 | 8 | 5.80 ± 0.63 |
| 8 | 7 | 60 | 7 | 6.13 ± 0.46 |
| 9 | 7 | 70 | 6 | 6.71 ± 0.72 |
| Optimum level | 5 | 60 | 7 | |
| R-value | 3.36 | 0.77 | 0.21 | |
| F-value | 37.354 | 1.254 | 0.969 | |
| *P*-value | <0.01 | 0.025 | 0.705 | |

**Table 7.** Effects of different processing techniques on the chemical composition of PKC

| Nutrient content | PKC[1] | PPKC[1] | FPKC[1] | EPKC[1] | *P*-value |
|---|---|---|---|---|---|
| DM, % | 92.59 ± 0.14 | 92.50 ± 0.39 | 92.51 ± 0.21 | 92.52 ± 0.11 | 0.962 |
| CP, % | 16.85 ± 0.13$^{C}$ | 16.51 ± 0.23$^{C}$ | 19.27 ± 0.14$^{A}$ | 18.79 ± 0.19$^{B}$ | <0.01 |
| Crude fiber, % | 17.38 ± 0.17$^{A}$ | 17.12 ± 0.11$^{B}$ | 16.33 ± 0.09$^{C}$ | 13.32 ± 0.06$^{D}$ | <0.01 |
| Ether extract, % | 6.65 ± 0.08$^{a}$ | 6.31 ± 0.12$^{b}$ | 6.55 ± 0.09$^{a}$ | 6.51 ± 0.09$^{a}$ | 0.012 |
| Ash, % | 4.04 ± 0.15$^{C}$ | 4.30 ± 0.04$^{A}$ | 5.18 ± 0.06$^{B}$ | 4.08 ± 0.08$^{C}$ | <0.01 |

[1]PKC, palm kernel cake; PPKC, extrusion palm kernel cake; FPKC, fermented palm kernel cake; EPKC, enzymatic palm kernel cake.
[a,b,A,B,C,D]Within a row, means without a common superscript differ ($P < 0.05$). Capital letters indicate significant differences ($P < 0.01$), lowercase letters represented a significant difference ($P < 0.05$).

## Effects of different processing techniques on conventional nutritional components of palm kernel cake

According to the data in Table 7, compared with untreated PKC, extrusion, fermentation, and enzymolysis had no significant effect on the dry matter content of PKC ($P > 0.05$). Fermentation and enzymolysis significantly increased PKC crude protein content ($P < 0.01$), extrusion treatment had no significant effect on PKC crude protein content ($P > 0.05$). The crude fiber content in PKC was decreased by extrusion, fermentation, and enzymolysis ($P < 0.01$), but the effect of enzymolysis was the best. Extrusion treatment significantly reduced the crude fat content in PKC ($P < 0.05$), but fermentation and enzymolysis had no significant effect on the content of crude fat in low PKC ($P > 0.05$). The content of PKC crude ash was significantly increased by extruding and fermenting treatment ($P < 0.01$), enzymolysis had no significant effect on PKC ash content ($P > 0.05$).

## Effects of different processing techniques of PKC on processing quality of pellet feed

According to the results in Table 8, compared with the control group, extrusion and fermentation treatment significantly increased the hardness of PKC pellet feed ($P < 0.05$), enzymolysis had no significant effect on PKC pellet feed ($P > 0.05$). Compared with the untreated PKC group, extrusion, fermentation, and enzymolysis significantly reduced the powder content and pulverization rate of the pellet feed ($P < 0.01$), there were no significant differences in powder content and pulverization rate between PKC pretreatment groups and control group ($P > 0.05$). In terms of starch gelatinization degree, compared with untreated PKC group, extrusion and fermentation treatments significantly increased the starch gelatinization degree of pellet feed ($P < 0.01$), enzymolysis had little effect on starch gelatinization degree of pellet feed ($P > 0.05$).

## Effects of different processing techniques of PKC on nutrient digestibility of growing–finishing pig

As shown in Table 9, compared with the control group, there was no significant difference in nutrient digestibility in PPKC group during the growth stage ($P > 0.05$), the nutrient digestibility of PKC, FPKC, and EPKC groups was significantly lower than that of control group ($P < 0.05$); Compared with PKC group, the digestibility of dry matter and ether extract in PPKC group was significantly higher than that in PKC group ($P < 0.05$), there was no significant difference in nutrient digestibility between FPKC group, EPKC group, and PKC group ($P > 0.05$).

At fattening stage, the apparent digestibility of DM, CP, and EE in PKC group was significantly lower than that in control group ($P < 0.05$), there were no significant differences between PPKC, FPKC, EPKC, and control groups ($P > 0.05$).

**Table 8.** Effects of different processing techniques of PKC on pelleting characteristics of pellet feed

| Items | Treatments[1] | | | | | *P*-value |
|---|---|---|---|---|---|---|
| | Control | 10%PKC | 10%PPKC | 10%FPKC | 10%EPKC | |
| Hardness, N | 42.63 ± 3.31$^{c}$ | 44.78 ± 1.73$^{bc}$ | 51.35 ± 2.22$^{a}$ | 49.56 ± 3.67$^{ab}$ | 46.84 ± 2.51$^{abc}$ | 0.020 |
| Pulverization rate, % | 6.93 ± 0.64$^{B}$ | 9.25 ± 1.00$^{A}$ | 5.86 ± 0.53$^{B}$ | 6.39 ± 0.40$^{B}$ | 7.06 ± 0.56$^{B}$ | <0.01 |
| Powder content, % | 3.28 ± 0.42$^{B}$ | 4.65 ± 0.37$^{A}$ | 2.80 ± 0.48$^{B}$ | 3.17 ± 0.29$^{B}$ | 3.50 ± 0.30$^{B}$ | <0.01 |
| Gelatinization starch, % | 41.07 ± 0.40$^{B}$ | 39.42 ± 0.27$^{D}$ | 43.37 ± 0.22$^{A}$ | 40.25 ± 0.12$^{C}$ | 39.83 ± 0.49$^{CD}$ | <0.01 |

[1]Control, corn–soybean meal diet;10%PKC, control plus 10% palm kernel cake;10%PPKC, control plus 10% extrusion palm kernel cake; 10%FPKC, control plus 10%fermented palm kernel cake; 10%EPKC, control plus 10% enzymatic palm kernel cake.
[a,b,c,A,B,C,D]Within a row, means without a common superscript differ ($P < 0.05$). Capital letters indicate significant differences ($P < 0.01$), lowercase letters represented a significant difference ($P < 0.05$).

**Table 9.** Effects of different processing techniques of PKC on nutrient digestibility of pig

| Items[1] | Treatments[2] | | | | | *P*-value |
|---|---|---|---|---|---|---|
| | Control | 10%PKC | 10%PPKC | 10%FPKC | 10%EPKC | |
| Growing | | | | | | |
| DM, % | 78.11 ± 0.99$^{a}$ | 75.18 ± 0.40$^{c}$ | 77.52 ± 0.76$^{ab}$ | 76.36 ± 1.13$^{bc}$ | 76.45 ± 0.74$^{bc}$ | 0.014 |
| CP, % | 79.78 ± 1.57$^{a}$ | 75.74 ± 0.67$^{b}$ | 78.68 ± 0.62$^{a}$ | 77.59 ± 1.08$^{ab}$ | 78.10 ± 1.47$^{a}$ | 0.017 |
| EE, % | 77.39 ± 1.10$^{a}$ | 73.17 ± 1.83$^{c}$ | 76.12 ± 1.53$^{ab}$ | 74.26 ± 1.16$^{bc}$ | 74.21 ± 1.15$^{bc}$ | 0.025 |
| Finishing | | | | | | |
| DM, % | 80.76 ± 1.80$^{a}$ | 76.70 ± 1.75$^{b}$ | 80.44 ± 1.29$^{a}$ | 78.65 ± 0.88$^{ab}$ | 79.06 ± 0.83$^{ab}$ | 0.031 |
| CP, % | 82.87 ± 1.16$^{a}$ | 78.13 ± 0.95$^{b}$ | 81.07 ± 1.87$^{a}$ | 80.14 ± 1.71$^{ab}$ | 80.66 ± 1.46$^{ab}$ | 0.033 |
| EE, % | 78.34 ± 1.03$^{a}$ | 75.29 ± 1.45$^{b}$ | 79.74 ± 1.48$^{a}$ | 78.88 ± 0.73$^{a}$ | 78.38 ± 1.44$^{a}$ | 0.015 |

[1]DM, dry matter; CP, crude protein; EE, ether extract.
[2]Control, corn–soybean meal diet; 10%PKC, control plus 10% palm kernel cake; 10%PPKC, control plus 10% extrusion palm kernel cake; 10%FPKC, control plus 10% fermented palm kernel cake; 10%EPKC, control plus 10% enzymatic palm kernel cake.
[a,b,c]Within a row, means without a common superscript differ ($P < 0.05$).

## Effects of different processing techniques of PKC on growth performance of growing–finishing pigs

According to the results in Table 10, there was no significant difference in ADFI among all groups at the growth stage ($P > 0.05$), but the feed intake of PKC group was the lowest. ADG of PKC group was significantly lower than the other groups ($P < 0.01$). F/G of control group and PKC processing groups was significantly lower than that of PKC group ($P < 0.05$), but no significant differences were found in F/G and ADG of PKC processing groups compared with control group ($P > 0.05$).

At fattening stage, the final weight of PKC group was significantly lower than that of control group and other PKC processing groups ($P < 0.05$). There was no significant difference in ADFI among all groups ($P > 0.05$). The ADG and F/G of PKC group were significantly lower than those of control group and other PKC processing groups, but there were no significant differences in F/G and ADG of PKC processing groups compared with control group ($P > 0.05$).

## Effects of different processing techniques of PKC on Blood indices of growing–finishing pigs

As shown in Table 11, the total protein and albumin contents showed no significant differences between control group and other groups in terms of growth stage, ($P > 0.05$). Urea content in PKC group was significantly higher than that in control group ($P < 0.05$), but there was no significant difference between other PKC processing groups and control group ($P > 0.05$). There were no significant differences in the activities of ALT and AST among all groups ($P > 0.05$), and PKC group was higher than control group and other PKC pretreatment groups. Compared with the control group, the activity of alkaline phosphatase in PKC group was significantly lower than that in the control group ($P < 0.01$), but there was no significant difference between the other PKC processing groups and the control group ($P > 0.05$).

At fattening stage, there were no significant differences in total protein and albumin contents between control group and other groups ($P > 0.05$). Urea content in PKC group was significantly higher than that in control group ($P < 0.05$), but there was no significant difference between other PKC processing groups and control group ($P > 0.05$). There were no significant differences in the activities of ALT and AST among all groups ($P > 0.05$). The activity of alkaline phosphatase in PKC group was significantly lower than that in control group ($P < 0.05$), but there was no significant difference between other PKC processing groups and control group ($P > 0.05$).

## Effects of different processing techniques of PKC on intestinal microflora of growing–finishing pigs

According to the data in Table 12, compared with the control group, the number of lactobacillus in cecum and colon of EPKC group was significantly higher than that of the control group ($P < 0.05$), there were no significant differences between PKC, PPKC, and FPKC groups and control group

**Table 10.** The effects of different processing techniques of PKC on growth performance of pigs

| Items[1] | Treatments[2] | | | | | *P*-value |
|---|---|---|---|---|---|---|
| | Control | 10%PKC | 10%PPKC | 10%FPKC | 10%EPKC | |
| Growing | | | | | | |
| IBW/kg | 28.06 ± 0.03 | 27.91 ± 0.07 | 27.82 ± 0.93 | 28.16 ± 0.04 | 28.10 ± 0.07 | 0.847 |
| FBW/kg | 50.47 ± 1.17$^{a}$ | 46.71 ± 0.55$^{b}$ | 50.92 ± 2.31$^{a}$ | 50.46 ± 1.72$^{a}$ | 50.60 ± 0.33$^{a}$ | 0.023 |
| ADFI (kg/d) | 1.74 ± 0.05 | 1.65 ± 0.05 | 1.67 ± 0.09 | 1.75 ± 0.18 | 1.78 ± 0.10 | 0.58 |
| ADG (kg/d) | 0.75 ± 0.04$^{A}$ | 0.63 ± 0.02$^{B}$ | 0.77 ± 0.05$^{A}$ | 0.74 ± 0.06$^{A}$ | 0.75 ± 0.01$^{A}$ | 0.007 |
| F/G | 2.34 ± 0.08$^{c}$ | 2.64 ± 0.06$^{a}$ | 2.18 ± 0.12$^{b}$ | 2.36 ± 0.20$^{c}$ | 2.38 ± 0.13$^{c}$ | 0.036 |
| Finishing | | | | | | |
| IBW/kg | 51.43 ± 0.09 | 51.49 ± 0.14 | 51.28 ± 0.13 | 51.56 ± 0.23 | 51.39 ± 0.07 | 0.334 |
| FBW/kg | 81.96 ± 0.63$^{a}$ | 77.80 ± 1.12$^{b}$ | 82.20 ± 1.28$^{a}$ | 82.23 ± 2.61$^{a}$ | 81.72 ± 1.97$^{a}$ | 0.048 |
| ADFI (kg/d) | 2.88 ± 0.07 | 2.67 ± 0.08 | 2.85 ± 0.06 | 2.81 ± 0.17 | 2.80 ± 0.10 | 0.203 |
| ADG (kg/d) | 1.02 ± 0.02$^{a}$ | 0.88 ± 0.04$^{b}$ | 1.03 ± 0.04$^{a}$ | 0.99 ± 0.08$^{a}$ | 1.01 ± 0.07$^{a}$ | 0.041 |
| F/G | 2.83 ± 0.08$^{b}$ | 3.04 ± 0.08$^{a}$ | 2.76 ± 0.08$^{b}$ | 2.85 ± 0.07$^{b}$ | 2.78 ± 0.11$^{b}$ | 0.014 |

[1]There were seven pigs per pen in growing phase and seven pigs per pen in finishing phases.
[2]Control, corn–soybean meal diet; 10%PKC, control plus 10% palm kernel cake; 10%PPKC, control plus 10% extrusion palm kernel cake; 10%FPKC, control plus 10% fermented palm kernel cake; 10%EPKC, control plus 10% enzymatic palm kernel cake.
$^{a,b,A,B}$Within a row, means without a common superscript differ ($P < 0.05$). Capital letters indicate significant differences ($P < 0.01$), lowercase letters represented a significant difference ($P < 0.05$).

**Table 11.** The effects of different processing techniques of PKC on blood indices of pigs

| Items[1] | Treatments[2] | | | | | *P*-value |
|---|---|---|---|---|---|---|
| | Control | 10%PKC | 10%PPKC | 10%FPKC | 10%EPKC | |
| Growing | | | | | | |
| TP, g/L | 59.28 ± 1.95$^{a}$ | 50.71 ± 2.02$^{b}$ | 57.26 ± 3.11$^{a}$ | 54.56 ± 3.42$^{ab}$ | 56.94 ± 1.87$^{a}$ | 0.019 |
| ALB, g/L | 32.07 ± 1.55$^{a}$ | 26.79 ± 2.32$^{b}$ | 30.64 ± 0.71$^{a}$ | 29.32 ± 1.69$^{ab}$ | 29.98 ± 1.30$^{a}$ | 0.025 |
| UREA, mmol/L | 4.17 ± 0.17$^{b}$ | 4.56 ± 0.14$^{a}$ | 4.13 ± 0.13$^{b}$ | 4.22 ± 0.18$^{b}$ | 4.15 ± 0.22$^{b}$ | 0.034 |
| ALT, U/L | 53.19 ± 2.51 | 55.82 ± 1.21 | 52.69 ± 1.54 | 51.29 ± 2.29 | 51.35 ± 3.67 | 0.211 |
| AST, U/L | 44.08 ± 1.63 | 44.78 ± 2.41 | 43.68 ± 1.73 | 44.10 ± 1.61 | 41.43 ± 1.00 | 0.230 |
| ALP, U/L | 157.39 ± 4.04$^{A}$ | 140.15 ± 1.40$^{B}$ | 155.14 ± 2.93$^{A}$ | 158.93 ± 4.56$^{A}$ | 154.79 ± 3.13$^{A}$ | <0.01 |
| Finishing | | | | | | |
| TP, g/L | 68.08 ± 2.33$^{a}$ | 58.20 ± 2.92$^{b}$ | 67.23 ± 3.32$^{a}$ | 65.92 ± 3.29$^{a}$ | 64.15 ± 1.58$^{a}$ | 0.010 |
| ALB, g/L | 39.89 ± 4.15$^{a}$ | 28.79 ± 4.00$^{b}$ | 38.41 ± 3.53$^{a}$ | 36.41 ± 3.31$^{a}$ | 35.86 ± 1.55$^{a}$ | 0.022 |
| UREA, mmol/L | 3.94 ± 0.08$^{b}$ | 4.37 ± 0.18$^{a}$ | 3.92 ± 0.20$^{b}$ | 3.87 ± 0.17$^{b}$ | 4.08 ± 0.10$^{b}$ | 0.015 |
| ALT, U/L | 52.58 ± 4.91 | 57.80 ± 5.53 | 49.70 ± 3.59 | 47.21 ± 4.61 | 47.52 ± 1.76 | 0.065 |
| AST, U/L | 42.80 ± 2.16 | 42.30 ± 1.94 | 42.17 ± 2.16 | 45.42 ± 1.33 | 44.53 ± 1.05 | 0.173 |
| ALP, U/L | 184.78 ± 10.6$^{a}$ | 149.89 ± 4.11$^{b}$ | 178.49 ± 16.4$^{a}$ | 187.97 ± 13.1$^{a}$ | 194.82 ± 24.0$^{a}$ | 0.036 |

[1]TP, total protein; ALB, Albumin; UREA, carbamide; ALT, alanine aminotransferase; AST, aspartate transaminase ALP, alkaline phosphatase.
[2]Control, corn–soybean meal diet; 10%PKC, control plus 10% palm kernel cake; 10%PPKC, control plus 10% extrusion palm kernel cake; 10%FPKC, control plus 10% fermented palm kernel cake; 10%EPKC, control plus 10% enzymatic palm kernel cake.
$^{a,b,A,B}$Within a row, means without a common superscript differ ($P < 0.05$). Capital letters indicate significant differences ($P < 0.01$), lowercase letters represented a significant difference ($P < 0.05$).

($P > 0.05$); There was no significant difference in the number of lactobacillus in feces among all groups ($P > 0.05$); It was obvious that processing of PKC increased the number of lactobacillus in pig intestines. There were no significant differences in the number of bifidobacterium in cecum, colon, and feces among all groups ($P > 0.05$). There was no significant difference in the number of *E. coli* in cecum among all groups ($P > 0.05$); Compared with the control group, there was no significant difference in the number of *E. coli* in colon and fecal matter in PKC processing group ($P > 0.05$), but significantly lower than that in PKC group ($P < 0.05$).

## Discussion

In this study, our reducing sugar results showed that all three processing reduced the NSP content in PKC, which was expected and consistent with previous results on the degradation of NSP in cotton meal (Chen et al., 2020). Previous studies showed that cellulolytic enzymes treatment of PKC significantly reduced the neutral detergent fiber, acid detergent fiber, but did not significantly affect the DM and crude fat content (Saenphoom et al., 2011). In addition, high temperature, high pressure, and high shear force during extrusion will crack the chemical bonds between cellulose and lignin

**Table 12.** The effects of different processing techniques of PKC on intestinal microflora of pigs [Log 10 (copies/g wet weight)].

| Items[1] | Treatments[2] | | | | | *P*-value |
|---|---|---|---|---|---|---|
| | Control | 10%PKC | 10%PPKC | 10%FPKC | 10%EPKC | |
| *Lactobacillus* sp. | | | | | | |
| Cecum | 8.04 ± 0.20$^{bc}$ | 7.87 ± 0.15$^{c}$ | 8.18 ± 0.16$^{ab}$ | 8.22 ± 0.07$^{ab}$ | 8.36 ± 0.05$^{a}$ | 0.013 |
| Colon | 8.11 ± 0.09$^{bc}$ | 7.91 ± 0.12$^{c}$ | 8.22 ± 0.20$^{ab}$ | 8.28 ± 0.03$^{ab}$ | 8.47 ± 0.14$^{a}$ | 0.016 |
| Feces | 8.23 ± 0.08 | 8.06 ± 0.17 | 8.29 ± 0.14 | 8.31 ± 0.10 | 8.52 ± 0.14 | 0.071 |
| *Bifidobacterium* sp. | | | | | | |
| Cecum | 7.84 ± 0.10 | 7.78 ± 0.15 | 7.80 ± 0.18 | 7.67 ± 0.07 | 7.87 ± 0.12 | 0.462 |
| Colon | 8.10 ± 0.08 | 7.64 ± 0.47 | 8.15 ± 0.11 | 8.12 ± 0.05 | 8.14 ± 0.03 | 0.075 |
| Feces | 8.17 ± 0.06 | 8.11 ± 0.08 | 8.25 ± 0.11 | 8.30 ± 0.12 | 8.36 ± 0.07 | 0.142 |
| *Escherichia coli* | | | | | | |
| Cecum | 7.94 ± 0.14 | 8.35 ± 0.16 | 7.98 ± 0.12 | 8.04 ± 0.20 | 8.01 ± 0.10 | 0.064 |
| Colon | 8.10 ± 0.07$^{abc}$ | 8.33 ± 0.24$^{a}$ | 8.23 ± 0.09$^{ab}$ | 8.05 ± 0.08$^{bc}$ | 7.97 ± 0.08$^{c}$ | 0.039 |
| Feces | 8.37 ± 0.09$^{ab}$ | 8.44 ± 0.10$^{a}$ | 8.27 ± 0.04$^{bc}$ | 8.17 ± 0.06$^{c}$ | 8.21 ± 0.13$^{bc}$ | 0.018 |

[1]Gut samples were randomly collected from 1 pig per pen, fecal samples were randomly collected from 6 pigs per pen, and the fecal samples from each pen were pooled.
[2]Control, corn–soybean meal diet; 10%PKC, control plus 10% palm kernel cake; 10%PPKC, control plus 10% extrusion palm kernel cake; 10%FPKC, control plus 10% fermented palm kernel cake; 10%EPKC, control plus 10% enzymatic palm kernel cake.
[a,b,c]Within a row, means without a common superscript differ ($P < 0.05$).

molecules, change the polarity, chemical properties, and biochemical properties of molecules, and increase the content of soluble dietary fiber (Gajula et al., 2008). Fermentation technology refers to the process of degrading nutrients and anti-nutrition factors in feed materials by using extracellular enzymes produced by microbial life metabolism. It has been reported that the content of hemicellulose and cellulose in the solid-state fermentation of PKC with lactobacillus was significantly reduced after fermentation, while the reducing sugar content was significantly increased (Alshelmani et al., 2021).

Hardness, pulverization rate, and powder content are common important indexes of particle quality evaluation (Mavromichalis et al., 2000). Our results show that the extrusion process can improve the hardness and reduce the flour content and pulverization rate of the palm-meal pellet feed. This result is consistent with the results of improving poultry particle quality with steam pressure and temperature regulation (Zang et al., 2009; Selle et al., 2010; Liu et al., 2013). In general, the high temperature, high pressure, and high shear force in the bulking process can cause starch gelatinization, protein denaturation, and viscosity enhancement of PKC, which is conducive to improving the quality of pellet feed (Chae et al., 1997). In addition, PKC contains a lot of coarse fiber, which is not only hard and poor adhesion, but also will reduce the binding force between feed particles, which may affect the ability of feed to absorb steam. The effect of enzymolysis or fermentation of PKC on pellet feed quality has not been reported. Our results show that when PKC is treated with cellulase or cellulase-producing microorganisms, the pulverization rate and flour content of pellet feed are reduced, it may because the texture of PKC becomes softer and the content of crude fiber is reduced, which is good for adhesion.

In addition, high NSP content prevents monogastric animals, especially pigs, from fully utilizing the nutrients in PKC and reduces feed efficiency (Sundu and Dingle, 2003) . This is consistent with the results of this study. The apparent digestibility of DM, CP, crude fat, calcium, and phosphorus in PKC group was significantly lower than that in the control group. Our results suggest that extrusion can improve nutrient digestibility by stretching the molecular structure of the protein, thereby increasing the area of contact between digestive enzymes and the protein, thereby improving the digestibility of the protein (Fernández-Garía et al., 1998). In addition, the improvement of nutrient digestibility by enzymolysis and fermentation was limited, possibly because the high temperature in the granulation process destroyed the activities of enzymes and microorganisms.

Processing of PKC can increase the average daily gain and decrease the feed/meat ratio of growing pigs, indicating that all the processing can improve the growth performance of growing pigs. These findings are consistent with previous studies which showed that partial supplementation of fermented cottonseed meal and extruded rapeseed meal in diets had no significant effect on the growth performance of livestock and poultry (Keady and O'Doherty, 2000; Gu et al., 2021). PKC contains high content of crude fiber and nonstarch polysaccharide such as mannan. Crude fiber reduces palatability and increases intestinal filling, thus reducing feed intake. Mannan dissolves in water, increases intestinal viscosity, interferes with nutrient digestion, absorption and utilization, and adversely affects animal performance (Davis et al., 2002; LeMieux et al., 2003). In this study, the contents of crude fiber and nonstarch polysaccharides such as mannan were reduced by extrusion, fermentation, and enzymolysis of PKC, which could partially explain the growth performance results.

Serum protein is an important marker of protein anabolism in vivo, and the contents of TP and albumin reflect the absorption and metabolism of protein in animals (Coma et al., 1995; Shim et al., 2003). Compared with untreated cottonseed meal group, extruded cottonseed meal could increase the serum total protein concentration of laying hens (Wang et al., 2020) . It can be seen that the concentration of total protein and albumin in animal serum can be increased by the processing of miscellaneous meals, which is consistent with our results. In this study, the content of total protein and albumin in blood of growing pigs can be increased by adding processing PKC in diet. Studies have suggested that changes in blood urea content can indirectly indicate the status of protein metabolism and amino acid metabolism in animals. When protein metabolism and amino acid metabolism

in animals are in a healthy dynamic balance, the blood urea content shows a downward trend (Rosebrough et al., 1983). In this study, the urea content of PKC group was the highest, indicating that untreated PKC destroyed the protein metabolism of growing–finishing pigs, and the urea content of each PKC pretreatment group was significantly decreased, indicating that processing of PKC could improve the protein metabolism of growing–finishing pigs. The enzyme activity of ALT and AST is considered to be an important indicator of heart and liver health in animals (Verma and Singh, 2014). It has been reported that Khmer phenol diets significantly increase serum AST enzyme activity and decrease globulin content in pigs (Braham et al., 1967). In this study, there were no significant differences in the enzyme activities of AST and ALT among all groups. Although the enzyme activities of PKC group were higher than those of all groups, they were in the normal range. ALP is one of the specific catalytic enzymes widely existing in bone and blood, and its activity is an important index reflecting the healthy growth of animals (Reyer et al., 2019). In this study, the ALP activity of PKC group was significantly lower than that of control group and other PKC pretreatment groups, which was consistent with the results of growth performance tests of pigs in previous experiments (Lei et al., 1993).

The composition and activity of mammalian gastrointestinal microbiota are important for animal health and development, and they play an important role in intestinal health and nutrient digestion and absorption (Leser et al., 2002; Richards et al., 2005; Hooper and Macpherson, 2010). A previous study showed that feed processing can alter the composition of the microbiome, affecting the total number of bacteria as well as genera and dominant species (Bao et al., 2016). Our PCR results showed that different processing of PKC caused changes in intestinal bacterial diversity of pigs. Interestingly, compared with the PKC group, the numbers of beneficial lactobacillus and bifidobacterium were significantly higher in the control and other PKC pretreatment groups, and the numbers of *E. coli* in the cecum, colon, and feces were reduced. This bacterial community phenomenon may be related to the crude fiber content in PKC. Untreated PKC contains high crude fiber content, and the crude fiber content is reduced by 10% to 20% after processing. At present, no studies have accurately and systematically described the mechanism of dietary high fiber content on intestinal microorganisms. Previous study found that diet supplemented with 1% inulin could increase the number of lactobacillus and bifidobacterium in the cecum of weaned pigs (Tako et al., 2008). It was also found that 7% crude fiber level increased the number of *Escherichia coli* in the cecum of rabbits, and salmonella was also detected (Esiegwu et al. (2013). Therefore, appropriate crude fiber level in animal diet can increase the number of beneficial bacteria such as lactobacillus and bifidobacterium, and decrease the number of harmful bacteria such as *Escherichia coli*.

## Conclusion

In conclusion, the optimized parameters of extrusion processing were as follows: extrusion temperature 100 °C, feeding speed 22.34 kg/min, water content 20%; the optimization parameters of fermentation processing were as follows: time 4 d, water content 55%, temperature 36 °C; the optimization parameters of PKC enzymolysis processing were as follows: pH 5, temperature 60 °C, time 8 h. Extrusion, fermentation, and enzymolysis of PKC had positively enhanced the pellet quality, growth performance, nutrient digestibility, and gut microbiota, while extrusion exhibited a superior feeding effect compared to fermentation and enzymolysis.

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## Conflict of Interest Statement

All authors declare that there are no conflicts of interest.

## Ethical Statement

The animal studies were approved by the Animal Care and Use Committee of College of Animal Sciences and Technology, Huazhong Agricultural University and performed in accordance with relevant regulations and guidelines.

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