Article

Not peer-reviewed version

Evaluation of the Available Energy Value and Amino Acid Digestibility of Brown Rice Stored for 6 Years and Its Application in Pig Diets

Beibei He , Jingjing Shi , Kuanbo Liu , Junlin Cheng , Weiwei Wang , Yongwei Wang * , Aike Li *

Posted Date: 21 September 2023

doi: 10.20944/preprints202309.1451.v1

Keywords: stored brown rice; energy and nutrient value; growth performance; physiological and metabolic characteristic; meat quality; pig

Article

Evaluation of the Available Energy Value and Amino Acid Digestibility of Brown Rice Stored for 6 Years and Its Application in Pig Diets

Beibei He, Jingjing Shi, Kuanbo Liu, Junlin Cheng, Weiwei Wang, Yongwei Wang * and Aike Li *

Institute of Grain Quality and Nutrition, Academy of National Food and Strategic Reserves Administration, Beijing, China, 100037; bgs@ags.ac.cn

* Correspondence: wyw@ags.ac.cn (W.W.); lak@ags.ac.cn (A.L.)

Simple Summary: The present study evaluated the available energy value and amino acid digestibility of brown rice stored for 6 years, and investigate its application in weaned piglet and growing-finishing pig diets. The results showed that the digestible energy, metabolizable energy and standardized ileal digestibility of amino acids were not changed in brown rice stored under good conditions for 6 years. Stored brown rice also had no significant effects on growth performance, nutrient apparent total tract digestibility, serum biochemical indices, carcass traits, meat quality and muscle fatty acids profiles of pigs, but reduced the activity of digestive enzymes in pig small intestine. Altogether, the brown rice stored under good conditions for 6 years can be used as a high-quality energy feed raw material in pig diet.

Abstract: Long-term storage may reduce the nutritional quality of brown rice, so the present study was aimed to evaluate the nutritive values and application in pig diets. In Exp.1, 18 Landrace × Yorkshire (L × Y) barrows with initial body weight (IBW) of 25.48 ± 3.21 kg were randomly assigned to three treatments, which included corn diet, one-year stored brown rice (BR1) diet and six-year stored brown rice (BR6) diet, to determine the digestible energy (DE) and metabolisable energy (ME) value of stored brown rice. In Exp. 2, 24 barrows (L × Y; IBW: 22.16 ± 2.42 kg) fixed with ileal T-cannula were randomly allotted to four dietary treatments, including corn diet, two stored brown rice diets and nitrogen-free diet, to evaluate the amino acid (AA) digestibility of stored brown rice. In Exp. 3 and 4, 108 crossbred weaned piglets (L × Y; IBW: 9.16 ± 0.89 kg) and 90 crossbred growing pigs (L × Y; IBW: 48.28 ± 3.51 kg) were allotted to 3 treatment diets, including control diet and two stored brown rice diets, respectively, to investigate the application of stored brown rice in weaned piglet and growing-finishing pig diets. Results showed that: 1) The DE and ME of corn and stored brown rice showed no significant differences ($P > 0.05$), while the apparent ileal digestibility (AID) of arginine, histidine, aspartic acid, and the standardized ileal digestibility (SID) of arginine and histidine were higher in stored brown rice compared to corn ($P < 0.05$). 2) Compared with the control diet, the stored brown rice diets showed no significant effects on growth performance, nutrient apparent total tract digestibility (ATTD) and serum biochemical indices ($P > 0.05$), but decreased digestive enzymes activities in duodenum, jejunum and ileum of piglets ($P < 0.05$). 3) Compared with the control diet, the stored brown rice diets showed no significant effects on growth performance, carcass traits, meat quality, fatty acids profiles in the longissimus dorsi muscle of growing-finishing pigs ($P > 0.05$). In conclusion, the brown rice stored for 6 years under good conditions has no obvious changes in available energy and nutrient values. Although it may reduce digestive enzyme activity in small intestine of the piglets, the stored brown rice showed no obvious adverse effect on growth performance and meat quality, and can be effectively used in pig diet.

Keywords: stored brown rice; energy and nutrient value; growth performance; physiological and metabolic characteristic; meat quality; pig

1. Introduction

Due to the rapid development of animal husbandry in recent years, the contradiction between supply and demand of corn has become increasingly prominent [1], so it is important to find alternative ingredients for livestock production. Rice is the most important grain crop and the annual output has reached more than 200 million tons in China. On the condition that food rations are absolutely safe, the overstocked grain can be considered as alternative energy sources. Therefore, research on the nutritional characteristics and utilization values of stored rice in animal feeding has great theoretical and practical significance.

During grain storage, the composition and structure of main nutrients (protein, starch and fat) will change due to its own respiration, oxidation and the action of microorganisms, causing the decrease of nutritional value [2,3]. Higher disulfide bond content and surface hydrophobicity and lower free sulfhydryl content were produced during storage, which leads to the deterioration of rice protein [4]. Compared to the fresh rice, the solubility of total starch and amylose was decreased, resulting in lower gelatinization temperature, higher hardness and lower viscosity of rice [5]. Besides, the lipid oxidation increased the contents of free fatty acids and volatile carbonyl compounds, such as glutaraldehyde and hexanal, which reduce the palatability of stored grain [6].

Several studies have reported the changes of energy value and nutrient digestibility of stored corn and wheat in animal diet. Zhang et al. reported that the nutrient availability of corn, including the digestible energy (DE) and metabolisable energy (ME) value was decreased after storage at room temperature for 10 months [7]. Although there were no significant changes of ME, the digestibility of crude protein, histidine and arginine, and starch, was decreased quadratically with storage time in corn stored for 4 years at room temperature [8]. Some studies have reported that the nutritional value of stored corn and wheat had little change under a standard storage conditions [9-11]. The major nutrients of brown rice obtained from rice hulling were higher than or equal to corn, and can be effectively used as energy ingredient [12,13]. However, few studies have been done on stored brown rice for the available energy values, amino acid digestibility and its application in animal diets.

The objective of present study was to evaluate the DE, ME and the apparent ileal digestibility (AID), standardized ileal digestibility (SID) of amino acids (AAs) of brown rice stored for 1 or 6 years, and to explore the effects of stored brown rice on growth performance, apparent total tract digestibility (ATTD) of nutrients, serum biochemical parameters, intestinal enzyme activities in weaned piglets, and carcass characteristics, meat quality in finishing pigs. The hypothesis was that if brown rice was stored under proper conditions, its available energy value and amino acid digestibility will not change significantly, and the growth performance and meat quality of pigs will not be influenced by inclusion of stored brown rice in the diets.

2. Materials and Methods

All animal experiments were conducted in accordance with the Chinese Guidelines for Animal Welfare and Experimental Protocol, and obtain prior approval by the Animal Care and Use Committee of Academy of National Food and Strategic Reserves Administration (ethical approval code: 20230316006).

The paddy rice used were stored in brick concrete barns for 1 or 6 years at National Grain Reserve Barn of Heilongjiang Province, as it has the greatest rice yield in China. The temperature in grain barn was controlled within 20 °C all year around. Before the start of the experiment, the paddy rice was hulled to obtain brown rice for subsequent experiments. The diet, fece and digesta samples collected in animal experiments were ground through a 1-mm sieve and thoroughly mixed for chemical analysis (Table A1). Dry matter (DM, method 934.01), crude protein (CP, method 990.03), ash (method 942.05), ether extract (EE, method 920.39), calcium (method 985.01), total phosphorus (method 985.01) and AAs (method 982.30) content were determined according the procedures of Association of Official Analytical Chemists (AOAC) International (2006). Neutral detergent fiber (NDF) and acid detergent fiber (ADF) were measured using the ANKOM200 Fibre Analyzer (Ankom Technology, NY, USA). Gross energy (GE) was analyzed using automatic isoperibol oxygen bomb

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 21 September 2023 doi:10.20944/preprints202309.1451.v1

calorimeter (IKA C6000; IKA, GER). The fatty acid value was analyzed according to the procedures of GB/T 20569-2006 (Guidelines for evaluation of paddy storage character).

Experiment 1: evaluating the DE and ME values of stored brown rice

Eighteen Landrace × Yorkshire (L × Y) barrows with initial body weight (IBW) of 25.48 ± 3.21 kg were randomly allotted to 3 diets with 6 replicated pigs per treatment. The diets were formulated to contain 96.9% of corn or brown rice stored for 1 or 6 year, and 3.1% minerals and vitamins to meet the nutritional requirements for growing pigs recommended by the National Research Council (NRC, 2012) (Table A2).

Pigs were individually raised in metabolism crates (1.4 m × 0.45 m × 0.6 m) and were free to water. The daily feed equivalent to 4% of initial body weight was divided into two equal parts and supplied at 08:30 and 16:30 to each pig.The room temperature was controlled at 23 ± 2 °C.

Before the start of animal trial, pigs were allowed 7 d to adapt to the metabolic crates and fed on a commercial diet. The animal experiment lasted for 12 d, of which 7 d were used to adapt to the experimental diets and 5 d were used to collect feces and urine [14]. Feces were collected from each metabolism crate and stored at - 20 °C.

Urine was collected separately in barrels containing 50 mL 6 mol/L HCl, and all barrels were placed under metabolism crates. 10% of the total urine collected daily was stored at - 20 °C. At the end of the experiment, the fecal and urine samples were thawed and merged. The fecal subsample was dried in a drying oven at 65 °C for 72 h, and 4 mL of urine sample was dropped into a crucibles containing quantitative filter paper and then dried in a drying oven at 65 °C for 8 h for further analysis of GE.

Experiment 2: evaluating the AA digestibility of stored brown rice

Eighteen barrows (L × Y) with T-cannula at the terminal ileum (IBW: 22.16 ± 2.42 kg) were allocated to 3 experiment diets in a completely randomized design with 6 replicated pigs per treatment. The diets were formulated to contain 96.6% of corn or brown rice stored for 1 or 6 year, and 3.1% minerals and vitamins to meet the nutritional requirements for growing pigs recommended by the NRC (2012). N-free diet containing 73.35% corn starch and 15% sucrose was used to evaluate the losses of basal ileal endogenous N and AAs. In addition, 0.3% chromic oxide was included as exogenous indicator (Table A2). Feeding management was the same as above.

After a 15 d recovery postoperative period, pigs were allowed 7 d to adapt to the environment and fed on a commercial diet. The animal experiment lasted for 7 d, of which 5 d were used to adapt to the experimental diets and 2 d to collection of digesta, which lasted for 9 h daily beginning at 08:30 [14]. The sample bag was fixed to the cannula to collect the digesta and then stored at - 20 °C. After the collection period, the digesta samples were thawed and merged to obtain the sub-sample by pig, then lyophilized by vacuum freeze drier.

Experiment 3: growth trail on weaned piglets

One hundred and eight weaned piglets (L × Y; IBW: 9.16 ± 0.89 kg) were selected from a commercial herd, and randomly allocated into 3 diet treatments with 6 replicate pens per treatment and 6 pigs per pen. The treatment included 1 control diet and 2 experimental diets formulated by completely replacing corn with brown rice stored for 1 or 6 years, respectively (Table A3). The diets were formulated based on the ME value in Exp. 1 and the SID AAs value in Exp. 2, and meet the nutritional requirements for weaned piglets recommended by NRC (2012). The ME and SID lysine, methionine, threonine, trptophan in 3 diets were kept the same. In the last 2 weeks of the experiment, 0.3% chromic oxide was added to each diet as an exogenous indicator.

Pigs were kept in pens with drinkers, feeders and slatted floors, and were provided water and feed freely. The environment temperature was controlled at 22 ± 2 °C. The experiment lasted for 28 days, and at the beginning (d 1) and the end of the experiment (d 28), .pigs and feed were weighed

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 21 September 2023 doi:10.20944/preprints202309.1451.v1

to calculate the average daily gain (ADG), average daily feed intake (ADFI) and feed conversion ratio (FCR).

From d 25 to d 27, approximately 100 g of fresh feces were collected daily from each pen and immediately stored at - 20 °C. All samples were pooled by pen and then dried at 65 °C in a drying oven for 72 h. After fasting for 16 hours, blood samples were collected though intravenous puncture on the morning (07: 00) of d 28 and then injected into a 10 mL vacuum tube. After centrifugation at 3, 000 × g for 15 min (4 °C), serum samples were collected and stored at -20 °C for the further determination of biochemical parameters. At the end of the experiment, 18 pigs with nearly average BW were selected from each pen. After overnight fasting, the pigs were slaughtered and the gastrointestinal tract was ligated, then the mucosa of duodenum, jejunal and ileum were scraped with a glass slide and stored in liquid nitrogen.

After fasting overnight, the pigs are slaughtered and the gastrointestinal tract of each pig is ligated. Then, the mucosa of the duodenum, jejunum, and ileum is scraped with a slide and stored in liquid nitrogen.

Experiment 4: growth trail on growing-finishing pigs

Ninety growing pigs (L × Y; IBW: 48.28 ± 3.51 kg) were randomly allotted into 3 dietary treatments with 6 replicate pens per treatments and 5 pigs per pen. The treatment diets included 1 control diet and 2 experimental diets formulated by replacing corn with brown rice stored for 1 or 6 years, respectively (Table A4). The diets were formulated based on the ME value in Exp. 1 and the SID AAs value in Exp. 2, and meet the nutritional requirements for pigs in different phases recommended by NRC (2012). The ME and SID lysine, methionine, threonine, trptophan in 3 diets were kept the same.

Pigs were kept in pens with drinkers, feeders and slatted floors, and were provided water and feed freely. The environment temperature was controlled at 18 ± 2 °C. The experiment lasted for 8 weeks and included 2 phases: I (d 1 to d 24), II (d 25 to d 56). At the beginning (d 1) and end of each phase (d 24 and d 56), pigs and feed were weighed to determine ADG, ADFI and FCR.

At the end of the experiment, 15 pigs with near average BW were selected from each pen. After overnight fasting, pigs were subjected to electric shock (250 V, 0.5 A, for 5-6 s), bleeding, and evisceration using standard commercial procedure. Approximately 10 g of longissimus dorsi muscle (LDM) were sampled from the left half of each carcass and stored at -20 °C.

Determination of serum biochemical indices and intestinal enzyme activities

Serum albumin (ALB), globulin (GLB), total protein (TP), triglyceride (TG), total cholesterol (TC), alanine aminotransferase (ALT), aspartate aminotransferase (AST) and urea nitrogen (UN) were measured by automatic biochemical analyzer (7020 series; Hitachi, Japan) and following the protocol of assay kits purchased from Nanjing Jiancheng Bioengineering Institute (Nanjing, China). The content of serum immunoglobulin (Ig) A, IgG and IgM were determined using enzyme linked immunosorbent assay (ELISA) using assay kits purchased from Takara Biomedical Technology Institute (Beijing, China). Serum catalase (CAT), total antioxidant capacity (T-AOC), glutathione (GSH), glutathione peroxidase (GSH-PX), malondialdehyde (MDA), superoxide dismutase (SOD) were measured using assay kits purchased from Nanjing Jiancheng Bioengineering Institute (Nanjing, China).

The duodenum, jejunal, ileum mucosa samples were homogenized in cold maleic acid buffer (0.1mol/L, pH = 6.8, 1: 10, w/v) and then centrifuged at 3, 000 × g for 10 min. Supernatants were collected to evaluate the activities of amylase, lipase, chymotrypsin, trypsin, lactase, maltase and surcrase, following the protocol of assay kits purchased from Nanjing Jiancheng Bioengineering Institute (Nanjing, China).

 doi:10.20944/preprints202309.1451.v1

Determination of carcass characteristics and meat quality

After slaughter and scraping, the head, hooves, tail and internal organs of pigs were removed, while the suet and kidneys were preserved to record carcass weight, and dressing percentage was calculated as dividing carcass weight by live body weight. Carcass straight length was measured from the first rib to the end of the public bone. Backfat thickness was recorded at the first rib, last rib and last lumbar vertebra, and Loin eye height and width were measured at the 10th rib following the equation: loin-eye area (cm^2) = 0.7 × loin eye height (cm) × loin eye width (cm), according to the NY/T 825-2004 (Technical Regulation for Testing of Carcass Traits in Lean-Type Pig).

The LDM on the left half of carcass between the 10th and 12th ribs was sampled for analysis of meat quality, including drip loss, shear force, pH and muscle color, according to the NY/T 821-2019 (Technical Code of Practice for Pork Quality Assessment) and NY/T 1180-2006 (Determination of Meat Tenderness Shear Force Method).

About 30 g of meat was hung in a plastic bag at 4 °C for 24 h and kept out of contact with the bag. Drip loss was calculated as a percentage of the droplet amount compared to the initial meat weight. Meat were cooked in a water bath at 70 °C for 20 min, and then ten cylindrical samples were obtained by cutting the meat parallel to the fiber direction, and shear force was determined by cutting the cylindrical sample vertically to the myofiber axis using a digital-display-muscle tenderness meter. At 45 min postmortem, initial pH of LDM was recorded using a glass penetration pH electrode, and pH of LDM was detected again at 24 h postmortem. The meet color was measured as L* (lightness), a* (redness) and b* (yellowness) using a tristimulus colorimeter for three times at 24 h postmortem. About 20 g meat sample was lyophilized to determine the fatty acids profile using classical gas chromatography (6890 series; Agilent Technologies, DE, USA) [15].

Statistical analysis

PROC UNIVERSATE program (SAS Inst. Inc., Carry, NC, USA) of SAS 9.2 was use the to check the normal and abnormal values of growth performance, nutrient digestibility, serum biochemical index, enzyme activity, carcass traits, and meat quality data. Cook's distance and abandoned was used to identify outliers. Then the PROC GLM program of SAS was used to analyze the data. The diet was the only fixed effect while each pig is considered an experimental unit (for growth performance data, each pen was considered an experimental unit). The LSMEANS statement was used to separate treatment means and the Tukey test was used to adjust the data. Significant difference was declared at $P<0.05$.

3. Results

The chemical composition of corn and brown rice stored for 1 or 6 years are shown in Table A1. The DM, ash, calcium and total phosphorus of stored brown rice had no differences compared with corn. The content of CP and GE were higher, while the EE, NDF and ADF were lower in stored brown rice. The chemical composition of brown rice stored for 1 or 6 years showed no differences. Moreover, compared to stored brown rice, the content of lysine, tryptophan and arginine were lower in corn, while the content of leucine, phenylalanine, glutamic acid and proline were higher in corn. The fatty acid value of brown rice stored for 6 years was higher compared to brown rice stored for 1 year (26.43 vs 19.73 mg KOH/100 g) (Table A1).

Available energy value and AA digestibility of stored brown rice

The DE values of brown rice stored for 1 or 6 years were 14.70 and 14.88 MJ/kg (as-fed basis), while the ME values of brown rice stored for 1 or 6 years were 14.22 and 14.31 MJ/kg, respectively. The DE and ME values of corn were 14.52 and 14.17 MJ/kg (Table 1). The AID and SID values of arginine and histidine in brown rice stored for 1 or 6 years, and the AID values of aspartic acid in brown rice stored for 1 or 6 years were higher than that of corn ($P < 0.05$). There was no difference in the digestibility of other amino acids between stored brown rice and corn ($P > 0.05$), and the

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 21 September 2023 doi:10.20944/preprints202309.1451.v1

digestibility of all the amino acids in brown rice stored for 1 or 6 years showed no significant difference ($P > 0.05$) (Table 2).

Table 1. Available energy concentration of stored brown rice (MJ/kg, Exp. 1) [1].

Item	Corn	BR1	BR6	SEM	*P* value
DE	14.52	14.70	14.88	0.14	0.06
ME	14.17	14.22	14.31	0.10	0.59
ME/DE	97.56	96.78	96.14	1.31	0.57

Values are the means of 6 observations. BR1 = Brown rice stored for 1 year; BR6 = Brown rice stored for 6 year; SEM = standard error of the mean; DE = digestible energy; ME = Metabolisable energy.

Table 2. Apparent and standardized ileal digestibility of crude protein and amino acids in stored brown rice (%, dry-matter basis, Exp. 2) [1].

Item	Apparent ileal digestibility					Standardized ileal digestibility				
	Corn	BR1	BR6	SE	*P*	Cor	BR1	BR6	SE	*P*
CP	72.17	80.0	81.94	3.98	0.06	79.15	89.71	88.0	5.11	0.12
Lysine	58.21	64.1	65.53	6.70	0.52	67.43	76.41	79.3	3.87	0.06
Methionine	75.98	82.8	77.57	4.66	0.33	79.55	87.08	81.4	3.71	0.26
Threonine	68.97	70.9	69.69	5.45	0.93	77.76	82.67	79.7	5.15	0.49
Trptophan	51.97	52.2	54.55	4.14	0.79	63.32	62.08	65.3	4.39	0.48
Leucine	79.62	76.2	77.03	3.09	0.53	86.21	84.58	83.4	2.56	0.65
Valine	69.01	75.8	77.55	3.97	0.11	77.59	81.97	81.7	2.90	0.51
Phenylalani	76.37	78.6	78.68	3.12	0.71	84.75	84.32	84.0	2.64	0.98
Isoleucine	69.49	74.3	73.45	4.23	0.49	78.10	80.16	78.9	3.48	0.91
Arginine	70.54	78.4	78.13	3.23	0.04	70.91	86.38	85.9	5.12	<0.01
Histidine	77.70	86.9	87.91	2.53	<0.01	86.20	97.31	96.0	3.51	<0.01
Glutamic	79.24	79.6	79.23	2.88	0.98	87.17	85.50	84.9	2.59	0.78
Tyrosine	73.17	84.9	82.05	5.96	0.16	82.44	91.39	88.1	5.13	0.44
Serine	68.01	71.3	70.94	4.30	0.70	78.58	79.02	78.6	3.43	1.00
Glycine	47.98	59.2	56.95	5.62	0.14	54.17	66.60	64.6	7.20	0.24
Proline	62.40	62.3	62.34	8.88	1.00	67.99	70.78	64.6	9.59	0.81
Cysteine	70.17	62.1	67.80	7.78	0.58	80.76	71.12	75.8	12.1	0.57
Alanine	67.23	63.7	64.85	5.16	0.79	77.44	71.48	72.8	4.94	0.59
Aspartic	66.80	77.0	77.07	4.83	<0.01	76.15	83.09	83.0	3.15	0.10

[1] Values are the means of 6 observations. BR1 = Brown rice stored for 1 year; BR = Brown rice stored for 6 year; SEM = standard error of the mean; CP = crude protein. [a, b] Different superscript letters mean significantly different ($P < 0.05$).

Growth performance and apparent total tract digestibility (ATTD) of nutrients of weaned piglets fed stored brown rice diets

The weaned piglets fed stored brown rice diets showed no significant differences on final BW, ADG, ADFI and FCR compared with the control diet ($P > 0.05$), and there was also no significant difference in growth performance between piglets fed brown rice stored for 1 or 6 years ($P > 0.05$) (Table 3). There were no differences in the ATTD of DM, GE, CP, OM, NDF, ADF, Ca and P between the weaned piglets fed stored brown rice diets or control diet ($P > 0.05$) (Table 4).

Table 3. Effects of stored brown rice on growth performance of the piglets (Exp. 3) [1].

Item	Control	BR1	BR6	SEM	*P* value
BW d 0, kg	9.12	9.33	9.04	0.20	0.15
BW d 28, kg	18.30	19.27	19.64	0.31	0.26
ADG, g/d	328.04	368.29	359.26	35.63	0.39
ADFI, g/d	554.78	609.80	610.36	80.32	0.18
FCR	1.71	1.66	1.70	0.26	0.92

[1] Values are the means of 6 observations. BR1 = Brown rice stored for 1 year; BR6 = Brown rice stored for 6 year; SEM = standard error of the mean; BW = body weight; ADG = average daily gain; ADFI = average daily feed intake; FCR = feed conversion ratio.

Table 4. Effects of stored brown rice on nutrients digestibility in the piglets (Exp. 3) [1].

Item	Control	BR1	BR6	SEM	*P* value
DM	86.79	86.98	87.95	0.65	0.19
GE	87.29	86.43	87.94	0.44	0.10
CP	81.40	81.12	81.50	0.47	0.71
OM	87.34	88.79	88.61	0.77	0.16
NDF	62.12	61.15	62.25	1.19	0.61
ADF	59.60	58.73	58.89	1.08	0.70
Calcium	43.57	41.57	41.82	1.78	0.74
Phosphorus	40.44	39.76	39.88	0.90	0.73

[1] Values are the means of 6 observations. BR1 = Brown rice stored for 1 year; BR6 = Brown rice stored for 6 year; SEM = standard error of the mean; DM = dry matter; GE = gross energy; CP = crude protein; OM = organic matter; NDF = neutral detergent fiber; ADF = acid detergent fiber.

Serum biochemical parameters

As shown in Table 5, no significant differences on concentrations of TP, ALB, GLB, TG, TC, AST, ALT, UREA were observed in serum of piglets fed the stored brown rice diets relative to the control diet ($P > 0.05$). And the activities of CAT, TAOC, GSH, GSH-PX, MDA and SOD were not different between the piglets fed the stored brown rice diets or control diet ($P > 0.05$). However, the concentration of serum IgG was decreased in weaned piglets fed stored brown rice diets ($P < 0.01$).

Table 5. Effects of stored brown rice on serum biochemical indices in the piglets (Exp. 3)[1].

Item	Control	BR1	BR6	SEM	*P*
Biochemical indices					
TP, g/L	37.63	36.06	36.00	3.61	0.88
ALB, g/L	18.56	16.56	17.73	1.45	0.42
GLB, g/L	19.06	19.50	18.27	2.55	0.89
TG, mmol/L	0.66	0.71	0.60	0.13	0.70
TC, mmol/L	1.35	1.65	1.68	0.24	0.36
AST, U/L	59.05	61.79	44.63	13.62	0.43
ALT, U/L	47.97	42.11	31.97	7.00	0.12
Urea, mmol/L	1.84	2.56	1.96	0.41	0.22
Immunity indices, g/L					

IgA	1.76	1.69	1.69	0.05	0.27
IgG	9.22[a]	8.53[b]	8.50[b]	0.14	< 0.01
IgM	0.75	0.72	0.72	0.03	0.53
Antioxidant indices					
CAT, U/ml	64.58	51.57	55.98	5.37	0.10
TAOC, U/mL	10.12	9.36	9.09	0.58	0.23
GSH, μmol/L	9.60	8.68	8.68	0.59	0.26
GSH-PX, U/mL	323.49	294.52	318.20	14.86	0.17
MDA, nmol/mL	3.43	3.89	3.75	0.24	0.21
SOD, U/mL	75.78	65.67	70.10	4.72	0.16

[1] Values are the means of 6 observations. BR1 = Brown rice stored for 1 year; BR6 = Brown rice stored for 6 year; SEM = standard error of the mean; TP = total protein; ALB = albumin; GLB = globulin; TG = total triglyceride; TC = total cholesterol; AST = aspartate aminotransferase; ALT = alanine aminotransferase; Ig = immunoglobulin; CAT = catalase; TAOC = total antioxidant capacity; GSH = glutathione; GSH-PX = glutathion peroxidase; MDA = malonaldehyde; SOD = superoxide dismutase.
[a, b] Different superscript letters mean significantly different ($P < 0.05$).

Digestive enzymes activities

As shown in Table 6, the activities of lipase, chymotrypsin, lactase, maltase, sucrase were decreased in duodenum of piglets fed the stored brown rice diets compared to the control diet ($P < 0.05$). And the activity of lactase in duodenum of piglets fed the brown rice stored for 6 years was lower compared to the piglets fed the brown rice stored for 1 year ($P < 0.05$). The activities of trypsin and sucrase were decreased in jejunum of piglets fed the brown rice stored for 6 years compared to the control diet ($P < 0.05$), and the activities of lactase and maltase were both decreased in jejunum of piglets fed the brown rice stored for 1 or 6 years compared the control diet ($P < 0.05$). The activity of lactase was decreased in ileum of piglets fed the stored brown rice diets compared control diet ($P < 0.05$), while the activity of lactase was lower in ileum of piglets fed the brown rice stored for 6 years compared to the brown rice stored for 1 year ($P < 0.05$). The activity of maltase was decreased in ileum of piglets fed the brown rice stored for 6 years compared the control diet ($P < 0.05$).

Table 6. Effects of stored brown rice on digestive enzymes in the piglets (Exp. 3) [1].

Item	Control	BR1	BR6	SEM	*P* value
Duodenum					
Amylase, U/g	79.62	70.95	56.71	28.62	0.73
Lipase, U/mg	15.10[a]	13.92[ab]	12.19[b]	0.86	0.02
Chymotrypsin, U/mg	39.61[a]	28.35[b]	24.67[b]	3.84	< 0.01
Trypsin, U/mg	16.89	14.72	13.55	1.27	0.07
Lactase, U/g	499.96[a]	334.62[b]	286.37[c]	7.14	< 0.01
Maltase, U/mg	215.53[a]	183.13[ab]	158.66[b]	19.54	0.05
Sucrase, U/mg	229.78[a]	191.51[b]	164.97[b]	12.55	< 0.01
Jejunum					
Amylase, U/g	44.03	42.21	40.06	19.99	0.98
Lipase, U/mg	15.53	14.01	12.59	1.32	0.14
Chymotrypsin, U/mg	31.65	26.83	22.88	3.18	0.06
Trypsin, U/mg	19.54[a]	15.64[ab]	12.95[b]	2.01	0.03

Lactase, U/g	316.86[a]	268.51[b]	259.26[b]	5.90	< 0.01
Maltase, U/mg	177.49[a]	146.19[b]	132.75[b]	8.99	< 0.01
Sucrase, U/mg	184.99[a]	163.38[ab]	143.78[b]	12.10	0.02
Ileum					
Amylase, U/g	99.78	49.93	34.29	29.47	0.12
Lipase, U/mg	14.24	13.27	11.83	0.98	0.10
Chymotrypsin, U/mg	10.41	9.71	8.56	0.98	0.22
Trypsin, U/mg	15.40	13.59	11.41	2.31	0.28
Lactase, U/g	565.69[a]	488.10[b]	423.99[c]	14.87	< 0.01
Maltase, U/mg	129.43[a]	104.59[ab]	91.61[b]	13.17	0.05
Sucrase, U/mg	133.28	107.31	95.96	13.54	0.06

[1] Values are the means of 6 observations. BR1 = Brown rice stored for 1 year; BR6 = Brown rice stored for 6 year; SEM = standard error of the mean. [a, b] Different superscript letters mean significantly different ($P < 0.05$).

Growth performance, carcass traits and meat quality of growing-finishing pigs fed stored brown rice diets

The final BW and ADFI in phase I, and ADFI in total phase were markedly decreased in pigs fed a corn diet ($P < 0.05$), and the BW, ADG, ADFI and FCR of growing-finishing pigs in all phases showed no significant differences between pigs fed brown rice stored for 1 or 6 years ($P > 0.05$) (Table 7).

Table 7. Effects of stored brown rice on growth performance in the growing-finishing pigs (Exp. 4) [1].

Item	Phase	Control	BR1	BR6	SEM	*P* value
BW, kg	Initial	47.85	48.55	48.43	0.38	0.19
	End Phase	71.55[b]	74.25[a]	72.95[ab]	0.72	< 0.01
	End Phase	97.63	98.00	98.83	1.56	0.74
ADG, kg/d	Phase I	0.91	0.99	0.94	0.03	0.10
	Phase II	0.97	0.88	0.96	0.07	0.45
	Total	0.94	0.93	0.95	0.03	0.84
ADFI,	Phase I	2.61[b]	2.89[a]	2.94[a]	0.08	< 0.01
	Phase II	3.05	3.10	3.20	0.08	0.23
	Total	2.90[a]	3.01[ab]	3.10[b]	0.07	0.03
FCR	Phase I	2.86	2.94	3.12	0.14	0.23
	Phase II	3.18	3.56	3.38	0.24	0.32
	Total	3.09	3.24	3.26	0.08	0.13

[1] Values are the means of 6 observations. BR1 = Brown rice stored for 1 year; BR6 = Brown rice stored for 6 year; SEM = standard error of the mean; BW = body weight; ADG = average daily gain; ADFI = average daily feed intake; FCR = feed conversion ratio. [a, b] Different superscript letters mean significantly different ($P < 0.05$).

The dressing percentage of LDM tended to decrease in pigs fed a corn diet compared with those fed the stored brown rice diet ($P = 0.06$), while other carcass traits including the carcass straight length, backfat thickness, loin-eye area, were not influenced by different diet treatment ($P > 0.05$) (Table 8). The meat quality including the drip loss, shear force, pH, and meat color were also not influenced by different diet treatment ($P > 0.05$) (Table 8). The corn diet or stored brown rice diet also had no significant effect on the fatty acid concentrations in LDM of finishing pigs ($P > 0.05$) (Table 9).

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 21 September 2023 doi:10.20944/preprints202309.1451.v1

Table 8. Effects of stored brown rice on carcass traits and meat quality of finishing pigs (Exp. 4) [1].

Item	Control	BR1	BR6	SEM	*P* value
Carcass traits					
Dressing percentage, %	65.67	69.20	70.02	1.67	0.06
Carcass straight length, cm	104.38	106.25	103	3.08	0.59
Backfat thickness, cm	2.39	1.98	2.47	0.26	0.19
Loin-eye area, cm2	46.95	54.46	49.81	8.11	0.66
Meat quality					
Drip loss, %	2.41	2.27	3.08	0.37	0.12
Shear force, N	36.97	40.88	37.85	4.99	0.72
pH45 min	6.39	6.54	6.18	0.21	0.26
pH24 h	5.92	5.84	5.81	0.16	0.81
L* (lightness)	42.28	42.35	43.60	1.20	0.50
a* (redness)	13.86	13.22	14.25	0.84	0.50
b* (yellowness)	2.02	2.40	1.85	0.47	0.52

[1] Values are the means of 6 observations. BR1 = Brown rice stored for 1 year; BR6 = Brown rice stored for 6 year; SEM = standard error of the mean; BW = body weight; ADG = average daily gain; ADFI = average daily feed intake; FCR = feed conversion ratio.

Table 9. Effects of stored brown rice on fatty acids profiles in the longissimus dorsi muscle of finishing pigs (mg/g, of fresh tissue) (Exp. 4) [1].

Item	Control	BR1	BR6	SEM	*P* value
Capric acid (C10: 0)	0.16	0.16	0.16	0.02	0.97
Lauric acid (C12: 0)	0.09	0.09	0.09	0.01	0.58
Myristic acid (C14: 0)	1.30	1.36	1.38	0.21	0.63
Palmitic acid (C16: 0)	21.62	22.15	23.03	8.36	0.98
Palmitoleic acid (C16: 1)	3.41	3.22	3.40	0.22	0.64
Heptadecanoic acid (C17: 0)	0.34	0.34	0.33	0.03	0.98
Stearic acid (C18: 0)	12.24	12.74	12.88	3.18	0.99
Oleic acid (C18: 1n-9c)	39.79	39.07	39.82	9.83	0.92
Linoleic acid (C18: 2n-6c)	7.72	7.98	7.98	1.14	0.84
Alpha-linolenic acid (C18: 3n-3)	0.37	0.39	0.36	0.03	0.87
Gama-linolenic acid (C18: 3n-6)	0.11	0.10	0.12	5.19	0.82
Icosanoic acid (C20: 0)	0.25	0.26	0.25	0.52	0.89
Eicosenoic acid (C20: 1)	0.74	0.72	0.75	0.05	0.93
Decosahedaenoic acid (C20: 2)	0.48	0.48	0.63	0.02	0.71
Dihomo-γ-linolenic (C20: 3n-6)	0.21	0.20	0.19	0.02	0.79
Arachidonic acid (C20: 4n-6)	1.53	1.45	1.69	0.06	0.60
Heneicosanoic acid (C21: 0)	0.34	0.33	0.35	0.20	0.87
n-6/n-3 PUFA	25.79	25.43	27.68	3.44	0.79
PUFA/SFA	0.33	0.33	0.34	0.11	0.99

[1] Values are the means of 6 observations. BR1 = Brown rice stored for 1 year; BR6 = Brown rice stored for 6 year; SEM = standard error of the mean; PUFA = polyunsaturated fatty acid; SFA = saturated fatty acid.

4. Discussion

Available energy values and amino acid digestibility of corn and stored brown rice

Rice is a grain variety that is not resistant to long-term storage, as aging and deteriorate could occur after the second year, and the warranty storage life is about three years. During rice storage, several physical and chemical properties were changed, which causing the reduction of nutritional value, especially under a high temperature and humidity environment [2,16]. Rehman et al. found that protein digestibility of maize grain was decreased when storage for 6 months at 25 °C or 45 °C, however, no significant changes occurred when storage at 10 °C [17]. In addition to the storage condition, the changes during storage is also related to the variety of grain, as Zhang et al. showed that the changes of nutrient value and storage characteristics during storage were complex between different varieties of corn [7]. The climate, soil and processing methods may also cause large variation in the storage quality of stored grain [18].

In the present study, the contents of CP, GE, total phosphorus, several essential amino acids, as well as the digestibility of arginine, histidine and aspartic acid in stored brown rice were higher than that of corn ($P < 0.05$) (Table A1 and Table 2), which was consistent with the previous studies [19-21]. There were no significant difference in main nutrient contents between two stored brown rices (Table A1), except for a increased fatty acid value in brown rice stored for 6 years, which indicated that long time storage did not change the total amount of protein, starch and fat, but may increase lipid oxidation in brown rice [22]. However, the changes in physical properties and structure of fat did not cause the decrease of available energy value and nutrient digestibility in stored brown rice when storage under proper conditions (Table 1 and Table 2). Mitchell and Beadles showed that the nutrient value of wheat and corn had no obviously change over long periods of time, when storage under a condition that preventing insect infestation and mold growth [11]. Bartov reported that the storage of maize for up to 110 months under good conditions had no adversely effect on the main nutrient contents and the ME value for young male broiler chicks [9]. Yin et al. also showed that the digestibility of starch, CP, and apparent metabolic energy value of corn in chicken was also not affected by storage for 5 years [8].

Effects of stored brown rice on growth performance and nutrient digestibility of piglets

When replaced 100% of corn with stored brown rice, we found that the growth performance and nutrient digestibility of weaned piglets showed no significant difference between the dietary treatments ($P > 0.05$) (Table 5 and Table 6). Kim et al. replaced 50% of corn with brown rice and found that the final BW, overall ADG, as well as ATTD of DM and GE were higher in weaned pigs fed brown rice diet [23]. Sittiya et al. showed that brown rice can totally replace corn in chicken diets without negative effect on growth performance [13]. Rice and broken rice can also be efficiently used in livestock and poultry diet [24,25]. Although brown rice are rich in various phytochemicals such as anthocyanins and tocopherols [26], however, it could be less palatable compared to corn, which may cause the decrease of FI in weaned piglet [27]. At the same time, some studies showed that the particle size, extrusion and heat treatment of brown rice had significant effect on its nutrient utilization, which should be paid attention to when brown rice is used in animal feed [28-31]. When corn stored for 4 years, it had no significant effect on FI, body weight gain and FCR of broilers from 0 to 6 weeks [8], which is consistent with our results (Table 5 and Table 6), and indicating that the growth performance of animals may be poorly correlated with the changes in nutrient value of stored grain [32,33].

Effects of stored brown rice on serum profiles and intestinal enzyme activities of piglets

There were no significant changes on serum profiles and antioxidant indexes between piglets fed the stored brown rice diets relative to the control diet (P > 0.05) (Table 7), which was consistent with previous researches [23,34]. Several researches showed that the concentration of plasma glucose and insulin, as well as the activities of maltase and aminopeptidase in the jejunum of rice-fed piglets were higher than that of corn-fed piglets [35-37], which may be due to a higher villus height and result in a higher ADG of piglets. In the present study, most of the activities of lipase, chymotrypsin, lactase, maltase, sucrase were decreased in the small intestine of piglets fed the stored brown rice diets compared to control diet (P < 0.05), and the activities of lactase in duodenum and lactase in ileum of piglets fed the brown rice stored for 6 years was lower compared to the brown rice stored for 1 year (P < 0.05) (Table 8). Although there were no related researches on the effect of brown rice on intestinal enzyme activities in pigs, previous study has showed that the fewer non-starch polysaccharides and resistant starch in brown rice making it easier to digest compared to corn [38], so the decrease in mucosal enzyme activities caused by feeding brown rice may not lead to the changes in energy and nutrient digestibility as well as the growth performance of piglets. He et al. found that extrusion or enzyme supplementation in stored brown rice diet significantly increased the carbohydrase activity in the digestive tract of piglets [39]. Dadalt et al. found that diets supplemented with multicarbohydrase and phytase improved the digestibility of energy and some nutrients of broken rice in postweaned piglet [40]. Therefore, we can appropriately extrude stored brown rice or supplement enzyme preparations to improve its nutrients digestibility.

Effects of stored brown rice on growth performance, carcass traits and meat quality of growing-finishing pigs

In the present study, the growth performance of growing-finishing pigs in all phases showed no differences between pigs fed brown rice stored for 1 or 6 years (P > 0.05) (Table 9). Kim et al. replaced corn with 50%, 75% and 100% of brown rice respectively, and found that there were no differences on growth performance, nutrient digestibility, and carcass characteristics among dietary treatments [23]. However, long time fed of brown rice may have an effect on intestinal microbiota of pig, as the low fiber contents in brown rice compared to corn providing less substrates for bacterial fermentation in the hindgut. Yin et al. showed that the catalase activity and peroxidase activity were decreased and the acidity of fatty acids was increased in corn stored for 5 years, which may result in significantly lower pH and increased drip loss in broiler breast muscle [8]. However, in the present study, although the fatty acid value was increased significantly in brown rice stored for 6 years compared to brown rice stored for 1 year (Table A1), the pigs fed the brown rice stored for 1 or 6 years showed no significant differences in carcass traits, meat quality and fatty acid concentrations in LDM (P < 0.05) (Table 8 and Table 9).

5. Conclusions

There were no significant changes in available energy value and amino acids digestibility of brown rice stored under good conditions for 6 years. Stored brown rice had no significant effects on growth performance, ATTD, serum biochemical indices, carcass traits, meat quality and muscle fatty acids profiles, but it would reduce the activity of digestive enzymes in pig small intestine. Therefore, the stored brown rice in good storage conditions can be used as a safe and high-quality energy feed raw material in pig diet.

Author Contributions: Conceptualization, H.B.; methodology, H.B and S.J.; validation, H.B. and L.K.; formal analysis, H.B. and S.J.; investigation, H.B. and L.K.; resources, C.J.; data curation, W.W.; writing—original draft preparation, H.B.; writing—review and editing, H.B. and W.Y.; visualization, H.B.; supervision, L.A.; project administration, W.Y.; funding acquisition, L.A. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by National Key Research and Development Program of China, grant number 2021YFD1300300.

 doi:10.20944/preprints202309.1451.v1

Institutional Review Board Statement: The animal study protocol was approved by the Ethics Committee of Academy of National Food and Strategic Reserves Administration (protocol code 20230316006 and date of approval 20230316).

Conflicts of Interest: The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript; or in the decision to publish the results.

Appendix A

Table A1. Analyzed nutrient content of corn and stored brown rice (%, as-fed basis)[1].

Item	Corn	BR1	BR6
DM	87.52	88.66	88.53
CP	7.83	8.06	8.32
GE, MJ/kg	16.75	17.40	17.39
EE	3.35	2.26	2.42
Ash	1.18	1.13	1.23
NDF	11.48	2.11	2.02
ADF	2.05	1.21	1.36
Calcium	0.02	0.02	0.02
Total phosphorus	0.28	0.31	0.32
Amino acid			
Essential amino acids			
Lysine	0.24	0.32	0.27
Methionine	0.18	0.21	0.19
Threonine	0.29	0.29	0.25
Tryptophan	0.06	0.12	0.10
Leucine	0.95	0.55	0.46
Valine	0.35	0.38	0.31
Phenylalanine	0.40	0.33	0.28
Isoleucine	0.25	0.23	0.20
Arginine	0.35	0.53	0.52
Histidine	0.20	0.13	0.18
Nonessential amino acids			
Glutamic acid	1.36	1.14	0.96
Tyrosine	0.23	0.22	0.23
Serine	0.36	0.33	0.28
Glycine	0.25	0.32	0.27
Proline	0.66	0.27	0.16
Cysteine	0.15	0.12	0.11
Alanine	0.49	0.41	0.35
Aspartic acid	0.51	0.62	0.52
Fatty acid value, mg KOH/100 g	55.43	19.73	26.43

[1] All values are the results of an analysis conducted in duplicate. BR1 = brown rice stored for 1 year; BR6 = brown rice stored for 6 year; DM = dry matter; CP = crude protein; GE = gross energy; EE = ether extract; NDF = neutral detergent fiber; ADF= acid detergent fiber.

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 21 September 2023 doi:10.20944/preprints202309.1451.v1

Table A2. Ingredients of the experimental diets used in Exp. 1 and 2 (%, as-fed basis) [1].

Item	Exp. 1			Exp. 2			
	Corn	BR1	BR6	Corn	BR1	BR6	N-free
Corn	96.90	-	-	96.60	-	-	-
Corn starch	-	-	-	-	-	-	73.35
Sucrose	-	-	-	-	-	-	15.00
BR	-	96.90	96.90	-	96.60	96.60	-
Soybean oil	-	-	-	-	-	-	3.00
Dicalcium	1.50	1.50	1.50	1.50	1.50	1.50	3.00
Limestone	0.80	0.80	0.80	0.80	0.80	0.80	-
Sodium chloride	0.30	0.30	0.30	0.30	0.30	0.30	0.45
Chromic oxide	-	-	-	0.30	0.30	0.30	0.30
Vitamin and mineral	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Cellulose acetate	-	-	-	-	-	-	4.00
Potassium carbonate	-	-	-	-	-	-	0.30
Magnesium oxide	-	-	-	-	-	-	0.10

[1] BR1 = Brown rice stored for 1 year; BR6 = Brown rice stored for 6 year. [2] Vitamin and mineral premix provided the following per kg of diet: vitamin A, 5512 IU; vitamin D3, 2 200 IU; vitamin E, 30 IU; vitamin K3, 2.2 mg; vitamin B12, 27.6 μg; vitamin B2, 27.6 mg; vitamin B1, 1.5 mg; vitamin B5, 14 mg; vitamin B6, 3 mg; niacin acid, 30 mg; choline chloride, 400 mg; folic acid, 0.7 mg; biotin, 44 μg; Mn, 40 mg (as manganese oxide); Fe, 75 mg (as ferrous sulfate); Zn, 75 mg (as zinc oxide); Cu, 100 mg (as copper sulfate); I, 0.3 mg (as potassium iodide) and Se, 0.3 mg (as sodium selenite).

Table A3. Ingredients and analyzed nutrient levels of the experimental diets used in Exp. 3 (%, as-fed basis) [1].

Item	Control	BR1	BR6
Ingredients			
Corn	62.78	-	-
Soybean meal (46% CP)	15.90	15.90	15.90
Extruded soybean	9.00	8.40	7.72
BR1	-	63.85	-
BR6	-	-	65.02
Fish meal	4.00	4.00	4.00
Whey powder	4.00	4.00	4.00
Soybean oil	1.00	0.60	0.60
Dicalcium phosphate	0.76	0.66	0.66
Limestone	0.68	0.78	0.78
Sodium chloride	0.30	0.30	0.30
Chromic oxide	0.30	0.30	0.30
Vitamin and mineral 2 permix	0.50	0.50	0.50
L-Lysine HCl	0.46	0.42	0.44
DL-Methionine	0.07	0.06	0.08
L-Threonine	0.21	0.21	0.24
L-Tryptophan	0.04	0.02	0.03
Analyzed nutrient levels			
DM	88.85	89.75	89.47
CP	18.82	18.66	18.70
GE, MJ/kg	16.35	16.52	16.43
Calcium	0.70	0.68	0.64
Total phosphorus	0.62	0.63	0.63

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 21 September 2023 doi:10.20944/preprints202309.1451.v1

Calculated nutrient levels 3			
ME, MJ/kg	13.72	13.73	13.71
SID Lysine	1.23	1.23	1.23
SID Methionine	0.36	0.36	0.36
SID Threonine	0.74	0.73	0.73
SID Tryptophan	0.21	0.21	0.21

[1] BR1 = Brown rice stored for 1 year; BR6 = Brown rice stored for 6 year; DM = dry matter; CP = crude protein; GE = gross energy; ME = Metabolisable energy; SID = Standardized ileal digestible. [2] Vitamin and mineral premix provided the following per kg of diet: vitamin A, 12 000 IU; vitamin D3, 2 000 IU; vitamin E, 24 IU; vitamin K3, 2 mg; vitamin B12, 24 µg; vitamin B2, 6 mg; vitamin B1, 2 mg; vitamin B5, 20 mg; vitamin B6, 3 mg; niacin acid, 30 mg; choline chloride, 0.4 mg; folic acid, 3.6 mg; biotin, 0.1 mg; Mn, 40 mg (as manganese oxide); Fe, 96 mg (as ferrous sulfate); Zn, 120 mg (as zinc oxide); Cu, 8 mg (as copper sulfate); I, 0.56 mg (as ethylenediamine dihydroiodide) and Se, 0.4 mg (as sodium selenite). [3] These values were calculated from data provided in Exp. 1 and 2.

Table A4. Ingredients and analyzed nutrient levels of the experimental diets used in Exp. 4 (%, as-fed basis) [1].

Item	Growing phase: 50 to 75 kg			Growing phase: 75 to 100 kg		
	Control	BR1	BR6	Control	BR1	BR6
Ingredients						
Corn	68.41	-	-	75.60	-	-
Soybean meal	21.00	21.00	21.00	16.68	16.05	16.00
Extruded soybean	3.10	2.30	1.60	-	-	-
Wheat bran	4.00	4.00	4.00	4.00	4.00	4.00
BR1	-	70.54	-	-	77.85	-
BR6	-	-	70.32	-	-	81.85
Soybean oil	1.20	-	-	1.58	0.10	-
Dicalcium phosphate	0.83	0.78	0.79	0.62	0.50	0.56
Limestone	0.80	0.82	0.82	0.80	0.88	0.86
Sodium chloride	0.35	0.35	0.35	0.35	0.35	0.35
Vitamin and mineral [2]	0.50	0.50	0.50	0.50	0.50	0.50
L-Lysine HCl	0.19	0.14	0.17	0.23	0.18	0.20
DL-Methionine	0.02	0.00	0.03	0.02	-	0.03
L-Threonine	0.02	0.02	0.06	0.04	0.04	0.08
L-Tryptophan	0.03	0.00	0.01	0.03	-	0.02
Analyzed nutrient levels						
DM	86.35	87.52	87.04	88.30	87.21	88.29
CP	16.46	16.40	16.39	14.11	14.08	14.12
GE, MJ/kg	16.13	16.05	16.09	16.03	16.06	16.18
Calcium	0.60	0.59	0.59	0.52	0.50	0.50
Total phosphorus	0.53	0.55	0.56	0.47	0.48	0.48
Calculated nutrient levels [3]						
ME, MJ/kg	13.25	13.25	13.25	13.10	13.10	13.10
SID Lysine	0.85	0.85	0.85	0.73	0.73	0.73
SID Methionine	0.26	0.26	0.26	0.24	0.24	0.24
SID Threonine	0.52	0.52	0.52	0.46	0.46	0.46
SID Tryptophan	0.19	0.19	0.19	0.16	0.16	0.16

[1] BR1 = Brown rice stored for 1 year; BR6 = Brown rice stored for 6 year; DM = dry matter; CP = crude protein; GE = gross energy; ME = Metabolisable energy; SID = Standardized ileal digestible. [2] Vitamin and mineral premix provided the following per kg of diet: vitamin A, 5512 IU; vitamin D3, 2 200 IU;

vitamin E, 30 IU; vitamin K3, 2.2 mg; vitamin B12, 27.6 μg; vitamin B2, 27.6 mg; vitamin B1, 1.5 mg; vitamin B5, 14 mg; vitamin B6, 3 mg; niacin acid, 30 mg; choline chloride, 400 mg; folic acid, 0.7 mg; biotin, 44 μg; Mn, 40 mg (as manganese oxide); Fe, 75 mg (as ferrous sulfate); Zn, 75 mg (as zinc oxide); Cu, 100 mg (as copper sulfate); I, 0.3 mg (as potassium iodide) and Se, 0.3 mg (as sodium selenite). [3] Nutrient levels were analyzed values, except metabolisable energy values were calculated.

References

1. Niu Y.N., Xie G.D., Xiao Y., Liu J.Y., Wang Y.Y., Luo Q., Zou H.X., Gan S.G., Qin K.Y., Huang M.D. Spatiotemporal patterns and determinants of grain self-sufficiency in China. *Foods* **2021**, *10*, 747.
2. Saikrishna A., Sayantani D., Vijayalakshmi S., Moses J.A., Anandharamakrishnan C. Ageing of rice: a review. *J. Cereal Sci.* **2018**, *81*, 161-170.
3. Kobayashi N., Sakurai K., Nakarai R., Shigaki K., Horikawa K., Honda M., Sugiura Y., Watanabe M., Takino M., Sugita-Konishi Y. Microflora of mycotoxigenic fungi in rice grains in Kyushu region of Japan and their changes during storage under non-controlled conditions. *Biocontrol. Sci.* **2019**, *24*, 161-166.
4. Zhao Q.Y., Lin J.H., Wang C., Yousaf L., Xue Y., Shen Q. Protein structural properties and proteomic analysis of rice during storage at different temperatures. Food Chem. 2021, 361:130028.
5. Wu P., Li C.F., Bai Y.M., Yu S.Y., Zhang X.A. A starch molecular basis for aging-induced changes in pasting and textural properties of waxy rice. *Food Chem*. **2019**, *284*, 270-278.
6. Zhang M.H., Liu K.L. Lipid and protein oxidation of brown rice and selenium-rich brown rice during storage. *Foods* **2022**, *11*, 3878.
7. Zhang L., Liu L., Li D.F., Li Q.F., Piao X.S., Thacker P.A., Brown M.A., Lai C.H. Effects of variety and storage duration on the nutrient digestibility and the digestible and metabolisable energy content of maize fed to growing pigs. *Arch. Anim. Nutr.* **2017**, *71*, 67-80.
8. Yin D.F., Yuan J.M., Guo Y.M., Chiba L.I. Effect of storage time on the characteristics of corn and efficiency of its utilization in broiler chickens. *Anim. Nutr.* **2017**, *3*, 252-257.
9. Bartov I. Effect of storage duration on the nutritional value of corn kernels for broiler chicks. *Poult. Sci.* **1996**, *75*, 1524-1527.
10. Cabell C.A., Ellis N.R. Feeding value of stored corn. J. Anim. Sci. 1955, 4:1167-1173.
11. Mitchell H.H., Beadles J.R. The effect of storage on the nutritional qualities of the proteins of wheat, corn and soybeans. *J. Nutr*.**1949**, *39*, 463-484.
12. Kim S., Cho J.H., Kim H.B., Song M. Evaluation of brown rice to replace corn in weanling pig diet. *J. Anim. Sci. Technol.* **2021**, *63*, 1344-1354.
13. Sittiya J., Yamauchi K., Takata K. Effect of replacing corn with whole-grain paddy rice and brown rice in broiler diets on growth performance and intestinal morphology. *J. Anim. Physiol. Anim. Nutr (Berl)*. **2016**, *100*, 381-390.
14. Ren P., Zhu Z.P., Dong B., Zang J.J., Gong L.M. Determination of energy and amino acid digestibility in growing pigs fed corn distillers' dried grains with solubles containing different lipid levels. *Arch. Anim. Nutr.* **2011**, *65*, 303-319.
15. Sukhija P.S., Palmquist D.L. Rapid method for determination of total fatty acid and composition of feedstuffs and feces. *J. Agric. Food Chem*. **1988**, *36*, 1202-1206.
16. Jood S., Kapoor A.C., Singh R. Available carbohydrates of cereal grains as affected by storage and insect infestation. *Plant Foods Hum. Nutr*. **1993**, *43*, 45-54.
17. Rehman Z.U., Habib F., Zafar S.I. Nutritional changes in maize (Zea mays) during storage at three temperatures. *Food Chem*. **2002**, *77*, 197-201.
18. Tran T.U., Suzuki K., Okadome H., Ikezaki H., Homma S., Ohtsubo K. Detection of changes in taste of japonica and indica brown and milled rice (Oryza sativa L.) during storage using physicochemical analyses and a taste sensing system. *J. Agric. Food Chem*. **2005**, *53*, 1108-1118.
19. Piao X.S., Li D.F., Han I.K., Chen Y., Lee J.H., Wang D.Y., Li J.B., Zhang D.F. Evaluation of Chinese brown rice as an alternative energy source in pig diets. *Asian-Austral. J. Anim*. **2002**, *15*, 89-93.
20. Cervantes-Pahm S.K., Liu Y., Stein H.H. Digestible indispensable amino acid score and digestible amino acids in eight cereal grains. *Br. J. Nutr.* **2014**, *111*, 1663-1672.
21. Cervantes-Pahm S.K., Liu Y., Stein H.H. Comparative digestibility of energy and nutrients and fermentability of dietary fiber in eight cereal grains fed to pigs. *J. Sci. Food Agric*. **2014**, *94*, 841-849.

22. Wang C.Y., Feng Y.C., Fu T.X., Sheng Y.N., Zhang S., Zhang Y.W., Jiang Y.J., Yu M., Zhan, D.J. Effect of storage on metabolites of brown rice. *J. Sci. Food Agric*. **2020**, *100*, 4364-4377.
23. Kim S., Cho J.H., Kim Y., Kim H.B., Song M. Effects of substitution of corn with ground brown rice on growth performance, nutrient digestibility, and gut microbiota of growing-finishing pigs. *Animals (Basal)* **2021**, *11*, 375.
24. Che T.M., Perez V.G., Song M., Pettigrew J.E. Effect of rice and other cereal grains on growth performance, pig removal, and antibiotic treatment of weaned pigs under commercial conditions. *J. Anim. Sci*. **2012**, *90*, 4916-4924.
25. Liu H., Wan H.F., Xu S.Y., Fang Z.F., Lin Y., Che L.Q., Li J., Li Y., Su X., Wu D. Influence of extrusion of corn and broken rice on energy content and growth performance of weaning pigs. *Anim. Sci. J*. **2016**, *87*, 1386-1395.
26. Ravichanthiran K., Ma Z.F., Zhang H., Cao Y., Wang C.W., Muhammad S., Aglago E.K., Zhang Y., Jin Y., Pan B. Phytochemical profile of brown rice and its nutrigenomic implications. *Antioxidants (Basel)* **2018**, *7*, 71.
27. He R.G., Ma Y.L., Wang Y.Q., Zhao J.Y., Wang H.X. Study of the brown rice nutritional value by the pig's digestion and metabolism trial. *J. Huazhong Agric. Univ*. **1994**, *13*, 268-273.
28. Bird A.R, Hayakawa T., Marsono Y., Gooden J.M., Record I.R., Correll R.L., Topping D.L. Coarse brown rice increases fecal and large bowel short-chain fatty acids and starch but lowers calcium in the large bowel of pigs. *J. Nutr*. **2000**, *130*, 1780-1787.
29. Li E.K., Zhu T., Dong W.X., Huang C.F. Effects of brown rice particle size on energy and nutrient digestibility in diets for young pigs and adult sows. *Anim. Sci. J*. **2019**, *90*, 108-116.
30. Torrallardona D., Andrés-Elias N., López-Soria S., Badiola I., Cerdà-Cuéllar M. Effect of feeding piglets with different extruded and nonextruded cereals on the gut mucosa and microbiota during the first postweaning week. *J. Anim. Sci*. **2012**, *90 Suppl 4*, 7-9.
31. Pluske J.R., Montagne L., Cavaney F.S., Mullan B.P., Pethick D.W., Hampson D.J. Feeding different types of cooked white rice to piglets after weaning influences starch digestion, digesta and fermentation characteristics and the faecal shedding of beta-haemolytic Escherichia coli. *Br. J. Nutr*. **2007**, *97*, 298-306.
32. Pirgozliev V.R., Rose S.P, Kettlewell P.S. Effect of ambient storage of wheat samples on their nutritive value for chickens. *Br. Poult. Sci*. **2006**, *47*, 342-349.
33. Pirgozliev V.R., Birch C.L., Rose S.P., Kettlewell P.S., Bedford M.R. Chemical composition and the nutritive quality of different wheat cultivars for broiler chickens. *Br. Poult. Sci*. **2003**, *44*, 464-475.
34. Lee J.J., Kim S., Cho J.H., Kyoung H., Lee S., Choe J., Liu Y., Ji P., Xiong X., Kim Y., Kim H.B., Song M. Potential use of ground brown rice for weanling pigs. *J. Anim. Sci*. **2021**, *99*, skab267.
35. Tasaka Y., Tachihara K., Kagawa R., Takada R. Effects of rice feeding on growth performance and protein (amino acids) metabolism in weanling piglets. *Anim. Sci. J*. **2020**, *91*, e13311.
36. Yagami K., Takada R. Dietary rice improves growth performance, mucosal enzyme activities and plasma urea nitrogen in weaning piglets. *Anim. Sci. J*. **2017**, *88*, 2010-2015.
37. Tsukahara T., Kishino E., Inoue R., Nakanishi N., Nakayama K., Ito T., Ushida K. Correlation between villous height and the disaccharidase activity in the small intestine of piglets from nursing to growing. *Anim. Sci. J*. **2013**, *84*, 54-59.
38. Pluske J.R., Siba P.M., Pethick D.W., Durmic Z., Mullan B.P., Hampson D.J. The incidence of swine dysentery in pigs can be reduced by feeding diets that limit the amount of fermentable substrate entering the large intestine. *J. Nutr*. **1996**, *126*, 2920-2933.
39. He J., Liu C., Fu C, Li J. Effects of extrusion and supplementation of exogenous enzymes to diets containing Chinese storage brown rice on the carbohydrase activity in the digestive tract of piglets. *J. Anim. Physiol. Anim. Nutr. (Berl.)* **2010**, *94*, 146-153.
40. Dadalt J.C., Gallardo C., Polycarpo G.V., Budiño F.E., Rogiewicz A., Berto D.A., Trindade Neto M.A. Ileal amino acid digestibility of broken rice fed to postweaned piglets with or without multicarbohydrase and phytase supplementation. *Asian-Australas J. Anim. Sci.* **2016**, *29*, 1483-1489.