

Effects of feeding solid-state fermented wheat bran on growth performance and nutrient digestibility in broiler chickens

A. R. Zhang,^{*,†,1} M. Wei,^{*,1} L. Yan,^{*} G. L. Zhou,^{*} Y. Li,^{*} H. M. Wang,^{*} Y. Y. Yang,^{*} W. Yin,^{*}
J. Q. Guo,^{*} X. H. Cai,^{*} J. X. Li,^{*} H. Zhou,^{*} and Y. X. Liang^{†,2}

^{*}New Hope Liuhe Co., Ltd, Key Laboratory of Feed and Livestock and Poultry Products Quality & Safety Control, Ministry of Agriculture, Chengdu, Sichuan 610023, China; and [†]State Key Laboratory of Agricultural Microbiology, College of Life Science and Technology, Huazhong Agricultural University, Wuhan 430070, China

ABSTRACT Solid-state fermentation has been used to improve the nutritive value of feed ingredients. In the present study, we investigated the effects of solid-state fermented wheat bran (FWB) on growth performance and apparent digestibility in broiler chickens. We measured the growth performance (ADFI, ADG, feed conversion, livability, and European performance efficiency factor) over 38 d in chicks fed a corn-soybean meal control diet (CON) or CON plus wet FWB (25 g/kg [T₁]; 50 g/kg [T₂]); or T₁ plus 3 g/kg (T₃); or T₂ plus 6 g/kg (T₄) soybean oil). The same diets were used to determine nutrient availability in chicks aged 20 d. Regression equations for AME and AME_n were obtained using 20-day-old chicks fed either the corn-soybean meal basal diet only or basal diet partially substituted with 50, 150, or 300 g/kg DM FWB. Diets containing 25 or 50 g/kg wet FBW did not affect the growth performance of

broiler chickens, nor the apparent DM, energy, and nitrogen digestibility of the feeds, compared with the control diets (all $P > 0.05$). Further supplementation with oil did not improve the growth performance of broiler chickens compared with controls or chickens fed FBW. However, chickens fed diets containing soybean oil (T₃ or T₄) had lower ($P = 0.005$ and $P = 0.040$, respectively) apparent DM and energy digestibility than the control and FWB groups. The regression equations for AME and AME_n with the substitution of FWB produced values of 1,854.3 and 1,743.9 kcal/kg DM, respectively, and the equations were $Y = 1854.3X + 52.7$ ($R^2 = 0.971$, $n = 24$, $P < 0.001$), and $Y = 1743.9X + 44.6$ ($R^2 = 0.978$, $n = 24$, $P < 0.001$), respectively. Supplementation with wet FWB did not affect the growth performance of broiler chickens. Therefore, FWB is a suitable feed component for broilers.

Key words: fermented wheat bran, broiler, growth performance, nutrient digestibility, metabolizable energy

2022 Poultry Science 101:101402

<https://doi.org/10.1016/j.psj.2021.101402>

INTRODUCTION

In commercial poultry production, feed contributes to up to 70% of the total production cost (Sugiharto and Ranjitkar, 2019). Because of global increases in feed prices, the poultry industry is using alternative or unconventional feed ingredients to increase profitability (Supriyati et al., 2015). Wheat bran (WB) is among the most abundant agricultural by-products in wheat-based agricultural countries such as China (Feng et al., 2020). However, the direct use of WB for some monogastric animals is limited because of its low protein and high fiber content. In addition, antinutritional factors such as

phytic acid, hemicellulose xylan (Hemery et al., 2007; Olukosi and Adeola, 2008), and other non-starch polysaccharides (NSP) have been identified in WB; these components may inhibit nutrient digestion and absorption (Koropatkin et al., 2012; Leo, 2012).

Solid-state fermentation has been reported as an effective approach for improving the nutritive value of by-products by reducing the cellulose content and improving the acid-soluble protein content (Teng et al., 2017; Yeh et al., 2018). In addition to improving the nutritional properties, fermentation results in numerous beneficial properties, including by increasing the number of lactic acid bacteria, decreasing pH, and increasing the concentration of organic acids (Engberg et al., 2009; Chiou et al., 2010; Canibe and Jensen, 2012). During the fermentation process, various essential nutrients are produced, such as vitamins, organic acids, amino acids, and small-size peptides, which may further increase the nutritional value of by-products (Feng et al., 2007; Chen, 2010), thereby extending the use of by-products

© 2021 Published by Elsevier Inc. on behalf of Poultry Science Association Inc. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

Received April 6, 2021.

Accepted July 20, 2021.

¹These authors contributed equally to this work.

²Corresponding author: liangyunxiang@mail.hzau.edu.cn

such as WB in the poultry industry. Previous studies of fermented WB mainly focused on indicators such as growth performance, intestinal health, immune performance, and economic benefits, whereas systematic evaluation of the nutritional value of fermented bran in poultry has not been widely conducted (Wanzenböck et al., 2020; Lin and Lee, 2020). Accurately quantifying the efficiency of metabolizable energy utilization of fermented soybean meal has led to the wide use of this material in the industry (Soumeh et al., 2019; Ping et al., 2020). However, no precise data are available for the change in the energy value of fermented WB, limiting the use of this raw material in the industry. In this study, systematically evaluated the potential metabolizable energy of fermented WB in poultry to provide information on the use of alternative feed ingredients for broiler chickens. Our study reveals changes in the energy and nutritional values of fermented WB. Moreover, the solid-state fermentation of raw materials is an effective method for increasing their nutrient value and providing higher fiber feeds for monogastrics.

The bacterial strains NHB-La13, NHB-Bs32, grx10, and NHF-Sc9 were used to prepare FWB. A feed trial was conducted to evaluate the nutritive value and nutrient digestibility of a solid-state FWB feed and its effects on growth performance in broiler chickens. Soluble carbon in WB is partially consumed during microbial activity causing energy loss during the fermentation process. We hypothesized that the NSP enzymes produced via microbial activity degrade indigestible fiber in WB and contribute to improving nutrient digestion and utilization.

MATERIALS AND METHODS

The experimental protocols, including the management and care of the birds, were reviewed and approved by the Animal Care and Use Committee of New Hope Liuhe Co., Ltd (Chengdu, China). The ethical inspection code is IAS 2019-53.

Preparation of FWB

WB was purchased from Wudeli Industrial Co., Ltd (Shandong, China). *Lactobacillus acidophilus* (NHB-La13), *Bacillus subtilis* (NHB-Bs32), and *Saccharomyces cerevisiae* (NHF-Sc9) were obtained from Chengdu Fenglan Technology Co., Ltd. (Chengdu, China). *Lactobacillus rhamnosus* (grx10) was obtained from Newhope Dairy Co., Ltd. (Sichuan, China).

The WB was inoculated with strains NHB-La13, NHB-Bs32, grx10, and NHF-Sc9 in a vacuum tank to produce FWB. These strains have been patented by China National Intellectual Property Administration and are used to ferment dairy products for human consumption. The main feature of the strains is that they secrete cellulase and protease. The patent numbers of these strains are as follows: C12N 1/20 (2006.01), C12Q 1/04 (2006.01), A23L 1/29 (2006.01), A23C 9/12

Table 1. Nutrient content of wheat bran and fermented wheat bran used as raw materials in broiler diets.

Item	Wheat bran	Fermented wheat bran
DM, %	88.50	60.60
CP, % DM	16.62	18.50
Starch, % DM	7.3	6.2
Ether extract, % DM	3.2	1.9
Crude fiber, % DM	8.8	7.2
Neutral detergent fiber, % DM	36.3	28.9
Acid detergent fiber, % DM	10.2	9.8
Crude ash, % DM	5.3	4.0
Calcium, % DM	0.10	0.09
Total phosphorous, % DM	1.08	1.07
pH	6.7	4.7
Total acid, mg/g	0.1	45.1
Acid soluble protein, % DM	1.21	8.34
Lactic acid bacteria, log ₁₀ cfu/g	NA	8.27
<i>Bacillus subtilis</i> , log ₁₀ cfu/g	NA	7.45
Gross energy, kcal/kg	3,986	3,786

(2006.01), A61K 35/74 (2006.01), and A61P 3/06 (2006.01). Solid-state fermentation was conducted for 72 h at 30°C under anaerobic conditions. The changes in pH and chemical composition before and after fermentation are shown in Table 1.

Trial 1

Experimental Broiler Chickens and Diets A total of 1,800 one-day-old Ross 308 broiler chicks with an average initial BW of 45.47 ± 0.16 g was randomly divided into 5 treatment groups. Each treatment was performed with 10 replicates with 36 birds (18 males, and 18 females per replicate) in a completely randomized design. The groups were fed a control corn-soybean meal basal diet (CON) or basal diet supplemented with 25 (T₁) or 50 (T₂) g/kg fermented FWB, or T₁ plus 3 g/kg soybean oil (T₃), or T₂ plus 6 g/kg soybean oil (T₄). The composition of the experimental diets and nutrient levels for the starter (1–14 d of age), grower (14–25 d of age), and finisher (25–38 d of age) periods were formulated according to the NRC, 1994 to meet the nutrient requirements of broilers (Table 2).

All chicks were housed in an environmentally controlled room with continuous light and allowed ad libitum access to water and feed. The temperature was maintained at 32°C during the first 3 d. Thereafter, it was gradually lowered by 3°C per week until it reached 25°C. This temperature was maintained until the end of the 38-d experiment period. The experimental protocols describing the management and care of the animals were reviewed and approved by the Animal Care and Use Committee of New Hope Liuhe Co., Ltd., following the requirements of the “Regulations for the Administration of Affairs Concerning Experimental Animals of China”.

Analyses of Growth Performance Bird weight and feed consumption were recorded on a per-pen basis at 1, 14, 25, and 38 d of age. Growth performance was evaluated based on ADG, ADFI, and feed conversion ratio (FCR, g feed/g gain). Mortality was recorded daily to calculate the mortality rate and to adjust the FCR.

Table 2. Composition and calculated nutrient and energy contents of the trial 1 basal diet (as-fed basis).

Item	Starter diet (1–14 d)	Grower diet (15–25 d)	Finisher diet (26–37 d)
Ingredient (%)			
Corn	57.90	61.43	62.06
Soybean meal	27.40	20.10	15.20
Peanut meal	3.00	4.00	5.00
Corn gluten meal	2.50	3.00	4.00
Cottonseed meal	2.50	4.00	4.50
Dried distillers' grains with solubles	2.00	3.00	3.00
Lysine	0.51	0.67	0.76
Calcium hydrogen phosphate	1.49	1.08	0.74
Stone powder	1.16	1.10	1.06
Soybean oil	0.54	0.62	2.68
Premix ¹	1.00	1.00	1.00
Total	100	100	100
Calculated value (%)			
DM	88.10	88.78	88.98
CP	21.98	21.02	19.99
Ether extract	2.75	3.09	5.09
ME, kcal/kg	3,000	3,050	3,150
Crude ash	5.80	5.21	4.60
Calcium	0.85	0.75	0.65
Total phosphorus	0.62	0.57	0.50

¹Supplied per kilogram of diet: retinol, 12,000 IU; cholecalciferol, 2,400 IU; α -tocopherol, 40 mg; menadione, 4 mg; thiamine, 3 mg; riboflavin, 6 mg; nicotinic acid, 25 mg; pantothenic acid, 10 mg; pyridoxine, 5 mg; cyanocobalamin, 0.03 mg; biotin, 0.05 mg; folic acid, 1 mg; Mn, 80 mg; Zn, 60 mg; Fe, 60 mg; Cu, 5 mg; Co, 0.2 mg; I, 1 mg; Se, 0.15 mg; choline chloride, 200 mg.

Trial 2

Analysis of Nutrient Digestibility At 20 d of age, 180 broiler chicks with similar BW (1.39 ± 0.15 kg) were selected from the CON and FWB groups before the trial. These birds were divided into 5 dietary treatment groups, each comprising 6 replicates with 6 broilers (3 males, and 3 females per replicate) for the nutrient availability trial. The birds in each replicate were housed in an individual cage ($65 \times 50 \times 41$ cm). Excreta were collected for 4 d after a 4-d adaptation period and weighed daily, and then individually homogenized during the 4-d collection period (Anne et al., 1990). Water and feed were provided ad libitum during the adaptation period. The excreta and feed samples were dried at 60 to 65°C, ground by passing through a 0.5-mm screen using a mill grinder (Retsch ZM 100; Retsch GmbH and Co., K.C.,

Haan, Germany), and then stored at 4°C until analysis. The moisture (oven-drying method, method 930.15) and CP content (method 990.03) were determined according to AOAC (2006) methods, and gross energy was measured using an adiabatic bomb calorimeter (model 356; Parr Instrument Company, Moline, IL). Twenty grams of each fresh FWB sample was mixed with 180 mL of distilled water, shaken for 1 h, and then filtered through 4 layers of cheesecloth. The filtrate was used to measure the pH (FiveEasy Plus™, Mettler-Toledo International, Inc., Columbus, OH). Ten grams of each fresh FWB sample, used to measure the microbial composition, was completely immersed in 90 mL of sterilized water, shaken for 30 min, and serially diluted (10^{-1} to 10^{-6}) with sterilized water. The number of lactic acid bacteria was measured by plate counting on MRS agar incubated at 37°C for 48 h in an anaerobic box (TEHER Hard Anaerobox, ANX-1, Hirosawa Ltd., Tokyo, Japan). Bacilli were counted on nutrient agar (Nissui Ltd., Tokyo, Japan) incubated at 30°C for 24 h under aerobic conditions. Colonies were counted as viable numbers of microorganisms in colony-forming units per gram of fresh matter.

Trial 3

Analysis of Available AME Value of FWB A total of 144 broiler chicks at 20-d-old with similar BW (1.38 ± 0.14 kg) was randomly allocated to 4 dietary treatments each consisting of 6 replicates of 6 broilers (3 males, and 3 females per replicate) in a randomized complete block design. The 4 dietary treatments included a corn-soybean meal basal diet and 3 test diets. For the 3 test diets, corn and soybean meal in the basal diet were partially replaced with FWB at concentrations of 50, 150, and 300 g/kg DM (Table 3). The sample collection process and chemical analyses were the same as those used in Trial 2.

The energy-yielding ingredients contributed 960.4 g/kg to the gross energy content of the basal diet. Energy was corrected for non-energy-yielding ingredients, and the substitution rate was corrected for the energy contribution of basal ingredients and FWB to the total dietary energy. The product of the FWB energy contribution-corrected substitution rate and

Table 3. Formulation and analyzed composition of the trial 3 basal diet (as-fed basis).

Item	Basal diet	Level of fermented wheat bran substitution (g/kg)		
		50	150	300
Ingredient (%)				
Corn	64.14	60.05	50.77	37.40
Soybean meal	31.90	29.33	25.28	18.67
FWB	0	6.66	19.99	39.97
Premix ¹	3.96	3.96	3.96	3.96
Total	100	100	100	100
Analyzed composition (%)				
Dry matter	87.0	86.3	84.8	82.6
CP	20.17	19.83	19.12	18.00
Gross energy, kcal/kg DM	4,338	4,293	4,334	4,310

¹Premix provided per kilogram of diet: vitamin A, 5,484 IU; vitamin D3, 2,643 IU; vitamin E, 11 IU; menadione sodium bisulfate, 4.38 mg; riboflavin, 5.49 mg; d-pantothenic acid, 11 mg; niacin, 44.1 mg; choline chloride, 771 mg; vitamin B12, 13.2 μ g; biotin, 55.2 μ g; thiamine mononitrate, 2.2 mg; folic acid, 990 μ g; pyridoxine hydrochloride, 3.3 mg; I, 1.11 mg; Mn, 66.06 mg; Cu, 4.44 mg; Fe, 44.1 mg; Zn, 44.1 mg; Se, 300 μ g.

dietary AME content is the FWB-associated AME intake. Because catabolic compounds in excreted nitrogen can contribute to energy loss, AME was corrected to zero nitrogen retention using a factor of 8.22 kcal/g (Hill and Anderson, 1958).

The AME value in FWB can be determined from the regression of FWB-associated AME intake in the test diets against the FWB substitution in the test diets, and the slope of the regression equation represents the AME concentration in kcal/kg DM of FWB (Adeola and Ileleji, 2009). The test ingredient-associated energy was calculated by correcting the substitution ratio for the energy contribution of basal ingredients and test ingredient to the total dietary energy (Equation 1):

FWB-associated AM Eintage (M J)

$$= ME_{td} - (1 - P_{ti}) \times ME_{bd}, \quad (1)$$

where ME_{td} and ME_{bd} are the AME values of the test diet and basal diet, respectively, and P_{ti} is the substitution rate of FWB in the basal diet.

Statistical Analysis

All data were analyzed using the one-way ANOVA with post hoc Duncan's multiple range comparison test using SPSS statistical software (version 21.0 for Windows, SPSS, Inc., Chicago, IL). All data were analyzed by a regression including the linear and quadratic effects of dietary supplementation of FWB.

The FWB-associated AME or AME_n intake (kcal) was regressed against the FWB intake (kg) for each cage of birds, and the solutions option was used to generate the

intercept and slope using the general linear model procedures in SPSS Inc., 2012. There were 4 cages for 0, 50, 150, or 300 g FWB substitution/kg in each block, and 6 blocks in the metabolic trial. The intercept and slope data of the 6 blocks were analyzed by one-way ANOVA in a completely randomized design. Values in the tables are presented as the mean and pooled SEM. The statistical significance level for the difference was set at $P < 0.05$.

RESULTS

Solid-State FWB

Table 1 shows the nutrient composition and total microbe content of WB under fermentation conditions. The FWB slightly increased the contents of CP (18.50 vs. 16.62%) and ash (5.5 vs. 5.3%) but decreased the contents of starch (6.2 vs. 7.3%), crude fiber (7.2 vs. 8.8%), neutral detergent fiber (NDF) (28.9 vs. 36.3%), and acid detergent fiber (9.8 vs. 10.2%) compared with raw WB. The greatest change was observed for the content of NDF, which decreased from 36.3 to 28.9% on a DM basis. The fermentation of WB caused an approximately 7-fold increase in the content of acid soluble protein (1.21 vs. 8.34%) compared to in raw WB. In addition, the pH of WB decreased from 6.7 to 4.7, and the lactic acid bacteria and *Bacillus subtilis* counts rapidly increased during fermentation.

Trial 1

Growth Performance The effect of dietary treatments on the growth performance of broiler chickens is

Table 4. Effect of dietary fermented wheat bran (FWB) on the growth performance of broilers.

Item	Diet treatment ¹					SEM	<i>P</i> -value		
	CON ²	T ₁ ²	T ₂ ²	T ₃ ²	T ₄ ²		Treatment	Linear	Quadratic
Starter (1–14 d)									
ADFI, g/d	37.1	36.9	37.1	36.5	37.0	0.2	0.775	0.633	0.726
ADG, g/d	31.1	30.8	30.8	30.6	30.7	0.2	0.935	0.447	0.735
Feed conversion ratio	1.194	1.199	1.206	1.193	1.206	0.003	0.396	0.354	0.816
Livability, %	99.5	99.5	99.5	98.9	98.9	0.2	0.756	0.239	0.732
EPEF	258.9	255.8	254.1	253.6	252.0	1.8	0.810	0.231	0.791
Grower (15–25 d)									
ADFI, g	107.5	108.5	109.4	107.6	108.9	0.9	0.962	0.775	0.796
ADG, g	77.2	77.0	77.9	77.4	78.6	2.1	0.984	0.632	0.859
Feed conversion ratio	1.395	1.412	1.408	1.393	1.389	0.005	0.554	0.393	0.229
Livability, %	100.0	100.0	100.0	100.0	99.7	0.3	0.418	0.164	0.238
EPEF	554.7	546.4	554.8	556.7	565.1	8.1	0.972	0.602	0.705
Finisher (26–37 d)									
ADFI, g	150.2	155.3	152.5	149.7	155.2	1.8	0.805	0.741	0.945
ADG, g	88.2	91.3	88.5	86.0	90.1	1.3	0.770	0.872	0.840
Feed conversion ratio	1.705	1.706	1.726	1.750	1.727	0.009	0.548	0.193	0.584
Livability, %	99.2	99.7	99.2	99.7	100.0	0.0	0.264	0.109	0.635
EPEF	513.7	536.0	509.8	494.5	522.9	9.7	0.748	0.743	0.781
Overall period (1–37 d)									
ADFI, g	93.2	95.0	94.5	92.8	95.1	0.9	0.892	0.785	0.988
ADG, g	62.6	63.5	62.8	61.8	63.5	0.7	0.948	0.986	0.837
Feed conversion ratio	1.489	1.500	1.505	1.503	1.500	0.004	0.797	0.406	0.340
Livability, %	98.7	99.2	98.7	98.7	98.6	0.2	0.948	0.762	0.790
EPEF	415.3	420.5	412.4	406.6	417.7	5.8	0.960	0.833	0.785

Abbreviation: EPEF, European performance efficiency factor.

¹Diet treatments consisted of corn-soybean meal control diet (CON) or CON plus wet FWB (25 g/kg [T₁]; 50 g/kg [T₂]); or T₁ plus 3 g/kg (T₃); or T₂ plus 6 g/kg (T₄) soybean oil).

²n = 10 replicate cages of 36 birds per cage.

presented in Table 4. Diets containing 25 (T₁) or 50 g/kg (T₂) wet fermented bran (moisture 40%) did not affect the ADFI, ADG, FCR, livability, and European performance efficiency factor (**EPEF**) of broiler chickens compared with the control during the starter period (1–14 d; $P > 0.05$), grower period (15–25 d; $P > 0.05$), finisher period (26–37 d; $P > 0.05$), and overall period (1–37 d; $P > 0.05$). Moreover, diets supplemented with 3 g/kg (T₃), or 6 g/kg (T₄) oil did not improve the growth performance of broiler chickens compared with the T₁, T₂, and CON diets.

Trial 2

Nutrient Digestibility The apparent nutrient digestibility of the tested diets in broiler chickens is presented in Table 5. Diets containing 25 g/kg (T₁), or 50 g/kg (T₂) wet fermented bran did not differ in their apparent DM and energy digestibility from the CON diet. However, broilers fed diets supplemented with oil (T₃ or T₄) had lower ($P = 0.005$ and $P = 0.040$, respectively) apparent DM and energy digestibility than those fed the T₁, T₂, and CON diets. There was no difference in apparent nitrogen digestibility among the treatments.

Trial 3

Analysis of Available AME Value of FWB The effects of FWB substitution levels on intake and apparent nutrient digestibility of feed in broiler chickens are presented in Table 6. There was a linear increase in FWB intake and the associated AME or AME_n as FWB substitution in the basal diet increased from 0 to 300 g/kg ($P < 0.001$). The apparent digestibility of DM and energy for broilers progressively decreased as FWB substitution increased ($P < 0.001$). The diet with 300 g/kg FWB substitution had a lower ($P < 0.05$) nitrogen digestibility than the other diets; there was no difference ($P > 0.05$) among treatments with 0, 50, or 150 g/kg FWB substitution.

The regression of FWB-associated energy intake (Y , kcal) against the FWB substitution intake (X , kg) in broilers fed the test diets is shown in Figure 1. The regression equations for AME and AME_n were $Y = 1854.3X + 52.7$ ($R^2 = 0.971$, $n = 24$, $P < 0.001$), and $Y = 1743.9X + 44.6$ ($R^2 = 0.978$, $n = 24$, $P <$

0.001), respectively, and the slopes for both AME and AME_n regressions differed ($P < 0.001$) among the six blocks (Table 7). The slopes indicate that the AME and AME_n values of FWB used for broiler chickens were 1,854.3 and 1,743.9 kcal/kg DM, respectively.

DISCUSSION

Chemical Composition of FWB

After fermentation, the NDF content of WB decreased from 33 to 27%, whereas the content of CP and acid soluble protein increased. Moreover, the organic acid content of WB increased from to 3%. The decrease in the NDF content of FWB was due to the cellulase or NSP enzyme activities during fermentation. Supriyati et al. (2015) and Wizna et al. (2009) observed a similar decrease in the crude fiber content when rice bran was fermented with *B. amyloliquefaciens*. *Bacillus subtilis* produces several types of enzymes, such as α -amylase and protease, β -endoglucanase, and β -exoglucanase (Wizna et al., 2007). These enzymes can transform complex molecules, particularly lignocelluloses (which are limiting factors in animal feed), into simpler molecular components.

The increase (1.88 percentage units) in CP content observed in this study was likely due to decreases in the levels of other nutrients rather than an actual increase in protein content, particularly with respect to the fermentable carbohydrate content, such that protein content increased proportionately. The CP content of WB slightly increased from 16.62 to 18.50% on a DM basis after fermentation, which is similar to the result for fermented rice bran reported by Supriyati et al. (2015) and fermented rapeseed meal reported by Chiang et al. (2010). However, Supriyati and Kompiang (2002) reported a considerable increase in the protein content after the fermentation of by-products. This difference was most likely related to differences in the fermentation conditions, addition of inorganic nitrogen sources, and strains of microorganisms used. There was no additional nitrogen source in the present study.

Growth Performance and Digestibility

By-products such as rapeseed meal and wheat or barley bran contain considerable amounts of NSP that

Table 5. Effects of dietary fermented wheat bran (FWB) on dietary ME and feed apparent nutrient digestibility in broiler chickens.

Item	Diet treatment ¹					SEM	P-value		
	CON ²	T ₁ ²	T ₂ ²	T ₃ ²	T ₄ ²		Treatment	Linear	Quadratic
AME, kcal/kg DM	3462.8 ^b	3439.4 ^{bc}	3396.5 ^c	3552.0 ^a	3493.5 ^b	12.6	<0.001	0.010	0.087
AME _n , kcal/kg DM	3267.9 ^b	3250.5 ^{bc}	3197.3 ^c	3352.4 ^a	3298.1 ^b	12.3	<0.001	0.010	0.063
DM digestibility, %	74.35 ^a	74.41 ^a	74.36 ^a	73.48 ^b	73.05 ^b	0.16	0.005	0.007	0.390
Energy digestibility, %	76.73 ^{ab}	76.76 ^{ab}	77.33 ^a	75.76 ^b	75.62 ^b	0.21	0.040	0.230	0.563
Nitrogen digestibility, %	65.87	64.74	66.39	65.95	65.39	0.29	0.452	0.908	0.693

¹Diet treatments consisted of corn-soybean meal control diet (CON) or CON plus wet FWB (25 g/kg [T₁]; 50 g/kg [T₂]); or T₁ plus 3 g/kg (T₃); or T₂ plus 6 g/kg (T₄) soybean oil.

²n = 6 replicate cages of 6 birds per cage.

^{a,b,c}Within rows, different letters indicate significant differences ($P < 0.05$).

Table 6. Effects of fermented wheat bran substitution levels on intake and feed apparent nutrient digestibility in broiler chickens.

Item	Fermented wheat bran substitution ¹ g/kg				SEM	P-value		
	0	50	150	300		Treatment	Linear	Quadratic
Intake and energy contributions of FWB								
Intake, kg	0 ^d	0.134 ^c	0.411 ^b	0.863 ^a	0.069	<0.001	<0.001	<0.001
Associated-ME, kcal	0 ^d	307.6 ^c	885.8 ^b	1613.4 ^a	129.5	<0.001	<0.001	<0.001
Associated-ME _n , kcal	0 ^d	283.7 ^c	821.9 ^b	1516.8 ^a	121.4	<0.001	<0.001	<0.001
Digestibility (%)								
DM	71.78 ^a	71.07 ^a	68.40 ^b	62.80 ^c	0.75	<0.001	<0.001	<0.001
Energy	74.44 ^a	73.83 ^a	70.80 ^b	64.29 ^c	0.85	<0.001	<0.001	<0.001
Nitrogen	64.04 ^a	64.31 ^a	63.90 ^a	59.44 ^b	0.52	<0.001	<0.001	0.002

¹n = 6 replicate cages of 6 birds per cage.

^{a,b,c}Within rows, different letters indicate significant differences ($P < 0.05$).

cannot be digested by poultry because they lack of endogenous hydrolyzing enzymes. Soluble NSP can increase digesta viscosity and reduce nutrient digestibility in the small intestine of chicken. Previous studies showed that fermentation increases the CP content but decreases several antinutritional factors and toxic compounds in feed ingredients. Chiang et al. (2010) reported that broiler chickens fed fermented rapeseed meal led to weight gain and FCR improvement in the feeding trial. As demonstrated by Skrede et al. (2003), weight gain in broilers fed fermented barley and fermented wheat was higher than that in broilers fed control diets. These reports suggest that the improvement in the nutritive values and digestibility of feed ingredients through soluble NSP degradation during fermentation is a major factor determining improved broiler performance.

It is a common practice to dry conventional, commercial fermented feedstuffs to a final moisture content of less than 10%. However, the drying process negatively affects volatile substances and probiotics in the fermented feedstuff, such as organic acids, *Lactobacillus* spp., and yeast. The drying process also increases the cost of fermented feedstuffs. In the current study, broilers were fed diets containing wet FWB (moisture 40%). This did not affect the growth performance parameters of broilers (ADFI, ADG, and FCR) compared with those of the control group, which can be

attributed to the increased nutritive value of WB during fermentation. The improved nutrient digestibility (dry matter, energy, and nitrogen) of the diets containing FWB resulted in similar broiler performances to that on the corn-soybean meal diet. Chiang et al. (2010) and Ashayerizadeh et al. (2018) reported similar nutrient digestibility in fermented rapeseed meal. The beneficial effects of fermentation on nutrient digestibility and energy utilization have been demonstrated in mink and salmon (Skrede et al., 2001; Skrede et al., 2002). Apart from improved nutritional metrics and increased digestibility from fermentation processes, the mechanisms by which fermented feeds promote growth in broilers are largely unknown. Because fermentation may improve nutritional quality, digestibility, and palatability of feed ingredients such as WB for poultry, inclusion of these feedstuffs after fermentation in poultry rations can be expected to reduce feed costs without impairing broiler performance.

AME and AMEn Values of Wet FWB

A substitution method should overcome the limitations of the direct method (confined to some high-quality feedstuffs whose chemical composition is relatively balanced) by feeding the test feed in conjunction with a suitable basal diet of known dietary energy value. However, the substitution ratio of test ingredients is directly related to the accuracy of feed evaluation (Villamide, 1996; Yu et al., 2021). Thus, the regression method based on multiple substitution ratios is more accurate than the substitution method at a given substitution ratio. Recent studies focused on evaluating the energy available to poultry from a given feedstuff using the regression method. The AME content of the following were determined: corn distillers' dried grains with solubles for broiler chickens (Adeola and Ileleji, 2009), corn distillers' grains (Adeola et al., 2010), wheat, and barley (Bolarinwa and Adeola, 2012b). Similarly, Bolarinwa and Adeola (2012a, 2016) determined the digestible energy and AME values of barley, sorghum, and wheat for pigs using the regression method. Villamide et al. (2003) reported that multilevel assays to estimate the energy value of feeds by regression were more reliable. Furthermore, Bolarinwa and

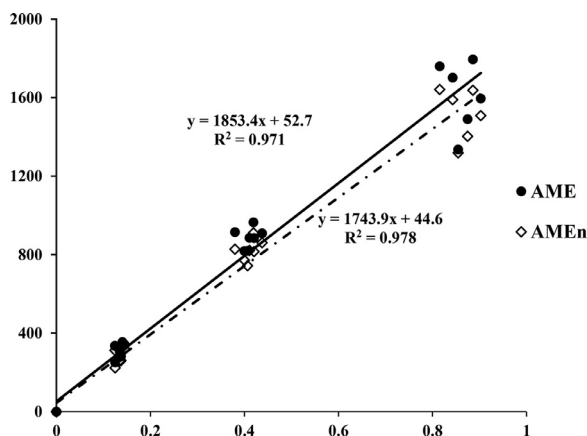


Figure 1. Correlation between FWB-associated energy intake (Y, kcal) against the FWB substitution intake (X, kg) in broilers fed the test diets differing in AME and AMEn: $Y = 1853.4X + 52.7$, $R^2 = 0.971$, $Y = 1743.9X + 44.6$, $R^2 = 0.978$. Abbreviation: FWB, fermented wheat bran.

Table 7. Slopes and intercepts of regressions in determination of AME and AME_n of fermented wheat bran for broilers.

Item	Slope kcal/kg	Intercept kcal	R^2	P-value		
				Slope	Intercept	Regression
AME	1853.4 ± 68.5	52.7 ± 33.0	0.971	<0.001	0.144	<0.001
AME _n	1743.9 ± 44.6	52.7 ± 33.0	0.978	<0.001	0.133	<0.001

Adeola (2016) suggested that multiple-point substitution and regression-derived energy utilization are more robust than single-point substitution.

In conclusion, diets supplemented with wet FWB had no influence on broiler growth performance or the apparent nutrient digestibility of the feed. The respective AME and AME_n values of FWB for broiler chickens were 1854.3 and 1743.9 kcal/kg of DM. Therefore, FWB is a suitable feed component for broilers.

ACKNOWLEDGMENTS

The authors are grateful to all members of Pingdu R & D base of New Hope Liuhe Co., Ltd., Shandong Branch, Qingdao, China, for their support and assistance.

This work was supported by the Major Science and Technology Special Project of Sichuan Province, China [grant number 18ZDZX0048]

DISCLOSURES

The authors have no conflicts of interest to report.

REFERENCES

- Adeola, O., and K. E. Ileleji. 2009. Comparison of two diet types in the determination of metabolizable energy content of corn distillers dried grains with solubles for broiler chickens by the regression method. *Poult. Sci.* 88:579–585.
- Adeola, O., J. A. Jendza, L. L. Southern, S. Powell, and A. Owusu-Asiedu. 2010. Contribution of exogenous dietary carbohydrates to the metabolizable energy value of corn distillers grains for broiler chickens. *Poult. Sci.* 89:1947–1954.
- AOAC International. 2006. *Official Methods of Analysis*. Assoc. Anal. Chem., Arlington, VA.
- Anne, B., B. Carré, L. Conan, J. Duperray, G. Huyghebaert, B. Leclercq, M. Lessire, J. McNab, and J. Wiseman. 1990. European reference method for the in vivo determination of metabolizable energy with adult cockerels: reproducibility, effect of food intake and comparison with individual laboratory methods. *Br. Poult. Sci.* 31:557–565.
- Ashayerizadeh, A., B. Dastar, M. Shams Shargh, A. R. Sadeghi Mahoonak, and S. Zerehdaran. 2018. Effects of feeding fermented rapeseed meal on growth performance, gastrointestinal microflora population, blood metabolites, meat quality, and lipid metabolism in broiler chickens. *Livest. Sci.* 216:183–190.
- Bolarinwa, O. A., and O. Adeola. 2012a. Direct and regression methods do not give different estimates of digestible and metabolizable energy of wheat for pigs. *J. Anim. Sci.* 90:390–392.
- Bolarinwa, O. A., and O. Adeola. 2012b. Energy value of wheat, barley, and wheat dried distillers grains with solubles for broiler chickens determined using the regression method. *Poult. Sci.* 91:1928–1935.
- Bolarinwa, O. A., and O. Adeola. 2016. Regression and direct methods do not give different estimates of digestible and metabolizable energy values of barley, sorghum, and wheat for pigs. *J. Anim. Sci.* 94:610–618.
- Canibe, N., and B. B. Jensen. 2012. Fermented liquid feed: microbial and nutritional aspects and impact on enteric diseases in pigs. *Anim. Feed Sci. Technol.* 173:17–40.
- Chiou, P. W. S., B. Yu, C. C. Chen, and Y. C. Shih. 2010. Evaluating nutritional quality of single stage- and two stage-fermented soybean meal. *Asian-Aust. J. Anim. Sci.* 23:598–606.
- Chen, Q. T. 2010. Application of fermented palm kernel meal and coconut meal in diet of laying hens. *Feed Res.* 10:11–12.
- Chiang, G., W. Q. Lu, X. S. Piao, J. K. Hu, L. M. Gong, and P. A. Thacker. 2010. Effects of feeding solid-state fermented rapeseed meal on performance, nutrient digestibility, intestinal ecology, and intestinal morphology of broiler chickens. *Asian-Aust. J. Anim. Sci.* 23:263–271.
- Engberg, R. M., M. Hammershj, N. F. Johansen, M. S. Abousekken, S. Steinfeldt, and B. B. Jensen. 2009. Fermented feed for laying hens: effects on egg production, egg quality, plumage condition, and composition and activity of the intestinal microflora. *Br. Poult. Sci.* 50:228–239.
- Feng, J., X. Liu, Z. R. Xu, Y. P. Lu, and Y. Y. Liu. 2007. Effect of fermented soybean meal on intestinal morphology and digestive enzyme activities in weaned piglets. *Digest. Dis. Sci.* 52:1845–1850.
- Feng, Y., L. Wang, A. Khan, R. Zhao, S. Wei, and X. Jing. 2020. Fermented wheat bran by xylanase-producing *Bacillus cereus* boosts the intestinal microflora of broiler chickens. *Poult. Sci.* 99:263–271.
- Hemery, Y., X. Rouau, V. Lullien-Pellerin, C. Barron, and J. Abecassis. 2007. Dry processes to develop wheat fractions and products with enhanced nutritional quality. *J. Cereal Sci.* 46:327–347.
- Hill, F. W., and D. L. Anderson. 1958. Comparison of metabolizable energy and productive determinations with growing chicks. *J. Nutr.* 64:587–603.
- Koropatkin, N. M., E. A. Cameron, and E. C. Martens. 2012. How glycan metabolism shapes the human gut microbiota. *Nat. Rev. Microbiol.* 10:323–335.
- Leo, S. 2012. Frankie P., Kathryn O., Jenny W.. 2012. Wheat bran: its composition and benefits to health, a European perspective. *Int. J. Food Sci. Nutr.* 8:1001.
- Lin, W. C., and T. T. Lee. 2020. Effects of *Laetiporus sulphureus*-fermented wheat bran on growth performance, intestinal microbiota and digesta characteristics in broiler chickens. *Animals* 10:1457.
- NRC (National Research Council). *Nutrient Requirements of Poultry*, 9th ed, 1994, National Academies Press; Washington, DC.
- Olukosi, O. A., and O. Adeola. 2008. Whole body nutrient accretion, growth performance, and total tract nutrient retention responses of broilers to supplementation of xylanase and phytase individually or in combination in wheat-soybean meal-based diets. *J. Poult. Sci.* 45:192–198.
- Ping, W., G. M. Kwaku, G. Yiting, M. Haile, H. Ronghai, L. Xuan, L. Shilong, Z. Cheng, Z. Liuping, and Z. Jinhua. 2020. Effect of partial replacement of soybean meal with high-temperature fermented soybean meal in antibiotic-growth-promoter-free diets on growth performance, organ weights, serum indexes, intestinal flora and histomorphology of broiler chickens. *Anim. Feed Sci. Technol.* 269:114–616.
- Skrede, G., S. Sahlstrøm, A. Skrede, A. Holck, and E. Slinde. 2001. Effect of lactic acid fermentation of wheat and barley whole meal flour on carbohydrate composition and digestibility in mink (*Mustela vison*). *Anim. Feed Sci. Technol.* 90:199–212.
- Skrede, G., T. Storebakken, A. Skrede, S. Sahlstrøm, M. Sørensen, K. D. Shearer, and E. Slinde. 2002. Lactic acid fermentation of wheat and barley whole meal flours improves digestibility of nutrients and energy in Atlantic salmon (*Salmo salar* L.) diets. *Aquaculture* 210:305–321.

- Skrede, G., O. Herstad, S. Sahlstrøm, A. Holck, E. Slinde, and A. Skrede. 2003. Effects of lactic acid fermentation on wheat and barley carbohydrate composition and production performance in the chicken. *Anim. Feed Sci. Technol.* 105:135–148.
- Soumei, E. A., H. Mohebodini, M. Toghyani, A. Shabani, A. Ashayerizadeh, and V. Jazi. 2019. Synergistic effects of fermented soybean meal and mannan-oligosaccharide on growth performance, digestive functions, and hepatic gene expression in broiler chickens. *Poult. Sci.* 98:6797–6807.
- SPSS Inc., *SPSS statistical software*. Version 21.0 for Windows, 2012, IBM; Chicago, IL.
- Sugiharto, S., and S. Ranjitkar. 2019. Recent advances in fermented feeds towards improved broiler chicken performance, gastrointestinal tract microecology, and immune responses: a review. *Anim. Nutr.* 5:1–10.
- Supriyati, and dan I. P. Kompiang. 2002. The chemical changing during fermentation of cassava tuber skin and its utilization in broiler chicken rations. *J. Ilmu Ternak Vet.* 7:150–154.
- Supriyati, T. Haryati, T. Susanti, and I. W. R. Susana. 2015. Nutritional value of rice bran fermented by *Bacillus amyloliquefaciens* and humic substances and its utilization as a feed ingredient for broiler chickens. *Asian-Aust. J. Anim. Sci.* 28:231–238.
- Teng, P. Y., C. L. Chang, C. M. Huang, S. C. Chang, and T. T. Lee. 2017. Effects of solid-state fermented wheat bran by *Bacillus amyloliquefaciens* and *Saccharomyces cerevisiae* on growth performance and intestinal microbiota in broiler chickens. *Ital. J. Anim. Sci.* 16:552–562.
- Villamide, M. J. 1996. Methods of energy evaluation of feed ingredients for rabbits and their accuracy. *Anim. Feed Sci. Technol.* 57:211–223.
- Villamide, M. J., J. García, C. Cervera, E. Blas, L. Maertens, and J. M. Perez. 2003. Comparison among methods of nutritional evaluation of dietary ingredients for rabbits. *Anim. Feed Sci. Technol.* 109:195–207.
- Wanzenböck, E., U. Zitz, C. Steinbauer, W. Kneifel, K. J. Domig, and K. Schedle. 2020. A diet containing native or fermented wheat bran does not interfere with natural microbiota of laying hens. *Animals* 14:1147–1155.
- Wizna, W., H. Abbas, Y. Rizal, A. Dharma, and I. P. Kompiang. 2007. Selection and identification of cellulase-producing bacteria isolated from the litter of mountain and swampy forest. *Microbiol. Indonesia* 1:135–139.
- Wizna, H. Abbas, Y. Rizal, A. Dharma, and I. P. Kompiang. 2009. Improving the quality of tapioca by-products (onggok) as poultry feed through fermentation by *Bacillus amyloliquefaciens*. *Pak. J. Nutr.* 8:1636–1640.
- Yeh, R., H. C. W. Hsieh, and K. L. Chen. 2018. Screening lactic acid bacteria to manufacture two-stage fermented feed and pelleting to investigate the feeding effect on broilers. *Poult. Sci.* 97:236–246.
- Yu, Y., F. Zhao, J. Chen, Y. Zou, S. L. Zeng, S. B. Liu, and H. Z. Tan. 2021. Sensitivity of in vitro digestible energy determined with computer-controlled simulated digestion system and its accuracy to predict dietary metabolizable energy for roosters. *Poult. Sci.* 100:206–214.