Energy content and nutrient digestibility of diets containing *Lactobacillus*-fermented barley or wheat fed to weaned pigs

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ABSTRACT: This study was conducted to determine the energy content and apparent total tract digestibility (**ATTD**) of nutrients of diets containing *Lactobacillus*-fermented barley or wheat fed to weaned pigs. Thirty-six weaned pigs (8.14 ± 0.65 kg of body weight) were randomly assigned to 1 of 6 diets in a completely randomized design to give 6 replicates per diet. Pigs were individually housed in metabolism crates to determine digestible energy and metabolizable energy contents. Net energy was also calculated from the average of 2 equations published by Noblet et al. (1994). Diets were fed at 2.5 times the maintenance energy requirement for 10 d of adaptation and 5 d of total but separate urine and fecal collection. Samples of barley or wheat were fermented for 90 d under anaerobic conditions with an inoculum of either homofermentative *Lactobacillus plantarum* (**Homo**) or heterofermentative *L. buchneri* (**Hetero**). Three diets were formulated based on either barley or wheat to consist of a control diet containing 42% unfermented grain and 2 diets containing either Homo-fermented or Hetero-fermented grain. Preplanned contrasts were used to evaluate the effects of the inclusion of fermented barley or wheat and to compare the effects of Homo-fermented with Hetero-fermented grains. Fermented wheat inclusion in a diet increased ATTD of gross energy and phosphorus, and retention of gross energy by 1.9%, 6.8%, and 6.3%, respectively. Also, fermented wheat diets had greater ($P < 0.05$) metabolizable energy content and tended to have greater ($P \leq 0.10$) net energy content than unfermented wheat diets. However, inclusion of fermented barley did not increase nutrient and energy digestibility. Hetero-fermented diets contained greater ($P < 0.05$) digestible energy and net energy content (DM basis) than Homo-fermented diets. Pigs fed barley-based diets showed less ($P < 0.05$) ATTD of DM, nitrogen, and gross energy than those fed wheat-based diets. In conclusion, wheat fermented with *Lactobacillus*-inoculum can be beneficially substituted for unfermented wheat, improving the ATTD of nutrient and energy, nitrogen retention, and energy content. Also, Hetero-inoculum is preferable to Homo-inoculum for grain fermentation considering greater energy content in weaned pigs.

Key words: digestibility, energy content, fermented grain, *Lactobacillus*, weaned pigs

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INTRODUCTION

The inclusion of highly digestible ingredients in weaner pig diets is imperative (Mahan and Newton, 1993) due to their immature digestive and immune systems (Pluske et al., 2018). In this regard, the use of grains, the main component of

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swine diets, fermented with *Lactobacillus* might be a beneficial strategy for weaner diets. There is a strong consensus that the content of antinutritional factors, such as dietary fiber and phytate content, are reduced after *Lactobacillus*-fermentation (Skrede et al., 2003; Skrede et al., 2007; Wang et al., 2007), thereby improving nutrient and energy digestibility (Hackl et al., 2010; Pieper et al., 2011). Also, microbial metabolites (e.g., short-chain fatty acids) and lactic acid bacteria from fermentation can be delivered to the gut of weaned pigs and may positively affect gut microbiota (van Winsen et al., 2001; Dibner and Buttin, 2002) and digestibility (Dibner and Buttin, 2002; Zhao and Kim, 2015), which possibly alters energy utilization in pigs. The homofermentative *Lactobacillus* (**Homo**) produce lactic acid as a final product, and thus Homo-inoculation can rapidly lower pH at the initial stage of fermentation (Giraffa et al., 2010). The heterofermentative *Lactobacillus* (**Hetero**) can metabolize the lactic acid into volatile fatty acids, which have effective antifungal activities (Holzer et al., 2003). However, no studies have focused on energy utilization in pigs fed diets containing fermented grains and a comparison between Homo- and Hetero-fermented grains for a swine diet. Therefore, it was hypothesized that fermented grains substituted for unfermented grains would increase the energy content of nursery pig diets. The objectives of this study were to 1) determine the energy content and nutrient digestibility of diets containing fermented barley or wheat, and 2) compare the effects of feeding Homo-fermented grain with those of feeding Hetero-fermented grains to pigs on digestibility and energy content.

MATERIALS AND METHODS

All experimental procedures were reviewed and approved by the University of Manitoba Animal Care Committee (Protocol #, AC11013), and pigs were cared for according to the guidelines of the Canadian Council on Animal Care (CCAC, 2009).

Animals, Housing, and Experimental Design

Thirty-six male piglets ([Yorkshire × Landrace] × Duroc) with an initial body weight of 8.14 ± 0.65 kg and weaned at 21 d of age were obtained from the Glenlea Swine Research Unit at the University of Manitoba. Pigs were randomly allotted to 1 of 6 experimental diets in a completely randomized design, with 6 replicates per diet, and were individually housed for 10 d in adjustable metabolism crates (1.80 × 0.60 m) with smooth, transparent plastic sides and plastic-covered expanded metal-sheet flooring. Each crate was equipped with a stainless steel feeder and a nipple drinker, which allowed the pigs ad libitum access to water. Room temperature was maintained at 29 ± 1 °C during week 1; thereafter, it was reduced by 1.5 °C per week. The experiment was conducted in 2 consecutive periods (18 pigs per period) using the same facility and similar experimental conditions and procedures. Daily feed allowance was set at 2.5 times the energy requirement for maintenance (106 kcal metabolizable energy [**ME**] per kg of body weight$^{0.75}$) per day based on body weight on day 1 and 10, which was close to voluntary feed intake (NRC, 2012). Two equal portions were offered at 0800 and 1600 h as a dry mash.

Fermentation Procedures and Experimental Diets

Commercial barley and wheat ground to pass through 3-mm sieve were separately inoculated with 1:1 mixture of *L. plantarum* DSMZ 8862 and DSMZ 8866 (BIO-SIL, Dr. Pieper, Technology and Product Development Ltd, Wuthenow, Germany) or *L. buchneri* NCIMB 40788 (Biotal, Lallemand Animal Nutrition, Montreal, QC, Canada). Water was added to adjust the moisture content to 27%; the inoculants were added at the rate of 6×10^5 colony forming unit per g of fresh barley or wheat. The mixture was transferred into 55-gallon polyethylene barrels, compacted, sealed tightly to create an anaerobic environment, and fermented for 90 d at room temperature. On day 90, the fermented wheat and barley were taken out and mixed with other ingredients to formulate the experimental diets (Table 1). Each grain used for both fermentation and the control diet were from the same batch. All experimental diets were stored at −20 °C until fed, to inhibit microbial proliferation.

Experimental Procedure, Sample Preparation, and Chemical Analyses

Pigs were fed experimental diets for 16 d, including 10 d for adaptation to experimental diets and environmental conditions. During the last 6 d of each feeding period, total but separate fecal and urine collection was performed for the estimation of digestible energy (**DE**) and ME. To mark the beginning and the end of fecal collection, 3 g of ferric oxide was fed as indigestible marker (Sigma number, 310050; Sigma-Aldrich, St. Louis, MO). From day 11 to 16, feces were collected once daily

Table 1. Composition of experimental diets, % (as-fed basis)

	Experimental diets			
	Barley		Wheat	
Item	Unfermented	Fermented	Unfermented	Fermented
Ingredient				
Barley	41.36	–	–	–
Fermented barley[1]	–	41.36	–	–
Wheat	–	–	43.88	–
Fermented wheat[1]	–	–	–	43.88
Soybean meal	22.96	22.96	21.68	21.68
Whey permeate	11.76	11.76	11.76	11.76
Canola meal	10.00	10.00	10.00	10.00
Peas	5.00	5.00	5.00	5.00
Vegetable oil	3.84	3.84	2.68	2.68
Limestone	1.32	1.32	1.39	1.39
Dicalcium phosphate	1.31	1.31	1.15	1.15
Salt	0.64	0.64	0.66	0.66
Celite	0.40	0.40	0.40	0.40
Choline chloride	0.11	0.11	0.11	0.11
L-Lys	0.67	0.67	0.69	0.69
L-Thr	0.24	0.24	0.23	0.23
DL-Met	0.24	0.24	0.21	0.21
Vit-Min premix[2]	0.15	0.15	0.15	0.15

[1]Inoculated with either homofermentative *Lactobacillus plantarum* or heterofermentative *L. buchneri*.

[2]Supplied the following per kilogram of diet: 2,000 IU vitamin A, 200 IU D_3, 40 mg E, 2 mg K, 1.5 mg B_1, 7 mg B_2, 2.5 mg B_6, 25 µg B_{12}, 14 mg calcium pantothenate, 1 mg folic acid, 21 mg niacin, 70 µg biotin, 10 mg Cu (as copper sulphate), 0.4 mg iodine (as potassium iodine), 120 mg iron (as ferrous sulphate), 10 mg Mn (as manganous oxide), 0.3 mg Se (as sodium selenite), and 110 mg Zn (as zinc oxide).

in the morning and were stored at −20 °C. Urine was collected into jugs containing 10 mL of 6 N hydrochloric acid to minimize nitrogen (**N**) losses once daily in the morning. The collected urine was weighed for 5 d from day 11 to day 16, and the subsample of the urine was obtained, strained through glass wool, and stored at −20 °C.

Fecal samples were dried in a forced-air oven at 60 °C for 3 d and finely ground before chemical analysis. Frozen urine samples were thawed and pooled independently for each pig, sieved through cotton gauze, and filtered with glass wool. Fermented grain and diet samples were analyzed for dry matter (**DM**), gross energy (**GE**), crude protein (**CP**), ether extracts (**EE**), ash, neutral detergent fiber (**NDF**), and acid detergent fiber (**ADF**). Diets samples were further analyzed for starch, nonstarch polysaccharides (**NSP**), total phosphorus (**P**), phytate, and calcium (**Ca**). Fecal samples were analyzed for DM, GE, N, Ca, and P, whereas GE and N contents in the urinal samples were analyzed.

Dry matter (method 934.01), EE (method 920.39A), and ash (942.05) were determined according to the Association of Official Analytical Chemists (AOAC, 2006). Nitrogen content was determined using the combustion analyzer (model CNC-2000; Leco Corporation, method 984.13A-D), and the N content was used to calculate the CP concentration (N × 6.25). The GE was determined using isoperibol bomb calorimeter (Parr Instrument Co., Moline, IL), which had been calibrated using benzoic acid as a standard. The Ca (method 968.08) and P (method 946.06) concentrations were analyzed according to the AOAC (2006) and read on an inductively coupled plasma mass spectrometer (Varian Inc., Palo Alto, CA). The ADF and NDF contents were analyzed according to the method of Goering and van Soest (1970) using α-amylase (Sigma number A3306; Sigma-Aldrich, St. Louis, MO) and sodium sulfite, and were corrected for ash adapted for an Ankom 200 Fiber Analyzer (Ankom Technology, Fairport, NY). The starch content was measured using an assay kit (Megazyme Total Starch assay kit; Megazyme International Ltd, Wicklow, Ireland). Total NSP was analyzed by using gas-liquid chromatography (component neutral sugars; Varian CP3380 gas chromatography, Varian Inc., Palo Alto, CA), colorimetry (uronic acids; Biochrom Ultrospec 50, Biochrom Ltd, Cambridge, United Kingdom), and the procedure described by Englyst and Cummings (1988) with some modifications (Slominski and Campbell, 1990). Phytate concentration was determined as described by Ellis et al.

(1977). The concentration of phytate-bound P was calculated as 28.2% of analyzed phytate (Tran and Sauvant, 2004). Nonphytate P was calculated by subtracting phytate-bound P from total P. To determine the GE of urine, 0.5 g of cellulose was dried at 100 °C for 24 h, 2 mL of urine sample were added over it, and the weight of the resulting mixture was recorded. The urine-cellulose mixture along with a sample of pure cellulose was again dried at 50 °C for 24 h and then weighed for estimation of urine DM. The GE of the dried urine-cellulose mixture and pure cellulose were determined using a bomb calorimeter as described above, from which the GE of urine samples were calculated by the difference method (Fleischer et al., 1981).

Calculations and Statistical Analysis

The apparent total tract digestibility (**ATTD**) of nutrients and nutrient retention (%) were determined by the total collection method using the following equation:

$$\text{ATTD (\%)} = 100 \times [(\text{NI} - \text{NO}_{\text{feces}}) / \text{NI}]$$

where NI is the nutrient intake (g) and NO_{feces} is the nutrient output in feces (g).

$$\text{Nutrient retention (\%)} = 100 \times \left[\frac{(\text{NI} - \text{NO}_{\text{feces+urine}})}{\text{NI}}\right]$$

where $\text{NO}_{\text{feces+urine}}$ is the total amount of output both in feces and urine (g).

The DE and ME contents of experimental diets were determined using the following equations:

$$\text{DE (kcal/kg)} = \frac{[(\text{ATTD of GE, \%}) \times (\text{GE content in experimental diets})]}{100}$$

and

$$\text{ME (kcal/kg)} = \frac{[(\text{GE retention, \%}) \times (\text{GE content in experimental diets})]}{100}$$

The net energy (**NE**) of experimental diets was calculated according to the equations established by Noblet et al. (1994):

$$\text{NE} = 0.700 \times \text{DE} + 1.61 \times \text{EE} + 0.48 \times \text{starch} - 0.91 \times \text{CP} - 0.87 \times \text{ADF}$$

and

$$\text{NE} = 0.726 \times \text{ME} + 1.33 \times \text{EE} + 0.39 \times \text{starch} - 0.62 \times \text{CP} - 0.83 \times \text{ADF}$$

where NE, DE, and ME are expressed in kilocalories per kilogram; EE, starch, CP, and ADF were expressed in gram per kilogram.

All data were analyzed using the MIXED procedure of SAS (version 9.4; SAS Inst. Inc., Cary, NC) with each animal used as the experimental unit. The model included experimental diet as the fixed variable and periods as the random variable. The following preplanned orthogonal contrasts were used to test differences between the treatments: 1) barley vs. fermented barley, 2) wheat vs. fermented wheat, 3) Homo vs. Hetero, and 4) barley vs. wheat.

RESULTS AND DISCUSSION

Wheat and barley are widely used ingredients to supply energy and nutrients in swine diets. However, antinutritional factors (e.g., arabinoxylan, β-glucan) in barley and wheat (Lin et al., 1987; Kim et al., 2005; NRC, 2012) lower availability of their energy for pigs, particularly weaned pigs whose digestive and immune systems are not fully developed (Pluske et al., 2018). Previous studies (Skrede et al., 2003; Skrede et al., 2007; Wang et al., 2007) reported that the antinutritional factors in cereal grains can be partially degraded during *Lactobacillus*-fermentation. Therefore, it is of interest to determine whether the inclusion of fermented barley or wheat substituted for respective unfermented grain in nursery diets can enhance nutrient digestibility and energy content. In the current study, homofermentative *L. plantarum* and heterofermentative *L. buchneri* were used as inoculum. The strains have been selected based on previous studies (Nkosi et al., 2009; Hackl et al., 2010; Pieper et al., 2011; dos Santos et al., 2015) showing their ability to facilitate successful fermentation in terms of preservability and to improve nutritive values of grains or fibrous feed ingredients for farm animals after fermentation.

The nutritional contents of unfermented wheat and barley were within the range of values published in NRC (2012; Table 2). During fermentation with the *Lactobacillus*-inoculum, the DM and GE (as-fed basis) content in barley and wheat were reduced. This was most likely due to the water added with the inoculum for fermentation diluting the energy concentrations. However, when the energy contents were expressed on a DM basis, fermented ingredients had approximately 100 kcal/kg numerically greater GE content than unfermented grains. This might be due to a concentrated CP content as the carbohydrate component was utilized for microbial metabolism during fermentation. In

Table 2. Nutrient content of experimental diets and ingredients (%, as-fed basis)

	Barley			Wheat		
		Fermented[1]			Fermented[1]	
Item	Unfermented	Homo	Hetero	Unfermented	Homo	Hetero
Experimental diet						
Dry matter	88.18	85.69	85.40	88.25	86.11	84.73
Gross energy, Mcal/kg	3.89	3.78	3.81	3.86	3.80	3.77
Crude protein	21.02	20.41	21.44	21.26	22.04	22.04
Ether extract	4.31	4.48	4.85	3.66	3.41	3.50
Starch	25.85	22.83	22.77	27.96	27.43	25.70
Ash	6.26	6.54	6.56	6.35	5.91	5.98
Neutral detergent fiber	15.25	10.45	11.75	11.94	9.10	10.02
Acid detergent fiber	6.82	6.16	6.40	5.97	4.99	5.63
Nonstarch polysaccharides						
Total	13.01	10.94	11.01	10.89	9.92	9.48
Arabinose	1.79	1.60	1.64	1.81	1.68	1.57
Xylose	2.51	1.89	1.86	2.09	1.65	1.55
Mannose	0.28	0.24	0.29	0.24	0.23	0.21
Galactose	1.54	1.57	1.60	1.45	1.47	1.50
Glucose	5.14	3.94	3.98	3.56	3.03	2.92
Uronic acids	1.75	1.71	1.65	1.74	1.86	1.73
Phosphorus (P)						
Total P	0.72	0.75	0.73	0.72	0.70	0.74
Phytate-bound P[2]	0.06	0.04	0.04	0.06	0.04	0.04
Phytate-bound P, % of total P	7.71	5.23	5.52	7.89	5.85	5.63
Nonphytate P[3]	0.67	0.71	0.69	0.67	0.65	0.70
Calcium	0.92	0.93	0.92	0.90	0.77	0.90
Ingredient						
Dry matter	88.49	73.34	73.60	88.61	74.38	73.69
Gross energy, Mcal/kg	3.74	3.18	3.25	3.82	3.27	3.29
Crude protein	9.30	8.87	9.06	12.26	11.53	11.18
Ether extract	1.43	1.43	1.14	1.30	0.74	1.00
Ash	1.25	1.84	1.77	1.54	1.03	1.26
Neutral detergent fiber	19.60	9.91	8.61	13.24	6.22	6.70
Acid detergent fiber	5.52	4.47	4.35	2.50	1.54	2.46

[1]Fermented with either homofermentative *Lactobacillus plantarum* or heterofermentative *L. buchneri*.

[2]Phytate-bound P was calculated as 28.2% of phytate (Tran and Sauvant, 2004).

[3]Nonphyate P was calculated as the difference between total P and phytate-bound P.

fact, the GE content of carbohydrates ranges from 3.7 to 4.2 kcal/kg, whereas that of protein is approximately 4.6 kcal/kg (van Milgen, 2002; NRC, 2012). However, CP concentration in fermented grains should be interpreted with caution because the single factor used for Kjeldahl method was 6.25, and this might be inappropriate due to possibly high production in nonprotein N compounds during microbial fermentation (Owens et al., 1999). The NDF content in the grains was substantially reduced after fermentation by an average of 42% (DM basis) and 52% (as-fed basis). Changes in nutritional components of diets, such as DM, GE, CP, EE, NDF, and ADF, were consistent with the composition of the grains used because the grains were the only different ingredients in the experimental diets. Interestingly, inclusion of fermented barley or wheat substituted for respective unfermented grain numerically decreased the NSP content by 13% and 8% (DM basis), respectively. Our previous study (Koo et al., 2018) showed that fermented wheat-based diets had 13% lower total NSP (DM basis) content than unfermented wheat-based diets mostly due to a reduction in water-soluble NSP content in fermented diets. This might have been because water added to make a high moisture content for fermentation dissolved water-soluble contents in grains and microbes readily attached to the substrates, enabling their breakdown during fermentation. Also, in the present study, numerically less concentration of arabinose, xylose, and glucose in fermented diets than in unfermented diets. This

implies possible degradation of arabinoxylan and cellulose in barley and wheat during fermentation. This is partially supported by Skrede et al. (2001), who found that total dietary fiber in wheat and barley was reduced by 9% and 14% (as-fed basis), respectively, after *Lactobacillus*-fermentation. Similarly, fermented barley and wheat diets contained 27% and 25% (DM basis) less phytate-bound P content, respectively, compared to unfermented diets. Skrede et al. (2007) also found that phytate content in barley was significantly decreased from 9.73 to 0.51 mmol/kg after fermentation with *L. plantarum*.

Pigs fed fermented wheat diets showed greater ($P < 0.05$) ATTD of DM, N, Ca, P, and GE than those fed unfermented wheat diets (Table 3). The present results confirmed our previous study (Koo et al., 2018), in which ATTD of DM, GE, and P were improved when wheat was substituted for fermented wheat in ileal-cannulated weanling pigs. Also, this is partially in agreement with other studies where digestibility of DM, N, GE, EE, P, or AA were improved (Hackl et al., 2010; Pieper et al., 2011; Le et al., 2016) when pigs were fed *Lactobacillus*-fermented diets compared to unfermented diets. The reason for the improvement of nutrient digestibility might be degradation of cell-wall carbohydrates of grain by lactic acid bacteria (Lyberg et al., 2006), which possibly made encapsulated nutrients available to pigs (Bedford and Schulze, 1998). Indeed, it has been reported that lactic acid bacteria are capable of secreting a variety of extracellular enzymes (e.g., α-amylase, ferulate esterase, peptitase, β-glucanase, β-glucosidase, peptidase, and phytase), hydrolyzing various substrates (Christensen et al., 1999; Aguilar et al., 2000; Crittenden et al., 2002; Abdel-Rahman et al., 2011; Fischer et al., 2014). Furthermore, endogenous phytase activity is optimized at pH 4 (Brejnholt et al., 2011), which is known to be reached by fermentation with *Lactobacillus*-inoculum (Pieper et al., 2011). This might have contributed to a reduction in phytate-bound P content, causing increased P digestibility. Also, lactic acid in fermented diet is known to lead to a low gastric pH (Canibe and Jensen, 2003), and the acidic condition may stimulate dietary protein hydrolysis and reduce gastric emptying rate (Humer et al., 2014). This might have partially contributed to the improvement of CP digestibility in pigs fed fermented diets in the present study. However, it is unclear why fermentation benefits were not shown with barley. This was also observed by Hackl et al. (2010) and Pieper et al. (2011), where greater nutrient digestibility was found in pigs fed fermented wheat, but not in those fed fermented barley compared to unfermented grain. This might be due to a lack of extracellular enzymes secreted by lactic

Table 3. Apparent total tract digestibility (ATTD), nitrogen balance, and phosphorus utilization in weaned pigs fed diets containing *Lactobacillus*-fermented grains[1]

	Barley			Wheat				*P*-values[2]			
		Fermented			Fermented						
Item	Control	Homo	Hetero	Control	Homo	Hetero	SEM	Ferm. barley	Ferm. wheat	Inocul.	Grain
ATTD, %											
Dry matter	88.1	87.3	88.4	88.3	90.5	90.4	0.60	0.769	0.003	0.374	0.001
Nitrogen	87.4	86.0	89.6	87.8	90.4	90.3	0.87	0.685	0.015	0.045	0.010
Calcium	78.9	75.0	75.6	69.1	77.3	79.8	2.46	0.204	0.004	0.514	0.571
Phosphorus	71.4	72.0	72.8	68.0	74.1	75.5	1.61	0.602	0.001	0.475	0.703
Phosphorus utilization											
Intake, g/d	3.6	4.0	3.7	3.8	3.4	3.7	0.18	0.254	0.258	0.650	0.368
Fecal output, g/d	1.0	1.1	1.0	1.2	0.9	0.9	0.09	0.853	0.003	0.769	0.554
Nitrogen balance											
Intake, g/d	16.7	17.1	17.6	17.8	17.0	17.8	0.86	0.510	0.692	0.478	0.522
Fecal output, g/d	2.1	2.4	1.8	2.2	1.8	1.7	0.16	0.954	0.034	0.031	0.137
Urinary output, g/d	2.4	1.7	1.7	2.8	1.9	2.2	0.40	0.131	0.107	0.696	0.258
Retained, %	72.3	76.2	79.8	72.3	78.1	78.1	2.78	0.080	0.070	0.496	0.978
Retained, g/d	12.2	13.1	14.0	12.8	13.3	13.9	0.89	0.190	0.460	0.362	0.700

[1]Control, unfermented grains; Homo, homofermentative *L. plantarum*; Hetero, heterofermentative *L. buchneri*.

[2]*P*-values of following contrasts: ferm. barley = control barley vs. fermented barley; ferm. wheat = control wheat vs. fermented wheat; inocul. = Homo-fermented vs. Hetero-fermented; and grain = barley vs. wheat.

acid bacteria for the specific structure of cell-wall components and configuration of the phytate-mineral-nutrient complexes present in barley; however, further research is warranted.

Greater ATTD of N and P in pigs fed fermented wheat led to a reduction in their fecal output ($P < 0.05$). Similarly, Kraler et al. (2014) also found that inclusion of fermented wheat bran substituting for unfermented wheat bran significantly decreased fecal excretion of P in pigs. This implies that feeding fermented wheat diets to weaned pigs might be a nutritional strategy to pursue a sustainable livestock industry by reducing the discharge of N and P into the environment. However, because various gasses such as CO_2, CO, CH_4, and H_2 produced during microbial fermentation can pose a environmental risk (Monteny et al., 2006), life-cycle assessment must be conducted to investigate the effects of fermented swine diets on the environment (Mackenzie et al., 2016).

The replacement of unfermented barley with fermented barley reduced DE ($P < 0.05$) because there was less GE content in fermented barley (Table 4). However, no differences in ME and NE content were observed between fermented and unfermented barley diets. Similarly, although ATTD of GE was greater ($P < 0.05$) in the fermented wheat diet than in the unfermented wheat diet, no difference in DE content was observed between fermented and unfermented wheat diets. However, greater ($P < 0.05$) ME and a trend ($P < 0.10$) for greater NE were found in fermented wheat diets than in unfermented diets. Substituting fermented barley diets for unfermented barley diets tended to result in a greater ME:DE ratio ($P < 0.10$), whereas pigs fed fermented wheat showed a greater ($P < 0.05$) ME:DE ratio than in those fed the control wheat diet. Given that there was a trend for greater ($P < 0.10$) N retention in pigs fed fermented grain diets, compared to control diets, pigs seemed to retain energy as protein more efficiently when they were fed fermented grain diets. The gut immunity might have been fortified by the fermented metabolites and lactic acid bacteria, reducing protein turnover. In fact, *Lactobacillus* delivered to the gut is known to reduce pro-inflammatory cytokines (Hou et al., 2015) and crypt depth (Le et al., 2016), which may decrease unnecessary protein turnover and increase energy retention as protein. In contrast, a lower ($P < 0.05$) ME:NE ratio with fermented wheat and

Table 4. Energy balance, digestible energy (DE), metabolizable energy (ME), and net energy (NE) contents of diets containing *Lactobacillus*-fermented grains fed to weaned pigs[1]

	Barley			Wheat				*P*-value[2]			
		Fermented			Fermented						
Item[3]	Control	Homo	Hetero	Control	Homo	Hetero	SEM	Ferm. barley	Ferm. wheat	Inocul.	Grain
GE, kcal/d											
Intake	1,926	1,985	1,946	2,019	1,833	1,900	96.6	0.721	0.167	0.871	0.635
Fecal output	245	267	228	241	183	193	14.5	0.883	0.003	0.283	0.001
Urinary output	249	201	187	291	211	157	30.4	0.122	0.004	0.249	0.747
ATTD, %	87.3	86.5	88.1	88.1	90.0	89.9	0.66	0.938	0.016	0.216	0.001
Retention, %	74.0	76.2	78.5	73.8	78.6	81.6	1.92	0.129	0.006	0.150	0.230
Diet DE, kcal/kg											
As-fed basis	3,390	3,274	3,357	3,402	3,416	3,389	25.1	0.013	0.986	0.243	0.003
DM basis	3,845	3,820	3,931	3,855	3,967	3,999	29.0	0.357	0.001	0.014	0.002
Diet ME, kcal/kg											
As-fed basis	2,874	2,885	2,989	2,850	2,983	3,076	73.6	0.453	0.037	0.161	0.338
DM basis	3,259	3,366	3,500	3,230	3,464	3,631	84.4	0.079	0.002	0.067	0.305
Diet NE[4], kcal/kg											
As-fed basis	2,224	2,188	2,254	2,221	2,274	2,297	33.1	0.927	0.092	0.158	0.110
DM basis	2,522	2,552	2,640	2,516	2,640	2,710	38.1	0.099	0.001	0.036	0.090
ME:DE ratio	0.85	0.87	0.85	0.84	0.86	0.90	0.02	0.076	0.015	0.205	0.999
NE:ME ratio	0.78	0.76	0.76	0.78	0.76	0.75	0.01	0.079	0.019	0.245	0.981

[1]Control, unfermented grains; Homo, homofermentative *L. plantarum*; Hetero, heterofermentative *L. buchneri*.

[2]*P*-values of following contrasts: ferm. barley = control barley vs. fermented barley; ferm. wheat = control wheat vs. fermented wheat; inocul. = Homo-fermented vs. Hetero-fermented; and grain = barley vs. wheat.

[3]GE, gross energy; ATTD, apparent total tract digestibility; DM, dry matter.

[4]The average of 2 calculated NE values using the following equations published by Noblet et al. (1994): 1) NE = 0.700 × DE + 1.61 × ether extract + 0.48 × starch − 0.91 × crude protein − 0.87 × acid detergent fiber and 2) NE = 0.726 × ME + 1.33 × ether extract + 0.39 × starch − 0.62 × crude protein − 0.83 × acid detergent fiber.

a trend for a lower ($P < 0.10$) ME:NE ratio with fermented barley were determined than the ratio with respective unfermented grain. This was mostly because of concentrated CP contents with reduced starch or EE contents in fermented grain diets. In fact, van Milgen et al. (2001) demonstrated that starch and lipids have greater energetic efficiencies of ME utilization (0.84 and 0.88, respectively) than protein (0.42).

Pigs fed Hetero-fermented diets showed greater ($P < 0.05$) ATTD of N and lower ($P < 0.05$) N fecal output than those fed Homo-fermented diets. Hetero-fermented diets also showed greater DE and NE ($P < 0.05$) and a trend for greater ME ($P < 0.10$) compared to Homo-fermented diets when energy contents were expressed on a DM basis. Our previous study (Koo et al., 2018) also showed that Hetero-fermented wheat diets had greater DE than Homo-fermented wheat diets. To the best of our knowledge, no studies have compared the effects of Homo-inoculum with those of Hetero-inoculum for fermentation on the energy content of grains. Presumably, acetic acid produced from Hetero efficiently suppressed the growth of yeasts and molds by which proteolysis and energy loss occurs, releasing ammonia-N and CO_2 (Holzer et al., 2003; Wang et al., 2014).

Dietary composition is the main factor affecting nutrient digestibility and energy content of diets (Noblet and Perez, 1993). For instance, the content of dietary fiber is well known to be negatively related to nutrient and energy digestibility (Agyekum and Nyachoti, 2017). In the present study, barley diets contained numerically 17%, 14%, and 13% greater NDF, ADF, and NSP (as-fed basis) content than wheat-based diets. These differences were mainly due to the difference in fiber content (e.g., NDF and ADF) between barley and wheat because the composition of all other ingredients was identical or similar across experimental diets. In this regard, pigs fed barley-based diets showed lower ($P < 0.05$) ATTD of DM, N, and GE and DE than those fed wheat-based diets. This is partially supported by Lynch et al. (2007), who reported that increasing levels of barley at the expense of wheat in a diet linearly reduced ATTD of DM, N, and GE and linearly increased fecal N excretion. Similarly, previous studies (Skrede et al., 2001; Jørgensen et al., 2010) reported that feeding wheat-based diets (unfermented + fermented) showed greater nutrient digestibility than barley-based diets in chicken or pigs.

In conclusion, wheat fermented with *Lactobacillus*-inoculum can be beneficially substituted for unfermented wheat, improving the ATTD of nutrients and energy, N retention, and energy content. However, the effects with fermented barley diets were shown only when the energy contents were expressed on a DM basis. Also, Hetero-inoculum is preferable to Homo-inoculum for grain fermentation in terms of energy content and N digestibility. However, further studies are needed to investigate the interaction between fermentation metabolites and energy utilization in pigs. Furthermore, life-cycle assessment is warranted to evaluate whether grain fermentation can be an environmentally friendly strategy within livestock industry.

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