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Digestibility of Energy and Crude Protein in Korean Rice Wine Residues Fed to Pigs

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Abstract: Two experiments were conducted to determine the digestibility of energy and Crude Protein (CP) in Korean Rice Wine Residues (RWR) using *in vitro* and *in vivo* approaches. In the *in vitro* experiment, 2-step and 3-step *in vitro* disappearance procedures were employed to determine *in vitro* Ileal Disappearance (IVID) and *in vitro* Total Tract Disappearance (IVTTD) of nutrients, respectively, in corn, a Soybean Meal-Wheat Mixture (SBMW), RWR and the experimental diets used in the *in vivo* experiment. In the *in vivo* experiment, 3 Jeju Island native pigs fitted with a T-cannula in the distal ileum with an initial mean body weight of 74.8±5.4 kg were individually housed in pens equipped with a feeder and a nipple drinker. A replicated 3×3 Latin square design was employed with 3 dietary treatments, 6 periods and 3 animals. Three experimental diets based on the corn and SBMW with a constant ratio (corn:SBMW = 3.06:1) were formulated to contain 0, 15 or 30% of RWR. All diets contained 0.5% chromic oxide as an indigestible index. Korean RWR had greater IVID of Dry Matter (DM) than corn and SBMW ($p<0.05$). The IVID of CP in RWR was less than that of corn and SBMW ($p<0.05$). The IVTTD of DM in RWR was greater than that of corn but less than that of SBMW ($p<0.05$). The IVID of CP linearly decreased as the inclusion rate of RWR increased ($p<0.001$). The digestible energy concentration (kcal/kg DM) in diets increased linearly with an increase in RWR in the diets ($p = 0.001$). The apparent and standardized ileal digestibility of CP in the diets showed a linear decrease ($p<0.001$) as the inclusion rate of RWR increased. In conclusion, Korean rice wine residues have a high digestible energy concentration and may be used in swine diets with attention to protein utilization.

Keywords: Digestible Energy, Makgeolli By-Product, Pigs, Rice Wine Residues

Introduction

The use of by-products for livestock feeding has increased over the last few decades due to the fluctuations in the price of conventional feed ingredients. When using the by-products in animal feeds, the knowledge of energy and nutrient availability in the by-product feed ingredients is crucial (Rijal *et al*., 2009; NRC, 2012). Accordingly, a number of data have been reported to provide biological availability of energy and nutrients (Son *et al*., 2014; Casas *et al*., 2015; 2018; Sung *et al*., 2019).

Korean Rice Wine Residues (RWR) called "Makgeolli by-products" are the residues after the fermentation of rice to produce wine. The RWR have been studied as an ingredient for cattle feeds (Piao *et al*., 2012; Jeong *et al*., 2016). To our knowledge, however, there is no information on the nutritional values of RWR as an ingredient for swine diets.

While animal experiments are often used for determining the digestibility of energy and nutrients in feedstuffs, animal experimentation is expensive and time-consuming. As an alternative method, *in vitro* procedures are available for routine determination of the digestibility of energy (Boisen and Fernández, 1997) and protein (Boisen and Fernández, 1995) in swine feedstuffs. Therefore, the objective of this study was to determine the Digestible Energy (DE) and digestibility of Crude Protein (CP) in Korean RWR fed to pigs using *in vitro* and *in vivo* approaches.

Materials and Methods

The protocol for the experiment was approved by the Institutional Animal Care and Use Committee at Konkuk University (KU18126).

Preparation of Korean Rice Wine Residues

Korean RWR from a Korean traditional wine (Makgeolli) processing company were dried in a forced-air drying oven at 55°C for 4 days to contain 15.9% moisture (Table 1).

In Vitro Ileal Disappearance Experiment

The *in vitro* Ileal Disappearance (IVID) of Dry Matter (DM) and CP were determined using a 2-step *in vitro* digestibility procedure described by Boisen and Fernández (1995) with minor modifications.

One g of a ground (1-mm sieve) ingredient or a diet sample was transferred into 100-mL conical flasks. A 25 mL of sodium phosphate buffer solution (0.1 M, pH 6.0) and 10 mL of HCl (0.2 M, pH 0.7) were added to the sample. To simulate digestion conditions in the stomach, 1 M HCl or NaOH was used to adjust the pH to 2.0 and 1 mL of freshly prepared pepsin solution (10 mg/mL; ≥250 units/mg solid, P7000, pepsin from porcine gastric mucosa, Sigma-Aldrich, St. Louis, MO, USA) was added to the samples. To avoid bacterial contamination, 0.5 mL of chloramphenicol (C0378, Chloramphenicol, Sigma-Aldrich, St. Louis, MO, USA) solution (5 g/L ethanol) was also added. Test flasks were closed with a silicon stopper and incubated in a shaking incubator at 39°C for 6 h.

After incubation, the second step of the procedure simulated the digestion in the small intestine. Firstly, 10 mL of sodium phosphate buffer solution (0.2 M, pH 6.8) and 5 mL of NaOH (0.6 M, pH 13.8) were added to the samples. Then, the pH was adjusted to 6.8 using 1 M HCl or NaOH. Thereafter, 1 mL of freshly prepared pancreatin solution (50 mg/mL; 4× USP, P1750, pancreatin from porcine pancreas, Sigma-Aldrich, St. Louis, MO, USA) was added. After incubation in a shaking incubator at 39°C for 18 h, 5 mL of 20% sulfosalicylic acid solution was added and samples were left for 30 min at room temperature to precipitate the indigestible protein. The samples were then filtered through pre-dried and pre-weighed glass filter crucibles (Filter Crucibles CFE Por. 2, Robu, Hattert, Germany) containing 400 mg of celite using the Fibertec System (Fibertec System 1021 Cold Extractor, Tecator, Höganäs, Sweden). Test flasks were rinsed twice with 1% sulfosalicylic acid solution and 10 mL of 95% ethanol and 99.5% acetone were added twice to the glass filter crucibles. Glass filter crucibles with undigested residues were dried at 80°C for 24 h. The undigested materials together with the celite were wrapped into a piece of nitrogen-free paper and kept for further analysis.

Table 1: Analyzed nutrient composition of corn, Soybean Meal-Wheat Mixture (SBMW) and Rice Wine Residues (RWR), as-fed basis

Item	Corn	SBMW[1]	RWR
Dry matter, %	86.40	89.02	84.10
Gross energy, kcal/kg	3,737	4,105	3,977
Crude protein, %	8.57	38.00	15.30
Ash, %	1.70	5.93	0.53
Neutral detergent fiber, %	8.33	5.55	4.18
Acid detergent fiber, %	2.56	3.29	2.54

[1]The ratio of soybean meal and wheat was 65:35 in the mixture

In Vitro Total Tract Disappearance Experiment

The *in vitro* Total Tract Disappearance (IVTTD) of DM and CP was determined using 3-step *in vitro* digestibility techniques described by Boisen and Fernández (1997) with minor modifications.

The first and second steps were similar to the IVID procedure, except for the weight of the samples, the concentration of the enzymes and incubation time. For IVTTD, 0.5 g of each ingredient sample was used and the concentrations of pepsin and pancreatin solutions were increased to 25 and 100 mg/mL, while the incubation periods were reduced to 2 and 4 h, respectively. In the third step of the IVTTD procedure, 10 mL of 0.2 M EDTA solution was added to the samples. The pH was then adjusted to 4.8 by adding acetic acid 30% or 1 M NaOH. Samples were supplemented with 0.5 mL of multi-enzyme (V2010, Viscozyme®, Sigma-Aldrich, St. Louis, MO, USA) as a substitute for microbial enzymes and incubated in a shaking incubator for 18 h at 39°C. After incubation, the undigested residues were collected and dried as previously described in the IVID procedure except for the drying condition (at 130°C for 6 h) and kept for further analysis.

In Vivo Digestibility Experiment

Three Jeju Island native pigs fitted with a T-cannula in the distal ileum with an initial mean Body Weight (BW) of 74.8±5.4 kg were individually housed in pens equipped with a feeder and a nipple drinker. The pigs were surgically fitted with a T-cannula in the distal ileum using the procedures adapted from Stein *et al*. (1998). A replicated 3 × 3 Latin square design was employed with 3 dietary treatments, 6 periods and 3 animals. The amount of daily feed intake was calculated at approximately 1.5 times the estimated energy requirement for maintenance (i.e., 197 kcal of metabolizable energy per kg of $BW^{0.60}$; NRC, 2012) and divided into 2 equal meals and fed to pigs at 0800 and 1700 h. Water was available at all times. The basal diet contained mainly corn and a Soybean Meal-Wheat Mixture (SBMW) at the ratio of 65:35 while the other 2 additional diets were formulated to contain 15 or 30% of RWR at the expense of corn and SBMW with a constant ratio (3.06:1) of corn:SBMW (Table 2).

Vitamins and minerals were included in all diets to meet or exceed requirement estimates for growing pigs (NRC, 2012). All diets contained 0.5% chromic oxide (Cr_2O_3) as an indigestible index.

An experimental period consisted of a 7-d adaptation and a 4-d collection periods composed of 2 d of fecal collection followed by 2 d of ileal digesta collection. Fecal samples were collected by the grab sampling procedure for the index method (Kong and Adeola, 2014). The ileal digesta samples were collected from 0800 to 1700 h. A plastic sample bag with wire was fixed to the T-cannula for the ileal digesta collection. A sample bag was changed at least once every 30 min, or whenever the bag was filled with ileal digesta. The collected ileal digesta samples for each animal were pooled together and then subsampled. These samples were immediately stored at –20°C. At the end of each period, BW was measured to determine the amount of feed allowance during the next period.

Chemical Analysis

The undigested residues from the 2-step *in vitro* experiment were analyzed for CP (AOAC, 2005; method 990.03) for the calculation of IVID of CP. The fecal samples were dried in a forced-air drying oven at 55°C and ground before analysis. The frozen ileal digesta samples were dried in a freeze dryer. Ingredients, diets, fecal and ileal digesta samples from the *in vivo* experiment were analyzed for Gross Energy (GE) using a bomb calorimeter (Parr 1261 bomb calorimeter; Parr Instruments Co., Moline, IL, USA) and analyzed for DM (AOAC, 2005; method 930.15) and CP. Samples of ingredients and diets were also analyzed for ash (AOAC, 2005; method 942.05), Neutral Detergent Fiber (NDF; Van Soest *et al*., 1991) and Acid Detergent Fiber (ADF; AOAC, 2005; method 973.18). Chromium (Cr) concentrations of the diet, feces and ileal digesta samples were analyzed by using spectrophotometer (Optizen 2120UV, Mecasys Inc., Daejeon, Republic of Korea).

Calculations and Statistical Analysis

The IVID or IVTTD of DM (%) was calculated using the following equation (Kong *et al*., 2015):

$$IVID\ or\ IVTTD\ of\ DM(\%) = \left[\left(DM_{TI} - DM_{RS}\right) \div DM_{TI}\right] \times 100$$

where, DM_{TI} and DM_{RS} (g) are the weight of *DM* in the test ingredient and the undigested residues, respectively, collected from IVID or IVTTD procedure. The IVID of CP was also calculated using the same equation.

The Apparent Total Tract Digestibility (ATTD) of GE was calculated based on the GE and Cr concentrations in feces and diet. The DE concentration of diets was calculated by multiplying ATTD of GE to GE in diets. The Apparent Ileal Digestibility (AID) and Standardized Ileal Digestibility (SID) of CP were calculated based on CP and Cr concentrations of the diet and ileal digesta (Stein *et al*., 2007; Kong and Adeola, 2014). Values for the ATTD of GE and AID and SID of CP were calculated according to the following equations (Son *et al*., 2014):

$$ATTD\ of\ GE(\%) = \left[1 - \left(GE_{feces} \div GE_{diet}\right) \times \left(Cr_{diet} \div Cr_{feces}\right)\right] \times 100$$

$$AID\ of\ CP(\%) = \left[1 - \left(CP_{ileal} \div CP_{diet}\right) \times \left(Cr_{diet} \div Cr_{ileal}\right)\right] \times 100$$

$$SID\ of\ CP(\%) = AID\ of\ CP + \left[\left(BEL\ of\ CP \div CP_{diet}\right) \times 100\right]$$

where, GE_{feces} is GE concentration in the feces (kcal/kg of DM), GE_{diet} is GE concentration in the diet (kcal/kg of DM), CP_{ileal} is the concentration of CP in the ileal digesta, CP_{diet} is the concentration of CP in the diet, Cr_{diet} is the concentration of Cr in the diet and Cr_{ileal} is the concentration of Cr in the ileal digesta. The CP and Cr concentrations were expressed as g/kg of DM. The Basal Endogenous Losses (BEL) of CP were assumed to be 17.2 g/kg of DM intake (Park *et al*., 2013).

Data were analyzed by the MIXED procedure of SAS (SAS Inst. Inc., Cary, NC, USA). The model included dietary treatment as fixed variable and animal and period as random variables. Differences between least squares means were determined by the PDIFF option with the Tukey's adjustment. Preplanned orthogonal contrasts were used to evaluate linear and quadratic effect of dietary inclusion rate of RWR. The experimental unit was a flask in *in vitro* experiment and a pig in *in vivo* experiment and the statistical significance was set at $p<0.05$.

Results

Korean RWR contain greater GE and CP compared with corn but less values than SBMW (Table 1). Moreover, RWR had the lowest values for ash and NDF concentrations. Analyzed diet nutrient concentrations were generally in agreement with the calculated values (Table 2).

In Vitro Disappearance Experiment

Korean RWR had greater IVID of DM ($p<0.05$) than corn or SBMW (Table 3). However, the IVID of CP in RWR was less than that of corn and SBMW ($p<0.05$). The IVTTD of DM in RWR was greater ($p<0.05$) than that of corn but less than that of SBMW.

The IVID of DM showed a quadratic response ranging from 84.9 to 85.7% as the inclusion rate of RWR increased ($p = 0.024$; Table 4). The IVID of CP decreased linearly as the inclusion rate of RWR increased ($p<0.001$).

Table 2: Ingredient composition of experimental diets, as-fed basis

	Diet		
Item	Basal diet	15% RWR	30% RWR
Ingredient, %			
Ground corn	73.40	62.13	50.83
Soybean meal-wheat mixture[1]	24.00	20.32	16.62
Rice Wine Residues (RWR)	-	15.00	30.00
Limestone	0.70	0.60	0.45
Dicalcium phosphate	0.80	0.85	1.00
Sodium chloride	0.30	0.30	0.30
Vitamin-trace mineral premix[2]	0.30	0.30	0.30
Chromic oxide	0.50	0.50	0.50
Nutrient composition			
Dry matter, %	87.68	87.19	86.65
Gross energy, kcal/kg	3,874	3,894	3,921
Crude protein, %	15.45	15.55	15.75
Ash, %	4.80	4.55	4.12
Neutral detergent fiber, %	8.08	6.64	6.31
Acid detergent fiber, %	3.26	2.60	2.63

[1]The ratio of soybean meal and wheat was 65:35 in the mixture
[2]Provided the following quantities per kg of complete diet: vitamin A, 6,000 IU; vitamin D_3, 1,200 IU; vitamin E, 0.75 IU; vitamin K, 0.3 mg; thiamin, 0.29 mg; riboflavin, 0.9 mg; pyridoxine, 0.6 mg; vitamin B_{12}, 3.6 µg; pantothenic acid, 3.0 mg; folic acid, 0.6 mg; niacin, 6.0 mg; biotin, 0.06 mg; Cu, 1.5 mg as copper sulfate; Fe, 12 mg as iron sulfate; I, 0.75 mg as calcium iodate; Mn, 36 mg as manganese sulfate; Zn, 45 mg as zinc sulfate; Co, 0.3 mg as cobaltous carbonate; Mg, 6.0 mg as magnesium oxide; and choline chloride, 75 mg

Table 3: *In vitro* Ileal Disappearance (IVID) of Dry Matter (DM), IVID of Crude Protein (CP) and *in vitro* Total Tract Disappearance (IVTTD) of DM in corn, a Soybean Meal-Wheat Mixture (SBMW) and Rice Wine Residues (RWR)[1]

Item, %	Corn	SBMW[2]	RWR	SEM[3]	p-value
IVID of DM	85.8[b]	82.5[c]	89.9[a]	0.3	< 0.001
IVID of CP	77.7[b]	91.1[a]	73.2[c]	0.5	< 0.001
IVTTD of DM	88.8[c]	95.6[a]	93.6[b]	0.3	< 0.001

[a–c]Least squares means within a row without a common superscript differ (p<0.05)
[1]Each least squares mean represents 3 observations
[2]The ratio of soybean meal and wheat was 65:35 in the mixture
[3]SEM = standard error of the mean

In Vivo Experiment

All pigs remained healthy throughout the experiment and consumed all of their designated diets. The DE concentration (kcal/kg DM) in diets increased linearly with an increase in RWR in the diets (Table 5; p = 0.001). As the concentration of RWR increased in the diet, the AID and SID of CP in the diets linearly decreased (p<0.001).

Discussion

The CP, ash, NDF and ADF concentrations in RWR used (Table 1) in the present work were less than the values reported by Piao *et al*. (2012). These differences are likely due to the differences in the processing, fermentation techniques and the additives included during the rice wine production procedure.

The IVID and IVTTD of DM in corn (Table 3) were consistent with the previous studies (Kong *et al*., 2015; Park *et al*., 2016). However, the IVID of DM in SBMW was slightly greater than the reported value (Navarro *et al*., 2018) while the IVID of CP and IVTTD of DM in SBMW were close to the reported values (Boisen and Fernández, 1995; Navarro *et al*., 2018). The nutrient concentrations in Soybean Meal (SBM) are often variable (Douglas *et al*., 2000) depending on the genotype (Palacios *et al*., 2004) and the growing environment (van Kempen *et al*., 2002). Heat processing may also affect the nutrient concentrations and bioavailability of nutrients in SBM (Goebel and Stein, 2011). Therefore, the discrepancy in IVID of nutrients of SBM among the studies may be at least partially due to different growing and production conditions of each SBM.

Rice, which is the major cereal in the production of rice wine, contains about 75% of carbohydrates as starch (NRC, 2012). Starch is highly digestible at the small intestine (Bach Knudsen, 1997), which explains the high IVID of DM in RWR (Table 3) in the present work.

A greater value of IVTTD of DM compared with the IVID of DM is in consistent with the previous studies (Kong *et al*., 2015; Navarro *et al*., 2018). In step 3 of *in vitro* disappearance procedure, multi-enzyme complex (Viscozyme®) containing various NSP-degrading enzymes was used for the digestion of large intestine.

Table 4: *In vitro* Ileal Disappearance (IVID) of Dry Matter (DM), IVID of Crude Protein (CP) digestibility and *in vitro* Total Tract Disappearance (IVTTD) of DM of diets used in the *in vivo* experiment[1]

	Rice wine residues, %				p-value	
Item, %	0	15	30	SEM[2]	Linear	Quadratic
IVID of DM	84.9	86.7	85.7	0.4	0.194	0.024
IVID of CP	86.0	83.0	77.1	0.5	< 0.001	0.068
IVTTD of DM	91.1	91.7	91.8	0.5	0.341	0.695

[1]Each least squares mean represents 3 observations except for IVID of CP in diet containing 30% rice wine residues (2 observations)
[2]SEM = standard error of the mean

Table 5: Digestible Energy to Gross Energy ratio (DE:GE), Digestible Energy (DE), Apparent Ileal Digestibility (AID) of Crude Protein (CP) and Standardized Ileal Digestibility (SID) of CP of experimental diets containing graded rice wine residues fed to pigs[1]

	Rice wine residues, %				p-value	
Item	0	15	30	SEM[2]	Linear	Quadratic
DE:GE	0.90	0.90	0.89	0.01	0.238	0.055
DE[3], kcal/kg	3,959	4,025	4,039	25	0.001	0.119
AID of CP, %	71.3	63.4	56.3	2.0	< 0.001	0.852
SID of CP, %	81.0	73.0	65.8	2.0	< 0.001	0.866

[1]Each least square mean represents 6 observations
[2]SEM = standard error of the mean
[3]Diet DE concentration is expressed in a dry matter basis

Therefore, IVTTD of DM was greater than IVID of DM (Table 4) in the current experiment due to the additional digestion process which simulates the hindgut fermentation. Moreover, greater IVTTD of DM in SBMW than in RWR may be due to the greater fiber concentration in SBMW than that in RWR.

Energy digestibility is highly correlated with the IVTTD of DM (Park *et al*., 2013; Son *et al*., 2017). In the present study, neither linear nor quadratic effect of RWR inclusion rate was observed in both of IVTTD of DM and DE:GE (Tables 4 and 5), indicating the consistency among the previous studies and the present study.

While the DE of most diets fed to pigs varies ranging from 70 to 90% of GE in the diet (Sauvant *et al*., 2004), the 3 experimental diets in the present work had very comparable DE:GE (Table 5). Thus, the linear effect of the RWR inclusion rate on DE (kcal/kg DM) is most likely due to the GE concentrations in the experimental diets (Table 2). In addition, dietary fiber is also a reason for variation in DE values among diets (Noblet and van Milgen, 2004) as the studies showed that the energy digestibility decreases with increasing dietary fiber concentrations (Le Goff and Noblet, 2001). In the present work, however, the fiber contents in the diets do not appear to be sufficiently variable to cause deviations in energy digestibility.

In the present work, the IVID of CP and ileal digestibility of CP linearly decreased with increasing RWR inclusion rate which primarily indicates low ileal digestibility of CP in RWR. Additionally, RWR may have potentially lowered ileal CP digestibility of corn and SBMW in the experimental diets. The low ileal CP digestibility in RWR is likely due to the relatively long drying period (4 days) in this work. The original RWR were wet and thus, had sufficient moisture and sugar to undergo Maillard reactions at a high temperature during drying process (Nursten, 2005; Kim *et al*., 2012; Ahn *et al*., 2014). Development of brown color is an indication of the formation of advanced Maillard products (Nursten, 2005; Kim *et al*., 2012). In the present study, a brown color was observed in RWR after oven-dried at 55°C for 4 days. Therefore, the low protein ileal digestibility in RWR is likely due to the Maillard reactions. Further research is warranted to improve protein digestibility in RWR.

Conclusion

Korean rice wine residues have a quite high digestible energy concentration and may be used in swine diets with attention to protein utilization.

Author's Contributions

Mariette Bessem Akonjuen: Conducted the animal experiment and drafted most of the manuscript.

Bokyung Hong: Assisted animal care, performed statistical analysis and critically revised statistical and discussion parts of the manuscript.

Hyunjun Choi: Designed the animal experiment, involved *in vitro* experiments and critically reviewed the manuscript.

Beob Gyun Kim: Supervised the experimental work and manuscript preparation and revised the manuscript.

Ethics

This article is original and contains unpublished material. The corresponding author confirms that all of the other authors have read and approved the manuscript and no ethical issues involved.

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