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Short communication

The concentration of energy in urine and the concentration of metabolizable energy in diets fed to pigs are influenced by urine preparation and drying method

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A B S T R A C T

An experiment was conducted to test the null hypothesis that gross energy (GE) in urine and metabolizable energy (ME) in diets fed to pigs are not impacted by the procedure used to determine GE in urine. To test the hypothesis, results obtained using the freeze-dried cotton-plastic bag method were compared with results obtained by dripping urine on undried cellulose pellets, by calculating GE from urine nitrogen using four different equations, and by comparing with results from oven-dried urine samples. Data for the concentration of GE in 11 diets and in feces from pigs fed these diets were used to calculate the digestible energy in the diets. The GE in urine from pigs fed these diets was determined using the above methods and ME in each diet was calculated. Results indicated that using the undried cellulose pellet method resulted in greater ($P < 0.01$) GE in urine and reduced ($P < 0.001$) calculated ME and ME:DE compared with the freeze-dried cotton-plastic bag. In contrast, if GE was calculated from the nitrogen in urine, regardless of the equation used in the calculation, GE was less ($P < 0.05$), and the calculated ME and ME:DE were greater ($P < 0.05$) than values from the freeze-dried cotton-plastic bag method. However, no differences between freeze-drying and oven-drying of urine for GE or calculated ME or ME:DE were observed. In conclusion, oven-drying of urine samples results in analyzed GE in urine that is not different from GE analyzed after freeze-drying, but using cellulose pellets or calculating GE from urine nitrogen results in estimates for GE that are less accurate.

1. Introduction

Accurate measurement of gross energy (GE) in urine is critical for the calculation of metabolizable energy (ME) in diets and feed ingredients. The concentration of GE in urine of pigs is determined by combusting a sample using a bomb calorimeter. A common method for preparing urine samples for bomb calorimetry includes freeze-drying urine that has been dripped on a cotton ball placed in a plastic bag (Kim et al., 2009). This procedure has been used to determine urine GE and calculate diet ME in multiple experiments (Ibagon et al., 2023; Lee et al., 2024; Cristobal et al., 2025). However, the concentration of GE in urine can also be determined by

Abbreviations: DDGS, distillers dried grains with solubles; DE, digestible energy; DFRB, defatted rice bran; DMI, dry matter intake; FFRB, full-fat rice bran; FSBM, fermented soybean meal; GE, gross energy; ME, metabolizable energy; SBM, soybean meal; WM, wheat middlings.

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dripping urine on a cellulose pellet, which is then ignited in a bomb calorimeter (Fent et al., 2000). Alternatively, GE can be calculated based on the nitrogen concentration in the urine assuming that all urinary energy originates from nitrogen-containing compounds (Etheridge et al., 1998; Sauvant et al., 2004; Do et al., 2025). Calculating urinary GE by using the undried cellulose pellet method or from nitrogen-based calculation may be less time-consuming than using the freeze-dried cotton-plastic bag approach. Likewise, oven-drying instead of freeze-drying of cotton-plastic bag urine samples may also reduce the time required for the drying procedure and does not require the use of a freeze-drier.

No difference in analyzed GE of pig urine was observed when using undried cellulose pellets, freeze-dried samples, or oven-dried samples (Jacobs et al., 2011), and no energy was lost due to oven-drying poultry excreta (Sibbald, 1979; Wallis and Balnave, 1983). However, to our knowledge, no data comparing values for GE in urine and ME in diets between the conventional freeze-dried cotton-plastic bag procedure and other sample preparation methods are available. Therefore, the objective of this work was to test the hypothesis that there are no differences in the analyzed GE in urine and the calculated ME of diets between the conventional freeze-dried cotton-plastic bag method and the undried cellulose pellet method, the nitrogen method, or the oven-drying method.

2. Materials and methods

Data for the concentration of GE in 11 diets (Tables 1 and 2) and in feces from pigs fed these diets were used to calculate the concentration of digestible energy (DE) in all diets. In each experiment, urine was collected for 4 or 5 days following a diet adaptation period of 5–7 days. In all experiments, urine was collected over a preservative of HCl, and on each collection day, urine buckets were emptied, the quantity of total urine voided was recorded, and 20% of the voided urine was stored at −20 °C. All urine samples were thawed and filtered before being used to determine effects of different sample preparation and drying methods on urine GE.

Table 1

Ingredient composition of experimental diets containing corn, corn and soybean meal (SBM), corn and fermented SBM (FSBM), corn, SBM, distillers dried grains with solubles (DDGS), and wheat middlings (WM), corn, SBM, and full-fat rice bran (FFRB), or corn, SBM, and defatted rice bran (DFRB).

	Urine sample size for Kjeldahl analysis			Sample preparation method					
				Conventional vs. cellulose pellet			Conventional vs. nitrogen value		
Ingredient, g/kg	Corn	Corn, SBM	Corn, SBM, DDGS, WM	Corn	Corn, FSBM	Corn, SBM	Corn, SBM	Corn, SBM, FFRB	Corn, SBM, DFRB
Ground corn	974.5	771.9	465.0	975.5	722.5	697.5	636.0	371.1	371.1
SBM	-	185.0	200.0	-	-	280.0	322.7	190.5	190.5
FSBM	-	-	-	-	255.0	-	-	-	-
DDGS	-	-	200.0	-	-	-	-	-	-
WM	-	-	100.0	-	-	-	-	-	-
FFRB	-	-	-	-	-	-	-	400.0	-
DFRB	-	-	-	-	-	-	-	-	400.0
Soybean oil	-	17.8	10.0	-	-	-	-	-	-
Ground limestone	6.0	8.6	12.3	11.0	10.0	10.0	7.8	16.4	16.4
Dicalcium phosphate	14.0	-	4.5	-	-	-	11.5	-	-
Monocalcium phosphate	-	1.5	-	8.0	7.0	7.0	-	-	-
Sodium bicarbonate	-	1.0	-	-	-	-	-	-	-
L- Lys·HCl, 780 g/kg Lys	-	2.8	2.7	-	-	-	-	-	-
DL-Met, 980 g/kg Met	-	0.3	-	-	-	-	-	-	-
L-Thr, 980 g/kg Thr	-	0.6	-	-	-	-	-	-	-
Salt	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0
Enzyme premix	-	5.0	-	-	-	-	10.0	10.0	10.0
Indigestible marker	-	-	-	-	-	-	5.0	5.0	5.0
Vitamin mineral premix[1]	1.5	1.5	1.5	1.5	1.5	1.5	-	-	-
Vitamin mineral premix[2]	-	-	-	-	-	-	3.0	3.0	3.0

[1]Provided the following quantities of vitamins and micro-minerals per kilogram of complete diet: Vitamin A as retinyl acetate, 11,150 IU; vitamin D_3 as cholecalciferol, 2210 IU; vitamin E as DL-alpha tocopherol acetate, 66 IU; vitamin K as menadione nicotinamide bisulfate, 1.42 mg; thiamin as thiamine mononitrate, 1.10 mg; riboflavin, 6.59 mg; pyridoxine as pyridoxine hydrochloride, 1.00 mg; vitamin B_{12}, 0.03 mg; D-pantothenic acid as D-calcium pantothenate, 23.6 mg; niacin, 44.1 mg; folic acid, 1.59 mg; biotin, 0.44 mg; Cu, 20 mg as copper hydroxychloride; Fe, 125 mg as iron sulfate; I, 1.26 mg as ethylenediamine hydroiodide; Mn, 60.2 mg as manganese hydroxychloride; Se, 0.30 mg (0.15 mg as sodium selenite and 0.15 mg as selenium yeast); and Zn, 125.1 mg as zinc hydroxychloride.

[2]The vitamin-micromineral premix provided the following quantities of vitamins and micro minerals per kilogram of complete diet: vitamin A as retinyl acetate, 11,136 IU; vitamin D_3 as cholecalciferol, 2208 IU; vitamin E as DL-alpha tocopherol acetate, 66 IU; vitamin K as menadione dimethylprimidinol bisulfite, 1.42 mg; thiamin as thiamine mononitrate, 0.24 mg; riboflavin, 6.59 mg; pyridoxine as pyridoxine hydrochloride, 0.24 mg; vitamin B_{12}, 0.03 mg; D-pantothenic acid as D-calcium pantothenate, 23.5 mg; niacin, 44.1 mg; folic acid, 1.59 mg; biotin, 0.44 mg; Cu, 20 mg as copper sulfate and copper chloride; Fe, 126 mg as ferrous sulfate; I, 1.26 mg as ethylenediamine hydroiodide; Mn, 60.2 mg as manganese sulfate; Se, 0.3 mg as sodium selenite and selenium yeast; and Zn, 125.1 mg as zinc sulfate.

Table 2
Ingredient composition of experimental diets containing corn and soybean meal (SBM), corn, SBM, distillers dried grains with solubles (DDGS), and wheat middlings (WM), corn, SBM, and full-fat rice bran (FFRB), or corn, SBM, and defatted rice bran (DFRB).

	Drying method				
	Experiment 1		Experiment 2		
Ingredient, g/kg	Corn SBM	Corn, SBM, DDGS, WM	Corn, SBM	Corn, SBM, FFRB	Corn, SBM, DFRB
Ground corn	670.0	460.0	636.0	371.1	371.1
SBM	281.5	200.0	322.7	190.5	190.5
DDGS	-	200.0	-	-	-
WM	-	100.0	-	-	-
FFRB	-	-	-	400.0	-
DFRB	-	-	-	-	400.0
Soybean oil	20.0	10.0	-	-	-
Ground limestone	9.0	12.3	7.8	16.4	16.4
Dicalcium phosphate	10.0	4.5	11.5	-	-
L- Lys·HCl, 780 g/kg Lys	-	2.7	-	-	-
Salt	4.0	4.0	4.0	4.0	4.0
Enzyme premix	-	5.0	10.0	10.0	10.0
Indigestible marker	4.0	-	5.0	5.0	5.0
Vitamin mineral premix[1]	1.5	1.5	-	-	-
Vitamin mineral premix[2]	-	-	3.0	3.0	3.0

[1]Provided the following quantities of vitamins and micro-minerals per kilogram of complete diet: Vitamin A as retinyl acetate, 11,150 IU; vitamin D_3 as cholecalciferol, 2210 IU; vitamin E as DL-alpha tocopherol acetate, 66 IU; vitamin K as menadione nicotinamide bisulfate, 1.42 mg; thiamin as thiamine mononitrate, 1.10 mg; riboflavin, 6.59 mg; pyridoxine as pyridoxine hydrochloride, 1.00 mg; vitamin B_{12}, 0.03 mg; D-pantothenic acid as D-calcium pantothenate, 23.6 mg; niacin, 44.1 mg; folic acid, 1.59 mg; biotin, 0.44 mg; Cu, 20 mg as copper hydroxychloride; Fe, 125 mg as iron sulfate; I, 1.26 mg as ethylenediamine hydroiodide; Mn, 60.2 mg as manganese hydroxychloride; Se, 0.30 mg (0.15 mg as sodium selenite and 0.15 mg as selenium yeast); and Zn, 125.1 mg as zinc hydroxychloride.
[2]The vitamin-micromineral premix provided the following quantities of vitamins and micro minerals per kilogram of complete diet: vitamin A as retinyl acetate, 11,136 IU; vitamin D_3 as cholecalciferol, 2208 IU; vitamin E as DL-alpha tocopherol acetate, 66 IU; vitamin K as menadione dimethylprimidinol bisulfite, 1.42 mg; thiamin as thiamine mononitrate, 0.24 mg; riboflavin, 6.59 mg; pyridoxine as pyridoxine hydrochloride, 0.24 mg; vitamin B_{12}, 0.03 mg; D-pantothenic acid as D-calcium pantothenate, 23.5 mg; niacin, 44.1 mg; folic acid, 1.59 mg; biotin, 0.44 mg; Cu, 20 mg as copper sulfate and copper chloride; Fe, 126 mg as ferrous sulfate; I, 1.26 mg as ethylenediamine hydroiodide; Mn, 60.2 mg as manganese sulfate; Se, 0.3 mg as sodium selenite and selenium yeast; and Zn, 125.1 mg as zinc sulfate.

2.1. Sample preparation methods

2.1.1. Conventional method vs. cellulose pellets

Urine samples from pigs fed diets containing corn, corn and fermented soybean meal (FSBM), or corn and soybean meal (SBM) were used. Samples were thawed and filtered, and for each sample, four sub-samples were prepared using the conventional freeze-dried cotton-plastic bag method (Kim et al., 2009). A cotton ball was placed in a small plastic bag after the weights of the cotton ball and the plastic bag had been recorded. Ten mL of urine was then dripped on the cotton ball in the bag (Kim et al., 2009). Samples were placed in a small plastic container and lyophilized in a freeze dryer (Gamma 1–16 LCSplus, IMA Life, Tonawanda, NY, USA) for eight days. Four additional sub-samples were prepared by dripping 1 mL of urine on cellulose pellets that were produced by using 0.50–0.75 g of α-Cellulose (SIGMA-ALDRICH, Milwaukee, WI, USA) and a pellet press (Model 2811; Parr Instruments, Moline, IL, USA). Cellulose pellets were then covered and placed at room temperature for no longer than 48 h. Samples from the freeze-dried cotton-plastic bag and the undried cellulose pellet methods were analyzed for GE using a bomb calorimeter (Model 6400; Parr Instruments, Moline, IL, USA), and the concentration of ME in the diets was calculated by the direct approach using the following equation (Kong and Adeola, 2014):

$$ME\ (kcal/kg) = [GE\ intake\ (kcal) - GE\ feces\ output\ (kcal) - GE\ urine\ output\ (kcal)]/total\ feed\ intake\ (kg)$$

The ME:DE ratio was calculated by dividing the calculated ME by the DE in each diet.

Data were analyzed using the PROC MIXED of SAS (SAS Inst. Inc., 2016) with pig as the experimental unit. The statistical model included the effect of diet and preparation method, and the interaction between diet and preparation method. Treatment means were calculated using the LSMEANS statement, and if significant, LSmeans were separated using the PDIFF option of SAS. Statistical significance and tendency were considered at $P \leq 0.05$ and $0.05 < P < 0.10$, respectively.

2.1.2. Conventional method vs. nitrogen value

Before conducting this experiment, a small preliminary experiment was conducted to determine the effect of urine sample size on the analyzed concentration of nitrogen in urine. Diets containing corn, corn and SBM, or corn, SBM, distillers dried grains with solubles (DDGS), and wheat middlings (WM) were used. Urine samples from pigs fed these diets were thawed and filtered, and for each sample, 5 or 15 mL of urine were analyzed in duplicate for nitrogen using the Kjeldahl procedure (Method 984.13; AOAC Int., 2019) on a Kjeltec™ 8400 apparatus (FOSS, Eden Prairie, MN, USA). Data for the concentration of nitrogen in urine were analyzed as described for the conventional and cellulose experiment, but the statistical model included the effects of diet and sample size, as well as the interaction between diet and sample size. Results of the preliminary experiment demonstrated that GE in urine was not impacted by sample size, and 5 mL of urine was, therefore, used in the main experiment.

In the main experiment, urine samples collected from pigs fed three diets were used. Diets contained corn and SBM, corn, SBM, and full-fat rice bran (FFRB), or corn, SBM, and defatted rice bran (DFRB). Samples were thawed and filtered, and six sub-samples were collected from each urine sample. Four of the six sub-samples were prepared using the freeze-dried cotton-plastic bag method and analyzed for GE using a bomb calorimeter, as described above. The remaining two sub-samples were used to determine nitrogen concentration in 5 mL of urine using the Kjeldahl procedure. The nitrogen in urine expressed as g/kg and the body weight of the pigs fed the diets used in the conventional vs. nitrogen value experiment were used to calculate the concentration of GE in urine using equations developed by Do et al. (2025):

$$\text{GE in urine (kcal/kg)} = -7.51 + (12.83 \times \text{nitrogen in urine [g/kg]}) \tag{1}$$

$$\text{GE in urine (kcal/kg)} = -8.89 + (12.91 \times \text{nitrogen in urine [g/kg]}) + (0.022 \times \text{body weight [kg]}) \tag{2}$$

$$\text{GE in urine (kcal/kg)} = -16.33 + (14.00 \times \text{nitrogen in urine [g/kg]}) + (0.192 \times \text{body weight [kg]}) - (0.030 \times \text{nitrogen in urine [g/kg]} \times \text{body weight [kg]}) \tag{3}$$

Additionally, the percentage of nitrogen in urine was multiplied by the amount of urine (g) excreted and then divided by the dry matter intake (DMI) in kg to calculate the amount of nitrogen (g) excreted per kilogram of DMI. This value was then used in the following equation to calculate the concentration of GE in urine (Sauvant et al., 2004):

$$\text{GE in urine (kcal/kg DMI)} = 192 + (31 \times \text{nitrogen in urine [g/kg DMI]})\ /4.184$$

The concentration of GE in urine expressed as kcal/kg urine was calculated using the following equation:

$$\text{GE in urine (kcal/kg urine)} = \text{GE in urine (kcal/kg DMI)} \times \text{DMI (kg)}\ /\ \text{urine output (kg)} \tag{4}$$

The concentration of ME and ME:DE in diets were calculated as previously described. Data were analyzed as described for the experiment comparing the conventional and the cellulose pellet method.

2.2. Conventional vs. oven-drying methods

2.2.1. Corn and wheat co-products

Urine samples collected from pigs fed diets that contained corn and SBM or corn, SBM, DDGS, and WM were thawed and filtered. Eight sub-samples of each sample of urine were prepared as described for the conventional freeze-dried cotton-plastic bag method. Four of the eight prepared sub-samples were lyophilized for eight days, and the other four sub-samples were oven-dried at 60 °C for five days in a forced-air oven (METALAB, Equipment Corp., Hicksville, NY, USA). All samples were analyzed for GE using a bomb calorimeter, and the concentration of ME and ME:DE in diets were calculated, as explained above. Data were analyzed as described for the cellulose experiment.

2.2.2. Rice co-products

To determine if the effect of the drying method on GE in urine and ME in diets is consistent among diets containing different feed ingredients, urine samples from pigs fed three diets containing rice coproducts were used. These samples were the same as those used to compare the conventional and nitrogen value methods. Therefore, eight additional sub-samples from each diet (i.e., corn and SBM; corn, SBM, and FFRB; and corn, SBM, and DFRB) were prepared as described for the conventional freeze-dried cotton-plastic bag method. Four of the samples were freeze-dried, and the other four were oven-dried, as described above. Analysis of GE in urine, calculations of ME and ME:DE in diets, and data analysis were conducted following the procedures described above.

Table 3
Main effect of sample preparation method (freeze-dried cotton-plastic bag or undried cellulose pellet) and diet composition on concentrations of gross energy (GE) in urine and digestible energy (DE) and metabolizable energy (ME) in diets containing corn, corn and fermented soybean meal (FSBM), or corn and soybean meal (SBM) fed to pigs.

	Diet					Method			
Item, MJ/kg	Corn	Corn, FSBM	Corn, SBM	SEM	P-value	Cotton plastic bag	Cellulose pellet	SEM	P-value
n	16	16	16	-	-	24	24	-	-
GE urine	0.20[b]	0.33[a]	0.21[b]	0.03	0.007	0.19	0.30	0.02	0.004
DE diet	13.81[c]	14.33[a]	14.07[b]	0.05	< 0.001	14.07	14.07	0.04	1.000
ME diet	13.49	13.54	13.36	0.08	0.238	13.63	13.30	0.06	< 0.001
ME:DE	97.65[a]	94.49[b]	94.95[b]	0.47	< 0.001	96.9	94.50	0.38	< 0.001

[a-c] Values without a common superscript letter are different ($P < 0.05$).

3. Results

3.1. Sample preparation methods

3.1.1. Conventional method vs. cellulose pellets

There were no differences among diets for ME in diets, but DE was greater (P < 0.05) in the diet containing FSBM compared with the corn diet and the corn-SBM diet, whereas ME:DE was greater (P < 0.05) in the corn diet compared with the other diets (Table 3). However, GE in urine obtained from undried cellulose pellets was greater (P < 0.01) than the value obtained from the freeze-drying cotton ball method. Therefore, the calculated ME in diets and ME:DE were less (P < 0.001) if the undried cellulose pellet method was used compared with the freeze-dried cotton ball method.

3.1.2. Conventional method vs. nitrogen method

Because no interactions between diet and sample size were observed, only the main effects of diet and sample size were included in the final model. The analyzed concentration of nitrogen in urine from pigs fed the corn diet was greater (P < 0.05) than in urine from pigs fed the two diets containing corn and SBM or corn, SBM, DDGS, and WM (Table 4). However, there was no sample size effect on nitrogen concentration in urine, as nitrogen analyzed from the 5 mL sample did not differ from that analyzed from the 15 mL sample.

For comparison of the conventional procedure and the nitrogen method, there were no interactions between diet and sample preparation method, and the final analysis, therefore, included only the main effects of diet and sample preparation method. The GE in urine was greater (P < 0.05) for the corn-SBM diet than from the two rice bran diets, but DE and ME were greater (P < 0.05) in the diet with full-fat rice bran than in the other two diets, whereas the diet with DFRB had the least (P < 0.05) DE and ME of all diets (Table 5). The ME:DE was, however, not different among diets. The GE in urine obtained by the conventional freeze-dried cotton-plastic bag technique was greater (P < 0.05) than when determined with the nitrogen method regardless of the equation used to calculate urine GE. All four nitrogen methods, therefore, resulted in greater (P < 0.05) calculated ME and ME:DE in diets compared with the conventional freeze-dried cotton-plastic bag method, but no differences among the nitrogen methods were observed.

3.2. Freeze-drying vs. oven-drying methods

The final models only included main effects of diet and drying methods because no interactions between diet and drying methods were observed, regardless of diet type.

3.2.1. Corn and wheat co-products

There were no differences between dietary treatments for GE in the urine, but concentrations of DE and ME in the diet containing corn, SBM, DDGS, and WM were greater (P < 0.05) than in the diet containing corn and SBM but ME:DE was not different between diets (Table 6). There was, however, no effect of the drying method on the concentration of GE in urine or the calculated ME or ME:DE in diets.

3.2.2. Rice co-products

Pigs fed the corn-SBM diet had greater (P < 0.05) GE in urine than pigs fed the FFRB diet, but DE and ME were greater (P < 0.05) in the FFRB diet than in the other diets whereas the DFRB had the least (P < 0.05) DE and ME, but ME:DE were not different among the three diets (Table 7). However, no differences between drying methods were observed for GE in urine or ME and ME:DE in diets.

4. Discussion

Energy is the most important and costly component in swine diets (Patience et al., 2015). Therefore, an accurate determination of energy in feed ingredients is critical for optimizing the economic efficiency of diet formulation. The ME or the net energy systems are commonly used in formulation of diets for pigs because these systems provide a more accurate reflection of energy utilization compared with the digestible energy system (NRC, 2012). To determine ME or net energy values, energy losses in feces and urine must be subtracted from GE intake (Adeola, 2001; NRC, 2012). It is, therefore, important that GE in urine be accurately determined. However, different methods have been used to obtain GE values in urine for calculating ME of diets and feed ingredients.

Table 4
Effect of sample size (5 or 15 mL) on nitrogen concentration in urine samples from pigs fed diets containing corn, corn and soybean meal (SBM), and corn, SBM, distiller's dried grains with solubles (DDGS), and wheat middlings (WM).

	Diet					Size (mL)			
Item, g/kg	Corn	Corn, SBM	Corn, SBM, DDGS, WM	SEM	P-value	5	15	SEM	P-value
n	16	20	12	-	-	24	24	-	-
Nitrogen	4.80[a]	2.20[b]	1.70[b]	0.03	< 0.001	3.00	2.80	0.30	0.573

[a-b] Values without a common superscript letter are different (P < 0.05).

Table 5
Effect of sample preparation method (freeze-dried cotton-plastic bag or nitrogen value) on concentrations of gross energy (GE) in urine, and digestible energy (DE) and metabolizable energy (ME) in diets containing corn and soybean meal (SBM), corn, SBM and full-fat rice bran (FFRB), or corn, SBM, and defatted rice bran (DFRB) fed to pigs.

Item, MJ/kg	Diet			SEM	P-value	Method					SEM	P-value
	Corn, SBM	Corn, SBM, FFRB	Corn, SBM, DFRB			Cotton plastic bag	Equation[1] 1	Equation[1] 2	Equation[1] 3	Equation[2] 4		
n	40	40	35	-	-	23	23	23	23	23	-	-
GE urine	0.18[a]	0.13[b]	0.12[b]	0.01	0.008	0.22[a]	0.11[b]	0.12[b]	0.11[b]	0.15[b]	0.02	< 0.001
DE diet	14.00[b]	14.78[a]	12.90[c]	0.03	< 0.001	13.89	13.89	13.89	13.89	13.89	0.04	1.000
ME diet	13.61[b]	14.40[a]	12.64[c]	0.04	< 0.001	13.30[b]	13.65[a]	13.63[a]	13.65[a]	13.52[a]	0.06	< 0.001
ME:DE	97.19	97.51	97.88	0.25	0.165	95.79[a]	98.21[b]	98.14[b]	98.17[b]	97.32[b]	0.33	< 0.001

[a-c] Values without a common superscript letter are different (P < 0.05).
[1]Values obtained using the Do et al. (2025) equations for estimation of GE in urine.
[2] Values obtained using the Sauvant et al. (2004) equation for estimation of GE in urine.

Table 6
Effect of drying method when using the cotton-plastic bag procedure on concentrations of gross energy (GE) in urine, and digestible energy (DE) and metabolizable energy (ME) in diets containing corn and soybean meal (SBM) or corn, SBM, distillers dried grains with solubles (DDGS), and wheat middlings (WM) fed to pigs, experiment 1.

Item, MJ/kg	Diet		SEM	P-value	Method		SEM	P-value
	Corn, SBM	Corn, SBM, DDGS, WM			Freeze-drying	Oven-drying		
n	14	12	-	-	13	13	-	-
GE urine	0.31	0.25	0.05	0.423	0.31	0.25	0.06	0.924
DE diet	13.85	14.58	0.12	< 0.001	14.21	14.21	0.12	1.000
ME diet	13.32	14.07	0.14	0.001	13.69	13.71	0.14	0.941
ME:DE	96.18	96.55	0.69	0.715	96.31	96.42	0.69	0.913

Table 7
Effect of drying method when using the cotton-plastic bag procedure on concentrations of gross energy (GE) in urine, and digestible energy (DE) and metabolizable energy (ME) in diets containing corn and soybean meal (SBM), corn, SBM, and full-fat rice bran (FFRB), or corn, SBM, and defatted rice bran (DFRB) fed to pigs, experiment 2.

Item, MJ /kg	Diet			SEM	P-value	Method		SEM	P-value
	Corn, SBM	Corn, SBM, FFRB	Corn, SBM, DFRB			Freeze-drying	Oven-drying		
n	16	16	16	-	-	24	24	-	-
GE urine	0.25[a]	0.18[b]	0.21[ab]	0.02	0.034	0.22	0.21	0.02	0.677
DE diet	14.00[b]	14.78[a]	12.90[c]	0.04	< 0.001	13.89	13.89	0.04	1.000
ME diet	13.35[b]	14.27[a]	12.29[c]	0.07	< 0.001	13.28	13.32	0.05	0.662
ME:DE	95.33	96.58	95.28	0.54	0.169	95.61	95.85	0.44	0.705

[a-c] Values without a common superscript letter are different (P < 0.05).

4.1. Conventional vs. cellulose method

Dripping urine on a cellulose pellet to be ignited by the bomb calorimeter has been used for urine GE analysis to calculate the ME of glycerol, corn, corn co-products, protein byproducts, and oil seeds (Fent et al., 2000; Kovács et al., 2011; Anderson et al., 2012; Kerr et al., 2017; Kim et al., 2018). However, the observation that GE of urine was greater if the cellulose pellet method was used than if the cotton-plastic bag method was used, and also that the ME in the diets was reduced when using the cellulose pellet method, demonstrates that ME is underestimated if the cellulose pellet method is used to determine GE in urine. Indeed, an ME value of only 3477 kcal/kg dry matter in corn was calculated when urine GE was estimated from the cellulose method (Kerr et al., 2013), which is much less than ME calculated after freeze-drying of urine (i.e., 3800–3900 kcal/kg dry matter; Rojas and Stein, 2015; Rodriguez et al., 2020). Likewise, the ME in corn in feed tables has been reported as 3322, 3395, and 3340 (Sauvant et al., 2004; NRC, 2012; Rostagno et al., 2011) corresponding to 3845, 3844, and 3818 on a dry matter basis.

It is not clear why the cellulose method results in greater urine GE values, and subsequently lower calculated ME values, than the conventional method, but it is possible that the smaller amount of urine used in this procedure results in greater variability, and therefore, reduced accuracy of the estimates for GE. Whereas it is possible to use 10 mL urine in the conventional method because this urine is being lyophilized before analyzed for GE, only a limited amount of urine can be dripped on the cellulose pellets and still maintain the pellet dry enough to be ignited. It is, therefore, possible that the greater GE obtained in urine for the cellulose method compared with the conventional method simply is due to reduced accuracy of the analysis. Nevertheless, it was concluded that the cellulose pellet method is not as accurate as the cotton-plastic bag method and that the calculated values for ME obtained using the cellulose pellet method may be underestimated. As a consequence, the initial hypothesis that there is no difference between the conventional freeze-drying method and the cellulose method was rejected.

4.2. Conventional vs. nitrogen method

The Kjeldahl method allows for estimation of GE in urine based on its nitrogen concentration using a fixed energy equivalent per unit of nitrogen, assuming all energy in urine originates from nitrogenous compounds (Sauvant et al., 2004; Do et al., 2025). Indeed, equations based on the nitrogen concentration of urine have been used to determine GE in urine for determination of ME in feed ingredients and diets (Le Bellego et al., 2001; Do et al., 2025).

A standard sample volume of 5 mL is typically used when determining nitrogen concentration in urine via the Kjeldahl method (Etheridge et al., 1998), but to our knowledge, no data were published comparing the accuracy of nitrogen values obtained from this standard volume with those from larger sample sizes. However, the observation that there is no difference in nitrogen concentration between the standard 5 mL sample and a 15 mL sample indicates that the 5 mL standard sample is sufficient to obtain accurate results when using Kjeldahl analysis to determine nitrogen in urine. The reason the GE in urine was greater when analyzed using the conventional freeze-drying method compared with the nitrogen value method is likely that compounds other than nitrogen contribute energy to urine because urine may contain carbohydrates and metabolites of fat metabolism in addition to nitrogen (Chwalibog and Thorbek, 2000). Likewise, GE in urine estimated from nitrogen can also be influenced by the body weight of pigs and the protein content of the diet (Do et al., 2025). However, even when the equations by Do et al. (2025) that take pig body weight into account were used, GE in urine still was less than obtained using the conventional freeze-drying method further indicating that there are energy containing compounds other than nitrogen in the urine. Therefore, the nitrogen method for determining GE in urine is less accurate than the cotton-plastic bag method and will underestimate urine GE. As a consequence, diet ME values are overestimated if GE in urine is calculated from nitrogen regardless of the equation used to estimate GE in urine.

Hydrochloric acid should be added to urine during collection to avoid nitrogen losses due to volatilization of urea (Lee et al., 2021; Kim et al., 2023). If nitrogen is volatilized, GE in urine will be underestimated, and the ME in diets will be overestimated, as was demonstrated in the current experiment. Indeed, a value of 4756 kcal/kg of ME (as-is basis) for full-fat soybeans was reported if an equation based on the nitrogen content of urine was used to calculate GE in urine (Kim et al., 2022). However, values of 4159–4676 kcal/kg of ME (as-is basis) in various sources of full-fat soybean meal were obtained when using the cotton-plastic bag method to estimate urine GE (Yoon and Stein, 2013; Ruiz-Arias et al., 2025). Therefore, we reject the hypothesis that there are no differences between GE in urine or calculated ME in diets if urine GE is analyzed following the freeze-drying method compared with the nitrogen method. It is therefore not recommended to use the urine nitrogen method to calculate ME in ingredients or diets fed to pigs.

4.3. Freeze-drying vs. oven-drying

Oven-drying of urine samples has been evaluated as an alternative to the freeze-drying method, and it was demonstrated that GE obtained after oven-drying is not different from GE analyzed after freeze-drying (Jacobs et al., 2011). However, no values have been reported for the calculated ME of feed ingredients or diets when GE in urine was prepared using the oven-drying method compared with values obtained using the freeze-drying method. The freeze-drying method is currently the accepted method for preparing urine for GE determination, but the laboratory equipment required to prepare the sample can be a limitation because access to a freeze dryer is needed (Kim et al., 2009). Therefore, while maintaining accuracy is critical, it remains unclear if alternative preparation methods for urine are equally efficient and accurate for the calculation of ME in diets or feed ingredients. However, if samples can be oven-dried instead of freeze-dried, the requirement to equipment is less, and the observation that there were no differences in GE in urine between the oven-drying method and the freeze-drying method, regardless of the diet and ingredients in the diet, is in agreement with data reported by Jacobs et al. (2011), who demonstrated that no volatilization of nitrogen occurs during the oven-drying process. Likewise, the lack of differences in calculated ME of diets between the oven-drying and freeze-drying methods indicates that urine samples that are dripped onto cotton balls that are placed in a small plastic bag can be oven-dried without losing accuracy in calculated ME values. The oven-drying method requires a shorter drying period of four to five days compared with eight days for the conventional freeze-drying method, and this method will, therefore, be faster than the freeze-drying method. The hypothesis that ME values in diets calculated after oven-drying of urine are not different from values calculated after freeze-drying of urine was, therefore, accepted.

5. Conclusion

Results of this work indicated that the nitrogen method for estimating GE in urine results in overestimation of ME in diets and ingredients due to the presence of energy-containing components other than nitrogen in urine. The cellulose pellet method overestimates GE in urine and results in an underestimation of the ME in diets and ingredients. However, oven-drying of urine dripped onto cotton placed in a plastic bag resulted in GE in urine that was not different from GE obtained after freeze-drying, and ME in diets was not affected by drying method. Therefore, there is no need to freeze-dry urine samples for obtaining GE in urine if a drying oven is available.

Author statement

HHS and LVL conceptualized the experiment. LVL conducted the laboratory part of the experiment, summarized the data, and analyzed the data. LVL also wrote the first draft of the manuscript. JAI contributed with data interpretation, and she wrote subsequent drafts of the manuscript. HHS and LVL edited the final version of the manuscript. HHS supervised the project.

CRediT authorship contribution statement

J. A. Ibagon: Writing – review & editing, Writing – original draft, Validation, Formal analysis, Data curation. **L. V. Lagos**: Writing – review & editing, Writing – original draft, Methodology, Investigation, Formal analysis, Conceptualization. **H. H. Stein**: Writing – review & editing, Project administration, Funding acquisition, Conceptualization.

Declaration of Competing Interest

The authors have no conflicts of interest.

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