Table 1 Ingredient and chemical composition of experimental diets fed to pigs (as-fed basis).

		Supplemental L-Met, %		Supplemental D-Met, %	
Item[†]	Basal diet	0.04	0.08	0.04	0.08
Ingredient composition, %					
Ground corn	55.00	55.00	55.00	55.00	55.00
Dried whey	10.00	10.00	10.00	10.00	10.00
Spray dried animal plasma	10.00	10.00	10.00	10.00	10.00
Corn starch	19.92	19.88	19.84	19.88	19.84
Soybean oil	2.00	2.00	2.00	2.00	2.00
L-Met	–	0.04	0.08	–	–
D-Met	–	–	–	0.04	0.08
L-Lys·HCl	0.32	0.32	0.32	0.32	0.32
L-Thr	0.04	0.04	0.04	0.04	0.04
L-Trp	0.03	0.03	0.03	0.03	0.03
L-Ile	0.14	0.14	0.14	0.14	0.14
Dicalcium phosphate	0.67	0.67	0.67	0.67	0.67
Ground limestone	1.18	1.18	1.18	1.18	1.18
Salt	0.20	0.20	0.20	0.20	0.20
Vitamin-mineral premix[‡]	0.50	0.50	0.50	0.50	0.50
Calculated composition					
Metabolizable energy, kcal/kg	3,552	3,551	3,549	3,551	3,549
CP, %	14.08	14.10	14.13	14.10	14.13
Ether extract, %	4.48	4.48	4.48	4.48	4.48
Met, %	0.18	0.22	0.26	0.22	0.26
Cys, %	0.41	0.41	0.41	0.41	0.41
Choline, %	0.32	0.32	0.32	0.32	0.32
Ca, %	0.72	0.72	0.72	0.72	0.72
Available P, %	0.34	0.34	0.34	0.34	0.34

Notes:

[†] Cys, cysteine; Met, methionine; Lys, lysine; Thr, threonine; Trp, tryptophan; Ile, isoleucine.

[‡] Provided the following quantities per kg of complete diet: vitamin A, 25,000 IU; vitamin D_3, 4,000 IU; vitamin E, 50 IU; vitamin K, 5.0 mg; thiamin, 4.9 mg; riboflavin, 10.0 mg; pyridoxine, 4.9 mg; vitamin B_{12}, 0.06 mg; pantothenic acid, 37.5 mg; folic acid, 1.10 mg; niacin, 62 mg; biotin, 0.06 mg; Cu, 25 mg as copper sulfate; Fe, 268 mg as iron sulfate; I, 5.0 mg as potassium iodate; Mn, 125 mg as manganese sulfate; Se, 0.38 mg as sodium selenite; Zn, 313 mg as zinc oxide; butylated hydroxytoluene, 50 mg.

Sample collection

For the N-balance study, the pigs were adapted to the experimental diets for 5 d and then total but separated collection of feces and urine was conducted for 4 d according to the marker-to-marker procedure (*Kong & Adeola, 2014*). The collected feces and urine were immediately stored in a freezer at −20 °C prior to further analyses.

Chemical analysis

At the completion of the study, the frozen fecal samples were dried in a forced-air oven at 55 °C and finely ground prior to chemical analyses. The experimental diets, fecal and urine samples were determined for crude protein (CP) content (N × 6.25) by the Kjeldahl method (Kjeltec 1035; Foss, Hillerod, Denmark).

Calculations and statistical analysis

Apparent total tract N digestibility and retention were calculated using the following equations:

$$\text{Apparent total tract N digestibility } (\%) = (N_I - N_F)/N_I \times 100,$$

$$\text{N retention } (\%\text{ of intake}) = (N_I - N_F - N_U)/N_I \times 100$$

where: N_I is the amount of N ingested (g); N_F and N_U are the amount of N voided via the feces (g) and urine (g), respectively.

Experimental data were analyzed using the MIXED procedures of SAS (SAS Institute Inc., Cary, NC, USA). The independent variables in the model included diet as a fixed effect and period and block nested within period as random effects. The orthogonal polynomial contrast was used to examine the relationship between N balance response criteria and graded concentrations of Met isomers. The relative bioavailability of D-Met to L-Met was estimated using a multiple regression model and the slope-ratio analysis described by *Littell et al. (1997)*. The statistical model used in the analysis as follows:

$$y = a + b_s x_s + b_t x_t + e,$$

in which y is response criterion; a is intercept; e is random error; b_s and b_t are the slopes for L- and D-Met, respectively; x_s and x_t are the concentrations of L- and D-Met intake, respectively. An individual pig served as the experimental unit and statistical significance was determined at $P < 0.05$.

RESULTS

The effects of supplemental L- or D-Met on N balance are shown in Table 2. Nitrogen intakes were similar across the treatments due to the restricted feeding based on the initial BW of pigs. Fecal N output was not affected by Met supplementation regardless of source and consequently apparent N digestibility did not change. In contrast, there was a linear response ($P < 0.05$) to Met supplementation from L- or D-Met in urinary N output, which resulted in increased ($P < 0.01$) retained N and N retention. No quadratic response was observed in any of the N-balance criteria. The estimated bioavailability of D-Met relative to L-Met from urinary N output (g/4 d) and N retention (% of intake) as dependent variables using supplemental Met intake (g/4 d) as an independent variable were 87.6 and 89.6%, respectively (Figs. 1 and 2), but approximate 95% fiducial limits for the relative bioavailability estimates for both dependent variables included 100%.

DISCUSSION

Nutrient bioavailability assay provides relative information on the capacity of feed ingredients to supply a nutrient capable of being digested, absorbed and available for use or storage (*Gabert, Jorgensen & Nyachoti, 2001*; *Adeola, 2009*). Growth performance has generally been used as response criteria for AA bioavailability assay (*Chung & Baker, 1992*; *Adeola, 2009*; *Shen, Weaver & Kim, 2014*). However, in the present study, growth responses to the supplemental Met were not significant among dietary treatments while

Table 2 Effects of dietary L-methionine (L-Met) and D-Met on nitrogen (N) balance of weaning pigs[†].

		Supplemental L-Met, %		Supplemental D-Met, %			*P*-values for contrast			
							Linear		Quadratic	
Item	Basal diet	0.04	0.08	0.04	0.08	SEM	L-Met	D-Met	L-Met	D-Met
BW, kg										
Initial	13.9	14.0	14.4	14.0	14.3	0.3	0.116	0.243	0.443	0.733
Final	15.1	15.1	15.6	15.4	15.3	0.4	0.142	0.537	0.277	0.548
Collection period (4 d)										
Feed intake, g	1,973	1,973	1,973	1,955	1,973	40	0.974	0.974	0.985	0.081
N intake, g	44.5	44.5	44.6	44.1	44.6	0.9	0.607	0.607	0.983	0.080
Fecal N output, g	8.33	7.85	8.07	8.24	7.70	0.43	0.630	0.243	0.450	0.612
N digestibility, %	81.3	82.4	82.0	81.4	82.7	0.8	0.537	0.234	0.480	0.555
Urinary N output, g	14.6	14.3	11.1	13.5	12.1	0.6	< 0.001	0.014	0.086	0.862
Retained N, g	21.4	22.4	25.4	22.4	24.8	0.7	< 0.001	< 0.001	0.170	0.337
N retention, % of intake	48.5	50.4	57.0	50.8	55.4	1.2	< 0.001	< 0.001	0.132	0.465

Note:
[†] Each least squares mean represents 10 observations except the basal diet (nine observations).

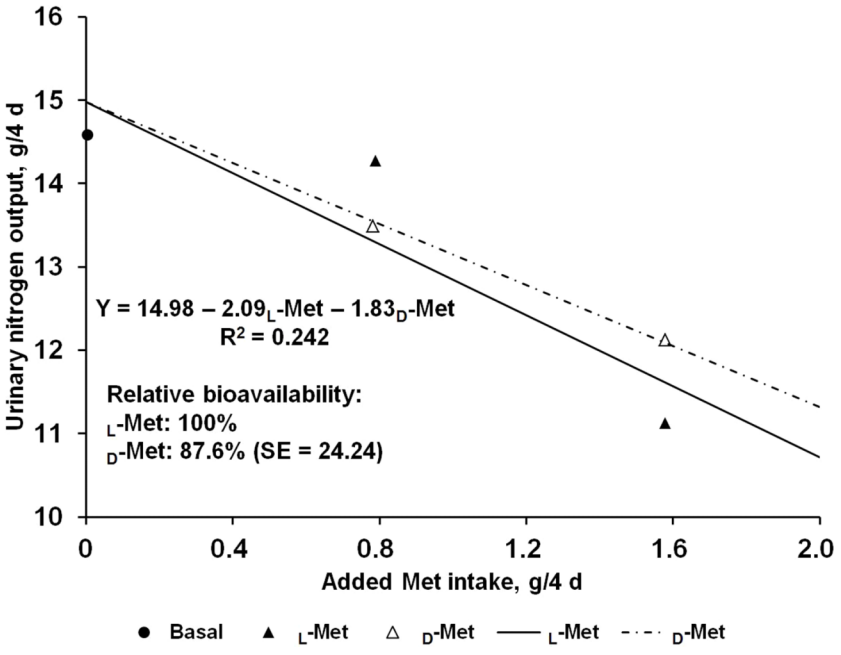

Figure 1 **Slope-ratio comparison based on the urinary nitrogen output (g/4 d) of nursery pigs fed diets with graded levels of D-methionine (D-Met) or L-Met.** Each data point represents least squares mean of 10 observations except the basal diet (nine observations).

N balance responses were affected by the supplemental Met. This may be attributed to the relatively greater sensitivity of N balance responses to AA adequacy compared to growth responses in a short-term experiment (*Figueroa et al., 2001*).

The results from the present study suggest that the relative bioavailability of D- to L-Met, using urinary N output (g/4 d) and N retention (% intake) as dependent

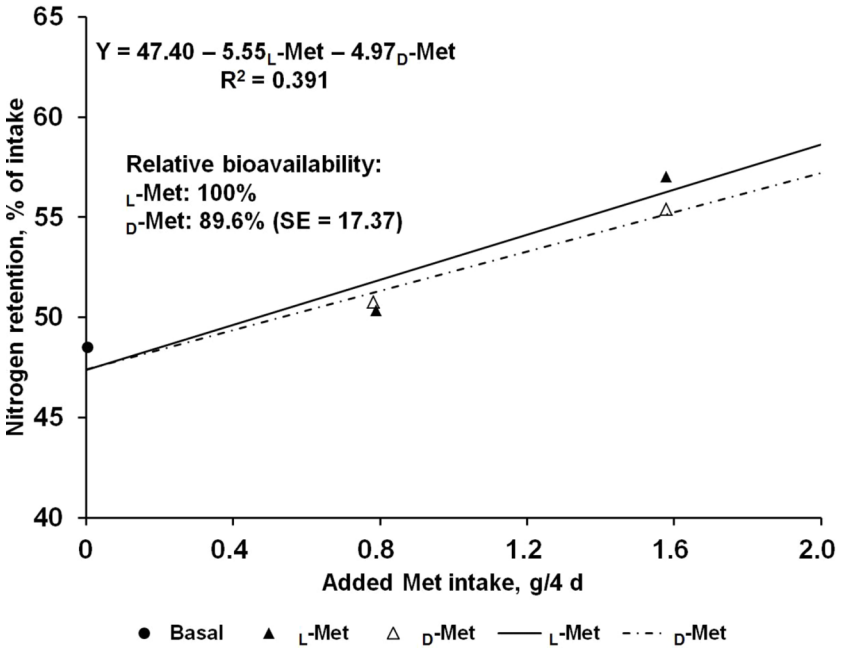

Figure 2 Slope-ratio comparison based on the nitrogen retention (%) of nursery pigs fed diets with graded levels of D-methionine (D-Met) or L-Met. Each data point represents least squares mean of 10 observations except the basal diet (nine observations).

variables, are 87.6% and 89.6%, respectively, and the 95% fiducial limits included 100%. To obtain accurate values from bioavailability assays, the assumptions for slope-ratio assay should be validated (*Littell et al., 1997*). This tested for linearity of the slopes and lack of curvature, and for intersection of responses to reference and test diets at the response to BD. For urinary N output (g/4 d) and N retention (% intake) in the present study, validity tests were performed and all assumptions were valid.

Due to the lack of D-transaminase, pigs are not able to directly utilize D-Met for protein synthesis and S-adenosylmethionine formation. To become bioavailable, D-Met has to be converted to α-keto-γ-methiolbutyrate in a process catalyzed by D-Met oxidase, with subsequent transamination to L-Met (*Lewis, 2003*). However, the efficiency of these additional enzymatic processes has not been so clear and little information is available on the relative bioefficacy of D- to L-Met. Using phenylalanine as an indicator AA, the relative bioefficacy of D- to L-Met was only 50% when 10 to 14-day-old pigs were used in an indicator AA oxidation study (*Kim & Bayley, 1983*). Recently, *Shen, Weaver & Kim (2014)* conducted a relative bioavailability study for a period of 20 d using growth performance as response criteria and reported that the bioavailability of DL- to L-Met was calculated as 69.4 and 81.3% for the average daily gain and gain:feed of nursery pigs, respectively. However, several other studies showed no differences in bioefficacy between D- and L-Met. In the present study, the bioavailability of D-Met was comparable with L-Met in nursery pigs when urinary N output (g/4 d) was used as the response criterion. This was in agreement with *Cho et al. (1980)* who determined the urinary Met excretion in six-week-old miniature pigs infused with solutions containing L- or DL-Met and reported no difference in utilization between Met isomers. Furthermore, no differences in BW gain or

plasma urea levels were observed when three-week-old pigs received low-protein-liquid diets containing either DL- or L-Met (0.51%) for seven days (*Reifsnyder, Young & Jones, 1984*) and *Chung & Baker (1992)* reported that D-Met was biologically equivalent to L-Met when the growth performance of pigs averaging 9.6 kg was used as response. It is difficult to explain the reason for discrepancy in the bioefficacy of Met isomers among studies but it may be due in part to the growth stage of animals (*Chung & Baker, 1992*). The activity of D-Met oxidase, the key enzyme that converts D- to L-Met, was determined to be greater in older animals than in younger animals (*D'Aniello et al., 1993*). In addition, the methods for measuring bioavailability also affect inconsistent results. As N retention is one of the most sensitive indices for AA utilization (*Kim et al., 2006*), the N balance technique was employed to determine the relative bioefficacy of D- to L-Met in the present work. In the study conducted by *Shen, Weaver & Kim (2014)*, the bioavailability of DL-Met was less than that of L-Met based on average daily gain and gain:feed, whereas the relative bioavailability of 100.9% was observed for plasma urea N, indicating that the contrary results may be attributed to the use of different response criteria for the estimates of relative bioavailability.

In conclusion, the relative bioavailability values for D- to L-Met in nursery pigs averaging 13.5 kg with the slope-ratio comparison of urinary N output (g/4 d) and N retention (% of intake) were 87.6% and 89.6%, respectively; but 95% fiducial limits for the relative bioavailability estimates included 100%.

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Competing Interests

The authors declare that they have no competing interests.

Author Contributions

- Changsu Kong conceived and designed the experiments, performed the experiments, analyzed the data, wrote the paper, prepared figures and/or tables.
- Jong Young Ahn performed the experiments, analyzed the data, wrote the paper.
- Beob G. Kim conceived and designed the experiments, contributed reagents/materials/analysis tools, reviewed drafts of the paper.

Animal Ethics

The following information was supplied relating to ethical approvals (i.e., approving body and any reference numbers):

The Institutional Animal Care and Use Committee of Konkuk University reviewed and approved all protocols (KU13188) used in the present study.

Data Deposition

The following information was supplied regarding data availability:

The raw data has been supplied as Supplemental Dataset Files.

Supplemental Information

Supplemental information for this article can be found online at http://dx.doi.org/10.7717/peerj.2368#supplemental-information.

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