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Non ruminant nutrition

Effect of the essential amino acid-nitrogen to total nitrogen ratio on lysine requirement for nitrogen retention in growing pigs

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Abstract

Low protein diets supplemented with essential amino acids (**EAA**) fed to pigs reduce the excess supply of EAA and nitrogen (**N**). However, low protein diets may become limiting in non-essential amino acids (**NEAA**) and N, thus affecting the utilization of EAA for N retention. It has been suggested that the EAA-N:total N (**E:T**) ratio can give an indication of dietary N sufficiency. An N-balance study was conducted to determine the effect of E:T ratio on the Lys requirement for maximum N retention. A total of 80 growing barrows (19.3 ± 0.21 kg initial body weight) were randomly assigned to 1 of 10 diets ($n = 8$) in 8 blocks in a 2 × 5 factorial arrangement. Diets consisted of a low ratio (**LR**; E:T of 0.33) or a high ratio (**HR**; E:T of 0.36) with graded Lys content (0.82%, 0.92%, 1.02%, 1.12%, and 1.22% standardized ileal digestible [**SID**]). After a 7-d adaptation, a 4-d N-balance collection was conducted. Blood samples were obtained on d 2 of the collection period 2 h after the morning meal for plasma urea N (**PUN**) analysis. Data were analyzed using the MIXED model procedure with fixed effects of ratio ($n = 2$), Lys ($n = 5$), and their interactions. The experimental block (room) was included as a random effect ($n = 8$). The SID Lys requirement was estimated using PROC NLIN linear broken-line breakpoint model. There was a significant interaction between E:T ratio and Lys ($P < 0.01$), where LR diets had a higher N retention than HR diets, while increasing Lys linearly increased N retention ($P = 0.01$) in both HR and LR diets. The marginal efficiency of utilizing SID Lys ($P < 0.01$) reduced with increasing Lys content, while the efficiency of utilizing N ($P < 0.05$) increased as Lys increased. The SID Lys required to maximize N retention of pigs fed HR diets was estimated at 1.08% ($R^2 = 0.61$) and LR diets at 1.21% ($R^2 = 0.80$). The current results indicate that N may be limiting in diets with a high E:T ratio, limiting N retention. Supplying additional dietary N, as intact protein, can increase N retention, resulting in a greater Lys requirement.

Lay Summary

Low protein diets supplemented with essential amino acids (**EAA**) can improve growth performance, but dietary non-essential amino acids (**NEAA**) and nitrogen (**N**) content may be limiting factors. This limitation may ultimately affect the efficient utilization of EAA for optimal N retention and growth performance. As a benchmark, appropriate quantities of EAA and total N (**TN**) must be provided, using the EAA-N to TN ratio (**E:T**) to indicate that both are supplied in sufficient amounts. The present study generally observed a linear increase in N retention with increasing dietary Lys, and N retention was greater in the low E:T as compared with high E:T diets. A greater Lys requirement was observed in the low E:T compared with the high E:T-fed pigs. A low E:T ratio with Lys above current recommendations is warranted to maximize N retention.

Key words: essential amino acids, low protein, growth performance, lysine, nitrogen, nitrogen retention

Abbreviations: AA, amino acid; CP, crude protein; DM, dry matter; EAA, essential amino acid; ET, essential amino acid-nitrogen:total nitrogen ratio; HP, high protein; HR, high ratio; LR, low ratio; Lys, lysine; ME, metabolizable energy; N, nitrogen; NE, net energy; NEAA, non-essential amino acid; PD, protein deposition; PROC NLIN, non-linear procedure; PUN, plasma urea nitrogen; SAS, Statistical Analysis Software; SID, standardized ileal digestible; TN, total nitrogen

Introduction

Reducing nitrogen (**N**) excretion is crucial for promoting sustainability animal agriculture and swine production. The use of low-protein diets supplemented with essential amino acids (**EAA**) has been used as a concept to improve feed efficiency while reducing N excretion and maintaining growth performance (Kerr et al., 1995; Peng et al., 2016; Wang et al., 2018; Spring et al., 2020). However, the total amount of N in the diet is important to maintain optimal performance. As only EAA are accounted for in low protein diets, non-essential amino acids (**NEAA**) and total N may be limiting. Furthermore, low-protein diets may limit the endogenous synthesis of NEAA (Mansilla et al., 2017a, 2018). Further, the utilization efficiency of EAA for N retention may be reduced as EAA may be catabolized to supply the N required for the endogenous synthesis of NEAA rather than be used for protein synthesis (Wang et al., 2018).

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The ideal protein concept focuses on meeting the EAA requirements of the pig, with little consideration given to NEAA requirements, even though NEAA supplies almost half of the total dietary N in practical diet formulations (Heger et al., 1998; Nitrayová et al., 2010). Consequently, the ratio between EAA-N and total N (**E:T**) has been suggested to describe the relationship between EAA and NEAA on an N basis and indicate a sufficient supply of both EAA and NEAA (Heger, 2003; Heger et al., 2008; Nitrayová et al., 2010). The ratio considers the amount of N coming from both EAA and NEAA sources, which may have implications on the efficiency of using EAA for N retention. It has been reported that a minimum amount of NEAA should be included in diets to improve N retention, with an E:T ratio of approximately 0.48 previously indicated as required to maximize N retention (Heger et al., 1998). While efforts have been made to determine the optimal E:T ratio, the impact of the E:T ratio, specifically through adjusting NEAA content, on EAA requirements has never been examined. Therefore, the objective of the present study was to determine the effect of the E:T ratio on Lys requirement in growing pigs for N retention. We hypothesized that the Lys requirement would be greater in pigs fed a diet with a high vs low E:T ratio when the ratio is adjusted by including additional NEAA from intact protein.

Materials and Methods

The experimental protocol was reviewed and approved by the University of Saskatchewan's Animal Research Ethics Board (AUP#20130054) and followed the Canadian Council on Animal Care guidelines (Canadian Council on Animal Care (CCAC), 2009).

Animals, Housing, and Diets

A total of 80 growing barrows of 19.3 ± 0.21 kg initial body weight (**BW**) were used in an N-balance experiment at the Prairie Swine Centre, Inc. (Saskatoon, SK). The pigs were housed individually in metabolism crates (1.4 m × 1.5 m) in a temperature-controlled room (22 ± 1 °C). Pigs were randomly assigned to 1 of 10 dietary treatments in a 2 × 5 factorial arrangement, with factors of E:T ratio and Lys content, over 8 blocks (groups of pigs) in a randomized complete block design ($n = 8$ pigs/treatment). Diets were formulated to have a high E:T ratio of 0.36 (**HR**) and a low E:T ratio of 0.33 (**LR**), representing an N-deficient diet and N-supplemented diet, respectively. Dietary Lys content was formulated at 0.82%, 0.92%, 1.02%, 1.12%, and 1.22% standard ileal digestible (**SID**) Lys. Previous ratio calculations included total EAA and only accounted for AA-N (Heger et al., 1998; Heger, 2003). However, advances in our knowledge of N utilization in the pig (Mansilla et al., 2017b) indicate that non-protein N can be utilized for NEAA synthesis. Therefore, in the current study, the E:T ratio was calculated as the SID EAA content (Arg, Val, His, Ile, Lys, Leu, Met + Cys, Phe + Tyr, Thr, Trp), supplied to the requirement (NRC, 2012), and the TN content of the diet, including both AA and non-protein N. This calculation, inclusion of EAA, and use of practical ingredients in diet formulation resulted in a lower ratio than previously indicated as optimal (Heger et al., 1998). The dietary Lys content was formulated to be 80, 90, 100, 110, and 120% of requirements, and all other EAA were formulated to 110% of requirements according to NRC (2012). Diets were formulated to meet or exceed requirements for all other nutrients and contained titanium dioxide as an indigestible marker (Table 1). The diets containing the lowest and highest levels of Lys were mixed and then blended in appropriate proportions to obtain the 0.92%, 1.02%, and 1.12% SID Lys diets. Feed was provided at 2.8 × maintenance metabolizable energy requirements [110 × $BW^{0.75}$(NRC, 2012)] for each individual pig and fed in equal meals twice daily at 0700 and 1500 hours with ad libitum access to water. Feed refusals were collected for each pig daily and weighed to determine daily feed intake.

Nitrogen Balance and Blood Sampling

The experimental period consisted of a 7-d dietary adaptation period followed by a 4-d collection period. During the 4-d collection period, urine was collected quantitatively daily over 24-h periods for each pig using metal trays and jugs placed underneath the metabolism crates. Nitrogen losses were minimized by adding an appropriate amount of 6N HCl to collection jugs to maintain pH <3 (de Lange et al., 2001). Following each daily collection, total urine was weighed, and a 10% subsample was obtained for each pig. Urine subsamples (10% of the daily collection) were pooled per pig over the collection period and stored at −20 °C until further analysis. Fresh fecal grab samples were collected daily and stored at −20 °C. At the end of each collection period, fecal samples were thawed, pooled for each pig, and homogenized. Subsamples were then stored at −20 °C until further analysis. Blood samples were collected from all pigs 2 h after the morning meal on day 2 of the collection period. Samples were obtained via jugular puncture into vacutainer tubes (BD, Mississauga, ON, Canada) containing heparin. Samples were centrifuged at 2,500 × *g* for 15 min, after which plasma was collected and stored at -20 °C to analyze for plasma urea nitrogen (**PUN**).

Analytical Procedures

Fecal samples were freeze-dried (Labconco Freeze Dry System, 18L; Kansas City, MO, USA) before grinding in a centrifugal mill (Grinder Retsch ZM 200 GmbH & Co. Rheinische Straße, Germany) through a 1-mm sieve. Diet and fecal dry matter (**DM**) content were analyzed in duplicate by oven drying at 135 °C for 2 h (forced air ovens, Thermo Fisher Scientific Isotemp 750F, Waltham, MA, USA) according to method 930.15 (AOAC, 2007). An automatic analyzer was used to determine N content in the diet, fecal, and urine samples (LECO FP 528; MI, USA; Method, 990.03; AOAC, 2007). Titanium dioxide was determined in diet and fecal samples as previously described (Myers et al., 2004). Diet samples were analyzed for AA composition (Table 2) at Central Testing Laboratories (Winnipeg, MB, Canada). Plasma urea nitrogen was analyzed using a commercially available kit (Invitrogen Urea Nitrogen Colorimetric Detection Kit #EIABUN (BUN), Thermo Fisher Scientific, Waltham, MA, USA).

Calculations

The apparent total tract digestibility (**ATTD**) of N was determined using the indicator method according to the following equation:

$$\text{N digestibility (\% ATTD)} = 100 - \left[\frac{\text{TiO}_{2D} \times \text{NF}}{\text{TiO}_{2F} \times \text{ND}}\right] \times 100\%$$

where TiO_{2D} and TiO_{2F} are the titanium dioxide concentrations in the diet and feces, respectively, and N_D and N_F are the N concentrations in the diet and feces, respectively.

Table 1. Ingredient composition of experimental diets (as-fed basis)[1]

	High E:T ratio[2]		Low E:T ratio[2]	
SID Lys, %	**0.82**	**1.22**	**0.82**	**1.22**
Soybean meal	5.0	5.0	10.0	10.0
Corn	79.9	79.4	75.7	75.2
Soy protein concentrate	8.0	8.0	8.0	8.0
Soybean oil	2.5	2.5	2.5	2.5
L-Lysine	0.297	0.807	0.135	0.645
L-Arginine	0.150	0.150	—	—
DL-Methionine	0.153	0.153	0.111	0.111
L-Threonine	0.242	0.242	0.172	0.172
L-Tryptophan	0.072	0.072	0.040	0.040
L-Isoleucine	0.091	0.091	—	—
L-Valine	0.082	0.082	0.002	0.002
L-Histidine	0.053	0.053	—	—
Monocalcium phosphate	1.38	1.38	1.32	1.32
Limestone	1.32	1.32	1.32	1.32
Salt	0.40	0.40	0.40	0.40
Titanium dioxide	0.10	0.10	0.10	0.10
Vitamin/mineral premix[3]	0.20	0.20	0.20	0.20
Calculated nutrient content[4]				
E:T	0.36	0.36	0.33	0.33
Dry matter, %	87.7	89.1	88.3	88.2
Crude protein, %	15.3	15.7	16.6	17.0
ME, kcal/kg	3461	3466	3449	3454
NE, kcal/kg	2663	2667	2630	2634
Ca, %	0.77	0.77	0.77	0.77
P, %	0.38	0.38	0.38	0.38
Amino acids, % SID				
Lys	0.82	1.22	0.82	1.22
Arg	0.95	0.95	0.95	0.95
His	0.40	0.40	0.40	0.40
Ile	0.59	0.59	0.59	0.59
Leu	1.20	1.20	1.32	1.32
Met + Cys	0.58	0.58	0.58	0.58
Phe + Tyr	1.02	1.02	1.17	1.17
Thr	0.67	0.67	0.67	0.67
Trp	0.19	0.19	0.19	0.19
Val	0.67	0.67	0.67	0.67

E:T, essential amino acid-nitrogen:total nitrogen ratio; ME, metabolizable energy; NE, net energy; SID, standardized ileal digestible.

[1] The lowest and highest Lys diets were blended in appropriate proportions to achieve diets containing the other graded levels of Lys (not shown).

[2] E:T ratios of 0.36 (high ratio diets) and 0.33 (low ratio diets) reflect the amount of nitrogen in the diets coming from essential amino acids (E) and from all other components (T), with the higher ratio having a larger contribution of nitrogen from essential amino acids.

[3] Supplied per kilogram of complete feed: vitamin A, 4,000 IU; vitamin D, 0.019 mg; vitamin E, 15 IU; vitamin B12, 0.01 mg; menadione, 1.0 mg; thiamine, 0.50 mg; riboflavin, 2.0 mg; pyridoxine, 1.0 mg; niacin, 10 mg; pantothenate, 6 mg; folic acid, 0.25 mg; biotin, 0.05 mg; Cu, 7.5 mg; Fe, 50 mg; Mg, 20 mg; I, 0.50 mg; Zn, 50 mg, and Se, 0.15 mg.

[3] Nutrient content of diets based on estimated nutrient contents of ingredients according to NRC (2012).

Nitrogen retention was determined with analyzed values using the following equation:

$$\text{N retained}\,(\text{g/d}) = \text{N intake}\ \ (\text{g/d}) - (\text{fecal N output} + \text{urinary N output})$$

Protein deposition (g/d) was calculated as N retained × 6.25.

Marginal efficiency of N intake above maintenance was calculated as

$$K_{\text{nitrogen}} = \frac{(\text{N retained in body protein})}{(\text{SID N intake} - \text{maintenance N requirements})}$$

where SID N intake and maintenance N requirement were according to calculated values (NRC, 2012) and determined feed intake and N retained based on analyzed values.

Marginal efficiency of SID Lys intake above maintenance was calculated as

Table 2. Analyzed nutrient content of experimental diets (as-fed basis)[1]

SID Lys, %	High E:T ratio[2]					Low E:T ratio[2]				
	0.82	**0.92**	**1.02**	**1.12**	**1.22**	**0.82**	**0.92**	**1.02**	**1.12**	**1.22**
Dry matter, %	90.2 (87.0)	90.1 (87.0)	90.6 (87.0)	90.5 (87.0)	90.3 (87.0)	90.3 (87.0)	90.1 (87.0)	90.5 (87.0)	90.6 (87.0)	90.3 (87.1)
Crude protein, %	16.0 (15.3)	15.7 (15.4)	16.4 (15.5)	16.4 (15.6)	16.6 (15.8)	17.1 (16.6)	17.3 (16.7)	17.2 (16.8)	17.1 (16.9)	17.2 (17.))
Total amino acid, %										
Lys	0.78 (0.91)	0.80 (1.02)	0.96 (1.12)	1.17 (1.22)	1.19 (1.31)	0.90 (0.92)	0.96 (1.03)	1.08 (1.13)	1.18 (1.23)	1.22 (1.32)
Met	0.36 (0.40)	0.37 (0.41)	0.31 (0.41)	0.29 (0.41)	0.33 (0.40)	0.31 (0.38)	0.35 (0.39)	0.25 (0.39)	0.34 (0.39)	0.29 (0.38)
Met + Cys	0.61 (0.66)	0.62 (0.67)	0.50 (0.67)	0.51 (0.67)	0.54 (0.66)	0.55 (0.67)	0.63 (0.68)	0.43 (0.68)	0.61 (0.68)	0.54 (0.66)
Thr	0.54 (0.76)	0.49 (0.71)	0.54 (0.70)	0.64 (0.70)	0.59 (0.76)	0.62 (0.77)	0.63 (0.72)	0.61 (0.72)	0.63 (0.72)	0.59 (0.77)
Arg	0.84 (0.85)	0.81 (0.85)	0.80 (0.85)	0.92 (0.85)	0.92 (0.85)	0.90 (1.01)	0.90 (1.00)	0.89 (1.00)	0.91 (1.00)	0.87 (1.00)
Ile	0.62 (0.65)	0.61 (0.65)	0.62 (0.65)	0.71 (0.65)	0.70 (0.65)	0.72 (0.67)	0.70 (0.66)	0.72 (0.66)	0.72 (0.66)	0.69 (0.66)
Leu	1.31 (1.36)	1.26 (1.36)	1.28 (1.36)	1.46 (1.36)	1.45 (1.36)	1.58 (1.50)	1.55 (1.50)	1.58 (1.50)	1.58 (1.50)	1.54 (1.50)
Val	0.72 (0.75)	0.71 (0.77)	0.72 (0.76)	0.83 (0.76)	0.81 (0.75)	0.82 (0.76)	0.80 (0.77)	0.82 (0.77)	0.82 (0.72)	0.80 (0.76)
His	0.35 (0.44)	0.35 (0.44)	0.37 (0.44)	0.43 (0.44)	0.36 (0.44)	0.41 (0.45)	0.42 (0.45)	0.37 (0.45)	0.43 (0.44)	0.41 (0.44)
Phe	0.66 (0.70)	0.65 (0.70)	0.65 (0.70)	0.76 (0.70)	0.76 (0.70)	0.82 (0.81)	0.83 (0.81)	0.82 (0.80)	0.84 (0.80)	0.81 (0.80)

[1] Analyzed total amino acid content with calculated values in parentheses.
[2] E:T ratios of 0.36 (high ratio diets) and 0.33 (low ratio diets) reflect the amount of N in the diets coming from essential amino acids (E) and from all other components (T), with the higher ratio having a larger contribution of N from essential amino acids.
E:T, essential amino acid-nitrogen:total nitrogen ratio.

$$K_{\text{lysine}} = \frac{(\text{protein deposition} \times \text{Lys \% of body protein})}{(\text{SID N intake} - \text{maintenance N requirements})}.$$

where SID Lys intake and maintenance lysine requirements were according to calculated values (NRC, 2012) and determined feed intake and analyzed protein deposition. Body protein was assumed to contain 7.10% lysine (NRC, 2012).

Statistical Analysis

Statistical analyses were conducted as a 2 × 5 factorial in a randomized complete block design using the MIXED model procedure of SAS version 9.4 (SAS Institute, Inc., Cary, NC, USA). The UNIVARIATE procedure of SAS was used to verify residual normality and identify outliers. The N balance parameters were analyzed with dietary Lys ($n = 5$), ratio ($n = 2$), and their interactions as fixed effects and (room) block ($n = 8$) as a random effect. The body weight at the start of the collection period was included in the model as a covariate. Orthogonal polynomial contrasts were used to determine the linear and quadratic response of increasing dietary Lys inclusion on N retention. The linear broken-line breakpoint model (PROC NLIN, SAS 9.4) was used to estimate Lys requirement in both HR-and-LR-fed pigs. The regression model (PROC REG) was used to determine the Lys utilization efficiency for N retention. The Tukey-Kramer mean separation test was used to determine significant differences. The significance level was defined as $P \leq 0.05$.

Results

Nitrogen Balance

Nitrogen balance data are presented in Table 3. There was no effect of treatment on initial BW ($P > 0.05$; data not shown). Collection period BW was greater in the LR-fed pigs than the HR-fed pigs (21.6 vs. 21.3; $P = 0.02$) and with increasing dietary Lys, regardless of ratio ($P < 0.01$). There was no effect of dietary treatment on feed intake ($P > 0.05$). Nitrogen intake was greater in LR-fed pigs than in HR-fed pigs and there was an interaction between E:T ratio and Lys content on N intake, with N intake generally increasing with increasing dietary Lys content in the HR diets but not LR diets. Fecal and urinary N output was lower in pigs fed the HR diets ($P < 0.01$) and urinary N output was decreased with increasing Lys content ($P < 0.01$). The ATTD of N was higher in the HR-fed pigs compared to the LR-fed pigs (83.1 vs. 81.6 %; $P < 0.01$). We did not observe any significant effect of dietary Lys content on the ATTD of N ($P > 0.50$). Nitrogen retention and PD were greater with increasing dietary Lys content; however, on average, the LR-fed group had higher N retention and PD than the HR-fed group ($P < 0.05$). As presented in Table 4, orthogonal contrasts indicated further that as the dietary Lys content increased, N retention and PD increased linearly in the LR-fed pigs ($P < 0.001$). However, we observed a linear and quadratic response in the HR-fed pigs on N retention and PD as Lys content increased ($P < 0.05$).

Effect of E:T ratio on Lys requirement

Broken line regression was used to estimate Lys requirement in the LR and HR-fed pigs using N retention as the output parameter. In HR-fed pigs, N retention was maximized at 17.8 g/d with a Lys requirement of 1.08 % SID ($R^2 = 0.61$; $P < 0.01$; Fig. 1A), and in LR-fed pigs, N retention was maximized at 19.3 g/d with a Lys requirement of 1.21 % SID ($R^2 = 0.80$; $P < 0.01$; Fig. 1B). Using the PUN concentration (Fig. 2A and 2B), we determined Lys requirement for minimizing PUN in the HR-fed pigs was 1.12% SID Lys ($R^2 = 0.14$) at 6.37 mg/dL PUN (Fig. 2A), while in the LR-fed pigs, we determined 1.17% SID Lys ($R^2 = 0.23$; Fig. 2B) required to minimize PUN was 4.03 mg/dL.

Table 3. Nitrogen balance in pigs fed a low or high E:T ratio diets with graded levels of lysine[1]

		SID Lys, %						*P*-value		
Item	E:T[2]	0.82	0.92	1.02	1.12	1.22	SEM	E:T	Lys	E:T × Lys
Body weight, kg	0.36	20.6	21.4	21.7	21.5	21.3	0.23	0.02	<0.01	0.44
	0.33	21.0	21.6	22.1	21.4	22.1				
Feed intake, g DM/d	0.36	802.7	792.8	808.1	806.1	802.4	3.70	0.14	0.20	0.31
	0.33	804.5	805.5	806.9	807.1	802.7				
N intake, g/d	0.36	24.4	24.3	24.7	25.3	25.0	0.13	<0.01	<0.01	<0.01
	0.33	26.1	25.8	26.4	26.1	26.9				
ATTD of N, %	0.36	83.4	84.3	82.5	83.3	81.7	0.69	<0.01	0.81	0.21
	0.33	81.4	81.2	81.8	81.9	82.0				
Fecal N output, g/d	0.36	4.2	3.8	4.3	4.3	4.5	0.19	<0.01	0.40	0.30
	0.33	4.7	4.9	4.8	4.8	5.0				
Urinary N output, g/d	0.36	4.9	4.1	3.6	3.0	2.5	0.20	<0.01	<0.01	0.43
	0.33	6.1	5.1	4.2	3.5	3.2				
N retained, % of N intake	0.36	54.3	57.8	62.1	64.1	62.9	1.12	<0.01	<0.01	0.10
	0.33	48.8	53.1	60.2	58.8	62.9				
N retained, g/d	0.36	14.9	16.0	17.1	17.9	17.8	0.26	0.02	<0.01	<0.01
	0.33	15.2	15.8	17.9	17.4	19.3				
PD[3], g/d	0.36	93.2	99.9	106.9	111.9	111.3	1.60	0.02	<0.01	<0.01
	0.33	94.8	98.9	111.9	109.0	120.8				
PUN, mg/dL	0.36	10.8	9.2	6.8	2.8	7.6	1.54	0.51	<0.01	0.48
	0.33	9.3	11.2	6.0	3.3	4.0				
K_{nitrogen}[3]	0.36	0.85	0.92	0.95	0.99	0.99	0.013	<0.01	<0.01	0.32
	0.33	0.78	0.82	0.88	0.91	0.96				
K_{lysine}[4]	0.36	1.09	1.05	0.98	0.93	0.88	0.015	0.86	<0.01	0.58
	0.33	1.07	1.02	0.99	0.93	0.90				

[1] Data presented are least-square means (*n* = 8 pigs/treatment).

[2] E:T ratios of 0.36 (high ratio diets) and 0.33 (low ratio diets) reflect the amount of N in the diets coming from essential amino acids (E) and from all other components (T), with the higher ratio having a larger contribution of N from essential amino acids.

[3] Protein deposition is calculated as N retained (g/d) × 6.25.

[3] Marginal efficiency of N intake above maintenance calculated as: (nitrogen retained in body protein)/ (standardized ileal digestible nitrogen intake—maintenance nitrogen requirements). Standardized ileal digestible nitrogen intake and maintenance nitrogen requirement according to NRC (2012).

[4] Marginal efficiency of Lys intake above maintenance calculated as: (protein deposition × lysine % of body protein)/ (standardized ileal digestible lysine intake—maintenance lysine requirements/efficiency of SID lysine utilization for maintenance). Standardized ileal digestible lysine intake and maintenance lysine requirements were calculated according to NRC (2012). Body protein was assumed to contain 7.10% lysine (NRC, 2012).

ATTD; apparent total tract digestibility; DM, dry matter; E:T, essential amino acid nitrogen to total nitrogen ratio; N, nitrogen; PD, protein deposition; PUN, plasma urea nitrogen; SEM, standard error of the mean; SID, standardized ileal digestible.

Table 4. Linear and quadratic relationship of dietary SID Lys and EAA:TN ratio on nitrogen (N) retention[1]

	Ratio[2]	SID Lys, %						*P*-value	
		0.82	0.92	1.02	1.12	1.22	SEM	Linear	Quadratic
N retained, g/d	0.36	14.5	15.9	17.1	17.8	17.7	0.36	<0.0001	0.02
	0.33	14.6	15.9	17.3	17.9	19.1	0.38	<0.0001	0.51

[1] Data presented are least-square means (*n* = 8 pigs/treatment).

[2] E:T ratios of 0.36 (high ratio diets) and 0.33 (low ratio diets) reflect the amount of N in the diets coming from essential amino acids (E) and from all other components (T), with the higher ratio having a larger contribution of N from essential amino acids.

E:T, essential amino acid nitrogen to total nitrogen ratio; N, nitrogen; SEM, standard error of the mean; SID, standardized ileal digestible.

Effect of E:T ratio on Lys and N utilization efficiency

The marginal efficiency of Lys (K_{lysine}) and N (K_{nitrogen}) intake for N retention is presented in Table 3. The K_{lysine} was lower with increasing Lys content ($P < 0.01$), while K_{nitrogen} efficiency was higher in pigs fed the HR diets and with greater Lys content ($P < 0.01$). There was no effect of ratio on K_{lysine} and no effect of the interaction between ratio and lysine on K_{nitrogen} and K_{lysine}.

The linear equations relating N retention (g/d) to SID Lys intake in both LR and HR-fed pigs are presented in Fig. 3. The intercept relating N retention to SID Lys intake was not different between the LR and HR diets ($P = 0.08$) but showed a tendency for high N retention in HR fed pigs when SID Lys intake was zero. The efficiency of Lys utilization for N retention, represented by the slope, was greater in pigs fed LR diets than in pigs fed the HR diets ($P = 0.04$).

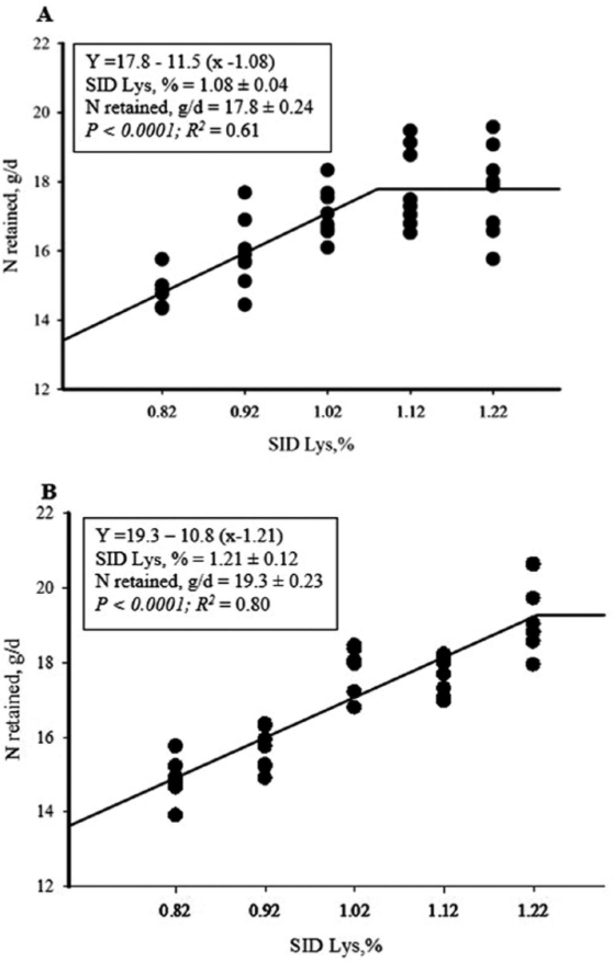

Figure 1. The linear broken-line model estimated nitrogen retention (N retention; g/d) in pigs fed high (**HR**; 0.36) or low (**LR**; 0.33) E:T ratio diet. A breakpoint was achieved at 1.08 SID Lys, % with a maximum N retention of 17.8 g/d for pigs fed the HR diets (**A**). While the breakpoint was achieved in pigs fed the LR diet at 1.21 SID Lys, % with a maximum N retention of 19.3 g/d (**B**).

Discussion

The objective of the current study was to determine the effect of E:T ratio on the Lys requirement and utilization of Lys and N for N retention in growing pigs. To achieve this objective, an N-balance study was conducted in which pigs were fed diets with either a high or low E:T ratio representing a low or high amount of total N, respectively.

Based on the N-balance data in this study, we estimated a higher Lys requirement when pigs were fed a diet with a low E:T ratio compared to a high E:T ratio-fed pigs. Moreover, pigs fed the LR diets had greater overall N retention compared to pigs fed the HR diets. This indicates that NEAA or N was deficient in HR diets and limited N retention and resulted in a lower need for lysine. Similar results were observed previously when growing pigs fed higher levels of ammonia-N (E:T ratio of 0.50) had greater N retention than pigs fed lower levels of ammonia-N (E:T ratio of 0.59) in an NEAA deficient diet (Mansilla et al., 2017a).

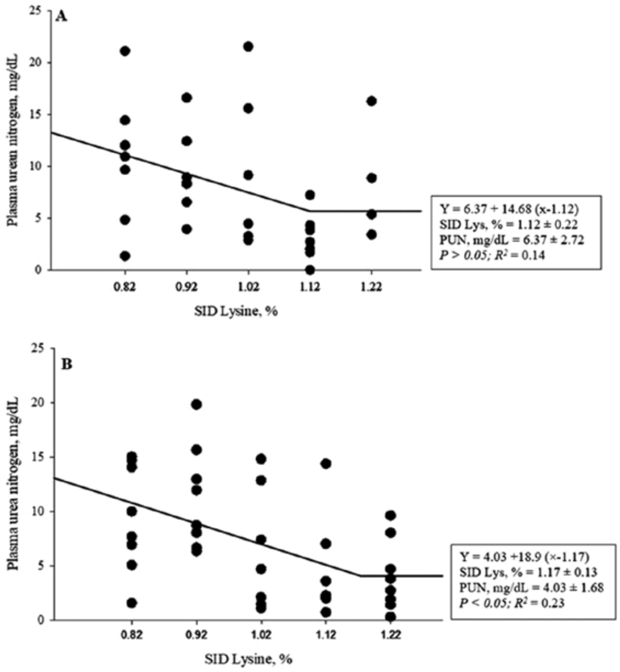

Figure 2. The linear broken-line model estimates for plasma urea nitrogen (**PUN**; mg/dL) in pigs fed a high (**HR;** 0.36) or a low (**LR;** 0.33) E:T ratio diet. A breakpoint was achieved at 1.12 SID Lys, %, with a maximum PUN of 6.37 mg/dL in pigs fed the HR diet (**A**). While in the LR diet, the breakpoint was achieved at 1.1% SID Lys, % with a maximum PUN of 4.03 mg/dL (**B**).

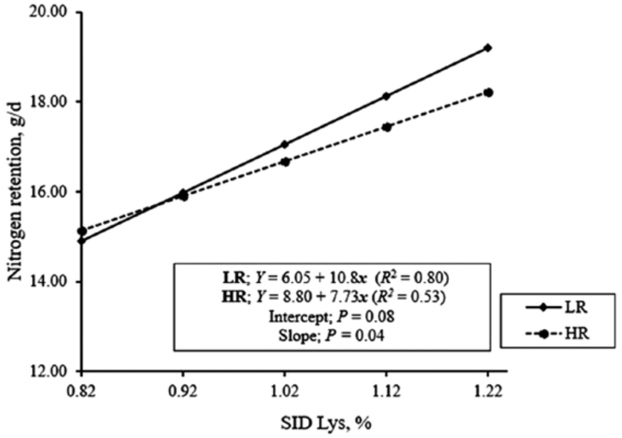

Figure 3. Regression analysis showing the efficiency of Lys intake on N retention in pigs fed a high (**HR;** 0.36) or a low (**LR;** 0.33) E:T ratio diet. The LR diet is represented in the figure with short dashes (LR; 0.33 ------), while the HR is presented in the figure with a solid line (HR; 0.36——). Data of the regression analysis using the one slope model: *Y = a + b(x)*, where ***Y*** is the N retention (g/d), ***a*** represents the intercept (extrapolating the maintenance requirement), ***b*** is the slope representing the efficiency of SID Lys utilization for N retention and ***x*** representing the SID Lys intake (g/d). The equations are as follows: **LR** diet ($Y = 6.05 + 10.8x$; $R^2 = 0.80$) while the HR diet ($Y = 8.80 + 7.73x$; $R^2 = 0.53$). The intercept $P = 0.08$ and slope $P = 0.04$.

As expected, increasing the dietary N content increased N intake in pigs fed the LR diet. The decrease in ATTD of N in the LR diets was most likely a result of the lower inclusion of crystalline AA in these diets, resulting in an increase in fecal N output. We also observed an overall increase in urinary N output in pigs fed the LR diet. While the higher N content supplied in the low E:T ratio diets resulted in greater N retention (g/d), the % of N intake retained was reduced. Both the E:T ratio and Lys content of the diet impacted the efficiency with which N was utilized for N retention. This is expected as N utilization is an inherently inefficient process, with an average of 30% to 60% of N intake retained by the pig (NRC, 2012). Likewise, the improved N retention, both in g/d and % of intake, with increasing Lys was expected as AA utilization for protein deposition will increase as EAA content approaches the requirement (Pencharz and Ball, 2003). Indeed, the % of N intake and the marginal efficiency of N (K_{nitrogen}) increased as the Lys increased, regardless of E:T ratio. Likewise, the overall efficiency of Lys for N retention was improved with supplemental N, providing further evidence that N is deficient in HR diets and that utilization of supplemental N will require a greater dietary Lys content. Improved N utilization is further supported by urinary N output and PUN, both indicators of N utilization. The decrease in PUN with increasing Lys content and no change in PUN with E:T ratio, despite an increase in N intake, suggest improved N utilization with supplemental N.

The observed effect of ratio on Lys requirement contradicts our hypothesis that the Lys requirement would be higher in diets deficient in N. This was based on the idea that when NEAA are limiting, EAA would be catabolized as a source of N for NEAA synthesis (Wang et al., 2018). We also assumed that the use of N from EAA would not depend on the EAA (i.e., the efficiency of N utilization is equivalent across EAA). However, it is possible that Lys is not an efficient source of N for NEAA synthesis. Although there is some evidence that all EAA can contribute to N supply for NEAA synthesis (Heger, 2003), the efficiency of converting some EAA may be lower than others. For example, the conversion of Arg and Lys (36% and 50%, respectively) to NEAA-N was shown to be less efficient than Gln, Pro, and Gly (>96%) in chicks (Allen and Baker, 1974). The role of Arg in urea synthesis may preclude it as an efficient source of N for NEAA synthesis. On the other hand, branched-chain amino acids (i.e., Leu, Ile, and Val) may contribute significantly to N supply and metabolism, given their extrahepatic deamination, which results in the production of Gln and Glu (Nie et al., 2018).

An optimum E:T ratio of 0.48 for achieving maximum N retention was previously determined in pigs (Heger et al., 1998). While a number of studies have examined this ratio (Heger, 2003), there has been a lack of consistency in how this ratio has been calculated. Various factors, such as total EAA, including excess above requirement, the selection of AA considered EAA and which sources of N (i.e., AA or non-protein N) are included in the NEAA-N fraction (Heger et al., 1998, 2008; Lenis et al., 1999; Mansilla et al., 2017a). For example, the inclusion of Arg as an EAA can have a significant impact on the ratio value given the high N content of Arg. Previous calculations have only included AA-N, with the assumption that other sources of N would not contribute to N supply for N retention. As a result, the total N fraction, as discussed by Heger, (2003), only includes N from NEAA and excess EAA. More recent advances in our understanding of N metabolism (Mansilla et al., 2015, 2018) have shown that non-protein N can contribute to both EAA and NEAA supply in the pig and non-protein N can be used as efficiently as NEAA supplementation in NEAA-deficient diets. Therefore, total N, regardless of source, should be accounted for in calculations of dietary N supply. These inconsistencies show that the estimated optimal ratio depends on the specific assumptions used in the calculation.

Thus, in the present study, we calculated the E:T ratio as the amount of EAA-N as the SID fraction up to the recommended requirement (NRC, 2012) and total dietary N as indicated by crude protein content. Consequently, our ratio represents the amount of balanced EAA (including Arg) available for protein synthesis and the total amount of N potentially available for the synthesis of NEAA. In addition to differences in E:T ratio calculation, diets in the current study were formulated using more practical ingredients, in contrast to the semi-synthetic diets utilized previously, which limited our ability to achieve higher ratios. We also adjusted the E:T ratio by including additional N from intact protein while keeping EAA content in the diet constant. Although this is the more relevant situation, as the overall goal of diet formulation is to limit the excess of EAA and total protein, many previous studies have estimated optimal E:T ratio in isonitrogenous diets where both the EAA and NEAA content are altered while total protein is kept constant (Heger, 2003). This has implications in the estimation and interpretation of studies examining E:T values, as Lenis et al. (1999) and Heger et al. (1998) showed that N retention and N utilization responses differ when EAA or N are kept constant and that E:T ratio is more important when dietary protein content is low.

The importance of NEAA content in diets has been receiving attention recently, especially with the observations that at very low dietary protein content, growth performance is negatively affected even when sufficient EAA are supplied to meet requirements. In current diet formulations, this is accounted for using SID Lys:CP ratio, with current recommendations being 7.45 for growing pigs. Based on formulated values, the high and low E:T ratio diets in the current study had 6.58 and 6.07 SID Lys:CP ratios, respectively, and 6.97 and 7.20 SID Lys:CP ratios based on estimated requirements. It is interesting that the estimated SID Lys:CP requirements in the current study are lower than the NRC (2012) recommended value, despite our diets having a higher protein content than what is assumed by NRC (2012)(i.e., 13.7%). While the SID Lys:CP ratio attempts to ensure equivalent EAA and NEAA, these values do not account for the source of N in diets, and estimates may have been generated utilizing diets with insufficient NEAA or N content. This was demonstrated in a recent meta-analysis in which Rocha et al. (2022) determined the minimum amount of protein required in diets before growth performance is reduced. They estimated a minimum of 18.4%, 16.1%, and 11.6% protein content for nursery, grower, and finisher pigs, respectively, which are greater than estimates in NRC, (2012). Moreover, a breakpoint for SID Lys:CP was only achieved for nursery pigs, suggesting a greater influence of protein on growth performance in grower and finisher pigs. While not likely, it is possible to formulate a diet with only EAA that meets the SID Lys:CP requirement, whereas the use of E:T ratio specifically requires the inclusion of NEAA or N in the diet. Therefore, we suggest that the use of E:T ratio represents an advancement in our characterization of nutrient requirements for pigs.

Conclusions

The current results suggest that N may become limiting in certain diets, as indicated by a high E:T ratio, even when EAA are formulated to meet requirements. Deficient dietary N results in a reduction in N retention and Lys requirement. Increasing the N content through the addition of intact protein, while maintaining EAA content, improves N retention and increases Lys requirement. Overall, NEAA should be accounted for when formulating diets for pigs, and the E:T ratio may be used as an indication of N sufficiency. Further research is required to determine the optimum E:T ratio as calculated in the present study and the effects of the ratio on EAA requirements.

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Conflict of Interest Statement

All authors declare no financial or personal conflicts of interest.

Data Availability

Data is available upon reasonable request to the corresponding author.

Author Contributions

D.A.C. designed the research with discussions on diet design with A.K.S. C.M.C., and J.C.P. conducted the research, C.M.C., M.O.W, L.A.R., A.K.S., and D.A.C. analyzed the data, C.M.C., M.O.W., A.K.S., and D.A.C. wrote the paper, and D.A.C. had primary responsibility for the final content. All authors read and approved the final manuscript.

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