Influence of dietary methionine to methionine plus cysteine ratios on nitrogen retention in gilts fed purified diets between 40 and 80 kg live body weight

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Gillis, A. M., Reijmers, A., Pluske, J. R. and de Lange, C. F. M. 2007. **Influence of dietary methionine to methionine plus cysteine ratios on nitrogen retention in gilts fed purified diets between 40 and 80 kg live body weight**. Can. J. Anim. Sci. **87**: 87–92. The relationship between the ratio of available methionine (MET) to methionine plus cysteine (TSAA) intake and whole-body protein deposition (PD) was established using the nitrogen (N) balance technique in gilts between 40 and 80 kg live body weight (BW), according to a repeated 5 × 5 Latin square design. Pigs were fed casein- and cornstarch-based diets that supplied equal moles of TSAA supporting a PD of approximately 80% of the gilts' PD potential. On a weight basis, the target ratios of MET to TSAA were 42, 47, 52, 57 and 67% for the five experimental diets, respectively. This calculated to 37, 42, 47, 52 and 62.5% on a molar basis. Total N excretion (urine plus feces) was reduced (linear; $P < 0.001$) and PD was increased (linear; $P < 0.001$) when the available MET to TSAA ratio was increased to 52%; these values did not change ($P > 0.05$) when the MET to TSAA ratio was further increased. A number of statistical models were fitted to the data to establish the best fit of the model parameters. The greatest proportion of the variation ($R^2 = 0.990$) was explained with an asymptotic model; based on this model the optimum available MET to TSAA ratio (supporting 90% of the asymptotic value for PD) was 55% on a weight basis or 50.5% on a molar basis. The results indicate that the minimum contribution of available MET to TSAA requirements of growing pigs is higher than the value currently suggested by the National Research Council (46 to 48% on a weight basis).

Key words: Methionine, cysteine, pigs, nitrogen balance, protein, deposition

Gillis, A. M., Reijmers, A., Pluske, J. R. et de Lange, C. F. M. 2007. **Incidence du ratio entre la méthionine des aliments et la combinaison méthionine-cystéine sur la rétention de l'azote chez les truies nullipares de 40 à 80 kg de poids vif recevant une ration purifiée**. Can. J. Anim. Sci. **87**: 87–92. Les auteurs ont étudié les liens qui existent entre le ratio de la méthionine disponible (MET) avec l'ingestion de méthionine et de cystéine (TSAA) et le dépôt de protéines dans l'organisme (PD) par la méthode du bilan azoté (N) chez les truies nullipares de 40 à 80 kg de poids vif. Pour cela, ils ont conçu une expérience en carré latin de 5 × 5 avec répétitions. Les sujets ont reçu des rations à base de caséine ou de fécule de maïs qui fournissaient une quantité molaire identique de TSAA conduisant à un PD égal à environ 80 % du PD potentiel des animaux. Exprimé en fonction du poids, le ratio visé entre la MET et la TSAA s'établissait respectivement à 42, 47, 52, 57 et 67 % pour les cinq expériences, soit 37, 42, 47, 52 et 62,5 % en valeur molaire. Les auteurs ont observé une baisse de l'excrétion totale de N (urine et fèces) (linéaire; $P < 0,001$) et une hausse du PD (linéaire également; $P < 0,001$) quand le ratio entre la MET disponible et la TSAA passe à 52 %; ces valeurs restent identiques ($P > 0,05$) quand le ratio entre la MET et la TSAA continue de s'accroître. Les auteurs ont appliqué les données à plusieurs modèles statistiques en vue d'établir celui qui accepterait le mieux les paramètres. Un modèle asymptotique explique la majeure partie de la variation ($R^2 = 0,990$); selon ce modèle, le ratio optimal entre la MET et la TSAA (à savoir celui qui explique 90 % de la valeur asymptotique du PD) est de 55 % en poids ou de 50,5 % en valeur molaire. Les résultats indiquent que la contribution minimale de la MET aux besoins de TSAA des porcs en croissance est supérieure à ce que suggère actuellement le National Research Council (46 à 48 % selon le poids).

Mots clés: Méthionine, cystéine, porcs, bilan azoté, protéines, dépôt

The sum of the sulfur-containing amino acids (TSAA) methionine (MET) and cysteine (CYS) is generally limiting in practical diets for growing-finishing pigs [National Research Council (NRC) 1998], and requires addition of synthetic MET to satisfy pigs' demands for lean tissue growth and maintenance. Cysteine can be synthesized from MET (Stipanuk 1986), and CYS and its oxidation product, cystine, can satisfy approximately 50% of the need for total TSAA in the diet (Roth and Kirschgessner 1989; Chung and Baker 1992; Kirchgessner et al. 1994), thereby reducing the need for dietary MET. Since MET can be converted to CYS, but not vice versa (Stipanuk 1986), it is feasible that a minimum dietary MET to TSAA ratio occurs in pig diets, at

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Abbreviations: **BW**, body weight; **CYS**, cysteine; **MET**, methionine; **PD**, whole body protein deposition; **TSAA**, sum of sulfur containing amino acids which MET and TSAA utilization for PD is maximized. Estimates of the minimum ratio between MET and TSAA vary and are affected by many factors including diet composition and amino acid sources, response criteria, pig body weight (BW), errors associated with chemical analysis, and total TSAA intake (Agricultural Research Council 1981; Roth and Kirchgessner 1989; Chung and Baker 1992; Kirchgessner et al. 1994; NRC 1998). Unfortunately, in most of the experiments conducted to assess the optimum MET to TSAA ratio, relatively few ratios have been evaluated or animals have been fed on an ad libitum basis, which will confound effects between diet MET and CYS levels, feed intake and pig performance. The MET to TSAA ratios are generally expressed on a weight basis (NRC 1998), but these ratios are better expressed on a molar basis. The potential conversion of MET to CYS is better represented on a molar basis than a weight basis, considering that the molar weight of MET (149 g) is higher than that of CYS (121 g).

The objective of the current study was to establish the minimum dietary available MET to TSAA ratio for growing pigs at which PD was maximized. Gilts were fed predetermined amounts of casein- and cornstarch-based diets and similar molar amounts of TSAA to closely control experimental conditions. The relationship between the MET to TSAA ratio and PD was established using the N-balance method.

MATERIALS AND METHODS

Experimental Design

Ten purebred Yorkshire gilts from the Arkell swine herd at the University of Guelph and with an initial live BW of approximately 40 kg were housed in metabolism crates and fed five different experimental diets according to a repeated 5 × 5 Latin Square design. In each experimental period, pigs were allowed to adjust to their respective dietary treatment for 5 d prior to a N-balance period that lasted another 5 d. The experiment finished when gilts reached approximately 80 kg BW. Full details of the procedures used for N-balance are provided by Möhn and de Lange (1998). The only alterations to these procedures were the omission of urinary catheters for the collection of urine and the use of a 5 d rather than a 7 d N-balance period. Given the large number of N balances that were conducted for each individual pig the risk of developing urinary infections was deemed too high when using urinary catheters. Rather, urine from each gilt was collected directly via a urine collection tray that then drained into collection containers, which contained 12 mL sulfuric acid added daily to avoid N losses by volatilization of ammonia (de Lange et al 2001).

The experiment was reviewed and approved by the University of Guelph Animal Care Committee, in accordance with guidelines set by the Canadian Council on Animal Care.

Experimental Diets and Feeding

To prepare experimental diets that were limiting in TSAA, a casein-, cornstarch- and synthetic amino acid-based "basal nutrient mix" [excluding added MET and CYS.HCl (67% CYS)] and a cornstarch-based N-free "energy mix" were formulated and prepared in single batches (Table 1). In the basal nutrient mix, the ratio of essential amino acids and total N to lysine was similar to that used previously by de Lange et al. (2001), with the exception of MET and CYS. The basal nutrient mix was divided into five equal parts and varying amounts of MET and CYS.HCl were added to these parts to produce the five different "nutrient mixes" (Table 2). This was done to achieve, on a molar basis, the same available TSAA content in all five dietary treatments, while varying on a weight basis the target available MET to TSAA ratios as follows: 42, 47, 52, 57 and 67% (equivalent to 37, 42, 47, 52, 62.5% on a molar basis) (Table 3). The true ileal MET and CYS digestibility values in casein were determined to be 99% (Nyachoti et al. 1997). True ileal digestible MET and CYS supplied by casein was assumed to be available for metabolism by the pig (Batterham 1992). The availability of MET and CYS supplied by synthetic amino acids was assumed to be 100% (Batterham 1992). Pigs were supplied equal moles of TSAA supporting a PD of approximately 80% of the gilts' maximum PD.

The amount of nutrient mix fed to gilts was maintained constant at 835 g d^{-1} across diets and experimental periods. The amount of energy mix was adjusted with BW to ensure that energy intake exceeded 2.8 × maintenance energy requirements so that energy intake did not limit PD (Möhn and de Lange 1998; de Lange et al. 2001). Feed wastage was collected daily on another tray and weighed for individual pigs at the completion of each N-balance period.

Chemical Analyses

Dry matter content in feed was measured by oven-drying for 2 h at 135°C [Association of Official Analytical Chemists (AOAC) 1990]. Dry matter contents were determined in the freeze-dried material, when samples were weighed to determine water loss during freeze-drying, and when sub-samples were taken for chemical analyses. The N contents in feed, feces and urine were determined using the Kjeldahl method (AOAC 1990). Representative samples of casein and the nutrient mixes were analyzed for amino acid composition using ion-exchange chromatography with post-column derivatization with ninhydrin in the laboratory of Degussa AG, Hanau, Germany (Llames and Fontaine 1994).

Calculations and Statistical Analyses

Nitrogen balances (g d^{-1}) were calculated as feed intake (feed allowance minus feed wastage) multiplied by the calculated mean N content in the feed minus N losses with feces, urine and wasted feed (Möhn and de Lange 1998). The mean N content in the feed was calculated from the analyzed N contents in the nutrient mix and the energy mix (Table 3) and the relative contribution of the nutrient mix and energy mix to the daily feed allowance. Nitrogen balances were converted to whole body PD (N × 6.25). Average molar amounts of available MET and CYS intakes were calculated based on determined feed intakes (feed allowance minus wastage) and calculated available MET and CYS levels in the nutrient mixes.

Statistical analyses were performed according to SAS Institute, Inc. (1996). Differences between treatment groups

Table 1. Ingredient composition of the "basal nutrient mix" and "energy mix"[z]

Ingredient	Basal nutrient mix	Energy mix
	($g\ kg^{-1}$)	
DL- Methionine	–	–
L- Cysteine.·HCL	–	–
Sodium caseinate	99.3	–
Cornstarch	288.0	669.25
Cellulose[y]	40.0	40.0
Sucrose	200.0	200.0
Corn oil	40.0	30.0
Limestone	8.0	11.0
Dicalcium phosphate	30.0	25.0
Salt	4.0	3.0
KCl	9.0	6.0
$MgSO_4$	3.5	5.0
Vitamin mix[x]	11.1	10
Mineral mix[x]	1.5	0.75
L-Lysine·HCl	10.3	–
L-Threonine	7.4	–
L-Tryptophan	2.5	–
L-Isoleucine	6.15	–
L-Valine	6.19	–
L-Histidine	3.24	–
L-Phenylalanine	9.8	–
L-Leucine	9.8	–
L-Aspartic acid	102.0	–
L-Glutamic acid	103.0	–
Choline chloride	0.60	–
Total	995.38[w]	1000.0

[z]As-fed.
[y]Avicel (Lee Chemicals Ltd., Toronto, ON).
[x]At a dietary inclusion level of 11.1 g kg^{-1} vitamin mix and 1.5 g kg^{-1} mineral mix the following amounts were supplied per kg of diet: vitamin A, 4440 I.U.; vitamin D3, 4444 I.U.; vitamin E, 17.8 I.U.; vitamin K, 1.11 mg; choline, 222 mg; pantothenic acid, 6.7 mg; riboflavin, 2.2 mg; folic acid, 0.89 mg; niacin, 11.1 mg; thiamin, 0.67 mg; vitamin B_6, 0.67 mg; biotin, 0.089 mg; vitamin B_{12}, 0.011 mg; Cu 15 mg (from $CuSO_4 \cdot 5H_20$); Zn 100 mg (from ZnO); Fe 100 mg (from $FeSO_4 \cdot 1H_20$) ; Mn 20 mg (from MnO); I 0.3 mg (from $Ca(IO_3)_2$); Se 0.3 mg (from Na_2SeO_3).
[w]The remainder was provided by methionine or cysteine (Table 2).

were established using mixed model procedures with BW (covariable), pig (n = 10; random effect), period (n = 5; random effect) and dietary treatment (n = 5; fixed effect) included in the model as sources of variation. Linear, linear-plateau, quadratic, and asymptotic models, as described by Möhn et al. (2000), were evaluated. Linear-plateau models were calculated as: $PD = a + b \times$ available MET:TSAA, when PD did not reach a plateau, and $PD = c$, when PD reached a plateau; a, b and c were model parameters. The break point ($a + b \times$ available MET:TSAA = c) was determined by iteration until the residual mean square error in the statistical model was minimized (Möhn et al. 2000). The parameter b in the model represents the increase (grams) in PD per gram increase in available MET:TSAA. Differences between treatment means were assessed using the least significant difference procedure of SAS, with significance accepted at $P < 0.05$.

RESULTS AND DISCUSSION

General Observations

Pigs generally appeared in good health and readily consumed their daily feed allowances. As a consequence, no results were excluded from the data set prior to statistical analyses.

Amino Acid Analyses of Nutrient Mixes

For amino acids other than MET and CYS, the analyzed amino acid contents were similar across nutrient mixes and in agreement with predicted values based on ingredient compositions and the amino acid composition of casein (Table 3). For lysine, threonine, tryptophan, isoleucine and leucine, the analyzed values were 0.4, 4.2, 1.6, 1.5 and 0.3% lower than the calculated values, respectively. In contrast, the analyzed MET, and to a lesser extend the CYS, contents were considerably lower than predicted contents. Across treatments, differences were 13% for MET and 14% for CYS. This discrepancy is difficult to explain given that extreme care was taken in diet manufacture; however, it may be (partly) attributable to the degradation of some free MET and CYS during the hydrolysis associated with amino acid analysis (Rutherfurd and Moughan 2000). For this reason, it is advisable to determine the free amino acid content in experimental diets prior protein hydrolysis in future studies. Nevertheless, the MET to TSAA ratios based on analyzed values were in general agreement with calculated values, being: 43, 45, 52, 55 and 66 versus 42, 47, 52, 57 and 67%, respectively. Given the care that was taken during diet preparation, the calculated, rather than analyzed, MET and CYS contents in the nutrient mixes were used in subsequent calculations and interpretation of the results. The calculated TSAA to lysine ratio in the diets varied between 41.3 and 43.6%, indicating TSAA were the first limiting amino acids in all diets.

Body Protein Deposition

Both linear and quadratic effects of the dietary MET to TSAA ratio on total N excretion (urine plus feces) and PD were highly significant ($P < 0.001$; Table 4), supporting the observation that plateau values for N excretion and PD were achieved at the highest dietary MET to TSAA ratio (Fig. 1). The plateau in PD in this study occurred at a daily intake of approximately 5.4 g of available TSAA, or about 40 mmol d^{-1} of the two amino acids. Higher levels of PD (> 135 g d^{-1}) were achieved by de Lange et al. (2001) and de Lange et al. (unpublished data) in a N-balance study using gilts of the same genotype and at a similar weight; however, in those studies the available TSAA intake was higher than in the current study. This allowed the gilts to better express their performance potential. In the current experiment, PD was thus sensitive to TSAA intake and hence to the dietary MET to TSAA ratio. Moreover, observed PD was reasonable representative of typical pig performance levels (NRC 1998).

A number of statistical models were evaluated to establish the relationship between the available MET to TSAA ratio and whole body PD (Fig. 1). Based on broken line-linear plateau regression analysis (R^2 = 0.893), a plateau in PD was achieved when the available MET to TSAA ratio was 53.5% on a weight basis, which was equivalent to 49% on a molar basis. A greater proportion of the variance (R^2 = 0.962) was explained when a curvilinear model was applied to the data,

Table 2. Added amounts of methionine (MET) and cysteine·HCl (CYS; 67% cysteine) ($g\ kg^{-1}$) to the basal "nutrient mix" to produce the five targeted MET to TSAA ratios in the nutrient mixes[z]

	Nutrient mix (targeted MET to TSAA ratios)				
Amino acid	42%	47%	52%	57%	67%
Methionine	–	0.35	0.72	1.07	1.85
Cysteine	4.84	4.45	4.00	3.58	2.66

[z]As-fed and on a weight basis.

Table 3. Analyzed and calculated amino acid content in nutrient mixes varying in methionine (MET) and cysteine (CYS) content[z]

	Nutrient mix (targeted MET to TSAA ratio)									
	42%		47%		52%		57%		67%	
Item	Anal.[y]	Calc.[y]	Anal.	Calc.	Anal.	Calc.	Anal.	Calc.	Anal.	Calc.
Dry matter (%)	95.6		95.4		95.7		95.8		95.7	
CP[w] (%)	26.7	24.8	26.6	24.8	26.9	24.8	27.0	24.8	27.2	24.8
MET	2.3	2.8	2.6	3.2	3.0	3.5	3.3	3.9	4.0	4.7
CYS	3.1	3.8	3.2	3.5	2.7	3.2	2.7	2.9	2.0	2.3
TSAA	5.3	6.6	5.8	6.7	5.9	6.8	6.0	6.8	6.0	7.0
MET:TSAA × 100	43	42	45	47	52	52	55	57	66	67
MET (mmoles)	15.2	18.8	17.5	21.2	20.6	23.8	22.1	26.2	26.7	31.5
CYS (mmoles)	25.4	31.5	26.3	29.3	22.5	26.7	22.5	24.2	16.9	18.9
TSAA (mmoles)	40.6	50.4	43.8	50.5	43.1	50.4	44.7	50.4	43.6	50.4
MET:TSAA × 100 (molar)	38	37	40	42	48	47	50	52	61	63
Lysine[x]	15.2	16.0	15.8		15.4		15.6		15.7	
Threonine	11.0	11.8	11.7		11.9		12.4		11.7	
Tryptophan	3.6	3.8	3.5		3.6		3.6		3.6	
Isoleucine	10.3	11.2	11.0		11.1		11.4		11.2	
Leucine	17.9	19.3	19.0		19.2		19.4		19.3	
Valine	12.0	12.7	12.9		13.3		12.7		12.5	
Histidine	5.6	6.2	5.9		6.0		6.0		5.9	
Phenylalanine	13.7	15.1	14.3		14.7		15.0		14.8	

[z]Values are given on a dry matter basis and as g/kg unless stated otherwise; MET to TSAA ratios are on a weight basis.
[y]Anal. = analyzed; Calc. = calculated.
[x]Calculated contents for amino acids other than MET and CYS are similar across nutrient mixes.
[w]CP, crude protein.

Table 4. Animal performance and whole body protein deposition (PD) in growing pigs fed diets with varying available methionine to methionine plus cysteine ratios (MET: TSAA; weight basis) based on N-balance data

	Targeted dietary available MET to TSAA ratio							
Item	42%	47%	52%	57%	67%	SEM[z]	L[y]	Q[x]
Number of pigs	10	10	10	10	10			
ME intake ($MJ\ d^{-1}$)	26.1	26.2	26.1	26.1	26.1	0.01		
TSAA intake ($g\ d^{-1}$)	5.22	5.30	5.36	5.40	5.51	0.035		
TSAA intake ($mmol\ d^{-1}$)	39.7	39.9	39.9	39.8	39.7	0.11		
MET intake ($g\ d^{-1}$)	2.21	2.49	2.80	3.08	3.74	0.067		
MET intake ($mmol\ d^{-1}$)	14.8	16.7	18.8	20.6	25.1	0.48		
CYS intake ($g\ d^{-1}$)	3.01	2.81	2.56	2.32	1.77	0.046		
CYS intake ($mmol\ d^{-1}$)	24.9	23.2	21.1	19.2	14.6	0.32		
N intake ($g\ d^{-1}$)	33.2	33.4	33.3	33.3	33.4	0.03	NS[w]	NS
N excretion ($g\ d^{-1}$)	15.9	14.7	14.5	14.2	14.0	0.19	< 0.001	< 0.001
PD ($g\ d^{-1}$)	108	116	118	120	121	1.4	< 0.001	< 0.001

[z]SEM, standard error of the mean.
[y]L, significance of linear effect.
[x]Q, significance of quadratic effect.
[w]NS, not significant ($P > 0.05$).

which resulted in PD being maximized when the available MET to TSAA ratio exceeded 57% on a weight basis, or 52% on a molar basis. The greatest proportion of the variation ($R^2 = 0.990$) in PD was explained by an asymptotic model that predicted an optimum ratio of 55%, or 51% on a molar basis, at 90% of the asymptotic response. In the asymptotic model, the value of 90% of the asymptotic response was chosen arbitrarily and based on the considerations presented by Baker (1985).

Despite the small differences between the statistical models used in the derivation of the optimum ratio between

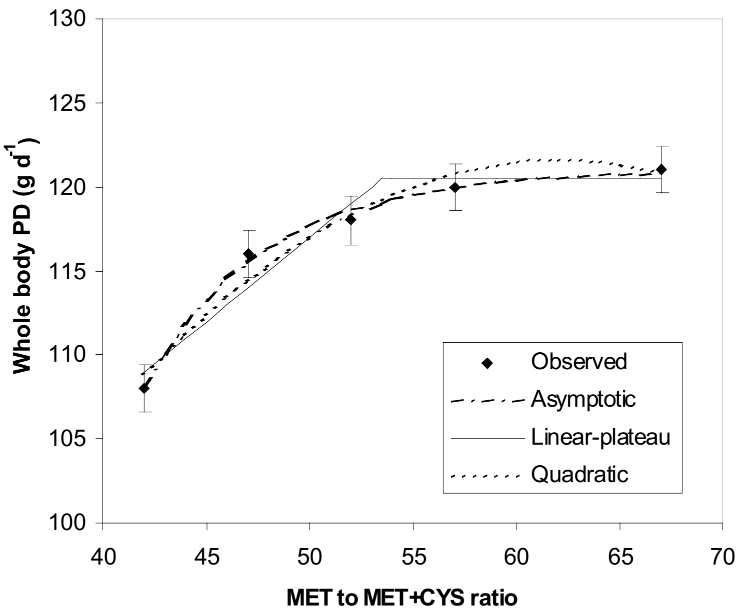

Fig. 1. Relationship between whole-body protein deposition (PD, g d^{-1}) and the calculated available methionine (MET) to methionine plus cysteine (TSAA) ratios (%), represented using a broken-line linear plateau model (plateau is 120.5, slope: $Y = 1.00\ X + 67.0$; $R^2 = 0.893$), a quadratic model ($Y = -0.0318\ X^2 + 3.9452\ X - 0.7838$; $R^2 = 0.962$), or an asymptotic model [$Y = 108.1 + 12.86 \times (1 - e^{-0.172 \times (X - 0.42)})$; $R^2 = 0.990$].

available MET and TSAA, the data from this study are consistent with values reported in the literature, although the criteria used for estimating this level differ between studies. For example, Roth and Kirchgessner (1987) showed that additions of MET to a diet significantly improved pig growth, feed conversion and carcass quality criteria, while additions of CYS (with constant dietary MET) decreased performance. These authors concluded that determination of the requirement for TSAA was possible only after the supply of a minimum amount of MET. In a follow-up study to investigate this minimum proportion of MET, Roth and Kirchgessner (1989) fed pigs between 30 and 60 kg and 60 and 90 kg BW diets that contained MET at 36, 40, 45, 50, 55, 60 or 64% (on a weight basis) of the total supply of TSAA in the diet, while maintaining the TSAA content slightly below the estimated requirement. Roth and Kirchgessner (1989) reported significant improvements in growth rate, feed conversion, area of eye muscle, and meat:fat ratio with a rising proportion of MET in the diet, with the optimum proportion of MET to TSAA being 55–57% based on a quadratic relationship. Fuller et al. (1989) estimated that CYS could supply approximately 0.8 of the total TSAA needs for maintenance, which can be explained primarily by keratin synthesis (Mitchell 1950), and close to half the total TSAA needs for body protein accretion. These authors surmised, therefore, that CYS, on a weight basis, could substitute for MET in supplying 54% of total TSAA needs of the pig. Furthermore, and given that the minimum proportion of MET required increases with an increasing level of performance due to the maintenance proportion decreasing relative to the pig's total TSAA requirement, Roth and Kirchgessner (1989) determined a theoretically optimum ratio of 60:40 MET:CYS, on a weight basis, in growing pigs for maintenance and protein accretion. This concurs with their experimentally determined optimum value.

Seemingly in contrast to our data and that of others, however, the NRC (1998) reported that, on a true ileal digestible amino acid and weight basis, the optimum dietary MET to TSAA ratios in pigs weighing 50–80 kg were approximately 46, 45 and 48% for pigs with lean growth rates of 300 (PD 118), 325 (PD 127) and 350 (PD 137) g d^{-1}, respectively. The NRC (1998) applied a factorial approach to establish this ratio without providing much empirical support, and suggested that this ratio is only influenced by BW and PD. Data from the current experiment indicate that the minimum ratio of available MET to TSAA required to maximize PD lies above this ratio.

In summary, the data from this study using N-balance studies showed that the minimum dietary available MET to TSAA ratio at which whole-body PD is maximized in gilts between 40 and 80 kg BW was 55% on a weight basis, or 51% on a molar basis, using an asymptotic model. The ratio using this particular model provided a better fit to the data than either a broken-line linear plateau or quadratic model. When the available MET to TSAA ratio was further increased, no changes in PD occurred. These values were consistent with previously determined values, but suggest that the current NRC (1998) estimate of the optimum MET to TSAA ratio for growing pigs is too low to maximize whole-body PD. The current findings allow refinement of the optimum dietary amino acid profile for growing pigs.

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