

# ***Commercial validation study for sulphur amino acid (SAA) requirement in finisher pigs***

**4B-120**

Report prepared for the  
Co-operative Research Centre for High Integrity Australian Pork

By

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## Executive Summary

The previous endotoxin model study (4B-109) demonstrated that pigs whose immune system is activated require approximately 26% more sulphur amino acids than healthy pigs (SID SAA:Lys ratio of 0.73 in comparison to NRC recommendation of 0.59) due to decreased protein utilization efficiency. To validate this finding in commercial farms, two independent commercial validation experiments were conducted (4B-109 and present study) using a range of diets containing varying levels of SID SAA:Lys ratios. A combined data set, which was statistically adjusted for differences in the two experimental batches, was fitted in a quadratic-plateau prediction model to estimate SAA requirement in commercially-housed finisher pigs.

The results are similar to the previous finding and the optimum SID SAA content for maximum feed utilization efficiency was 71% of the SID lysine in a finisher diet for commercially reared pigs. Considering current NRC recommendations of 56% of lysine for 50kg pigs to 59% of lysine for 100kg pigs, it is a 27% to 20% greater SID SAA requirement to negate the chronic exposure to stressors and bacterial/viral pathogens.

From the results of this research, a recommendation is made to formulate diets for finisher pigs to contain more than 70% of standardised ileal digestible sulphur amino acids in relation to standardised ileal digestible lysine content.

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# 1. Introduction

Pigs are continuously exposed to bacterial and viral challenges throughout their life. The pigs' response to these immune challenges is initiated by the release of cytokines which activate the cellular and humoral components of the immune system such as phagocytes and antibodies, respectively (Stahly, 1996). Immune responses, such as the high level of cytokine release, increase metabolic use of protein (i.e. synthesis of acute phase proteins in the liver) and hence decrease body protein accretion (van der Klis and Jansman, 2002). For example, Williams et al. (1997) demonstrated that pigs with high immune system activation reduced daily gain (11%), feed intake (29%) and body protein accretion (38%), and increased feed conversion ratio (FCR) (20%) compared with pigs with low immune system activation from 6 to 27 kg body weight. Moreover, Breuille et al. (1994, 1998) used an *E. coli* infection model in rats and reported that infected rats significantly increased liver protein synthesis (33% vs. 15%) whilst muscle protein synthesis was significantly decreased. Therefore, a mild bacterial disease challenge, which would be common in commercial production facilities, may significantly decrease feed efficiency by re-directing (partitioning) amino acids from body protein synthesis to immune activation to address this challenge.

Those amino acids that are used for synthesis of immune molecules may, therefore, be in short supply and hence be the reason for a limit to body protein deposition in commercial production systems. These amino acids include cysteine, threonine, serine, aspartate and asparagine (Rakhshandeh et al., 2010). Among those specific amino acids listed, sulphur amino acids (SAA), especially cysteine, are the most abundantly used amino acids for synthesis of immune molecules. For example, about 40% of glutathione and defensins are cysteine, and other acute-phase proteins also require high proportions of SAA.

In formulating diets for pigs, the supply of standardized ileal digestible (SID) lysine is determined relative to digestible energy (i.e. SID Lys/MJ DE). This is because lysine is the first limiting amino acid, or in other words the amino acid that is most likely to be deficient in a typical commercial diet. The levels of all other essential amino acids, including SAA, are set as a ratio to lysine. In this way, when the level of SID Lys/MJ DE is altered then the supply of all essential amino acids are automatically also changed. The current recommended SAA requirements for pigs are presented in Table 1 (NRC, 2012).

**Table 1 - NRC recommended sulphur amino acid requirements in pigs (NRC, 2012)**

Body weight, kg	5-25	25-50	50-75	75-100	100-135
SAA (% SID lysine)	55.0	56.0	56.0	58.0	59.0

A recent collaborative study between Murdoch University, DAFWA, Nutreco (The Netherlands), Evonic (Germany), University of Copenhagen (Denmark), and University of Guelph (Canada), investigated the impact of *E. coli* infection on SAA requirement in weaned pigs. The experiment was conducted in the Netherlands and found that weaner pigs challenged with an enterotoxigenic strain of *E. coli* increased SAA requirement by 30 % (72% of SID lysine compared with current recommendation of 55% of SID lysine for weaner pigs, Capozzalo et al., unpublished data).

Evidence from a pilot study conducted at the Medina Research Station (4B-109) supported the hypothesis that finisher pigs with immune system activation require diets containing higher concentrations of SAA compared to a healthy herd to achieve the similar level of performance. In this study, pigs were injected with an *E.coli* lipopolysaccharide (serotype 055:B5) to activate the immune system. Average daily gain (ADG) in pigs with chronic immune system activation increased linearly up to a SID SAA:Lys ratio of 0.75 for finisher 1 and 2 diets. In comparison, ADG in the control animals reached a plateau at a SID SAA:Lys ratio of 0.55. Similarly, FCR of the control animals reached a plateau at a SID SAA:Lys ratio of 0.55, while the corresponding ratio in the immune activated group was 0.65 (Kim et al., 2012).

A commercial validation study was conducted at Rivalea (4B-109). Elevated plasma haptoglobin levels were confirmed in these animals, indicating consistent exposure to various pathogens in the commercial herd. The outcomes from this study suggested that the SAA requirement for optimal FCR may be around 26% greater than current recommendations (SID SAA:Lys ratio of 0.73 compared with NRC recommendation of 0.58). However, diets used in the Rivalea experiment had significantly lower lysine contents than specified in the original formulations, which eventuated in increased SID SAA:Lys ratios that were above the current recommended levels. Before recommendations are delivered to industry, the dose response study needs to be reassessed with the target SID SAA:Lys ratios. Therefore the aim of this study was to validate the recommendation that a 26% increase in SAA requirement will minimise feed conversion ratio (FCR) in a commercial finisher herd.

The hypotheses tested in this experiment was that finisher pigs raised in a commercial facility will have a 26% higher SAA requirement for minimum FCR than the current dietary recommendation of 0.58 SID SAA:Lys ratio.

## 2. Methodology

### *Animals, diets and feeding*

This experiment was conducted at Rivalea Australia Pty Ltd (Corowa, NSW). A total of 672 female pigs weighing  $56.4 \pm 1.29$  kg (mean  $\pm$  SEM) were stratified to 48 pens (14 pigs/pen) and 6 dietary treatments (8 pens/treatment). The 8 diets were formulated to contain SID SAA:Lys ratios of 0.45, 0.55, 0.59, 0.66, 0.73 and 0.80. Diets were formulated to contain 13.8 MJ DE/kg, and 0.56 g SID lysine/MJ DE. Composition of the experimental diets is presented in Table 2.

Pigs received their respective experimental diets *ad libitum* for 6 weeks and fresh water was accessible via a nipple drinker. Water medication was supplied during the 19-20<sup>th</sup> days (CTC, 20 g/tonne/day) and the 33-34<sup>th</sup> days (Tylan in water, 23 g/tonne/day). Pigs were weighed and feed intake was measured at days 0, 21 and 42. At the end of the 42 days of the experiment, all pigs were slaughtered at a commercial abattoir and carcass composition was recorded. Blood samples were collected from three randomly selected pigs per pen at day 21 and analysed for plasma urea and haptoglobin levels.

Table 2 - Composition of experimental diets (g/kg as-fed basis).

SAA:Lys ratio	0.45	0.52	0.59	0.66	0.73	0.80
Wheat	470.5	469.7	469.5	468.7	468.4	467.6
Barley	396.7	396.9	396.7	396.9	396.6	396.8
Meat meal	76.5	76.5	76.4	76.5	76.4	76.5

SAA:Lys ratio	0.45	0.52	0.59	0.66	0.73	0.80	
Tallow	24.9	24.9	24.9	24.9	24.9	24.9	
Limestone	9.27	9.27	9.27	9.27	9.27	9.27	
Dicalcium phosphate	8.67	8.68	8.67	8.68	8.67	8.67	
Methionine	-	0.57	1.13	1.67	2.26	2.79	
Choline Cl 60%	0.57	0.57	0.57	0.57	0.57	0.57	
Lysine	4.49	4.49	4.49	4.49	4.48	4.49	
Threonine	1.53	1.53	1.52	1.53	1.52	1.53	
Tryptophan	0.13	0.13	0.13	0.13	0.13	0.13	
Salt	1.99	1.99	1.99	1.99	1.99	1.99	
Organic acid blend <sup>1</sup>	1.99	1.99	1.99	1.99	1.99	1.99	
Vit/Min <sup>2</sup>	2.22	2.22	2.22	2.22	2.22	2.22	
Salinomycin	0.50	0.50	0.50	0.50	0.50	0.50	
Total	1000	1000	1000	1000	1000	1000	
Calculated composition, g/kg							
DE, MJ/kg	13.76	13.76	13.76	13.76	13.76	13.77	
g SID Lys/MJ DE	0.56	0.56	0.56	0.56	0.56	0.56	
SID SAA: SID Lys	0.45	0.52	0.59	0.66	0.74	0.80	
Analysed composition, g/kg							
Lysine	8.0	7.9	8.0	7.8	8.0	8.0	
Methionine	2.3	2.6	3.1	3.6	3.9	4.5	
Cysteine	2.6	2.6	2.6	2.6	2.6	2.6	
SAA	4.9	5.2	5.7	6.2	6.5	7.1	
Threonine	5.9	6.0	5.5	6.2	6.2	6.0	
Isoleucine	3.7	4.2	3.7	4.1	3.8	4.2	
Leucine	9.0	9.6	9.2	9.0	9.1	9.4	
Valine	5.5	5.7	5.3	5.5	5.3	5.7	
Corrected SAA:Lys <sup>3</sup>	SID	0.54	0.60	0.66	0.76	0.78	0.87

<sup>1</sup>Fysal, Selko Feed Additives, the Netherland.

<sup>2</sup>Provided per kg of air-dry diet: vitamins: A 5000 IU, D3 1000 IU, E 30 IU, K 0.67 mg, B1 1.0 mg, B2 3.3 mg, B6 2.0 mg, B12 3.3 mg, pantothenic acid 10 mg, niacin 13 mg; minerals: Co 0.7 mg, Cu 50 mg, iron 50 mg, Mn 50 mg, Se 0.3 mg, Zn 75 mg, Cr 160 ppb.

<sup>3</sup>Corrected SID AA = (calculated SID AA x analysed AA content) / calculated AA content.

### **Chemical analysis**

The amino acid content of the diets was measured according to the method described by Ranyer (1985) and modified by Barkholt and Jensen (1989). Briefly, a 100 mg sample was hydrolysed with 6 M HCl, 0.5% phenol and 0.05% dithiodipropionic acid to convert protein-bound AA to free AA. The AA in the hydrolysate then underwent pre-column derivatisation with o-phthalaldehyde and fluorenylmethylchloroformate according to Hewlett Packard Technical Note PN 12-5966-311E. The AA derivatives were then separated and quantified by reverse phase HPLC (Hewlett Packard 1100 HPLC with Diode array detector). An Agilent Hypersill AA-ODS column (200 mm x 2.1 mm, 5 microns) with a pre-column was used for all analyses. The column temperature employed was 30 °C, detection was at 338 nm for primary and 262 nm for secondary AAs, and the flow rate was 0.3 mL/min. Plasma samples were deprotenised using 5-sulphosalicylic acid and determined amino acid profile using reverse phase HPLC (Hewlett Packard 1100 HPLC with Diode array detector).

Haptoglobin content in the plasma sample was determined using a modified method of Makimura and Suzuki (1982). Modifications are a higher concentration of sodium dihydrogen phosphate dihydrate (30 mM in reaction mix) and the use of a commercial supply of haemoglobin (Sigma-Aldrich, H2625) to produce the haemoglobin reagent (30 g/L in normal saline). The method was adapted onto an Olympus Au400 Autoanalyser (Olympus, Tokyo, Japan). Plasma urea content was measured using a urease kinetic method with an automatic analyser (Randox Daytona, Crumlin Co., Antrim, UK).

### ***Statistical analysis***

The present commercial validation study data (Experiment 2) was analysed by one-way analysis of variance procedure of Genstat 15. The pen was the experimental unit. Broken line analysis and quadratic regression analysis were used to estimate SAA requirement for finisher pigs.

The data collected from the previous study (Experiments 1, 4B-109) and the current study (Experiment 2, 4B-102) were then pooled for broken line analysis. As the experimental data were partly influenced by the two experimental batches and the experimental design turned to be unbalanced, the unbalanced analysis of variance was used with the experimental batches set as blocks. The pigs in the previous experiment (4B-109) were fed diets for 56 days while the present experiment fed the diets for 42 days. Therefore, performance data for 42 days were taken from Experiments 1 and 2 for statistical analysis. Average start and finish body weights were 47.0 and 86.2 kg for Experiment 1 and 56.4 and 95.3 kg for Experiment 2, respectively. The adjusted data were fitted to the linear-plateau and quadratic-plateau models to estimate SID SAA requirement in commercially-housed finisher pigs using the Nutritional Response Models 1.1 (University of Georgia).

## **3. Outcomes**

### ***3.1 Performance and SAA requirement in the current experiment***

A total of 11 pigs were removed (5 dead and 6 ill thrift) due to swine dysentery, ileitis and lameness during the 42 day feeding experiment and there was no statistical difference in the number of pigs removed from the experiment between treatments. Performance response to the increasing SID SAA:Lys ratios in the current study is presented in Table 2. In the first 21 days, average daily gain (ADG) was decreased ( $P < 0.01$ ) with increasing SID SAA:Lys ratios in the diet. However, during the 22-42 days, feed conversion ratio (FCR) was decreased with increasing SID SAA:Lys ratios ( $P < 0.01$ ), without affecting average daily feed intake (ADFI). Overall for 42 days, increasing SID SAA:Lys ratios linearly increased loin depth ( $P < 0.01$ ).

The optimum SID SAA:Lys ratio for minimum FCR was estimated using a linear-plateau and quadratic-plateau models (Figure 1). Requirement for SID SAA in relation to SID lysine content was 66 % of SID lysine and 69.5% of lysine, when the data were fitted to a linear-plateau and a quadratic-plateau model, respectively.

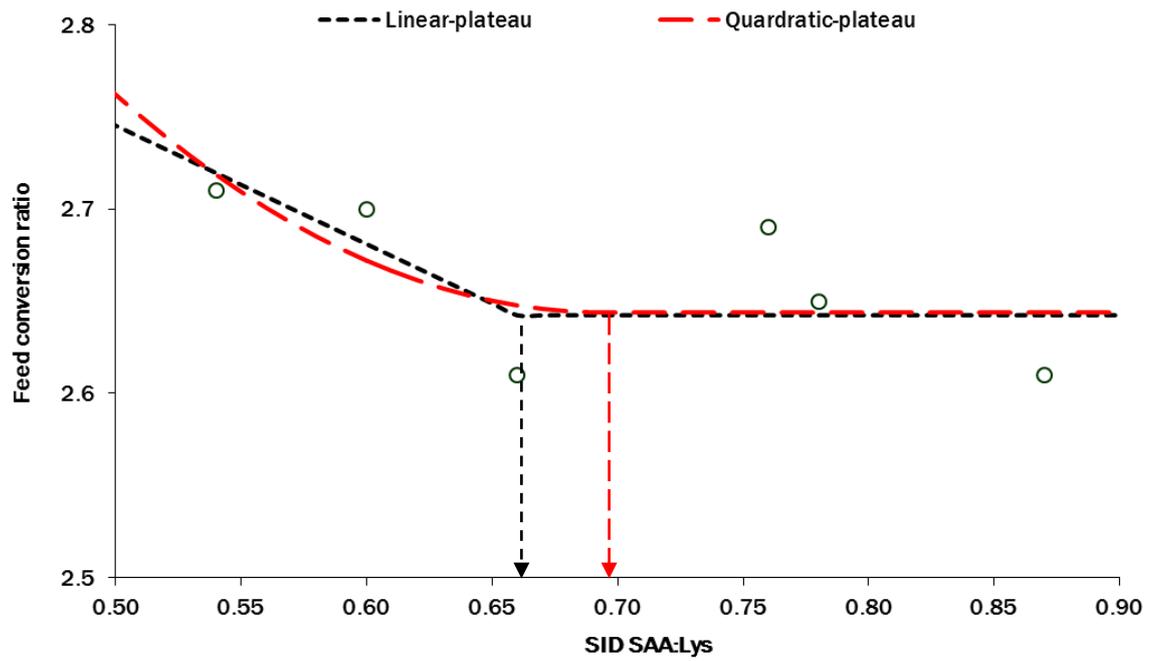


Figure1 - Requirement for SID SAA in relation to SID lysine content estimated using either a linear-plateau (SID SAA:Lys = 0.66,  $R^2 = 0.52$ ,  $P=0.019$ ) or a quadratic-plateau model (SID SAA:Lys = 0.695,  $R^2 = 0.45$ ,  $P=0.033$ ) in the finisher pigs.

**Table 3 - Performance and carcass responses of commercially-housed finisher pigs to increasing levels of standardized ileal digestible sulphur amino acids to lysine ratio fed for 42 days before-slaughter (Experiment 2).**

	Corrected SID SAA:Lys ratio						SEM	Significance	
	0.54	0.60	0.66	0.76	0.78	0.87		P=	Lin
Start weight, kg	56.4	56.3	56.5	56.5	56.5	56.3	1.29	1.000	0.988
Finish weight, kg	95.4	93.9	97.0	94.2	96.0	95.4	1.84	0.852	0.884
Day 0-21 <sup>1</sup>									
ADG, Kg	1.02	0.98	1.00	0.95	0.99	0.95	0.016	0.034	0.006
ADFI, kg	2.47	2.39	2.43	2.36	2.43	2.36	0.035	0.203	0.076
FCR	2.43	2.44	2.44	2.48	2.45	2.48	0.043	0.912	0.275
Day 21-42 <sup>1</sup>									
ADG, Kg	0.84	0.81	0.93	0.84	0.89	0.91	0.040	0.223	0.157
ADFI, kg	2.56	2.44	2.61	2.46	2.54	2.49	0.077	0.612	0.676
FCR	3.11	3.06	2.80	2.93	2.86	2.75	0.084	0.025	0.004
Day 0-42 <sup>1</sup>									
ADG, Kg	0.93	0.89	0.96	0.90	0.94	0.93	0.021	0.203	0.787
ADFI, kg	2.51	2.41	2.52	2.41	2.49	2.43	0.051	0.445	0.362
FCR	2.71	2.70	2.61	2.69	2.65	2.61	0.035	0.151	0.060
HSCW, kg <sup>2</sup>	74.5	74.8	74.6	74.6	74.7	75.2	0.24	0.489	0.135
Dressing, % <sup>2</sup>	78.3	79.0	77.9	79.2	78.1	78.5	0.84	0.864	0.922
P2 back fat, mm <sup>2</sup>	10.4	10.3	10.6	10.1	10.5	10.7	0.24	0.536	0.467
Loin depth, mm <sup>2</sup>	51.3	51.9	51.3	52.1	53.3	52.7	0.48	0.022	0.005

ADG: average daily gain; ADFI: average daily feed intake; FCR: feed conversion ratio; HSCW: hot standard carcass weight.

<sup>1</sup>Start weight was used as a covariate for statistical analysis.

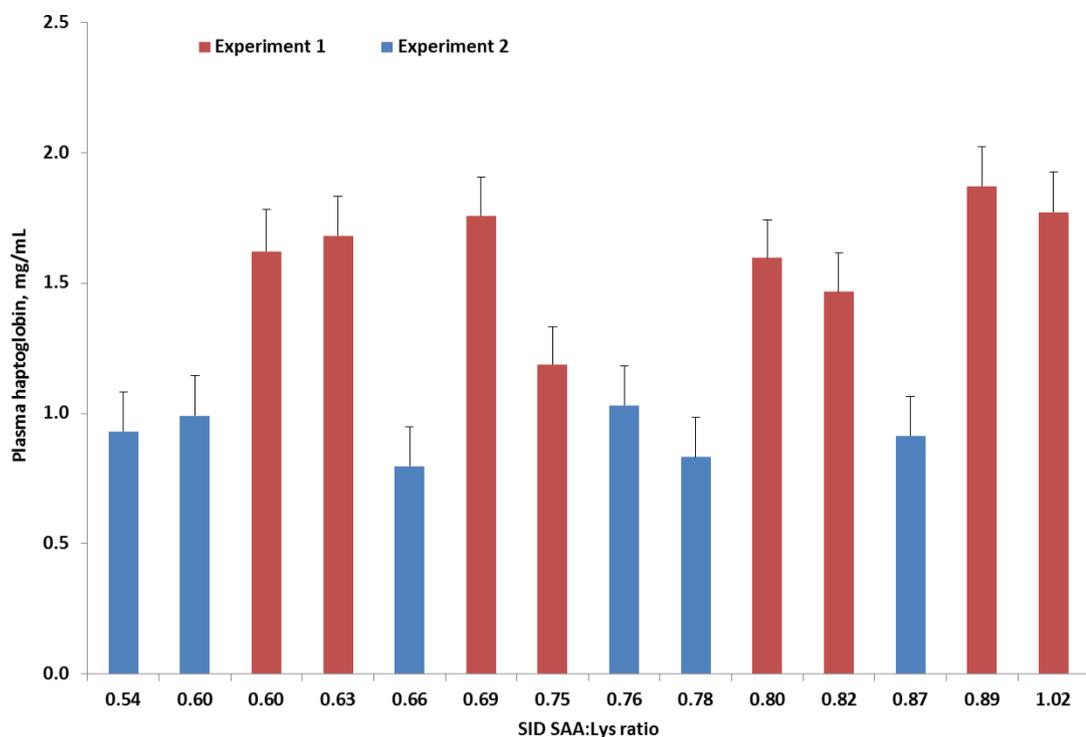
<sup>2</sup>Finish weight was used as a covariate for statistical analysis.

### 3.2. Plasma measures: the combined data set

Results of the present and the previous experiment (4B-109) were combined and analysed to estimate SAA requirement in the commercially-housed finisher herd.

Haptoglobin content was measured to gauge the level of immune system stimulation and presented in Figure 2.

The haptoglobin content between the two experiments were significantly different ( $P < 0.001$ ) and pigs used in Experiment 2 had a lower level of immune system stimulation than pigs used in Experiment 1. Use of in-feed antibiotic (Salinomycin) and water medications (CTC 20 g/tonne in 19-20<sup>th</sup> days and Tylan 23 g/tonne in 33-34 days) in Experiment 2 may have contributed to the lower level of haptoglobin compared with Experiment 1 which only used water medication (Tylan 10 mg/tonne, once every 14 days).



**Figure 2 - Plasma haptoglobin contents determined in the finisher pigs from the two experiments. Average haptoglobin levels were 1.61 and 0.92 mg/mL for Experiments 1 and 2, respectively (batch effect,  $P = 0.001$ ). Dietary treatment had no effect on plasma haptoglobin content ( $P > 0.05$ ).**

Plasma urea contents were measured to monitor amino acid utilization efficiency and presented in Figure 3. There were no differences in plasma urea content between the two experiments but dietary treatment had a significant effect ( $P < 0.01$ ). However, as diets from Experiment 1 and 2 had different lysine contents, and there was a different level of immune system activation, plasma urea could not be used as an index for protein utilization efficiency. Consequently, there was no linear relationship between plasma haptoglobin and urea ( $P > 0.05$ ).

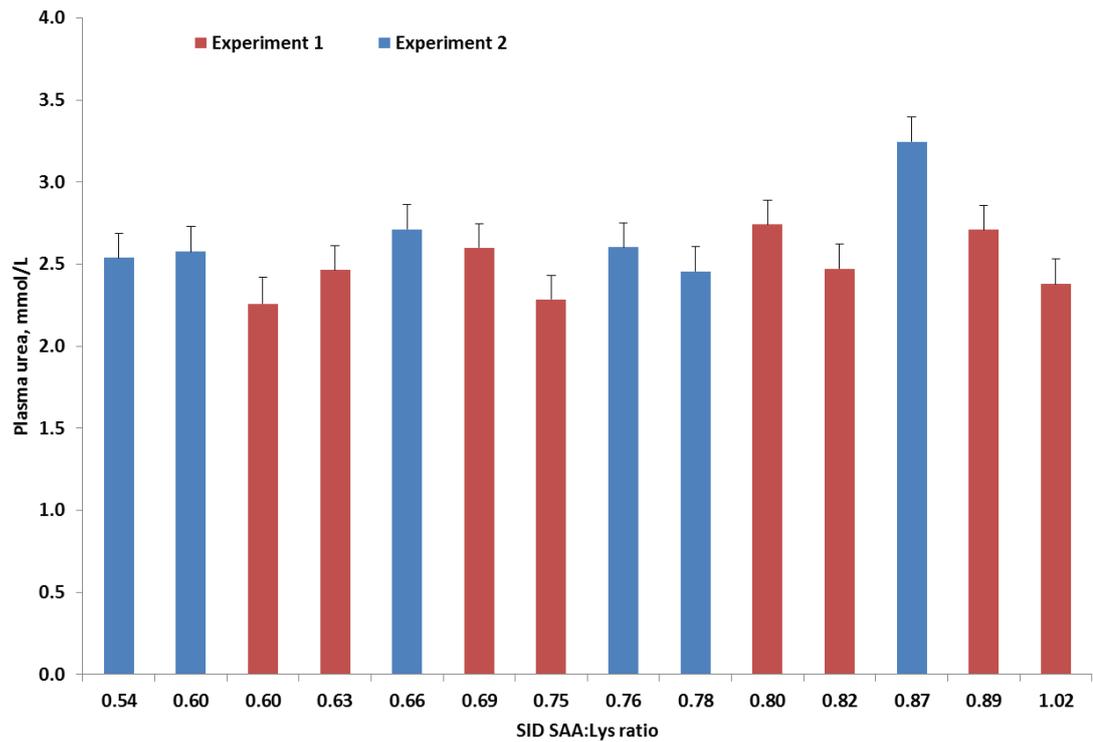


Figure 3 - Plasma urea contents determined in the finisher pigs from the two experiments. Average urea contents were 2.5 and 2.7 mmol/L for Experiments 1 and 2, respectively (no batch effect,  $P>0.05$ ). Dietary treatment significantly affected plasma haptoglobin contents ( $P<0.01$ ).

### 3.3. SAA requirement predicted from the combined data set

The combined data were fitted either to a linear-plateau model or a quadratic-plateau model and the results were presented in Figure 4. Based on the analysis, the minimum FCR of the commercial finisher herd was achieved at SID SAA:Lys ratio of 0.64 and 0.71 for linear-plateau and quadratic-plateau prediction models, respectively.

In the combined data, increasing SID SAA:Lys ratio had no relationship with ADG, however, increasing SID SAA:Lys ratio decreased ADFI in a linear-plateau manner (Figure 5) that was comparable to the pigs' FCR response to the increasing SID SAA:Lys ratios. The ADFI of the commercial finisher herd reached a plateau at a SID SAA:Lys ratio of 0.63 and 0.71 for linear-plateau and quadratic-plateau prediction models, respectively (Figure 5). These results suggest that under the experimental conditions, increasing dietary SID SAA:Lys ratios decreased feed intake but maintained daily growth rate via improving feed utilization efficiency.

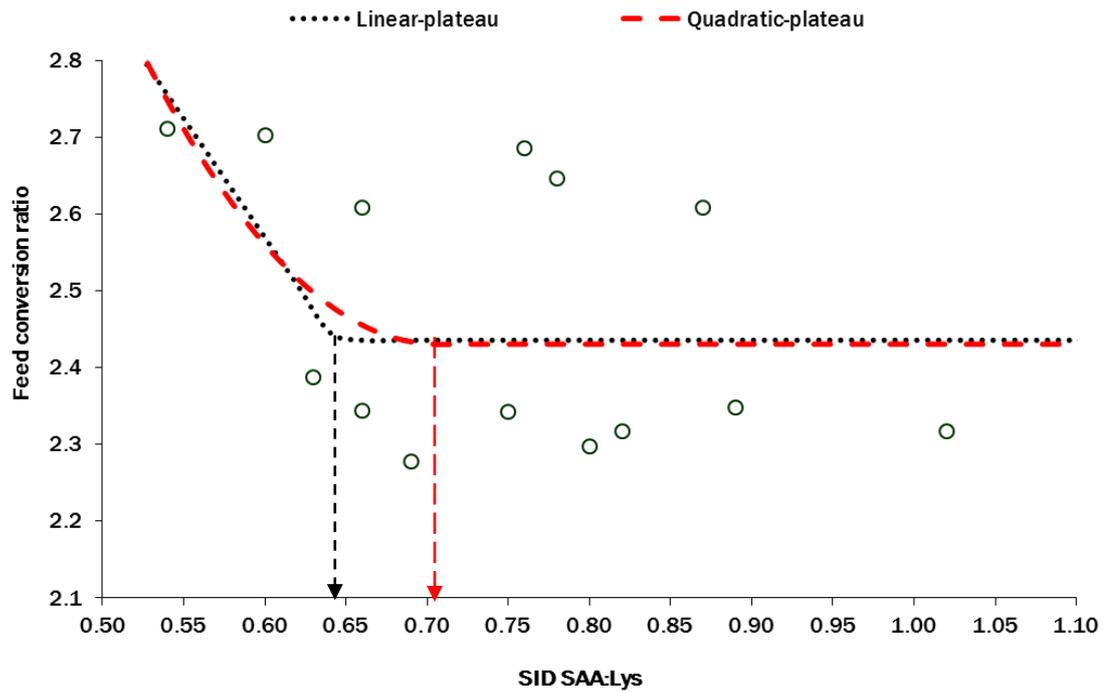


Figure 4 - Requirement for SID SAA in relation to SID lysine content estimated using either a linear-plateau (SID SAA:Lys = 0.64,  $R^2 = 0.26$ ,  $P < 0.001$ ) or a quadratic-plateau model (SID SAA:Lys = 0.71,  $R^2 = 0.25$ ,  $P < 0.001$ ) in the finisher pigs.

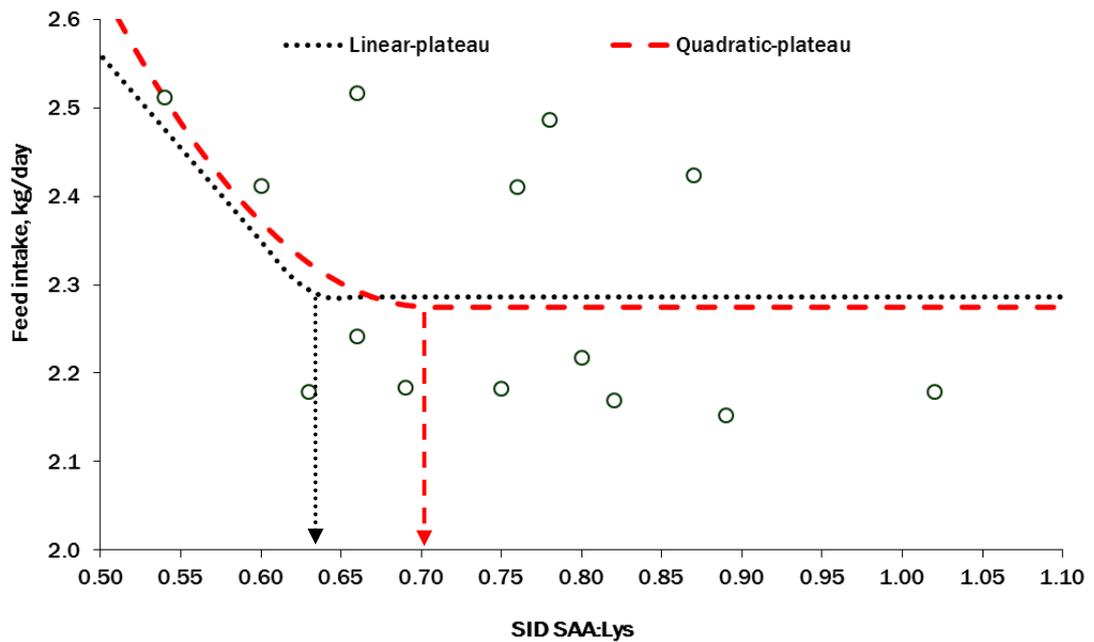


Figure 5 - Requirement for SID SAA in relation to SID lysine content estimated using either a linear-plateau (SID SAA:Lys = 0.63,  $R^2 = 0.21$ ,  $P < 0.001$ ) or a quadratic-plateau model (SID SAA:Lys = 0.71,  $R^2 = 0.20$ ,  $P < 0.001$ ) in the finisher pigs (Outliers removed).

## 4. Application of Research

The endotoxin model study (4B-109) demonstrated that the pigs whose immune system is activated require approximately 26% more sulphur amino acids (SAA) than healthy pigs due to decreased protein utilization efficiency. To validate this finding in commercial farms, two independent experiments were conducted (4B-109 and the present study) using a range of diets containing varying levels of SID SAA:Lys ratios. The combined data set which was statistically adjusted for differences in the two experimental batches was fitted in both linear-plateau and quadratic-plateau prediction models. The results are similar to the previous finding that the finisher pigs require 64% or 71% SID SAA in relation to SID lysine content in the diet to utilize feeds efficiently. Unlike the nutrient requirement estimated from individually-housed pigs, the nutrient requirement estimated from a population of pigs such as group housed pigs is recommended to use the quadratic-plateau model, which better integrates population variation (Pomar et al., 2003). Therefore, the ideal SID SAA content for maximum feed utilization efficiency is 71% of the SID lysine in a finisher diet for commercially reared pigs. Considering current NRC recommendations of 56% of lysine for 50kg pigs to 59% of lysine for 100kg pigs, it is a 27% to 20% greater SID SAA requirement, respectively, for 50kg and 100kg pigs that is likely required to negate the chronic exposure to stressors and bacterial/viral pathogens (Rakhshandeh et al., 2010).

An unexpected but interesting finding is that the improved FCR symmetrically observed in the endotoxin injection model and the commercial validation studies were driven by different factors. When the pig's immune system was activated by intramuscular injection of *E. coli* lipopolysaccharides the increased SAA content in the diet decreased FCR by increased growth rate (4B-109). In contrast, in the commercially housed pigs with natural exposure to pathogens through the oral and respiratory routes, increasing SAA content in the diet decreased FCR by maintaining the growth rate while feed intake was decreased most likely due to subclinical infection response (Present study). Therefore, pig's responses to the increasing dietary SAA content may differ dependent on the origin and severity of the immune system activation. The underlying mechanism for this physiological response is unknown and further clarification is required.

Nevertheless, after a series of experiments, it can be concluded that under the experimental conditions where pigs are continuously exposed to chronic immune system activation, SID SAA requirement for finisher pigs should be 20-27% higher (i.e., SID SAA:Lys ratio of 0.71) than current NRC recommendation. Increasing SID SAA may not improve daily gain or feed intake, however, it will ensure maximum feed conversion efficiency in immune system compromised pigs.

## 5. Conclusion

This research showed that increasing SAA:Lys ratio above the current NRC recommendation in commercially reared finisher pigs will maximise feed utilisation efficiency. From the results of this research, it is recommended formulating diets for finisher pigs to contain 71% of standardised ileal digestible sulphur amino acids in relation to standardised ileal digestible lysine content.

## 6. Limitations/Risks

The optimum SID SAA requirement can vary dependent on the origin and severity of immune system activation. The current recommendation is based on the experimental conditions; however, the variation in SAA requirement is expected to be low between farms.

## 7. Recommendations

As a result of the outcomes in this study the following recommendations have been made:

- Formulate diets for finisher pigs to contain more than 70% of standardised ileal digestible sulphur amino acids in relation to standardised ileal digestible lysine content.

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