SULPHUR AMINO ACID SUPPLEMENTATION TO IMPROVE HERD FEED CONVERSION EFFICIENCY IN COMMERCIAL GROWER PRODUCTION SYSTEM

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Report prepared for the

Co-operative Research Centre for High Integrity Australian Pork

By

Jae Cheol Kim[1], Hugh Payne[1], Cherie Collins[2], David Henman[2] and Bruce Mullan[1]

[1]Livestock Innovation, Department of Agriculture and Food, South Perth, WA 6151

[2]Rivalea Australia Pty Ltd., Corowa, NSW 2646

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Executive Summary

Pigs kept under commercial conditions are continuously exposed to microorganisms, and typically respond to these immune system challenges by elevated release of cytokines, increased metabolic use of protein (i.e. synthesis of acute phase proteins in the liver and production of antibodies), and decreased protein deposition. A mild bacterial disease challenge for example, may significantly alter nutrient partitioning from protein deposition to the synthesis of immune molecules, and hence the amino acids used for the synthesis of these molecules may become deficient in diets and limit maximum rates of protein deposition. Sulphur amino acids, especially cysteine, are one of the most abundantly used amino acids for synthesis of immune function-related molecules. A previous study indicated that when the pig's immune system is activated, a significant proportion of sulphur amino acids are re-directed and retained in non-protein compounds such as glutathione, while at the same time muscle protein anabolism is compromised. However, the role of sulphur amino acids on protein utilization efficiency of finisher pigs grown in commercial production facilities, where pigs are continuously exposed to immune system challenges, has not been explored as yet. Therefore, the rationale for this project was that current recommendation for SAA requirement based on empirical studies at a hygienic research facility may significantly underestimate the true SAA requirement for finisher pigs housed in less hygienic commercial facilities and significantly reduce herd feed conversion efficiency.

To determine SAA requirement of finisher pigs housed in commercial pig production systems, two experiments were conducted. The first pilot experiment was conducted in a research facility (Medina Research Station) using an immune system activation model. Pigs received twice-weekly intramuscular injection of either sterile saline or E. coli endotoxin, to simulate conditions in a hygienic research facility or continuous pathogen exposure under commercial conditions, respectively, to compare the role of increasing dietary SAA on protein utilization efficiency. This pilot study clearly demonstrated that SAA requirement expressed as a proportion of lysine for immune system activated pigs was 0.75 and was significantly higher than for healthy pigs at 0.55. Unlike healthy pigs, immune system activated pigs did not achieve maximum protein deposition (67 vs. 59 g/d, respectively) at the current recommended SAA level of 0.55. However, protein deposition rate in immune system activated pigs returned to the pre-infection level of 67 g/d when the dietary SAA:lysine ratio was increased to 0.75. Based on assumption that the E. coli endotoxin model represents the level of pathogen challenges in the commercial facility, the results of this pilot study indicate that increasing dietary SAA in commercial finisher pigs will significantly improve herd feed conversion ratio. To validate the findings of the pilot study, a commercial study using a dose-response design was conducted at Rivalea Australia Pty Ltd. Results indicate that minimum feed conversion ratio and maximum carcass gain were achieved at dietary SAA:Lys ratio of 0.71 and 0.73, respectively. Level of plasma haptoglobin in the Rivalea herd was greater than the healthy pigs at Medina but was lower than the E. coli endotoxin injected pigs. This finding suggests the severity of immune system activation may affect SAA requirement in pigs.

Evidence from this project suggests that a higher level of SAA is required for efficient utilization of protein in finisher pigs raised in commercial production facilities and a minimum SAA:Lys ratio of 0.71 be recommended for finisher pigs in commercial facilities. When the severity of immune system activation (i.e., level of pathogen load) is greater, SAA:Lys ratio may need to be increased further.

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The commercial validation study was designed and conducted by Dr Cherie Collins and Mr David Henman at Rivalea Australian Pty Ltd., Corowa, NSW.

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 (Beisel, 1991, Stahly, 1996, See Figure 1). 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 (Klis and Jansman, 2002). For example, Williams et al. (1997) demonstrated that pigs with high immune system activation had a reduction in daily gain (11%), feed intake (29%), body protein accretion (38%) and FCE (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, 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.

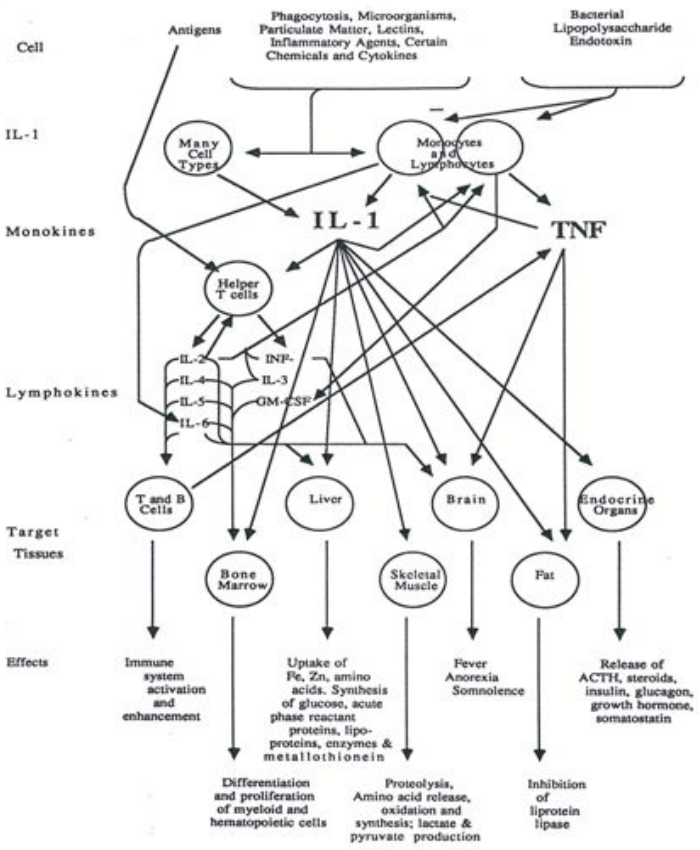


Figure 1 - Acute phase response to infection (After Beisel, 1991)

The amino acids that are used for synthesis of immune molecules may, therefore, be in short supply and hence be the reason for limiting body protein deposition in commercial production systems. These amino acids include the sulphur amino acids cysteine, threonine, serine, aspartate and asparagine (Rakhshandeh et al., 2010). Among those specific amino acids listed, sulphur amino acids, 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 sulphur amino acids.

A recent Canadian study (Rakhshandeh et al., 2010) used an E. coli lipopolysaccharide injection model to stimulate the immune system of 20 kg male pigs while feeding the pigs with gradually increased levels of dietary sulphur amino acids (1.3, 2.9 and 4.2 g/kg). The study found that (1) immune system stimulation decreased whole body N retention but increased whole body sulphur amino acid retention, thereby significantly decreasing whole body nitrogen:sulphur amino acid (N:S) balances; and (2) immune stimulation increased dietary requirements for SAA relative to other amino acids, as increasing dietary SAA significantly reduced the N:S balance compared with healthy pigs. These results indicate that when the pigs' immune system is activated, a significant proportion of SAA are re-directed and retained in non-protein compounds, such as glutathione, while at the same time muscle nitrogen anabolism is decreasing. A major advantage of using the E. coli lipopolysaccharide injection model to stimulate the immune system is that all animals will have a similar challenge and hence we are more likely to determine if there is a statistically significant effect of treatment. The E coli lipopolysaccharides injection model is a well established technique which stimulates the immune system but does not cause clinical disease or make the pig actually sick. Therefore, it is an excellent model to simulate conditions for a pig in a dirty environment or on a low health status farm. Furthermore, use of an E coli lipopolysaccharide injection has been demonstrated to consistently result in immune stimulation but does not result in clinical disease, causing only transient elevation in body temperature and mild discomfort at the injection site.

The aim of this project is, therefore, to determine the dietary requirement for SAA in growing pigs that are or are not subject to a bacterial challenge so that we can better understand the relationship between health and nutrient requirements.

2. Methodology

2.1. Infection study

A split plot experiment with 72 male pigs weighing 52.9 ± 0.39 kg (mean ± SEM) was conducted. The main plot was with and without IS activation, and 4 diets containing different amounts of standardized ileal digestible (**SID**) SAA (SAA to lysine ratios of 0.45, 0.55, 0.65 and 0.75, Table 1) were the subplots.

Table 1 - Composition of experimental diets used in the infection study (g/kg as-fed basis).

Phase	**Phase I (50-75 kg)**				**Phase II (75-100 kg)**			
SAA:Lys ratio/ Ingredients	**0.45**	**0.55**	**0.65**	**0.75**	**0.45**	**0.55**	**0.65**	**0.75**
Barley	672.8	660.8	659.9	658.9	725.7	715.0	714.3	713.5
Wheat bran	39.4	50.0	50.0	50.0	0.0	0.0	0.0	0.0
Lupin	131.2	132.8	134.5	136.3	189.7	200.0	200.0	200.0
Lupins kernels	83.9	80.1	79.2	78.3	0.0	0.0	0.0	0.0
Blood meal	1.1	4.5	3.6	2.8	0.0	0.0	0.0	0.0
Canola oil	35.0	35.0	35.0	35.0	48.4	48.4	48.4	48.4
L-Lysine	3.74	3.46	3.53	3.60	3.65	3.53	3.54	3.54
DL-Methionine	0.00	0.77	1.60	2.43	0.00	0.65	1.41	2.17

Phase	Phase I (50-75 kg)				Phase II (75-100 kg)			
SAA:Lys ratio/ Ingredients	**0.45**	**0.55**	**0.65**	**0.75**	**0.45**	**0.55**	**0.65**	**0.75**
L-Threonine	1.30	1.18	1.21	1.24	1.20	1.13	1.14	1.14
L-Tryptophan	0.24	0.20	0.21	0.22	0.24	0.23	0.23	0.23
Vit/Min[1]	0.70	0.70	0.70	0.70	0.70	0.70	0.70	0.70
Limestone	8.1	8.2	8.2	8.2	8.3	8.2	8.2	8.22
DCP	18.8	18.7	18.7	18.7	18.7	18.7	18.7	18.7
Salt	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.00
Choline chloride	1.7	1.7	1.7	1.7	1.4	1.4	1.4	1.4
Total	1000	1000	1000	1000	1000	1000	1000	1000
Calculated composition, g/kg								
DE, MJ/kg	13.5	13.5	13.5	13.5	13.5	13.5	13.5	13.6
g SID Lys/MJ DE	0.60	0.60	0.60	0.60	0.55	0.55	0.55	0.55
SID SAA: SID Lys	0.45	0.55	0.65	0.75	0.46	0.55	0.65	0.75
Analysed composition, g/kg								
Lysine	10.0	9.6	10.2	9.8	9.2	8.9	8.8	9.8
Methionine	1.9	2.9	4.7	5.3	1.9	2.9	3.7	4.2
Cysteine	2.4	2.5	2.8	2.4	2.4	2.2	2.5	2.4
SAA	4.4	5.4	7.5	7.7	4.3	5.1	6.2	6.6

[1]Provided per kg of air-dry diet: vitamins: A 4900 IU, D3 980 IU, E 14 mg, K 0.7 mg, B1 0.7 mg, B2 2.1 mg, B6 1.05 mg, B12 10.5 mg, calcium pantothenate 7.5 mg, folic acid 0.13 mg, niacin 8.4 mg, biotin 21 mg; minerals: Co 0.14 mg, Cu 7 mg, iodine 0.35 mg, iron 42 mg, Mn 28 mg, Se 0.21 mg, Zn 70 mg.

Increasing SAA to lysine ratio (**SAA:Lys**) was achieved by increasing the DL-methionine content in the diet. A two-phase feeding program was employed for Phase I (50-75 kg) and Phase II (75-95 kg), as essential amino acid requirements decrease as pigs grow from 50 to 75 kg. Phase I and II diets were formulated to contain 13.5 MJ DE/kg, and 0.60 and 0.55 g available lysine/MJ DE, respectively.

At the beginning, 8 pigs in the control group were slaughtered in a commercial abattoir and the carcasses were scanned to calculate body protein and fat content at the start of the experiment using dual energy x-ray absorptiometry (**DXA,** Hologic QDR 4500A fan beam X-ray bone densitometer, Hologic, Waltham, MA, USA), which was calibrated for pigs (Suster et al., 2003). Immune system (IS) activation was achieved by intramuscular injection of *E. coli* **LPS** (serotype 055:B5, Sigma, St. Louis, MO, USA; 30 μg/kg BW and increased by 15% in subsequent injections to counteract development of resistance) every Monday and Thursday while control pigs were injected with sterile saline. Daily rectal temperature was measured at 1100 h each day. Blood samples (3 x 8 mL tube) were collected at the end of weeks 2, 4, and 6 to measure plasma urea, blood immune cell count, albumin, plasma haptoglobin, homocysteine (**Hcy**) and plasma AA. All pigs were slaughtered at wk 6 as per commercial practice, and the left side of the carcass was collected and scanned by DXA for estimation of protein deposition rate between 50 – 100 kg.

2.2. Commercial validation study

This experiment was conducted at Rivalea Australia Pty Ltd (Corowa, NSW). Total of 1,344 pigs (672 females and 672 immunocastrates) weighing 47.0 ± 0.38 kg (mean ± SEM) were stratified to 96 pens (14 pigs/pen) and 8 dietary treatments (12 pens/treatment). The 8 diets were formulated to contain SID

SAA:Lys ratios of 0.45, 0.50, 0.55, 0.60, 0.65, 0.70, 0.75 and 0.80. Diets were formulated to contain 14 MJ DE/kg, and 0.60 g and 0.55 g SID lysine/MJ DE for finisher 1 and 2, respectively. Composition of the experimental diets is presented in Tables 2 and 3.

Pigs were acclimatized in the new environment while fed commercial grower pellet diet for 7 days and then received their respective experimental diets for 8 weeks. Pigs were fed the finisher 1 diets for first 21 days and then finisher 2 diets between 22 – 56 days. Water medication (Tylan in water, 10 mgs/tone) was supplied once every 14 days. Immunocastrates were vaccinated with Improvac at 10 and 17 weeks of age. Pigs were weighed and feed intake was measured at days 0, 21, 42 and 56. At the end of the 56 days of feeding trial, all pigs were slaughtered at commercial abattoir and carcass composition was recorded. Blood samples were collected from two randomly selected pigs per pen at day 37 and analysed for plasma urea and haptoglobin levels.

2.3. 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 (for reactive lysine assay, the dithiodipropionic acid was omitted) 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).

Table 2 - Composition of experimental Finisher 1 diets used in the commercial validation study (g/kg as-fed basis).

SAA:Lys ratio/ Ingredients	0.45	0.5	0.55	0.60	0.65	0.7	0.75	0.8
Wheat	390.4	341.1	327.3	312.8	298.3	284.3	266.6	289.2
Barley	267.7	314	327.3	340.7	353.3	366.7	388.3	360.3
Lupin kernels	134.3	133	132.7	132.3	132	131.7	132.7	132
Mill mix	100	100	100	100	100	100	93.3	100
Meat meal	30	30	30	30	30	30	30	30
Blood meal	10	10	10	10	10	10	10	10
Water	10	10	10	10	10	10	10	10
Tallow-enzyme	15	15	15	15	15	15	15	15
Tallow-mixer	16.7	20.3	21.3	22.3	23.3	24.3	25	24
Limestone	11	11	11	11	11	11	11	11
DCP	5	5	4.33	4.33	5	4.33	5	5
L-Lysine	3.67	3.73	3.73	3.73	3.77	3.87	3.77	3.77
DL-Methionine	0.2	0.7	1.13	1.57	2	2.47	2.9	3.3
L-Threonine	1.4	1.47	1.5	1.5	1.53	1.57	1.57	1.57
Tryptophan H/A	0.2	0.2	0.21	0.21	0.21	0.22	0.23	0.23
Isoleucine H/A	0.13	0.2	0.2	0.22	0.23	0.25	0.27	0.27

SAA:Lys ratio/ Ingredients	0.45	0.5	0.55	0.60	0.65	0.7	0.75	0.8
Cu proteinate	1	1	1	1	1	1	1	1
QAF Grower premix[1]	0.67	0.67	0.67	0.67	0.67	0.67	0.67	0.67
Phytase[2]	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Xylanase[3]	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
Choline chloride - 60%	0.33	0.33	0.33	0.33	0.33	0.33	0.33	0.33
Salt	2	2	2	2	2	2	2	2
Total	1000	1000	1000	1000	1000	1000	1000	1000
Calculated composition, g/kg								
DE, MJ/kg	14	14	14	14	14	14	14	14
g ID Lys/MJ DE	0.6	0.6	0.6	0.6	0.6	0.6	0.6	0.6
ID SAA: ID Lys	0.45	0.5	0.55	0.6	0.65	0.7	0.75	0.8
Analysed composition, g/kg								
Lysine	8.3	8.9	9.0	9.1	9.2	9.5	9.3	9.3
Methionine	2.9	3.3	4.0	3.6	4.5	4.8	5.3	5.9
Cysteine	3.1	3.2	3.2	3.3	3.3	3.4	3.4	3.7
SAA	6.0	6.5	7.2	6.8	7.7	8.2	8.7	9.6

[1]Provided per kg of air-dry diet: vitamins: A 4900 IU, D3 980 IU, E 14 mg, K 0.7 mg, B1 0.7 mg, B2 2.1 mg, B6 1.05 mg, B12 10.5 mg, calcium pantothenate 7.5 mg, folic acid 0.13 mg, niacin 8.4 mg, biotin 21 mg; minerals: Co 0.14 mg, Cu 7 mg, iodine 0.35 mg, iron 42 mg, Mn 28 mg, Se 0.21 mg, Zn 70 mg.

[2]Natuphos 5000

[3]Porzyme 9310

Table 3 - Composition of experimental Finisher 2 diets used in the commercial validation study (g/kg as-fed basis).

SAA:Lys ratio/ Ingredients	0.45	0.5	0.55	0.60	0.65	0.7	0.75	0.8
Wheat	344.3	349.8	341.3	314.9	306.1	301	305.2	368.9
Barley	303	314.3	330	338.3	354.7	366.7	395	300
Lupin kernels	110.3	100	100	100	100	99.3	100	86.7
Mill mix	100	91	83.3	98.3	90.33	83.3	51.7	95
Hominy	50	50	50	50	50	50	50	50
Blood meal	13.3	15.3	15.3	15	15	15.3	16.3	17.3
Water	10	10	10	10	10	10	10	10
Tallow-enzyme	15	15	15	15	15	15	15	15
Tallow-mixer	22	22	22	25	25	25	22	22.1
Limestone	16.7	16.7	16.7	16.7	16.7	16.7	16.7	16.7
DCP	5	5	5	5	5	5	5	5
L-Lysine	3.63	3.6	3.6	3.63	3.63	3.63	3.6	3.57
DL-Methionine	0.27	0.67	1.07	1.47	1.87	2.3	2.73	3
L-Threonine	1.4	1.4	1.43	1.43	1.47	1.47	1.47	1.4
Tryptophan H/A	0.17	0.2	0.2	0.2	0.2	0.2	0.2	0.2
Isoleucine H/A	0.33	0.43	0.47	0.47	0.47	0.5	0.53	0.57

SAA:Lys ratio/ Ingredients	0.45	0.5	0.55	0.60	0.65	0.7	0.75	0.8
Cu proteinate	1	1	1	1	1	1	1	1
QAF Grower premix[1]	0.67	0.67	0.67	0.67	0.67	0.67	0.67	0.67
Phytase[2]	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Xylanase[3]	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
Choline chloride - 60%	0.57	0.6	0.6	0.6	0.6	0.6	0.6	0.67
Salt	2	2	2	2	2	2	2	2
Total	1000	1000	1000	1000	1000	1000	1000	1000
Calculated composition, g/kg								
DE, MJ/kg	14	14	14	14	14	14	14	14
g ID Lys/MJ DE	0.55	0.55	0.55	0.55	0.55	0.55	0.55	0.55
ID SAA: ID Lys	0.45	0.5	0.55	0.6	0.65	0.7	0.75	0.8
Analysed composition, g/kg								
Lysine	8.0	8.0	8.5	8.4	8.7	8.4	8.4	8.2
Methionine	2.7	2.7	3.4	3.5	4.0	4.2	4.6	5.1
Cysteine	3.1	3.1	3.2	3.1	3.2	3.1	3.1	3.5
SAA	5.7	5.8	6.6	6.6	7.2	7.4	7.7	8.6

[1]Provided per kg of air-dry diet: vitamins: A 4900 IU, D3 980 IU, E 14 mg, K 0.7 mg, B1 0.7 mg, B2 2.1 mg, B6 1.05 mg, B12 10.5 mg, calcium pantothenate 7.5 mg, folic acid 0.13 mg, niacin 8.4 mg, biotin 21 mg; minerals: Co 0.14 mg, Cu 7 mg, iodine 0.35 mg, iron 42 mg, Mn 28 mg, Se 0.21 mg, Zn 70 mg.

[2]Natuphos 5000

[3]Porzyme 9310

An Aglient 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 ºC, 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).

Plasma urea and serum albumin contents were determined using a urease kinetic method and the bromocresol green method, respectively, with an automatic analyser (Randox Daytona, Crumlin, Co., Antrim, UK). Plasma haptoglobin content was determined using a haptoglobin detection kit (Tridelta Development Ltd., Greystones, Ireland) and plasma AA content was measured as described by Kim et al. (2009). Whole blood immune cell count was done using an automatic haematology analyzer (ADIVA 2120, Bayer Healthcare, Siemens, Germany). Plasma Hcy content was measured spectrophotometrically using an enzyme immune assay.

Homocysteine content was measured using an enzyme immunoassay (the Axis Shield Enzyme Immunoassay Homocysteine Test). Protein-bound homocysteine was reduced to free homocysteine, and was enzymatically converted to S-adenosyl-L-homocysteine (SAH) in a separate procedure prior to the immunoassay. The enzyme is specific for the L-form of homocysteine, which is the only form present in the blood. Disulfide and protein-bound forms of homocysteine were reduced to free homocysteine by use of dithiothreitol. Homocysteine in the test sample was then converted to S-adenosyl-L-homocysteine by the use of SAH hydrolase and excess adenosine. The subsequent solid-phase enzyme immunoassay was based on competition between SAH in the sample and immobilized SAH bound to the walls of the microtitre plate for

binding sites on a monoclonal anti-SAH antibody. After removal of unbound anti-SAH antibody, a secondary rabbit anti-mouse antibody labelled with the enzyme horseradish peroxidase was added. The peroxidise activity was measured spectrophotometrically after addition of substrate, with the absorbance inversely related to the concentration of homocysteine in the sample.

2.4. Statistical analysis

Differences between treatments were assessed by split plot ANOVA procedure of Genstat 12 (VSN International Ltd., Hertfordshire, UK). Linear or broken line regression analyses were conducted to determine SAA requirement in healthy and immune system activated pigs. The individual pig was the experimental unit for statistical analysis of Experiment 1 data. The commercial validation study data (Experiment 2) were analysed by Two-way ANOVA procedure of Genstat 12. The pen was the experimental unit. Broken line analysis and quadratic regression analysis were used to estimate SAA requirement for finisher pigs. Quadratic response curves were fitted to the data to predict the optimum dietary SAA:Lys ratio for maximum daily gain, minimum FCR and maximum carcass yield for finisher pigs. The quadratic curves were fitted using $y=ax^2+bx+d$, where y=either daily gain, FCR, or hot carcass weight, x= SID SAA:Lys ratio and a, b, and c were representative components of the equation.

3. Outcomes

3.1. Infection study

After first injection of 60 mg LPS/kg, two pigs severely reacted to the endotoxin injection and died overnight (1 pig each from the SAA:Lys 0.45 and 0.55 treatment) and one pig was removed from the experiment due to similar hypersensitivity to the endotoxin (from SAA:Lys 0.65 treatment). As a consequence the injection volume was adjusted to 30 μg/kg for the second injection and increased by 15% in every subsequent injection to counteract development of resistance to LPS.

3.1.1. Chronic immune system activation

Chronic immune system activation was successfully established as pigs injected with LPS showed a tendency to increase rectal temperature (38.6 vs. 40.7, $P = 0.09$, Figure 2). Injection of LPS also increased plasma haptoglobin (1.1 vs. 2.0 mg/mL, $P < 0.001$), the proportion of neutrophils (0.39 vs. 0.42, $P < 0.05$), and decreased serum albumin content (38.4 vs. 36.8 g/L, $P < 0.01$; Figure 3). It is reported that bacterial infection increases the neutrophil content in pigs (Zhang et al., 1997) and decreases the negative acute phase protein, albumin (Lampreave et al., 1994). Based on the assumption that the experimentally induced chronic immune system activation in the present study mimics pathogen exposure of pigs under commercial conditions, the role of increasing dietary SAA (as DL-methionine) on whole body PD rate, plasma urea content as an index for protein utilization efficiency, and plasma AA profiles were examined.

3.1.2. Performance

Performance response of pigs to dietary SAA:Lys ratio is presented in Table 4 and Figure 4. Due to the severe reaction to the first endotoxin introduction, many pigs did not eat and growth rate was low during the first week. Therefore, the performance data for the first week of the experiment was removed from the data set for statistical analysis. Body weight of pigs without and with chronic immune system activation responded differently to the dietary SAA:Lys ratios.

Pigs that received a saline injection showed a quadratic response to dietary SAA:Lys ratio with the heaviest body weight achieved at a SAA:Lys ratio of 0.55. In contrast, pigs that received the LPS injection showed a linear response to dietary SAA:Lys ratio and the heaviest body weight was achieved at a SAA:Lys ratio of 0.75 (Table 4).

Average daily gain of pigs without immune system stimulation reached a plateau at a SAA:Lys ratio of 0.55 (Quadratic $P<0.001$), while it was increased linearly up to the SAA:Lys ratio of 0.75 in the pigs with chronic immune system activation (Linear $P<0.001$). Voluntary feed intake of pigs without immune system activation peaked at a SAA:Lys ratio of 0.55 and gradually decreased thereafter with increasing SAA:Lys ratio (Quadratic $P=0.079$). In contrast voluntary feed intake of pigs with immune system activation was linearly increased to a SAA:Lys ratio of 0.75 (Linear $P<0.05$).

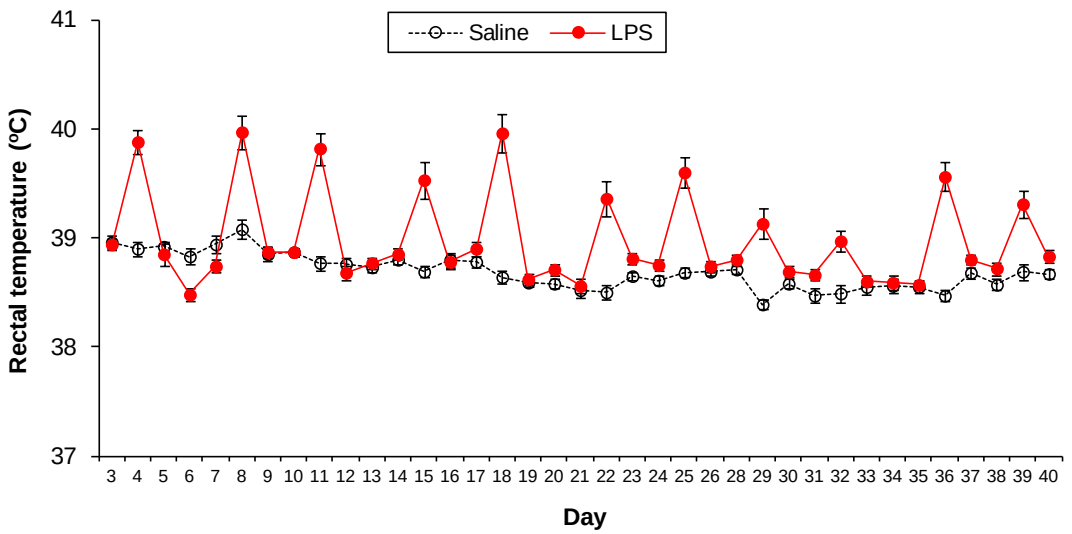


Figure 2 - Rectal temperature measured at 1100 h daily in pigs intramuscularly injected either saline (control) or E. coli lipopolysaccharides (LPS).

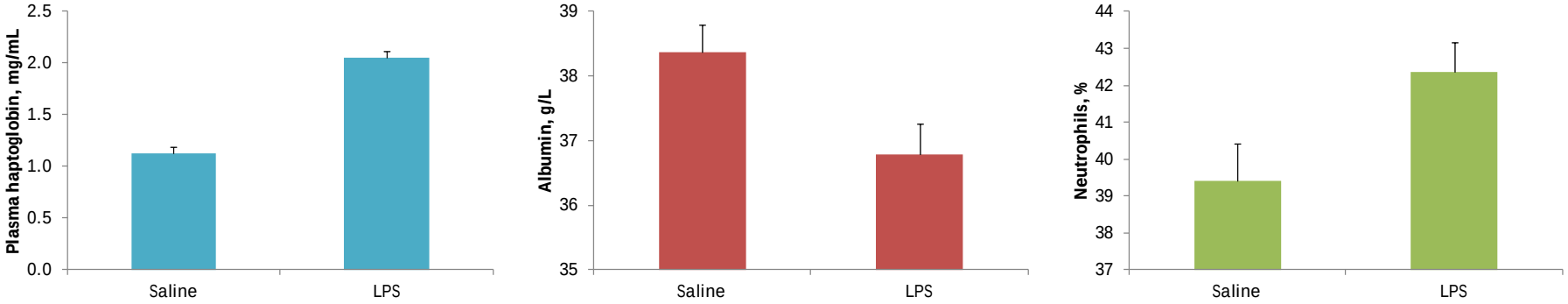


Figure 3 - Plasma haptoglobin, plasma albumin and proportion of neutrophils in pigs intramuscularly injected either saline (control) or E. coli lipopolysaccahrides (LPS).

Table 4 - Effect of dietary SAA:Lys ratio in pigs without and with chronic immune system activation on performance of pigs between 53 to 92 kg*

	Saline[a]								LPS[a]							
	SAA:Lys					Significance			SAA:Lys					Significance		
	0.45	0.55	0.65	0.75	SEM	Diet	Lin.	Quad.	0.45	0.55	0.65	0.75	SEM	Diet	Lin.	Quad.
Initial weight, kg	53.0	53.0	52.0	52.9	0.62	0.583	0.605	0.428	53.0	52.8	53.0	53.5	0.16	0.037	0.052	0.024
D 21 weight, kg	68.9	74.2	73.6	71.9	0.82	0.001	0.032	0.001	66.2	68.3	68.2	68.4	1.05	0.318	0.117	0.408
Day 42 weight, kg	87.0	97.7	96.5	94.7	1.14	0.001	0.001	0.001	84.7	88.8	89.3	91.1	1.45	0.028	0.005	0.486
ADG, g	870	1112	1075	1075	27.6	0.001	0.001	0.001	757	946	1009	1046	39.2	0.001	0.001	0.085
VFI, g	2691	2958	2898	2841	88.8	0.202	0.341	0.079	2341	2542	2518	2721	111.5	0.138	0.034	0.909
FCR, g/g	3.12	2.67	2.68	2.66	0.072	0.004	0.003	0.032	3.12	2.69	2.51	2.59	0.068	0.001	0.001	0.011

*Two pigs died and 1 pig was removed from the LPS treatment group due to hypersensitivity to the endotoxin injection. Therefore, initial body weight was uneven between dietary treatments and the difference was adjusted by using the initial body weight as a covariate for all performance analysis.
[a]Pigs received either intramuscular injection of saline or *E.coli* lipopolysaccharides (LPS, serotype O55:B5, Sigma) as per injection schedule.
[b]Feed conversion efficiency was calculated as g feed required per g body weight gain.

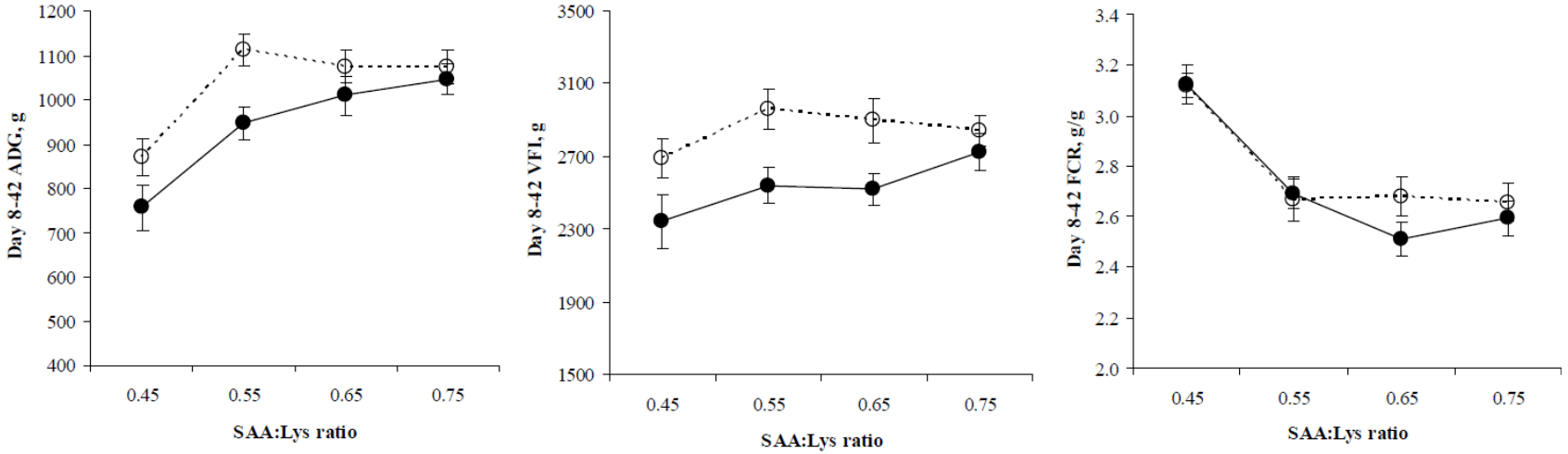


Figure 4 - Performance response of finisher pigs to dietary SAA:Lys ratio without and with immune system activation.

Feed conversion ratio of pigs without immune system activation reached a plateau at a SAA:Lys ratio of 0.55 (Linear P<0.001), while that of pigs with immune system activation reached a plateau at a SAA:Lys ratio of 0.65 (Linear P<0.001).

3.1.3. Protein utilization efficiency

Broken-line analysis showed that while whole body protein deposition rate reached a plateau of 67 g/d at a SAA:Lys ratio of 0.54 in saline-injected (control) pigs, immune system activated pigs linearly increased protein deposition rate as dietary SAA:Lys ratio increased to 0.75. Protein utilisation efficiency, measured as plasma urea content, showed exactly the same pattern to the protein deposition response and confirms that an increased amount of SAA is required when the pig's immune system becomes activated (Figure 5).

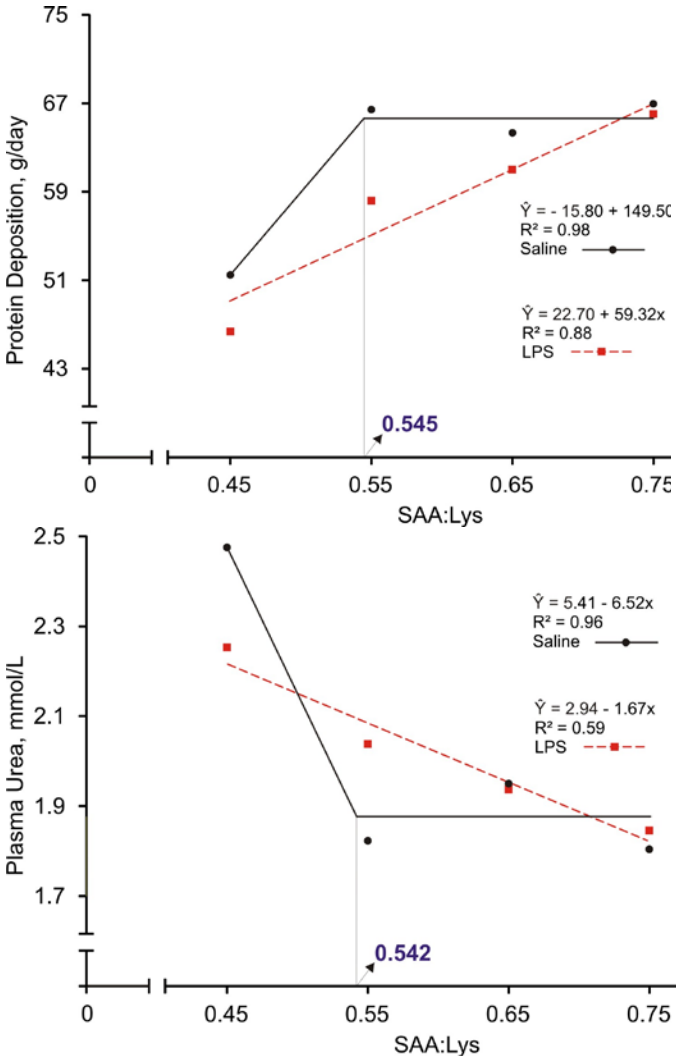


Figure 5 - Protein deposition (g/d) and plasma urea content (mmol/L) in response to increasing standardised ileal digestible sulphur AA to Lys ratio (SAA:Lys) in pigs intramuscularly injected with either saline (control) or E. coli lipopolysaccharides (LPS).

3.1.4. DXA carcass composition

Carcass composition data determined using DXA is presented in Table 5. Carcass weight, dressing per cent and daily lean gain were linearly increased as SAA:Lys ratios increased in the immune system activated pigs, while healthy pigs reached a plateau at a SAA:Lys ratio of 0.55. An interesting finding is that the

maximum carcass weight and daily lean gain that were achieved at the SAA:Lys ratio of 0.55 in healthy pigs were achieved in immune system activated pigs by increasing SAA:Lys ratio to 0.75. However, increasing SAA:Lys ratio in immune system activated pigs did not improve daily fat and bone gains, most likely due to decreased feed intake resulting from the immune system stimulation.

Table 5 - Carcass composition measures by DXA in pigs intramuscularly injected with either saline (control) or E. coli lipopolysaccharides (LPS) and fed diets contain increasing sulphur AA to Lys ratios (SAA:Lys)

	Saline				LPS				Pooled	*P* =[1]	
Item\SAA:Lys	**0.45**	**0.55**	**0.65**	**0.75**	**0.45**	**0.55**	**0.65**	**0.75**	**SEM**	**IS**	**SAA**
Carcass wt, kg	58.8	65.3	64.0	64.6	56.0	61.3	62.7	64.5	1.27	NS	***
Dressing, %	67.2	66.6	67.0	68.0	68.0	69.4	70.6	70.3	0.57	***	*
Lean gain, g/d	603	672	662	674	579	634	667	670	12.4	NS	***
Fat gain, g/d	74	87	75	69	73	61	58	73	9.7	NS	NS
Bone gain, g/d	16.9	17.6	18.0	17.7	15.5	15.3	16.0	15.5	0.54	**	NS

3.1.5. Plasma homocysteine and amino acid content

An increasing amount of methionine was provided to increase the total SAA content in the experimental diet, which allowed us to test the hypothesis that the rate of conversion from methionine to cysteine increases in immune system activated pigs with increasing dietary supply of methionine. Generally, immune system activated pigs showed decreased plasma amino acid contents ($P < 0.05$ – 0.001, Table 6), which was most likely due to increased hepatic amino acid demand for production of acute-phase proteins and increased production of antibodies. Immune system activated pigs had lower levels of the intermediate molecule homocysteine ($P < 0.001$, Figure 7) and lower serine contents ($P < 0.05$, Table 6), which is required for conversion of homocysteine to cysteine (Figure 6). Increasing dietary SAA:Lys ratio linearly increased plasma methionine, cysteine, homocysteine and taurine contents ($P < 0.001$, Table 6), while the serine content was linearly decreased. These results partly supported the hypothesis that the conversion of methionine to cysteine is increased in immune system activated pigs.

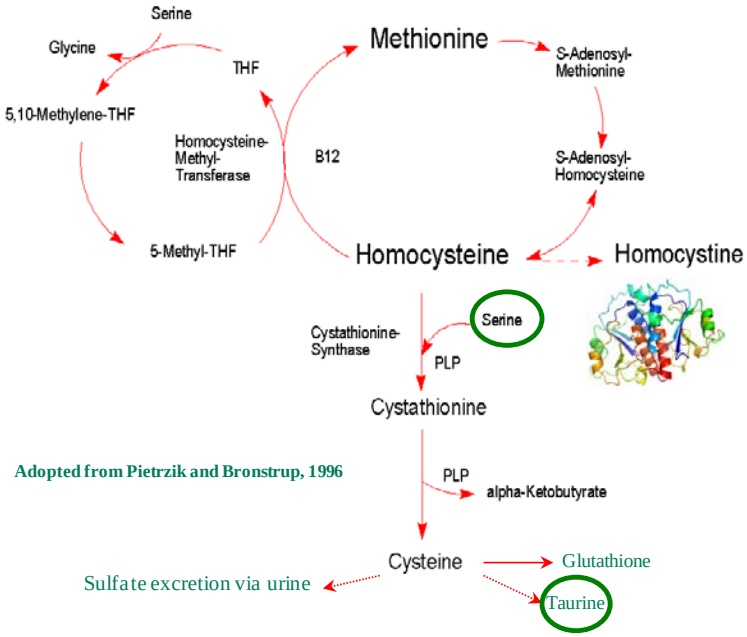


Figure 6 - Biosynthesis of cysteine from methionine (after Pietrzik and Bronstrup, 1996)

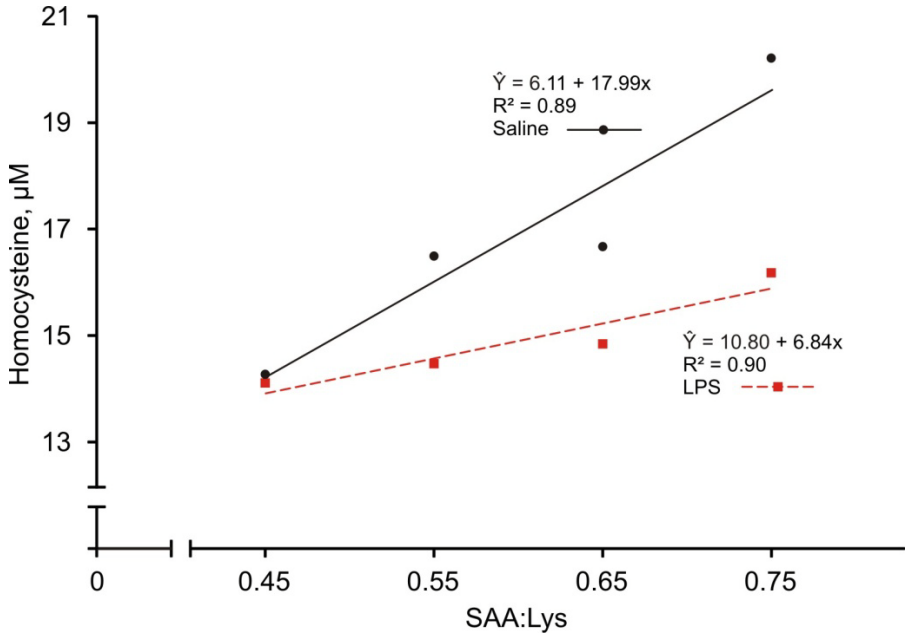


Figure 7 - Mean plasma homocysteine content in pigs intramuscularly injected with either saline (control) or E. coli lipopolysaccharides (LPS) and fed diets contain increasing sulphur AA to Lys ratios (SAA:Lys). Effect of LPS (P<0.001) and SAA (P<0.01) were significant but interaction was not significant.

Table 6 - Plasma AA (nmol/mL) contents in pigs intramuscularly injected with either saline (control) or E. coli lipopolysaccharides (LPS) and fed diets contain increasing sulphur AA to Lys ratios (SAA:Lys)

	Saline				LPS				Pooled	*P* =[1]	
AA\SAA:Lys	**0.45**	**0.55**	**0.65**	**0.75**	**0.45**	**0.55**	**0.65**	**0.75**	**SEM**	**IS**	**SAA**
Ala	805	820	795	941	820	793	756	700	39.8	*	NS
Arg	222	261	247	259	215	269	282	240	21.3	NS	NS
Asp	28	27	24	26	30	29	27	25	1.7	NS	†
Cys	45	81	92	101	63	79	97	108	8.61	NS	***
Glu	253	275	298	288	327	293	339	308	18.3	**	NS
Gly	1183	1189	1263	1294	1057	954	923	950	47.3	***	NS
His	127	113	105	99	119	100	99	91	4.2	**	***
Ile	180	185	180	184	175	176	153	142	5.5	***	**
Leu	544	533	522	454	476	498	446	432	27.7	*	*
Lys	462	423	351	375	466	395	348	302	31.6	NS	***
Met	17	44	65	86	17	37	60	79	3.4	†	***
Phe	105	96	92	91	109	99	99	91	3.8	NS	***
Pro	449	425	405	441	436	370	374	346	16.0	***	**
Ser	290	208	200	204	268	183	160	162	9.6	***	***
Taurine	94	83	100	111	89	90	106	116	5.0	NS	***
Thr	393	228	231	225	337	202	195	182	20.7	**	***
Trp	66	52	52	52	53	43	40	38	2.5	***	***
Tyr	134	120	119	148	117	102	112	118	6.0	***	**
Val	378	333	308	282	374	316	282	254	15.2	†	***

[1]NS; not significant; *P < 0.05; **P < 0.01; ***P < 0.001

3.2. Commercial validation study

3.2.1. Plasma haptoglobin and urea

Plasma haptoglobin level was measured from two randomly selected pigs per pen of 14 pigs. As the numbers of collected samples were not sufficient to represent the pen, interpretation of the data may be limited. However, mean plasma haptoglobin level of this commercial herd (1.61 ± 0.08 mg/mL) was significantly higher ($P<0.001$) than that of the control pigs used for the infection study at Medina Research Station (1.13 ± 0.05 mg/mL). However, plasma haptoglobin level in the commercial herd was significantly lower ($P<0.001$) than LPS injected pigs (2.04 ± 0.13 mg/mL; See Figure 3 and Table 7). Therefore, the herd used in the commercial validation study had a lower level of immune system activation than LPS injected pigs but had a greater level of immune system activation compared to pigs grown in the research facility at Medina, although the validity of the cross site comparison was not demonstrated in this study.

Plasma urea content was also measured as an indicator for protein utilization efficiency. However, unlike the result in Experiment 1, there was no significant dietary effect on plasma urea content because the within-treatment variation was higher than between-treatment variation. The small number of samples (2 pigs out of 14 pigs per pen) may be responsible for the high within-treatment variation. Therefore, it is inconclusive whether increasing SAA:Lys ratio decreased plasma urea content in a linear-plateau or quadratic manner in finisher pigs raised in the commercial facility.

3.2.2. Growth performance and carcass composition

Results of the ANOVA test are presented in Table 8. There were significant gender effects for all measured performance and carcass composition indices except back fat thickness. However, interactions between gender and dietary treatment for all measured variables were not significant. Therefore, only dietary effect on performance and carcass composition will be discussed. As indicated in the Table 8, average daily gain, feed conversion ratio, and hot carcass weight in pigs fed the diet containing SAA:Lys ratio of 0.75 were aberrant for reasons that could not be identified or explained. Analysis of diet samples showed that the diet contained correct amounts of amino acids and SAA:Lys ratio (Tables 2 and 3). Therefore, the SAA:Lys ratio 0.75 treatment was omitted in the subsequent regression analysis for estimation of SAA requirement. Unlike Experiment 1, which showed a linear-plateau response to dietary SAA:Lys ratio, performance and hot carcass weight responses to increased SAA:Lys ratio in the commercial validation study was quadratic. Therefore, estimation of SAA requirement using a broken line analysis would significantly underestimate the actual requirement (See Figure 8). Morris (1999) suggested that use of the broken line analysis for estimation of amino acid requirement is not suitable for data from populations of animals because individual animals vary in their maintenance requirements (the intercepts) and production potential (the plateau). Therefore, Morris (1999) recommended broken line analysis for individual pig data and quadratic, exponential or polynomial models for population data, depending on how the data fit the model. A quadratic model was selected and used to estimate the optimum SAA:Lys ratio for average daily gain (ADG), feed conversion ratio (FCR) and hot carcass weight (HCW). As demonstrated in Figure 8 and Table 9, maximum ADG, minimum FCR and maximum HCW were achieved at SAA:Lys ratios of 0.70, 0.71 and 0.73, respectively. This finding is similar to the estimated SAA:Lys requirement of 0.75 derived from the E. coli endotoxin model in the Experiment 1 and significantly higher requirement than current NRC recommendation of 0.59 for finisher pigs (NRC 1998).

The level of plasma haptoglobin in the Rivalea herd was greater than the healthy pigs at Medina but was lower than the E. coli endotoxin injected pigs. This finding may indicate that the severity of immune system activation may affect SAA requirement in pigs and further research is required for clarification.

Table 7 - Effect of gender and SAA:Lys ratio on plasma haptoglobin and urea content

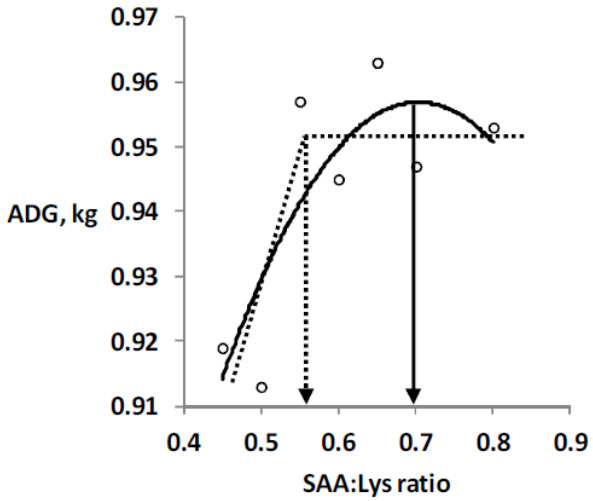
	Gender		**SAA:Lys ratio**									***P*=[1]**	
	Male	**Female**	**0.45**	**0.50**	**0.55**	**0.60**	**0.65**	**0.70**	**0.75**	**0.80**	**SEM**	**Gender**	**SAA**
Haptoglobin, mg/mL	1.6	1.6	1.7	1.6	1.8	1.2	1.6	1.5	1.9	1.8	0.15	0.550	0.050
Urea, mmol/L	2.4	2.6	2.5	2.2	2.6	2.3	2.7	2.5	2.7	2.4	0.15	0.011	0.195

[1]Gender x SAA interaction was not significant for all measured variables.

Table 8 - Effect of gender and SAA:Lys ratio on performance and carcass composition of finisher pigs

	Gender		SAA:Lys ratio									*P*=[1]	
	Male	Female	0.45	0.50	0.55	0.60	0.65	0.70	0.75	0.80	SEM	Gender	SAA
Growth performance													
Initial wt, kg	47.8	46.3	46.9	47.1	46.9	47.1	47.2	47.0	46.9	47.0	0.38	0.001	1.000
ADG, kg	0.966	0.913	0.919	0.913	0.957	0.945	0.963	0.947	0.923	0.953	0.0149	0.001	0.129
VFI, kg	2.31	2.27	2.28	2.29	2.33	2.27	2.30	2.29	2.29	2.31	0.029	0.038	0.870
FCR	2.40	2.49	2.48	2.51	2.43	2.41	2.39	2.42	2.47	2.41	0.025	0.001	0.017
Carcass composition													
Hot carcass wt, kg	78.3	76.2	76.3	76.0	77.8	77.8	78.8	77.0	76.3	78.3	0.71	0.001	0.040
Dressing, %	77.0	78.2	77.5	77.4	77.6	77.8	78.2	77.0	77.3	77.9	0.31	0.001	0.213
P2 back fat, mm	10.0	9.8	10.1	9.7	10.3	9.4	10.1	10.0	9.5	9.8	0.26	0.472	0.212

[1]Gender x SAA interaction was not significant for all measured variables.



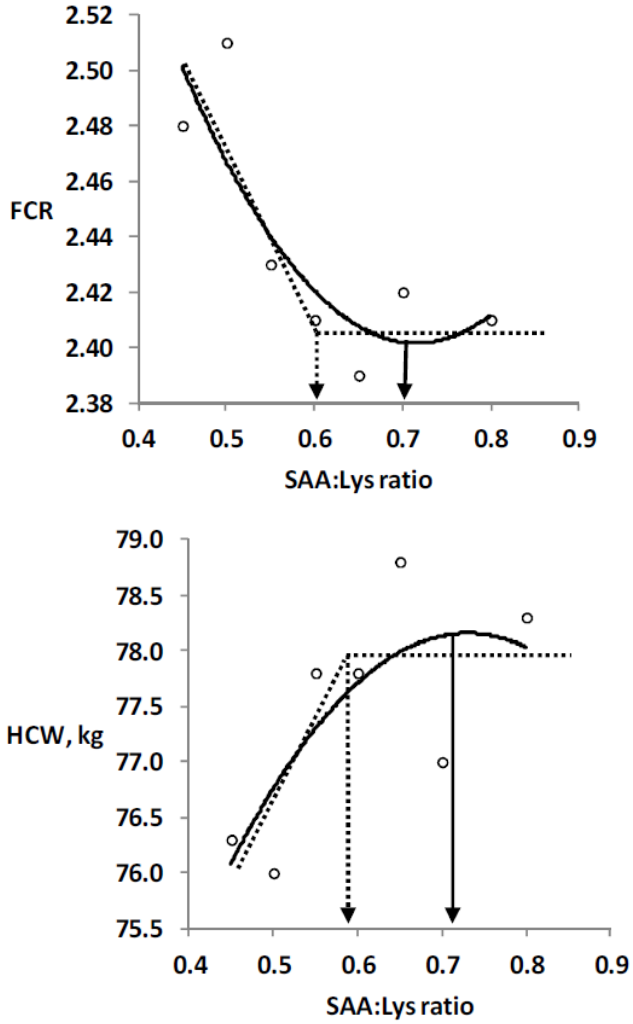


Figure 8 - Estimation of SAA requirement for finisher pigs using a broken line analysis (---) and quadratic regression model (—). The SAA requirements estimated using the broken line analysis (break point) for average daily gain (ADG), feed conversion ratio (FCR) and hot carcass weight (HCW) were 0.56, 0.61, and 0.59. The SAA requirements estimated using a quadratic regression model for ADG, FCR and HCE were 0.70, 0.71 and 0.73, respectively.

Table 9 - Quadratic response equations[1] used to predict the optimum SID SAA:Lys ratio for finisher pigs.

	Quadratic equation	R^2	Optimum SAA:Lys ratio
ADG	$y= -0.6656x^2 + 0.9364x + 0.6276$	0.67	0.70
FCR	$y= 1.4124x^2 - 2.0212x - 3.1247$	0.72	0.71
HCW	$y= -26.316x^2 + 38.452x - 64.111$	0.55	0.73

[1]Where y=either average daily gain (ADG), feed conversion ratio (FCR), or hot carcass weight (HCW), x= SID SAA:Lys ratio.

4. **Application of Research**

The endotoxin model study demonstrated that the pigs whose immune system is activated require significantly greater amount of sulphur amino acids than healthy pigs. The subsequent validation study on a commercial farm conducted under assumption that pigs in commercial farms are continuously exposed to infection pressure and experience a level of immune system activation that is greater than pigs in optimal conditions (e.g. in Medina Research Station), demonstrated that optimum SAA:Lys ratio for minimum FCR and maximum carcass

yield for pigs raised in commercial production facilities is greater than 0.71. Therefore, to counteract increased metabolic use of SAA in commercial herds, formulating diets with a SAA:Lys ratio of 0.71 is recommended for finisher pigs. Application of this novel nutrition concept in commercial diet formulation would significantly improve feed utilization efficiency and carcass yield of the Australian herd.

5. **Conclusion**

Evidences demonstrated in this project suggest that a greater amount of SAA is required for efficient utilization of protein in finisher pigs raised in commercial production facility and a SAA:Lys ratio of at least 0.71 is recommended to minimise feed conversion ratio and maximise carcass yield.

6. **Limitations/Risks**

Application of this novel nutrition concept in a healthy herd would not improve herd feed conversion efficiency and carcass yield and hence additional use of SAA will be wasted through the de-amination or trans-amination processes in the body. Development of an on-farm decision tool to measure herd health status or level of herd immune system activation could assist the effective use of this nutritional strategy.

Future research should be directed to clarify:

- SAA requirement in weaner and grower pigs in commercial production system.
- Effect of severity of immune system activation on SAA requirement.

7. **Recommendations**

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

- Nutritionists and feed mills use standardized ileal digestible SAA:Lys ratio of 0.71 for formulation of finisher pig diet to improve feed conversion efficiency and carcass yield.
- Further research is required to develop a robust tool to measure the level of immune system activation in commercial herds.

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