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Determination of the optimum standardised ileal digestible sulphur amino acids to lysine ratio in weaned pigs challenged with enterotoxigenic *Escherichia coli*

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Highlights:

- Overall average daily gain was optimized at 0.71 (SE = 0.073) standardised ileal digestible sulphur amino acid to lysine ratio (SID SAA:Lys)
- A SID SAA:Lys of 0.73 (SE = 0.065) optimised feed intake
- Overall gain to feed was optimized at 0.68 (SE = 0.090) SID SAA:Lys

ABSTRACT: This experiment tested the hypothesis that pigs challenged with an enterotoxigenic strain of *E. coli* (ETEC) would require a higher sulphur amino acids (SAA) to Lys ratio (SAA:Lys). Pigs (n=120) weighing 7.4 ± 0.52 kg (mean ± SD) and weaned at 27 d (Pietrain genotype, mixed sex) were stratified into 1 of 6 treatments based on weaning weight, sex and genotype for the F4 fimbria receptor (n=20). Five diets were formulated with increasing ratios of standardised ileal digestible (SID) sulphurSAA:Lys. Pigs were housed in pens of 4 during an adaptation period of 6 d after which time pigs were housed individually. Pigs fed different SID SAA:Lys levels were infected with ETEC (5 mL, 1.13 x 10^8 CFU/mL, serotype O149:K91:K88) on d 8, 9, and 10 after weaning. The sixth diet, which contained 0.55 SID SAA:Lys and corresponded to current NRC recommendations, was allocated to 2 groups of pigs either with or without ETEC infection, and was considered as the infected or non-infected control group respectively. Pigs were fed Phase 1 diets (10.2 MJ NE, 1.2% SID Lys) *ad libitum* until d 15 after weaning. Phase 2 diets (10.2 MJ NE, 1.1% SID Lys) were fed *ad libitum* for the following three weeks. Diets did not contain any antimicrobial compounds. Corrected SID SAA:Lys determined based on analysed amino acid content and the respective standardised ileal digestibility of

ingredients were found to be 0.47, 0.55, 0.61, 0.69 and 0.77 for Phase 1 diets, and 0.47, 0.55, 0.63, 0.71 and 0.78 for Phase 2 diets. Following infection, oedema disease was diagnosed in all groups including the non-infection control group, therefore data from non-infected pigs were combined with pigs infected and fed 0.55 SAA:Lys for analysis of production and plasma data. There were no dietary effects of SID SAA:Lys on days with diarrhoea or faecal shedding of F4 ETEC ($P > 0.05$). Overall, average daily gain (ADG), feed intake and G:F were optimised at 0.71 (SE = 0.073), 0.73 (SE = 0.065) and 0.68 (SE = 0.090) SID SAA:Lys, respectively. For pigs infected with ETEC and not provided with antimicrobial compounds, and under conditions of the current study, it is suggested that the SID SAA:Lys lies above the current NRC recommendation of 0.55 for pigs after weaning.

Key words: *E. coli*, pig, performance, sulphur amino acids

INTRODUCTION

Post-weaning diarrhoea (PWD), which is characterised by watery faeces within the first two weeks after weaning, is a multifactorial disease commonly associated with proliferation of certain strains of enterotoxigenic *Escherichia coli* (ETEC) that attach to epithelial receptors in the small intestine (Pluske et al., 1997; Hopwood and Hampson, 2003; Fairbrother et al., 2005). Weaning is also associated with activation of inflammatory cascades (Lallès et al., 2007) that are likely to increase requirements for specific essential amino acids (Melchior et al., 2004; Heo et al., 2013) such as Met and Cys (sulphur amino acids (SAA**)**), which are needed for immune function, glutathione synthesis and growth (Grimble, 2006). The conversion

rate of Met to Cys (which is irreversible) increases during immune system stimulation to meet the Cys needs for the immune system and thus increases the dietary Met requirements to satisfy the needs of protein synthesis (Rakhshandeh et al., 2014). Kim et al. (2012a), for example, demonstrated that immune system stimulation caused by *E. coli*-derived lipopolysaccharide (LPS) increased the SAA:Lys requirement of finisher pigs from 0.58 to 0.75, to optimize feed efficiency and protein deposition.

For pigs between 7-11 and 11-25 kg body weight, the recommended standardised ileal digestible (SID) SAA:Lys is 0.56 and 0.55, respectively (National Research Council, 2012). However and given the various inflammatory challenges faced by pigs in the post-weaning period, it is possible that the pigs' requirement for SAA lies above these currently recommended levels. In this regard, the hypothesis examined in this experiment was that, relative to Lys, the needs for SAA are increased under conditions of a pathogenic ETEC challenge after weaning, and that the negative effects of inflammation on pig productivity can be abridged when the dietary SAA:Lys is increased.

MATERIALS AND METHODS

The experimental protocol was reviewed and approved by the Animal Experiments Committee Utrecht (DEC Utrecht, Approval number 2009.III.02.018), and the Murdoch University Animal Ethics Committee (??).

Screening for F4 receptors

At birth, tail samples from piglets farrowed at the Trouw Nutrition Swine Research Centre, The Netherlands, were collected to test the pigs' susceptibility to the enterotoxigenic F4 *E. coli* (O149:K88) used in the infection model. The pigs were screened for the presence of F4ab/ac receptor genes using labeled primers and PCR as

described by Jensen et al. (2006). Pigs were then classified as homozygous resistant (RR), homozygous susceptible (SS) or heterozygous (RS) for F4ab/ac. Animals selected for the trial were RR or RS genotypes for F4ab/ac receptors.

Experimental diets, animal housing and management

A total of 120 pigs (Pietrain) weighing 7.4 ± 0.52 kg (mean ± SD) (1:1 male: female) were selected at weaning (27 d of age) and stratified according to weight, sex and genotype for the F4 receptor (RR:RS=7:3) into 1 of 6 treatments. Five treatment groups were infected with ETEC and fed varying dietary SID SAA:Lys, namely 0.47, 0.55, 0.61, 0.67 and 0.76. The sixth group was not infected with ETEC and was provided a diet containing 0.55 SID SAA:Lys, which is the current recommended by the National Research Council (2012). Diets were fed in 2 phases with the first phase fed from d 0-15, and the second from d 16-36, after weaning.

Diet composition and calculated energy and nutrient contents of experimental diets are presented in Tables 1 and 2. Analyzed energy, crude protein (CP) and amino acid (AA) content are presented in Tables 3 and 4. All diets were provided in pellet form with a diameter of 2.2 mm. All diets were formulated to meet the requirements for energy and all nutrients with the exception of Lys, which was marginally limiting (Boisen, 2003). Phase 1 diets were formulated to contain 10.25 MJ net energy (NE)/kg, 20.9% CP, and a SID Lys content of 1.17%. Phase 2 diets were formulated to 10.25 MJ NE/kg, 19.9% CP and a SID Lys content of 1.07%. As Cys can be synthesized in the body from Met (Li et al., 2007), only DL-Met was added to the diets to increase SAA:Lys. Increased DL-Met supplementation progressively increased Met to Cys ratios from 0.47 to 0.54, 0.59, 0.63, and 0.67. Samples of feed ingredients were sent for AA analysis (Evonik, Hanau, Germany) and diets were re-

formulated based on the analysed AA contents and values for SID prior to diet manufacture (Sauvant et al., 2004).

It is known that newly weaned pigs have a low and variable feed intake with piglets often not meeting their maintenance energy requirements until at least d 7 after weaning (Pluske et al., 1997). Therefore, an adaptation period was used to ensure that pigs were eating the treatment diets (and thus varying levels of SAA) before experimental infection with *E. coli*.

During the 6 d adaptation period, pigs were allocated to pens in groups of four (5 group pens/treatment). Pigs were supplied with experimental Phase 1 diets *ad libitum*. Each group pen had slatted floors with 0.4 m^2 per pig. Each pen had 2 nipple bowl drinkers and an upright, single-space feeder. The ambient temperature for the periods d 6-15, 16-22, 23-29 and 29-36 were 27.9 ºC, 26.7 ºC, 26.3 ºC and 26.0 ºC, respectively.

At d 6 after weaning, pigs were weighed and moved to individual pens where they remained for the rest of the study. Pens had slatted floors with a space allowance of 0.8 m^2 per pig. Each pen had a nipple bowl drinker and an upright single space feeder. Pens were located in three rooms with 40 pens per room. A sanitation bath was located outside each room. The pigs allocated in the control treatment were housed in the first room of workflow to minimise contamination with experimentally infected animals. Experimental diets and water were provided *ad libitum*. Feed wastage was recorded daily. Feed residual and piglet weight was measured on d 0, 6, 15, 22, 29 and 36. Feed intake was based on feed disappearance.

Diet analysis

Diet samples were analyzed using a ballistic bomb calorimeter to determine GE content (Invivo Labs, Binh Duong City, Vietnam). Diets were also analyzed for

CP and AA content by Evonik (Hanau, Germany) as described by Htoo et al. (2007). The dietary SID SAA: Lys ratios were then corrected based on the analyzed dietary AA contents using the following formula;

Corrected SID SAA:Lys = (calculated SID SAA:Lys x analyzed total SAA:Lys) / calculated total SAA:Lys.

Experimental infection with E. coli

A 900 µL aliquot of 15% glycerol ETEC (serotype O149:K91:F4ac) stock solution was used to inoculate 50 mL of sterile brain heart infusion medium. Cultures were then incubated at 37 ºC on an orbital shaker for 2 hours and 40 minutes so as to enter the mid-log phase of growth. This culture was then diluted 1:10 with a 2.5% sucrose solution before being transported on ice to the piggery. Experimental infection with ETEC was conducted at d 8, 9 and 10 after weaning via daily oral dosing using a syringe. Each infected pig received 5 mL/d of freshly prepared broth to provide 1.13 x 10^8 cfu/mL of *E. coli* per pig.

Faecal scoring and faecal swabs

A visual assessment of faeces was conducted daily. Faecal consistency was assessed on a four-point scale with scores 0 (solid), 1 (soft), 2 (sloppy), and 3 (liquid). Diarrhoea was defined as pigs having a score of 3. The incidence of PWD was defined as the number of days with a score of 3 over the time period. Faecal swabs were taken on d 8, 10, 12, 15, 17 and 20 by inserting a sterile cotton bud into the anus. Swabs were streaked out onto sheep blood agar plates and incubated overnight to ascertain the amount of faecal shedding of F4 E. coli as described by Heo et al. (2009). The presence of β- hemolytic E. coli was then scored using a subjective score on a six-point scale ranging from 0 to 5, where: 0 = no growth, 1 = hemolytic E. coli in 1st section, 2 = hemolytic E. coli in 2nd section, 3 = hemolytic E. coli in 3rd section,

4 = hemolytic E. coli in 4th section, 5 = hemolytic E. coli in 5th section, present right out to the 5th section of the plate.

Pure colonies of *E. coli* isolated from faecal swabs confirmed edema disease from the presence of F18 *E. coli*. Hemolytic F18 *E. coli* was morphologically undistinguishable from the F4 *E. coli*. Therefore a PCR technique was used to screen for F4 *E. coli* in the hemolytic colonies. Within each section of the plate, colonies were pooled and suspended in lysis buffer. Samples were incubated for 10 minutes at 99º C to lyse the cells. After incubation, samples were centrifuged for 2 minutes at 12,000 g at 4 ºC. Detection for F4 *E. coli* was performed using a CFX96 Real-time system, C1000 Thermal cycler (BioRad Laboratories Inc., Hercules, USA) with a primer specific for F4 *E. coli* (Forward primer: GGTTCAGTGAAAGTCAATGCATCT, reverse primer: CCCCGTCCGCAGAAGTAAC, probe: Cy5 CCACCTCTCCCAACACACCGGCAT-BHQ_2) and SYBR green dye (BioRad IQ Super Mix #170-8860; West et al., 2007). Samples were cycled 40 times (15 seconds at 95 ºC and 30 seconds at 60 ºC). Results were then assessed as either presence or absence of F4 *E. coli* for that section of the plate. This was referred to as the F4 swab score.

Blood sampling

Blood samples from each pig were taken on d 8, 10 and 20 after weaning. Samples were collected from the jugular vein into lithium heparin tubes. Blood was processed by centrifugation at 2,000 g at 4 ºC for 10 minutes to separate plasma from erythrocytes. Plasma samples were then aliquoted and stored at -20 ºC until analyzed.

Plasma analysis

Plasma urea (**PU**) on d 8, 10 and 20 were determined using an Olympus AU400 (Tokyo, Japan) analyzer (Olympus Reagent Kit OSR6134; Beckman Coulter Ireland Inc., Co. Clare. Ireland). An Olympus AU400 analyzer was also used to determine the plasma levels of haptoglobin (Makimura and Suzuki, 1982) at d 8, 10 and 20 after weaning. Plasma albumin was measured using a Randox Daytona analyzer (Crumin, UK) and a commercial kit (Cat #AB3800, Randox, Crumin, UK). Levels of tumor necrosis factor alpha (TNF-α) were measured using a commercial ELISA kit (Cat # DY690-B, R & D Systems, Minneapolis, USA).

Amino acids from plasma samples from d 10 were analyzed (Animal Health Laboratories, DAFWA, Perth, Western Australia) using HPLC on a reverse-phase C-18 column (Laich et al., 2002). Cysteine is unstable and readily oxidizes to cystine (Meister, 1988). The method used to determine AA in plasma used hydrolyzation to stop this from occurring, however the effect is not always immediate and thus initial reading of Cys cannot be determined. Therefore, results presented are a total of Cys and cystine.

Statistical analyses

Faecal F4 *E. coli* score and days with PWD data were analyzed using the GLM function in SPSS (Version 20, SPSS Institute Chicago, Illinois, USA) with pig as the experimental unit and treatment as the independent variable. Data were further analyzed using the repeated-measures function in GLM to determine if there was an interaction between time and diet. Mortality of pigs for the overall time period was analyzed using Pearson's Chi squared test in SPSS. As faecal F4 *E. coli* score, days with PWD and mortality of piglets did not differ between pigs infected or not infected with ETEC and fed 0.55 SID SAA:Lys, these groups were subsequently combined for all further analyses. Production data for the first 6 d were analyzed with pens as the

experimental unit for ADG, ADFI and G:F using the ANOVA function, to ensure no effect of treatment occurred within the first 6 d of weaning. Production data after d 7 and plasma data were then analyzed using the ANOVA function for linear and quadratic effects with pig as the individual unit and dietary SID SAA:Lys as the independent variable. Overall treatment means for ADG, ADFI and G:F were fitted to quadratic plateau broken line analysis using a Nutrition Response Model Program (version 1.1, Vedenov and Pesti, Georgia University, USA). Further analysis of plasma urea, haptoglobin, albumin and TNF-α were conducted using repeated measures ANOVA to examine sampling time by treatment interactions.

All means are reported as least square means. Statistical significance was accepted at $P < 0.05$ and $0.05 < P < 0.10$ was considered a trend.

RESULTS

Diet analysis

The analyzed GE and CP contents were similar between diets and close to calculated values (Tables 3 and 4). The final SID SAA:Lys (corrected after diet analysis) for Phase 1 diets were 0.46, 0.55, 0.61, 0.69 and 0.77 (Table 3). The final SID SAA:Lys (corrected after analysis) for Phase 2 diets were 0.47, 0.55, 0.63, 0.71, 0.78 (Table 4). All data presented hereafter will refer to the corrected SID SAA:Lys of Phase 2 diets as SID SAA:Lys.

Evaluation of infection with E. coli

Research staff observed that pigs displayed clinical signs of edema disease such as neurological symptoms and overt diarrhoea within one day of infection with ETEC. Edema disease diagnosis was confirmed by clinical analysis (serotyping of culture) and affected all treatment groups including the non-infection control with total mortality ranging from 15-40%, however it was not different between treatments

(P = 0.324; Table 5). Data for pigs that died were completely removed from the dataset.

The faecal swab score for F4 *E.* coli showed a significant time effect with scores on d 8 and 17 after weaning having the lowest values and d 13 having the highest value ($P < 0.001$). No effect of treatment on faecal swab scores for F4 *E. coli* was found at any time point ($P > 0.05$; Table 5).

There was an effect of time on days with PWD, with more diarrhoea occurring during d 7-15 after weaning than any other time period ($P = 0.011$). The number of days with PWD was not different between treatment groups for any time period ($P > 0.05$; Table 5).

Production traits

Due to F4 and F 18 *E.* coli being present and affecting the control as well as the experimentally-infected pigs, data for pigs fed 0.55 SAA:Lys regardless of experimental infection were combined; data did not differ (P > 0.10) between these two treatment groups. No differences between treatments were observed for any production traits during the adaptation period (d 0 – 6) after weaning ($P > 0.05$; data not shown).

On d 6 and 15 after weaning there were no linear or quadratic effects of increasing SID SAA:Lys on body weight (BW; $P > 0.05$). On d 22, d 29 and d 36 after weaning there were positive linear effects between BW and SID SAA:Lys (P = 0.035, P = 0.032 and P = 0.020, respectively; Table 6).

Increasing the SID SAA:Lys showed positive linear and quadratic effects for ADG during d 7-15 (P = 0.039 and 0.009, respectively) after weaning. The ADG increased linearly between d 16-22 post-weaning in response to increasing SID SAA:Lys (P = 0.030). No linear or quadratic effects were found for ADG between d

23-29 ($P > 0.05$) after weaning. The ADG between d 30-36 after weaning showed a positive linear effect ($P = 0.011$), whilst ADG for the overall time period showed positive linear effects and a trend for a quadratic response to SID SAA:Lys ($P = 0.007$ and 0.099, respectively; Table 6).

A positive quadratic effect for ADFI between d 7-15 ($P = 0.030$) after weaning in response to increasing SID SAA:Lys was observed. The ADFI showed a positive linear trend in response to increasing SID SAA:Lys between d 16-22 ($P = 0.086$) after weaning, but there were no linear or quadratic effects of ADFI in response to increasing SID SAA:Lys between d 23-29 ($P > 0.05$) after weaning. Feed intake showed a positive linear effect between d 30-36 after weaning in response to SID SAA:Lys ($P = 0.011$), and the overall ADFI from d 6-36 after weaning showed positive linear effects in response to increasing SID SAA:Lys ($P = 0.034$; Table 6).

Feed efficiency (as G:F) during d 7-15 after weaning increased linearly and quadratically in response to increasing dietary SID SAA:Lys ($P < 0.016$ and 0.009, respectively). A weak positive linear trend was found for G:F between d 16-22 after weaning in response to increasing SID SAA:Lys ($P = 0.094$). A positive quadratic trend was found for G:F between d 23-29 after weaning in response to increasing SID SAA:Lys ($P = 0.064$). No linear or quadratic effects were observed for G:F in response to SID SAA:Lys for d 30-36 after weaning ($P > 0.05$). For the overall time period (d 6-36 after weaning) both linear and quadratic positive effects were found for G:F in response to increasing SID SAA:Lys ($P = 0.002$ and 0.025 respectively; Table 6).

The dietary SID SAA:Lys to maximize the ADG of 8- to 20-kg pigs was estimated to be 0.71 (SE = 0.073, $R^2 = 0.94$) based on the quadratic broken-line model (Fig. 1). To maximize ADFI and G:F, the optimal SID SAA:Lys was estimated to be

0.73 (SE = 0.065, R^2 = 0.97) and 0.68 (SE = 0.090, R^2 = 0.90) by the quadratic broken-line regression, respectively (Fig. 2 and 3).

Plasma haptoglobin, albumin and TNF- α

There were no linear or quadratic effects of SID SAA:Lys on plasma haptoglobin at any time point sampled (P > 0.05). No time effects for plasma haptoglobin were found (P > 0.05). A negative linear trend was found of increasing SID SAA:Lys on plasma albumin on d 10 after weaning (P = 0.061). Plasma albumin was highest on d 8 and lowest on d 20 (P < 0.001) after weaning. No linear or quadratic effects were found between SID SAA:Lys and plasma albumin for any other time point sampled (P > 0.05).

No significant effect of time was found for TNF-α (P > 0.05), therefore plasma levels of each SID SAA:Lys between sampling days are presented. No linear or quadratic effects of SID SAA:Lys on TNF-α levels were found (P > 0.05; Table 7).

Plasma urea and amino acids

Plasma urea levels on d 8, 10 and 20 after weaning showed an interaction effect between time and SID SAA:Lys (P = 0.029). Plasma urea levels decreased linearly (P = 0.027) and quadratically (P = 0.007) by increasing SID SAA:Lys on d 8 after weaning. On d 10 after weaning there was a negative linear trend between increasing SID SAA:Lys and plasma urea (P = 0.063). No linear or quadratic effects were found on d 20 after weaning between SID SAA:Lys and plasma urea (P > 0.05; Fig. 4).

Increasing the dietary SID SAA:Lys linearly increased plasma levels of Met (P < 0.001), Phe (P = 0.003), Asp (P = 0.022), Glu (P = 0.011), Tau (P < 0.001), cysteine plus cystine (P < 0.001) and Pro (P = 0.035) on d 10 after weaning. A linear

trend for increasing dietary SID SAA:Lys to increase plasma levels of Arg (P = 0.077) and Trp (P = 0.091) was also found. Increasing dietary SID SAA:Lys had negative linear effects on plasma levels of Lys (P < 0.001), Thr (P < 0.001), Val (P = 0.021) and Ser (P < 0.001). A linear trend effect of increasing dietary SID SAA:Lys was found for plasma levels of Gly (P = 0.007). Positive quadratic effects of dietary SID SAA:Lys were found for plasma levels of Arg (P = 0.035), Ile (P = 0.016), Tau (P < 0.001) and cysteine plus cystine (P < 0.001). Negative quadratic effects of dietary SID SAA:Lys were found for plasma levels of Lys (P = 0.001), Thr (P = 0.001) and Ser (P = 0.020). Positive quadratic trends were found for increasing SID SAA:Lys for Glu (P = 0.057) and Gln (P = 0.076; Table 8).

DISCUSSION

The general hypothesis tested in the present study was that weaner pigs infected with ETEC and presumed to be under inflammatory stress would require greater levels of dietary SAA:Lys for modulation of inflammation responses as well as maintaining production performance than the level recommended by the National Research Council (2012) of 0.55 for pigs of this body weight range. It has been documented previously that inflammatory stress can increase the requirement for SAA in growing pigs (Li et al., 2007; Rakhshandeh et al., 2007; Kim et al., 2011b; Kim et al., 2012b). The present study confirmed the hypothesis and found that increasing dietary SID SAA:Lys to the level that are greater than currently recommended by National Research Council (2012) improved ADG, ADFI and G:F.

In accordance with previous statistical analysis of nutritional response modeling (Pomar et al., 2003; Pesti et al., 2009), these data were fitted to a quadratic plateau broken line to obtain optima of 0.71, 73 and 0.68 SID SAA:Lys for ADG, ADFI and G:F, respectively, which are above the currently recommended level of

0.55 SID SAA:Lys (National Research Council, 2012). The findings of the present study are similar, albeit with much younger pigs, to those of Yi et al. (2006) who found an optimum SAA:Lys of 0.64 for the ADG of 28–49 kg pigs, Gaines et al. (2005) who found an optimum of 0.60 for ADG of 29–45 kg pigs, and Zhang et al. (2015) who reported an optimum of 0.62 to optimize ADG and G:F of 25–50 kg pigs. In the present study pigs were likely to have experienced a greater level of challenge as a result of experimental infection in addition to edema disease, which may help to explain the differences in optimum ratios between the present study and other studies (Gaines et al., 2005; Owen et al., 1995; Peak, 2005; Yi et al., 2006).

Infection with E. coli and assessment of inflammation and immune stress

Escherichia coli (F18) causing oedema disease is known to colonize the small intestine, producing a toxin that causes vascular lesions in the intestine leading to diarrhoea (Imberechts et al., 1992). Pigs within the current study, regardless of treatment group, displayed symptoms of oedema disease, and this was confirmed by a strain of F18 *E. coli* that was isolated from these pigs. The outbreak of oedema disease was unexpected and not the focus of this work, however the oedema disease undoubtedly contributed to the total health challenge on the animals. Unlike F4 fimbriae, no genetic screening for F18 fimbriae was able to be conducted in these pigs before experimentation, thus receptors for F18 may not have been evenly distributed between treatments and may have, in part, caused the varying level of mortality between groups.

The non-infected control animals showed the same levels of F4 *E. coli* shedding and number of days with PWD both prior to (d 8) and after experimental infection with F4 *E. coli* as the animals in the infection groups. Mortality rates of pigs fed 0.55 SAA:Lys were the same between pigs infected with F4 *E. coli* and those not

infected with F4 *E. coli*, suggesting that pigs were equally affected by disease. Compared to Heo et al. (2009), who used a similar infection model with F4 *E. coli*, faecal swab plate scores had similar values as the present trial. Observations of piglets and mortality data also indicated all animals were exposed to oedema disease causing F18 *E. coli*.

Markers of inflammation and immune stress were measured to ascertain if the higher levels of SAA in the diet modulated the inflammatory response in response to infection with ETEC. Acute phase proteins (**APP**) are commonly used as indicators of herd health as they sharply respond to inflammation and immune stimulation (Eckersall et al., 1996). There are two types of APP: positive APP (e.g. haptoglobin), which increase under conditions of inflammation, and negative APP (e.g. albumin) that decrease under such conditions (Eckersall and Bell, 2010). Methionine and Cys make up 40 g/kg of protein for haptoglobin (a positive APP) and only 35 g/kg protein for muscle (Dahl, 1962; Peters, 1985; Reeds et al., 1994), thus an inflammatory response would require greater SAA relative to other amino acids than protein deposition. Haptoglobin levels in the present study were higher than the upper threshold for inflammation (induced by 0.3 mL turpentine/kg BW injection) as described by Heegaard et al. (2011). Levels of haptoglobin in the present study were also higher than levels reported by Kim et al. (2011a) in weaner pigs also infected with ETEC, further supporting the notion that pigs in the present study were suffering from a marked inflammatory challenge. However this was not attenuated by increasing SID SAA:Lys. The effect of time on haptoglobin level was expected as Pomorska-Mól et al. (2012) showed a trend for increased haptoglobin levels in pigs from 4 to 6 weeks of age.

Due to the high cysteine content in albumin (Reeds and Jahoor, 2001), it was expected that higher dietary levels of SAA would maintain albumin levels during an inflammatory challenge. However, the present study found a decreasing trend of plasma albumin with increasing SID SAA:Lys immediately after infection. These findings are in contradiction with work by Litvak et al. (2013) who found that higher Met:SAA increased plasma albumin. Albumin catabolism produces AA and it is thought that the decrease albumin in animals suffering from infection is due to increased breakdown rather than decreased synthesis (Reeds and Jahoor, 2001). Baynes and Thorpe (1981) used labeled serum albumin in rats to determine the major catabolic sites and found the 40-60% of the albumin dose was catabolized in muscle and skin. The present study found an increase in daily gain with increasing SID SAA:Lys, which would also increase muscle and thus the catabolism of albumin which may explain this unexpected finding. Plasma albumin also decreased over time. Nevertheless, literature on plasma albumin is contrasting where some authors (Heegaard et al., 2011; Rakhshandeh and de Lange, 2012) showed no response of immune stimulation (using an LPS injection) on plasma albumin, however, Litvak et al. (2013) found that immune stimulation caused by LPS injection decreased plasma albumin. Rothschild et al. (1979) found that in cases of myxedema there was evidence of tissue trapping of albumin. Thus, the decrease found in the present study in plasma albumin over time could be a result of increased tissue retention of albumin caused by oedema, increased catabolism caused by infection, or increased muscle mass also facilitating albumin catabolism.

The pro-inflammatory cytokine, TNF-α, did not show a response to increased SID SAA:Lys in the diet, further supporting the notion that SID SAA:Lys was not

modulating the inflammatory/immune response in the present study. This may have been due the unknown effect of mixed infection with both F4 and F18 *E. coli.*

Plasma urea and amino acids

Excess amino acids cannot be stored and are degraded with the production of urea, hence PU levels are often used as an indicator of protein utilization efficiency and have been used to determine protein requirements (Chen et al., 1995; Heo et al., 2009). Conversely, lower PU levels can indicate that either nitrogen utilization efficiency is increased or muscle protein catabolism is decreased, which can be a result of anabolic factors such as growth hormone or protein synthesis or catabolic factors such as an immune response (Shen et al., 2012). The interaction effect on PU between sampling day and SID SAA:Lys indicates that stage of infection (pre-infection, during infection and after infection) alters the urea content in the plasma of weaner pigs.

It was unsurprising to find that plasma levels of Met, Cys-Cystine and Tau increased with increasing levels of dietary SAA:Lys as they are sulphur-containing AA. In the present study, increasing dietary SID SAA:Lys also decreased plasma Ser. As Ser is essential for the conversion of homocysteine to Cys (Kim et al., 2012b), it suggests that significant amounts of Met were converted to Cys to support whole body antioxidant capacity. These patterns of plasma AA in response to immune/inflammatory stress are in congruency with Kim et al. (2012a), who also reported similar patterns in Met, Tau and Ser in grower pigs given LPS to induce immune stimulation. Taurine is metabolized from Cys and represents an irreversible loss of Cys (Rakhshandeh and De Lange, 2011). Work by Malmezat et al. (1998) in rats using labeled ^{35}S found a 54% increase of ^{35}S in Tau but only a 30% increase in ^{35}S in SO_4 caused by an intravenous inoculation of live *E. coli*. This indicates that

under these immune stimulated conditions, there is an irreversible loss of Cys to taurine. In a review article by Rakhshandeh and De Lange (2011), the authors hypothesized that additional supply of Met would improve whole body protein homeostasis and the immune response. The increased level of plasma Tau in response to increasing levels of SAA:Lys in the diet in combination with improvements in ADG and FCR found in the present study are in agreement with this hypothesis.

Lysine levels in plasma decreased with increasing SAA:Lys. Lysine has high concentrations in haptoglobin and muscle (92 g Lys/g haptoglobin and 92 g Lys/g muscle; Dahl 1962; Reeds et al., 1994), and thus is expected to decrease as SAA:Lys in the diet increases. This is because once SAA are not limiting more Lys will be utilized for production of APP or protein deposition.

In conclusion, oedema disease and F4 *E. coli* affected all treatments equally as measured by mortality, faecal swab scores and days with PWD, and may have increased the requirement for SAA above recommended levels. Under the conditions of this experiment, quadratic broken-line analysis determined that feeding a diet with average SID SAA:Lys of 0.70 maximized ADG, and G:F of 8- to 20-kg pigs. These levels are above those currently recommended by the National Research Council (2012) for pigs of this BW, hence pigs subject to an ETEC challenge in the post-weaning period where diets are devoid of antimicrobials may require higher levels of SID SAA:Lys than currently recommended. However, and given the unavoidable lack of a non-infected control in the present study, then some caution with regard to the overall implications of the study is advised.

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Conflict of interest

The authors declare they do not have any conflict interest.

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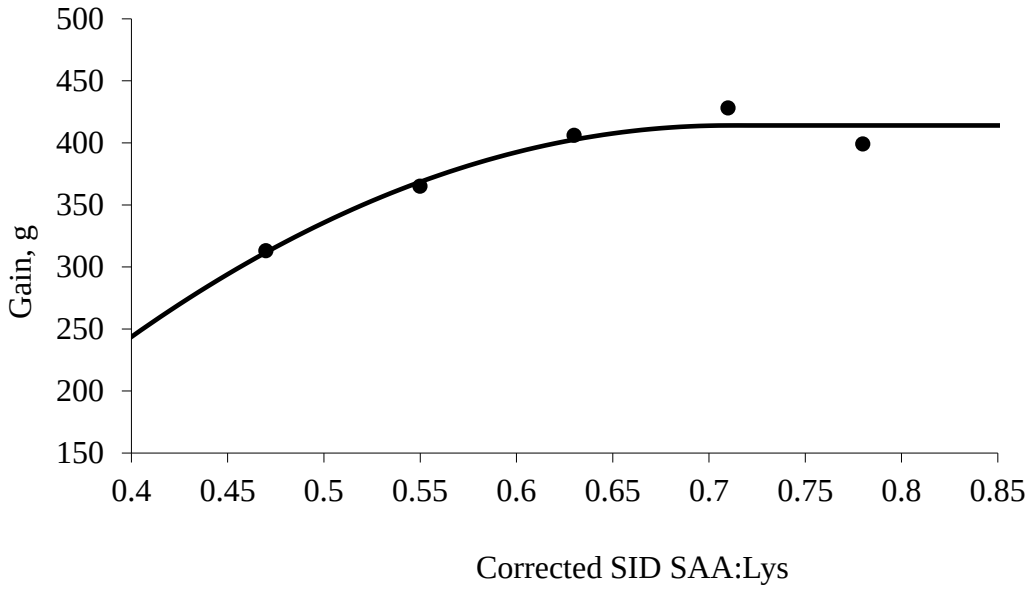

Fig. 1. The quadratic plateau broken-line model; Eq.: $y = 414 - 1{,}768.5\ (0.71 - x)^2$; breakpoint (BP) = 0.71(SE = 0.0.073, $R^2 = 0.94$). If SID SAA:Lys is > BP, then x = 0; if SID SAA:Lys is < BP, then x = SID SAA:Lys.

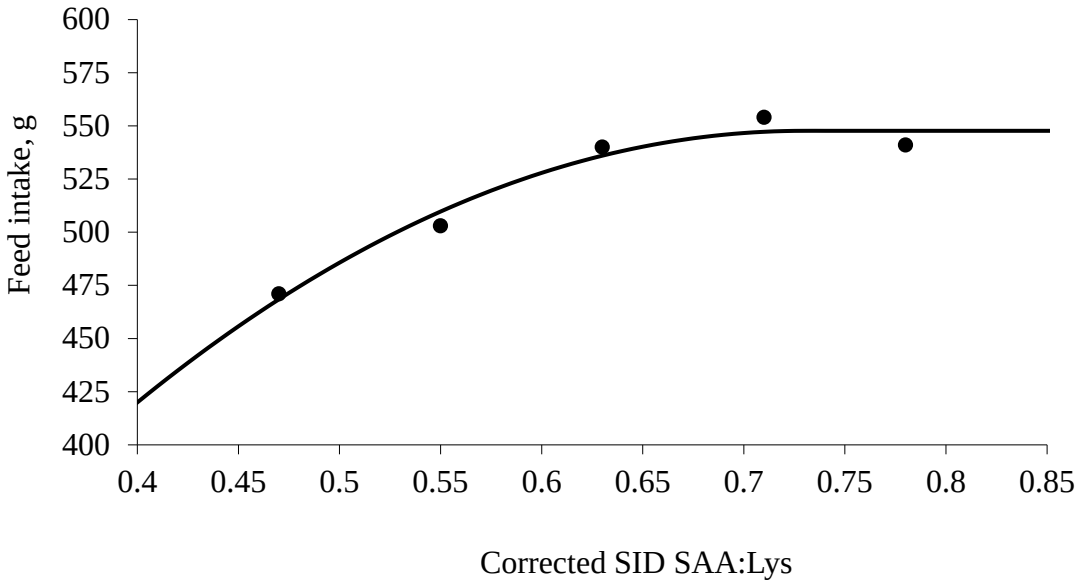

Fig. 2. The quadratic broken-line model; Eq.: $y = 548 - 1{,}174\ (0.73 - x)^2$; breakpoint (BP) = 0.63 (SE = 0.065, $R^2 = 0.97$). If SID SAA:Lys is > BP, then x = 0; if SID SAA:Lys is < BP, then x = SID SAA:Lys).

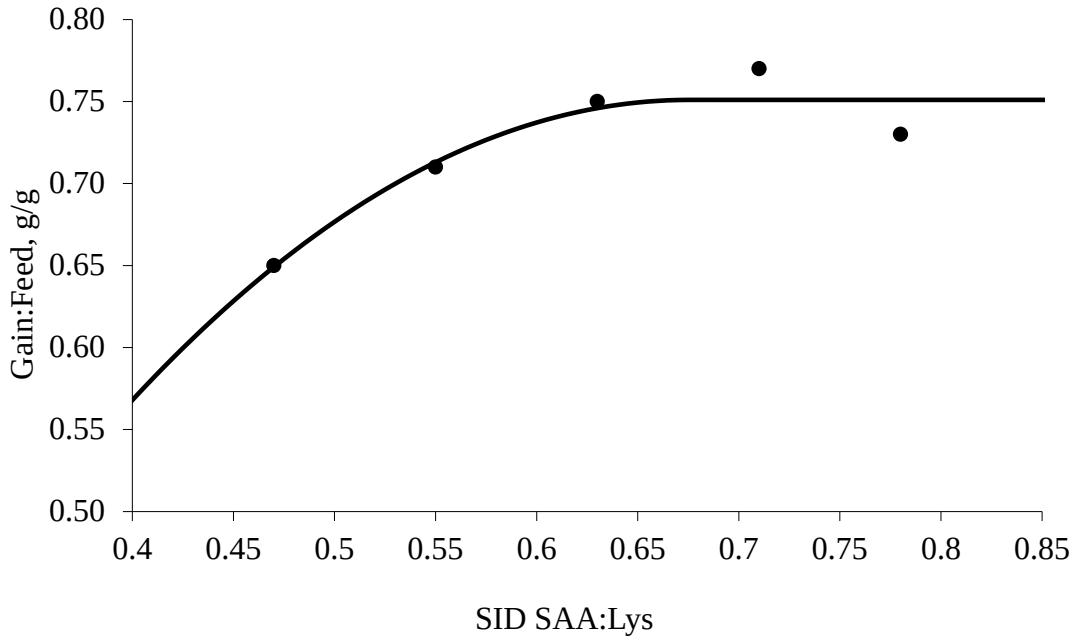

Fig. 3. The quadratic broken-line model; Eq.: $y = 0.75 – 2.41 (0.68 – x)^2$; breakpoint (BP) = 0.68 (SE = 0.090, $R^2 = 0.90$). If SID SAA:Lys is > BP, then x = 0; if SID SAA:Lys is < BP, then x = SID SAA:Lys).

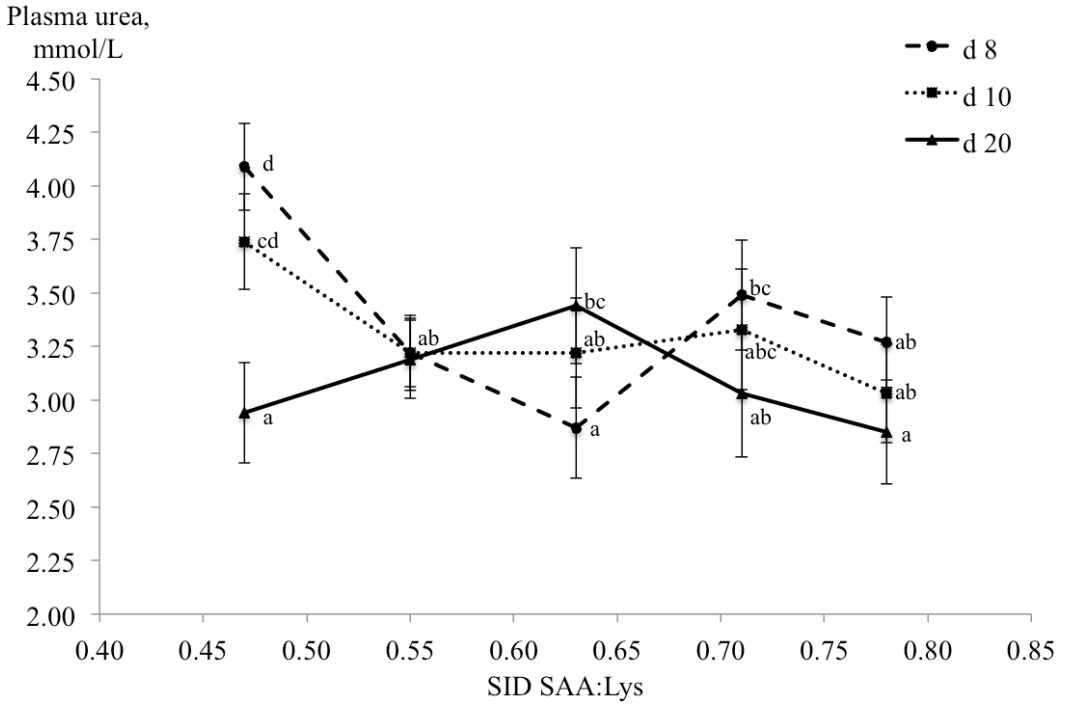

Fig. 4. Plasma urea levels on d 8, 10 and 20 after weaning of pigs fed varying ratios of SID SAA:Lys. An interaction effect of time and SAA level was found ($P = 0.029$), different letters next to data points denote statistical difference ($P < 0.05$).

Table 1. Composition of the Phase 1 experimental diets (as fed basis).

	Calculated SID[1] SAA:Lys				
Ingredients, g/kg	0.45	0.55	0.62	0.70	0.78
Wheat/barley mix	600.4	599.1	598.1	597.1	596.0
Soybean meal	216.0	216.0	216.0	216.0	216.0
Hamlet protein	50.0	50.0	50.0	50.0	50.0
Lactose	40.9	40.9	40.9	40.9	40.9
Soya oil	32.9	33.0	33.1	33.2	33.3
Sugar	20.0	20.0	20.0	20.0	20.0
Mono-calcium phosphate	14.4	14.4	14.4	14.4	14.4
Piglet premix[2]	10.0	10.0	10.0	10.0	10.0
Salt (NaCl)	5.9	5.9	5.9	5.9	5.9
Calcium formate	3.0	3.0	3.0	3.0	3.0
Choline chloride	0.3	0.3	0.3	0.3	0.3
Limestone	0.1	0.1	0.1	0.1	0.1
Vitamin E absorbate	0.7	0.7	0.7	0.7	0.7
L-Lys-HCl	3.4	3.4	3.4	3.4	3.4
L-Thr	1.4	1.4	1.4	1.4	1.4
L-Trp	0.4	0.4	0.4	0.4	0.4
DL-Met	0.0	1.2	2.1	3.0	4.0
L-Val	0.1	0.1	0.1	0.1	0.1
SID AA content, g/kg[1]					
Arg	12.2	12.2	12.2	12.2	12.2
His	4.3	4.3	4.3	4.3	4.3
Ile	7.6	7.6	7.6	7.6	7.6
Leu	13.1	13.1	13.0	13.0	13.0
Lys	12.0	12.0	12.0	12.0	12.0
Met	2.5	3.7	4.5	5.4	6.4
Met + Cys	5.4	6.6	7.5	8.4	9.4
Phe	8.5	8.5	8.5	8.5	8.5
Thr	7.7	7.7	7.7	7.7	7.7
Trp	2.6	2.6	2.6	2.6	2.6
Val	8.4	8.4	8.4	8.4	8.4
SAA:Lys	0.45	0.55	0.62	0.70	0.78
NE, MJ/kg	10.25	10.25	10.25	10.25	10.25
CP, g/kg	205	205.6	206	206.5	207
Total Ca, g/kg	6.0	6.0	6.0	6.0	6.0
Total P, g/kg	7.0	7.0	7.0	7.0	7.0

1 SID AA = standardised ileal digestible amino acid, calculated from analyzed AA content in feed ingredients and book values (Sauvant et al., 2004) for SID.

2 Provided the following nutrients (per kg of air-dry diet); Vitamins: A 15,000 IU, C 36 mg D3 2,000 IU, E 100 IU, K 2mg, thiamine 2 mg, riboflavin 6 mg, pyridoxine 2 mg, cyanocobalamin 30 µg, calcium pantothenate 13.8 mg, calcium-D-pantothenate 15 mg, nicotinic acid 32 mg, betaine hydrochloride 150mg, folic acid 1 mg, biotin 100 µg. Minerals: copper 150 mg (as cupric sulphate), iodine 1.5 mg (as potassium iodine), iron 160 mg (as ferrous sulphate), Mn 50 mg (as manganous oxide), Se 0.42 mg (as sodium selenite), Zn 105 mg (as zinc oxide), butylated hydroxytoluene 104 mg, propyl gallate 1.67 mg, 6-phytase 600 FTU (BigConc 2, Trouw Nutrition International, Putten, The

Netherlands)

Abbreviations: SAA:Lys = sulphur amino acid to lysine ratio.

Table 2. Composition of Phase 2 experimental diets (as fed basis).

	Calculated SID[1] SAA:Lys				
Ingredients, g/kg	0.47	0.55	0.62	0.70	0.78
Wheat/barley mix	678.8	678.0	677.3	676.5	675.7
Soybean meal	195.0	195.0	195.0	195.0	19.5
Hamlet protein	30.0	30.0	30.0	30.0	30.0
Soya oil	35.2	35.2	35.2	35.2	35.2
Sugar	20.0	20.0	20.0	20.0	20.0
Mono-calcium phosphate	13.7	13.7	13.7	13.8	13.8
Piglet premix[1]	10.0	10.0	10.0	10.0	10.0
Limestone	4.7	4.6	4.5	4.3	4.2
Salt (NaCl)	4.4	4.4	4.4	4.4	4.4
Sodium bicarbonate	2.0	2.0	2.0	2.0	2.0
Choline chloride	0.3	0.3	0.3	0.3	0.3
Vitamin E absorbate	0.7	0.7	0.7	0.7	0.7
L-Lys-HCl	3.5	3.5	3.5	3.5	3.5
L-Thr	1.3	1.3	1.3	1.3	1.3
L-Trp	0.3	0.3	0.3	0.3	0.3
DL-Met	0.0	0.9	1.7	2.6	3.5
SID AA content, g/kg[1]					
Arg	11.0	11.0	11.0	11.0	11.0
His	4.0	4.0	4.0	4.0	4.0
Ile	6.9	6.9	6.9	6.9	6.9
Leu	12.0	12.0	12.0	12.0	12.0
Lys	11.0	11.0	11.0	11.0	11.0
Met	2.3	3.2	4.0	4.9	5.8
Met + Cys	5.1	6.0	6.8	7.7	8.6
Phe	8.2	8.2	8.2	8.2	8.2
Thr	7.0	7.0	7.0	7.0	7.0
Trp	2.4	2.4	2.4	2.4	2.4
Val	7.7	7.7	7.7	7.7	7.7
SAA:Lys	0.47	0.55	0.62	0.70	0.78
NE, MJ/kg	10.25	10.25	10.25	10.25	10.25
CP, g/kg	192	193	193	194	194
Total Ca, g/kg	6.7	6.6	6.6	6.6	6.5
Total P, g/kg	6.8	6.8	6.8	6.8	6.8

[1] SID AA = standardised ileal digestible amino acid, calculated from analysed AA content in feed ingredients and book values (Sauvant et al., 2004) for SID.

[2] Provided the following nutrients (per kg of air-dry diet); Vitamins: A 15,000 IU, C 36 mg D_3 2,000 IU, E 100 IU, K 2mg, thiamine 2 mg, riboflavin 6 mg, pyridoxine 2 mg, cyanocobalamin 30 µg, calcium pantothenate 13.8 mg, calcium-D-pantothenate 15 mg, nicotinic acid 32 mg, betaine hydrochloride 150mg, folic acid 1 mg, biotin 100 µg. Minerals: copper 150 mg (as cupric sulphate), iodine 1.5 mg (as potassium iodine), iron 160 mg (as ferrous sulphate), Mn 50 mg (as manganous oxide), Se 0.42 mg (as sodium selenite), Zn 105 mg (as zinc oxide), butylated hydroxytoluene 104 mg, propyl gallate 1.67 mg, 6-phytase 600 FTU (BigConc 2, Trouw Nutrition International, Putten, The Netherlands)

Abbreviations: SAA:Lys = sulphur amino acid to lysine ratio.

Table 3. Analysed chemical composition of the experimental diets (as fed basis) for Phase 1.

	Corrected SID[1] SAA:Lys				
Analysed chemical composition, g/kg	0.46	0.55	0.61	0.69	0.77
GE, MJ/kg	17.04	17.12	17.04	17.08	17.12
CP, g/kg	209	210	211	211	210
Arg	12.9	13.2	13.1	13.3	13.1
His	4.8	4.9	4.9	4.9	4.9
Ile	8.4	8.8	8.6	8.8	8.7
Leu	14.9	15.0	15.0	15.1	15.0
Lys	12.6	13.0	12.9	13.1	13.0
Met	2.9	4.0	4.6	5.7	6.6
Met + Cys (SAA)	6.0	7.2	7.9	9.0	9.8
Phe	10.2	10.4	10.4	10.5	10.4
Thr	8.6	8.9	8.8	8.8	8.8
Trp	2.9	2.9	2.9	2.9	3.0
Val	9.3	9.6	9.6	9.7	9.5
Corrected SID SAA:Lys[1]	0.46	0.55	0.61	0.69	0.77

[1] Dietary SID SAA: Lys were corrected based on the analysed dietary AA contents using the following formula;

Corrected SID SAA:Lys = (calculated SID SAA:Lys x analysed total SAA:Lys) / calculated total SAA:Lys.

Abbreviations: SID = standardised ileal digestible, SID SAA:Lys.

Table 4. Analysed chemical composition of the experimental diets (as fed basis) for Phase 2.

	Corrected SID[1] SAA:Lys				
Analysed chemical composition, g/kg	0.47	0.55	0.63	0.71	0.78
GE, MJ/kg	17.12	17.04	17.21	17.16	17.08
CP, g/kg	193	193	193	196	180
Arg	11.8	11.6	11.8	11.8	11.7
His	4.5	4.4	4.4	4.5	4.4
Ile	7.8	7.6	7.8	7.7	7.6
Leu	13.7	13.4	13.6	13.6	13.5
Lys	11.8	11.7	11.7	11.7	11.7
Met	2.7	3.5	4.3	5.2	6.0
Met + Cys (SAA)	5.8	6.5	7.3	8.2	9.0
Phe	9.5	9.3	9.4	9.4	9.3
Thr	8.0	7.9	7.9	8.0	7.8
Trp	2.7	2.7	2.7	2.7	2.7
Val	8.7	8.6	8.6	8.6	8.5
Corrected SID SAA:Lys[1]	0.47	0.55	0.63	0.71	0.78

[1] Dietary SID SAA: Lys were corrected based on the analysed dietary AA contents using the following formula;
Corrected SID SAA:Lys = (calculated SID SAA:Lys x analysed total SAA:Lys) / calculated total SAA:Lys.

Abbreviations: SID = standardised ileal digestible.

Table 5. Mortality, faecal swabs' score, faecal swab score for F4 *E. coli*, and the incidence of post-weaning diarrhoea of pigs fed varying levels of SID SAA:Lys.

Infection	No	Yes						*P*-value		
Corrected SID SAA:Lys[1]	0.55	0.47	0.55	0.63	0.71	0.78	SEM	Treatment[2]	Time[3]	Time x Treatment
Mortality (%)	30	15	30	40	35	30		0.649		
Faecal F4 *E. coli* score[4]										
d 8	0.23	0.12	0.21	0.17	0.08	0.20	0.104	0.922	<0.001	0.973
d 10	0.54	0.44	1.36	1.08	0.92	0.93	0.359	0.476		
d 13	1.62	1.50	1.71	1.50	1.58	1.40	0.368	0.993		
d 15	0.77	1.00	0.93	0.75	0.92	0.80	0.361	0.995		
d 17	0.15	0.00	0.21	0.42	0.17	0.33	0.145	0.496		
Days with PWD[5], %										
d 7-15	4.6	5.6	7.9	5.6	5.6	4.4	1.23	0.970	0.011	0.483
d 16-22	5.9	0.0	0.0	1.2	1.2	0.9	0.68	0.151		
d 23-29	3.6	3.6	1.0	0.0	0.0	2.8	0.79	0.589		
d 7-29	4.7	3.3	3.4	2.5	2.5	2.9	0.64	0.943		

[1] SID = standardised ileal digestible
[2] Treatment = Effect of treatment
[3] Time = Effect of time
[4] Agar plates were scored from 0-5 according to the number of streaked sections that had visible growth of haemolytic *E. coli* where 0 = no growth, 1 = *E. coli* in first section, and so on 5 = heaviest growth (Heo et al., 2009).
[5] Days with PWD = percentage of days with diarrhoea (faecal score = 3).
Abbreviation: SAA = sulphur amino acid.

Table 6. Effect of SID SAA:Lys ratio on growth performance from day 6 to 36 after weaning.

	Corrected SID SAA:Lys[1]						*P*-value	
	0.47	0.55	0.63	0.71	0.78	SEM	Linear	Quadratic
BW, kg								
d 6	8.2	8.4	8.5	8.2	8.5	0.21	0.553	0.863
d 15	9.9	11.0	11.0	10.6	10.8	0.38	0.253	0.160
d 22	12.1	12.8	13.3	13.4	13.4	0.51	0.035	0.311
d 29	14.4	15.6	16.8	16.6	16.3	0.69	0.032	0.105
d 36	17.2	18.7	20.4	20.2	20.0	0.99	0.022	0.161
ADG, g/d								
d 7-15	190	291	297	301	269	25.9	0.039	0.009
d 16-22	312	286	359	437	365	37.1	0.030	0.543
d 23-29	373	428	480	440	422	38.8	0.370	0.117
d 30-36	384	439	500	556	534	51.4	0.011	0.431
d 6-36	313	365	406	428	399	27.7	0.007	0.099
ADFI, g/d								
d 7-15	279	333	347	330	314	19.9	0.275	0.030
d 16-22	467	445	463	528	491	35.3	0.086	0.761
d 23-29	579	564	647	615	599	40.1	0.470	0.449
d 30-36	618	653	730	775	772	52.9	0.011	0.596
d 6-36	471	503	540	554	541	28.5	0.034	0.320
G:F, g/g								
d 7-15	0.65	0.86	0.87	0.92	0.82	0.053	0.016	0.009
d 16-22	0.70	0.62	0.76	0.83	0.76	0.060	0.094	0.759
d 23-29	0.63	0.75	0.76	0.72	0.70	0.046	0.440	0.064
d 30-36	0.63	0.63	0.68	0.71	0.68	0.045	0.185	0.657
d 6-36	0.65	0.71	0.75	0.77	0.73	0.023	0.002	0.025

[1] SID = standardised ileal digestible.

Abbreviation: SAA = sulphur amino acid.

Table 7. Effect of SID SAA:Lys on plasma haptoglobin, albumin, and TNF- α on d 8, 10 and 20 after weaning.

	Corrected SID SAA:Lys[1]						*P*-value	
	0.47	0.55	0.63	0.71	0.78	SEM	Linear	Quadratic
Haptoglobin[2], mg/mL								
d 8	1.98	1.67	1.75	1.87	1.50	0.212	0.258	0.934
d 10	2.03	1.74	1.69	1.70	1.70	0.215	0.294	0.435
d 20	2.35	2.30	2.00	1.58	2.12	0.255	0.138	0.276
Albumin[3], mg/mL								
d 8	29.74	29.75	28.94	30.40	28.90	0.578	0.569	0.739
d 10	28.34	27.88	27.54	27.02	26.94	0.620	0.061	0.805
d 20	27.62	25.34	25.72	25.10	25.71	0.828	0.118	0.134
TNF- α[4], pg/mL	42.78	39.30	52.64	41.34	39.95	4.366	0.789	0.225

[1]SID = standardised ileal digestible.
[2] Time effect (P = 0.022) where d 8 = d 10 < d 20.
[3] Time effect (P < 0.001) where d 8 > d 10 > d 20.
[4] No effect of time (P >0.05) therefore means of SID SAA:Lys between sampling points are presented.
Abbreviation: SAA = sulphur amino acid, TNF-α = tumour necropsy factor-alpha.

Table 8. Effect of SID SAA:Lys on plasma amino acid profile for pigs on d 10 after weaning.

	Corrected SID SAA:Lys[1]						*P*-value	
	0.47	0.55	0.63	0.71	0.78	SEM	Linear	Quadratic
Essential AA (μmol/L)								
Arg	136.1	139.0	194.3	158.4	158.9	11.66	0.077	0.035
His	104.7	94.5	103.0	105.3	98.4	3.80	0.877	0.117
Ile	164.6	172.9	199.1	178.8	167.4	9.09	0.685	0.016
Leu	184.9	171.1	193.6	176.4	166.0	8.80	0.238	0.330
Lys	250.9	130.3	145.1	126.0	132.0	16.48	<0.001	0.001
Met	16.5	45.1	76.1	87.1	109.1	5.88	<0.001	0.145
Phe	101.6	106.3	123.6	117.9	121.5	5.34	0.003	0.221
Thr	478.5	237.0	245.6	229.9	215.5	32.03	<0.001	0.001
Trp	40.8	41.2	45.7	42.8	45.8	2.15	0.091	0.779
Val	378.5	325.2	383.8	329.1	303.0	20.06	0.021	0.445
Non-essential AA, μmol/L								
Asp	31.1	32.8	48.3	45.0	42.1	4.72	0.022	0.126
Glu	303.0	353.4	416.7	443.3	390.4	32.87	0.011	0.057
Asn	69.3	62.0	68.6	67.1	59.0	7.14	0.493	0.721
Ser	241.4	157.7	180.0	148.8	146.2	11.94	<0.001	0.020
Gln	122.2	192.5	133.7	145.5	126.0	15.87	0.426	0.076
Gly	1,137.4	1,000.4	1,068.1	950.4	950.6	76.43	0.077	0.760
1-methyl L-Histidine	19.6	20.6	20.6	20.2	21.5	0.72	0.129	0.897
Ala	580.9	607.1	694.7	643.7	674.5	44.16	0.107	0.446
Tau	36.1	42.8	52.7	59.7	103.6	3.66	<0.001	<0.001
Ty	83.2	89.5	101.9	97.7	94.0	6.	0.1	0.1

						73	57	58
Cys-Cystine	7.4	12.4	21.0	16.4	17.0	1.	<0.	0.0
						60	001	01
Pro	204.6	220.2	240.9	249.1	247.7	17.	0.0	0.4
						30	35	85

[a,b,c,d] Means in the same row with different superscripts differ (P<0.05).
[1] SID = standardised ileal digestible.
Abbreviation: SAA = sulphur amino acid.