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Effect of increasing the dietary tryptophan to lysine ratio on plasma levels of tryptophan, kynurenine and urea, and on production traits in weaner pigs experimentally infected with an enterotoxigenic strain of *Escherichia coli* [g]

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Effect of increasing the dietary tryptophan to lysine ratio on plasma levels of tryptophan, kynurenine and urea, and on production traits in weaner pigs experimentally infected with an enterotoxigenic strain of *Escherichia coli*

This experiment examined if immune system stimulation of weaner pigs, initiated using inoculation by an enterotoxigenic strain of *Escherichia coli* (ETEC), increased the requirement for dietary tryptophan (Trp) such that additional supplementation improved production indices, modulated the inflammatory response, and altered plasma levels of Trp and its metabolite kynurenine (Kyn). The effects on post-weaning diarrhoea were also evaluated. Individually-housed mixed-sex pigs (n = 72) weaned at 21 d of age (Landrace x Large White, initial BW of 6.3 ± 1.13 kg) were stratified into one of six treatments (n = 12) according to a two by three factorial arrangement of (i) with or without ETEC infection and (ii) three dietary ratios of standardised ileal digestible (SID) Trp to lysine (Lys; SID Trp:Lys) of 0.16, 0.20, or 0.24, in a completely randomised block design. Pigs had *ad libitum* access to diets (14.13 MJ ME/kg, 12.4 g/kg SID Lys, 195 g/kg crude protein) for three weeks after weaning. Pigs were infected with ETEC (O149:K98:K88) at 72, 96, and 120 h after weaning and then bled on d 3, 11 and 19. Infection did not affect plasma Trp and Kyn, but increasing the dietary Trp:Lys ratio increased ($p < 0.001$) plasma Trp and Kyn. Dietary SID Trp:Lys affected plasma urea (PU) levels on d 3 such that pigs fed 0.24 SID Trp:Lys had lower levels of PU than pigs fed 0.20 Trp:Lys ($p = 0.047$) On d 11, PU was lower in pigs fed 0.20 SID Trp:Lys than pigs fed 0.16 SID Trp:Lys ($p = 0.007$). Infection increased ($p = 0.039$) the diarrhoea index and deteriorated faecal consistency from d 4-10 ($p < 0.05$), however levels of haptoglobin and acid soluble glycoprotein levels were not affected by either infection or Trp treatments. Treatments did not affect daily gain or feed intake, however a SID Trp:Lys ratio of 0.24 improved ($p = 0.021$) feed efficiency over the study period compared to other ratios, without an infection effect. In conclusion, in the absence of dietary antibiotic growth promotants, increasing the dietary SID Trp:Lys ratio to 0.24 improved feed conversion ratio after weaning and increased plasma levels of Trp and Kyn regardless of infection with *E. coli*.

Keywords: acute phase proteins, *E. coli*, kynurenine, pig, tryptophan

1.0 Introduction

A number of recent reports have demonstrated that requirements for several essential amino acids are increased when the pig's immune system is activated (Li *et al.* 2007; de Ridder *et al.* 2012; Kim *et al.* 2012; Rakhshandeh *et al.* 2013). Amongst these, tryptophan (Trp) is associated with many important physiological functions. The degradation of Trp to kynurenine (Kyn) via 2,3 indoleamine dioxygenase (IDO), which increases during periods of inflammation, and the greater concentration of Trp in acute phase proteins (APP) indicate that under conditions of immune system stimulation the requirement for Trp will likely be greater (Moffett and Namboodiri 2003; de Ridder *et al.* 2012). Le Floc'h *et al.* (2006); (2009) found that unsanitary housing, which the authors predicted would cause an inflammatory response, decreased plasma levels of Trp but increased Kyn levels, and that pigs fed more Trp (0.15 versus 0.20 standardised ileal digestible (SID) Trp:Lys) were able to maintain plasma levels of Trp compared to unsupplemented pigs under inflammatory conditions. Further work by Trevisi *et al.* (2009) using an oral ETEC infection model found that increasing the Trp:Lys ratio from 0.20 to 0.28 increased feed intake and maintained growth in ETEC-susceptible pigs, but did not affect faecal consistency or shedding of ETEC, thereby partially compensating for immune system stimulation caused by ETEC infection. These data indicate an increased need for Trp during immune stress.

The National Research Council (2012) currently recommends a diet with a SID Trp:Lys of 0.16 for pigs between 7-25 kg however does not take into account health status of the pig. However as indicated above, current levels might not be sufficient for pigs raised in commercial production systems where clinical and subclinical infections occur, and particularly in the immediate post-weaning period where post-weaning diarrhoea (PWD) caused by enterotoxigenic strains of *E. coli* can be common. This disease is one of the factors contributing to the post-weaning growth check (Heo *et al*., 2009), and is a condition characterised by frequently discharged watery faeces within the first two weeks after weaning and is associated with the proliferation of some strains of enterotoxigenic *E. coli* (ETEC) (Fairbrother *et al*. 2005). Antimicrobial growth promotants (AGP) such as antibiotics and high levels of zinc oxide have been used to overcome the potential negative effects of *E. coli* (Fairbrother *et al.* 2005). However, alternative strategies are required due to restrictions or bans on AGP use and increasing concerns about antimicrobial resistance and the impact of zinc on the environment (Heo *et al.* 2013).

The overall aim of this experiment was to examine whether incorporating higher than recommended (National Research Council, 2012) levels of Trp into a weaner diet without AGP would improve indices associated with inflammation and the immune response following infection with ETEC, influence the frequency and duration of PWD, and have beneficial effects on production. The present study tested the hypothesis that pigs challenged with an ETEC would have a greater requirement for Trp than pigs without ETEC challenge as measured by plasma levels of acute-phase proteins (APP), Trp and its metabolite Kyn, and effects on production measurements.

2 Materials and Methods

The experimental protocol was reviewed and approved by the Animal Ethics Committee of the Department of Agriculture and Food Western Australia (AEC 3-11-17) and Murdoch University (R2436/11).

2.1 Experimental Design

A total of 72 pigs were stratified into one of six treatments (n = 12) according to a two by three factorial arrangement of (i) without and with ETEC infection and (ii) three dietary ratios of SID Trp to Lys of 0.16, 0.20, and 0.24, in a completely randomised block design. Pigs were fed experimental diets for three weeks after weaning and infection with ETEC occurred at 72, 96 and 120 h after weaning. Diets were formulated to meet the minimum recommendations according to National Research Council (2012) guidelines with the exception of Trp and Lys.

2.2 Animals, Housing and Diets

The 72 pigs (6.3 ± 1.13 kg; mean ± SD; Large White x Landrace, mixed sex) were sourced from a commercial piggery at weaning (21 d of age). Upon arrival, pigs were randomly allocated to treatments according to initial body weight and block within the facility. Pigs were housed individually with a space allowance of 0.4 m^2, with a feeder and a nipple bowl drinker situated in each pen. All pens were contained within the same room, with uninfected pigs located at the front of the room so that the workflow was directed from uninfected pigs to infected pigs to prevent possible contamination to the non-infected pigs. Ambient temperature was maintained at 29 ± 1 °C for the first week and decreased by 2 °C in subsequent weeks. Pigs had *ad libitum* access to water and the experimental diets for three weeks after weaning. Pigs were monitored twice daily and were weighed weekly until d 21. Feed was available to the piglets in excess, and feed intake was estimated as feed disappearance with feed wastage recorded daily and feed refusal recorded weekly per pen.

A basal diet based on wheat, barley, whey and soybean meal was formulated to contain 14.13 MJ ME and 10.4 MJ NE as calculated (Sauvant *et al.* 2004). Formulation of amino acids in the diet was based on analysed amino acid contents of ingredients and established SID coefficients to achieve sufficient levels of all nutrients, except Trp and Lys (Sauvant *et al.* 2004). Diets were formulated such that Lys was marginally limiting at 92% of requirement as outlined for 7-11 kg pigs (National Research Council, 2012). Two graded levels of L-Trp were added to basal diets to create three dietary levels of SID Trp:Lys (0.16, 0.20, and 0.24). Diets did not contain AGP, and were fed in meal form. The diet composition and analysed chemical composition are presented in Tables 1 and Table 2

2.3 Experimental infection with ETEC and Measurements of PWD

The inoculation culture of ETEC was grown as described by Heo *et al.* (2009). Infection with 6, 8, and 10 mL of ETEC (2.16 x 10^8 CFU/mL, β-haemolytic serotype O149:K98:K88; toxins LT, ST, and STb) occurred at 72, 96, and 120 h after weaning, respectively. The inoculation procedure involved mild restraint with inoculum administered orally via a drench gun in 2 mL aliquots. Faecal swabs were taken regularly after weaning to assess ETEC shedding (faecal ETEC score).

Faecal consistency was assessed daily for 14 d after weaning using a four-point scale. Faeces were given a score of (1) firm, (2) soft, spreads slightly, (3) very soft, spreads easily or (4) watery liquid consistency with the latter being considered diarrhoea. This score was then converted to percentiles such that 1 = 0%, 2 = 33.3%, 3 = 66.7% and 4 = 100%, to allow for statistical analysis. The diarrhoea index (DI) was calculated as the mean proportion of days with diarrhoea with respect to 14 d after weaning (Heo *et al.* 2009).

Faecal shedding of β-haemolytic ETEC was assessed on d 0, 3, 5, 7, 9, 11 and 14 after weaning by inserting a cotton swab into the anus. Swabs were then used to inoculate sheep blood (50 mL/L) agar plates (PathWest, Western Australia). Plates were incubated overnight at 37°C and were assessed based on morphology and haemolysis. Scores were given to plates on a six-point scale from 0 to 5 where 0 = no growth and 5 = growth in the fifth section of the plate (Heo *et al.* 2009).

2.4 Blood sampling and analysis

Blood was collected on d 3, 11 and 19 after weaning via jugular venipuncture into a 9 mL heparinised vacutainer. Samples were immediately placed on ice before being centrifuged for 15 min at 2000 x g. Plasma was then recovered and stored at -20 °C until analysis. Plasma Trp and Kyn levels on d 11 were determined using HPLC on a reverse-phase C-18 column (Laich *et al.* 2002). An Olympus AU400 analyser was used to determine plasma levels of the acute phase proteins (APP) haptoglobin (Makimura and Suzuki 1982) and acid soluble glycoprotein (ASG) (Tecles *et al.* 2007), and also plasma urea (PU; Olympus Reagent Kit OSR6134).

2.5 Statistical Analysis

All statistical analyses were performed using two-way ANOVA in GenStat (Version 15, VSN International) with SID Trp:Lys level and infection with ETEC as factors and the individual pig as the experimental unit. Where treatment effects were significant the means were separated using Fisher's protected least significant difference test. Faecal consistency and faecal shedding of ETEC were further analysed in GenStat using the repeated measures function. Statistical significance was accepted at $p < 0.05$ and $0.05 < p < 0.10$ was considered a trend. Sex was not included in the final statistical model as it had no effect.

3 Results

3.1 Plasma levels of Trp, Kyn, APP and PU

Plasma levels of Trp and Kyn were higher ($p < 0.001$) in pigs fed SID Trp:Lys ratios of 0.20 and 0.24 than 0.16. No effects of infection or interaction between the SID Trp:Lys ratio and infection were observed for plasma Trp. The PU levels before ETEC infection on d 3 were highest in pigs fed a 0.20 SID Trp:Lys ratio and lowest in pigs fed 0.24 SID Trp:Lys ($p = 0.046$). However and after ETEC infection on d 11, PU levels were highest in pigs fed 0.16 SID Trp:Lys and lowest in pigs fed 0.20 SID Trp:Lys ($p = 0.007$). Haptoglobin levels were not affected by the Trp:Lys ratio, infection, or the interaction between SID Trp:Lys and infection for any time point (Table 3).

Levels of ASG were not affected by infection for any time point. No treatment effects were observed for ASG levels before ETEC infection on d 3. After ETEC infection on d 11, a trend for an interaction between SID Trp:Lys and infection was observed such that ASG levels were higher in pigs infected and fed 0.24 SID Trp:Lys than pigs not infected and fed the same diet as well as pigs fed a diet of 0.16 SID Trp:Lys and infected ($p = 0.079$). By d 19, ASG levels were influenced by the SID Trp:Lys ratio such that pigs fed a 0.20 Trp:Lys ratio had higher levels than pigs fed other levels ($p = 0.028$; Table 3).

3.2 PWD, faecal shedding of ETEC and production indices

Infection increased the DI index ($p = 0.039$) in the first 14 d after weaning and increased faecal consistency (more liquid faeces) after infection (d 4-7 and 8-10, $p < 0.001$). There was an interaction effect of time and infection on faecal consistency score and faecal shedding of ETEC ($p < 0.001$). Faecal consistency score increased (became more liquid) in pigs infected with ETEC over time up until d 4-7 and then declined. Similarly, faecal ETEC score increased in pigs infected with ETEC until d 5 then decreased. There were no effects of the SID Trp:Lys ratio nor an interaction between the Trp:Lys ratio and infection for DI or faecal consistency. Faecal shedding of β-haemolytic ETEC was not different between groups at d 0 but was increased by infection on d 5, 9, 11 and 14 ($p <$ 0.05 – 0.01). On d 11, an interaction between the SID Trp:Lys ratio and infection was seen for faecal ETEC shedding such that pigs infected and fed 0.20 SID Trp:Lys ratio had higher scores than any other treatment group ($p = 0.029$; Table 4).

Pigs fed the diet containing a SID Trp:Lys ratio of 0.24 had a better FCR compared to pigs fed other diets for the overall period ($p = 0.021$). A trend was found for ADG from d 8 to d 14 for SID Trp:Lys and interaction with infection ($p = 0.066$). The SID Trp:Lys ratio did not affect ADFI. (Table 5).

4 Discussion

This study was conducted to test the hypothesis that the dietary requirement for Trp of weaner pigs would be greater when the immune system was stimulated, as indicated by an enhanced inflammatory state. To test this hypothesis a model of PWD, in conjunction with the absence of any AGP in the diet, was used to induce immune system stimulation, with measurements of APP and production taken. Any effects on PWD were also examined. In order to test this hypothesis, diets were formulated such that Lys was made the second limiting amino acid after Trp in the basal diet. With Lys as the second limitng amino acid, then the breakpoint can be considered the requirement of the tested amino acid (in this case Trp) relative to Lys (Boisen 2003). It is then assumed that the optimal Trp to Lys ratio is not affected by concentration Lys in the diet (Boisen 2003).

Haptoglobin and ASG are both positively linked to immune stress and are both used as indicators of immune system activation during the “acute” phase (Eckersall *et al.* 1996). In the present study, neither haptoglobin nor ASG were affected by infection with ETEC. This could be due to the nature of the acute phase response. During the acute phase response, the levels of these APP show a sharp increase immediately after infection and decline rapidly thereafter, as their biological half-lives are relatively short (Eckersall and Bell 2010). In a study comparing immune stress models in grower pigs, Rakhshandeh and de Lange (2012) found that turpentine injections caused the most severe immune system activation, lipopolysaccharide (LPS) toxin injections stemmed a sustained chronic inflammation response, and feedstuffs compromised of mycotoxins yielded a minimal immune response. Using the turpentine model, which is more likely to elicit an immune response than the ETEC model used in the present study, Eckersall *et al.* (1996) found that haptoglobin and ASG were increased two-fold soon after infection, but decreased to baseline levels on d 8 and 12 after injection, respectively. Similarly, Jacobson *et al.* (2004) used an experimental model of swine dysentery in weaner pigs and found that haptoglobin returned to baseline levels within 7 days after infection. Furthermore, the use of LPS injection in pigs shows clear elevations in APP and markers of the immune response (de Ridder *et al.* 2012; Kim *et al.* 2012; Rakhshandeh and de Lange 2012). The present study sampled pigs 5 d after infection and elevated levels of APP were expected, however this did not occur. This suggests that the degree of inflammation induced by this particular model of PWD was of insufficient magnitude relative to other models of inflammation used in pigs and (or) the timing of sampling was not within the acute phase.

Further support for this notion comes from the PWD data. A study by Kim *et al.* (2011) used the same oral infection procedure and fed diets with varying levels of protein and crystalline amino acids compared with a basal diet with AGP (control group) in an effort to reduce the incidence, severity and duration of PWD. The DI found in the present study was similar to the DI found in the control group fed AGP in the study by Kim *et al.* (2011), which indicates that a minimal response, even in DI, was elicited by the ETEC infection model used in the present study. Furthermore, Trevisi *et al*. (2009) used an oral ETEC infection model and two levels of Trp and similarly did not find significant differences in faecal consistency between Trp levels. The lack of response to infection in the present study in terms of faecal consistency score and the APP response supports the notion that whilst the infection model caused a low level of PWD (deteriorated faecal consistency and higher DI), it was most likely not severe enough to elicit an inflammatory response in the gastrointestinal tract.

The PU level is used as an indicator of protein utilisation efficiency as PU is known to increase when there is an imbalance of amino acids, as excess amino acids cannot be stored and therefore are degraded with the production of urea (Heo *et al.* 2009). Decreasing levels of PU can therefore indicate that either nitrogen use efficiency is increased or protein breakdown 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). Work by de Ridder *et al.* (2012) using a LPS infection model found that increasing dietary Trp decreased excreted nitrogen, lowered urinary nitrogen and increased protein deposition, which indicates that Trp utilisation efficiency was improved with increasing dietary Trp. In the present study, increasing the dietary ratio of SID Trp:Lys to 0.24 improved FCR. The decreased level of PU on d 11 in the pigs fed diets with a higher SID Trp:Lys ratio suggests that increasing this ratio above 0.20 improved amino acid utilisation efficiency, which is consistent with the observations for an improved FCR (Table 3). These data are also in congruence with the meta-analysis conducted by Simongiovanni *et al.* (2010), which concluded that the optima SID Trp:Lys ratio for pigs between 8 to 20 kg was 0.22 using a curve-linear plateau model and 0.26 using an asymptotic model with ADG and ADFI as the response criteria. Simongiovanni *et al.* (2010) found that the meta-analysis using feed efficiency as a response criteria yielded lower optimums (0.20 for curve linear plateau and asymptotic) than models with ADG and ADFI as the response criteria. This was explained by dietary Trp influencing feed intake (and hence feed efficiency), which is decreased in cases of imbalance of amino acids in the diet (Ettle and Roth 2004). Thus a large decrease in feed intake results in more nutrients being partitioned for maintenance, lower growth and poorer feed efficiency while a smaller decrease in feed intake will comparatively result in only a slight decrease in growth and better feed efficiency (Simongiovanni *et al.* 2010).

Increased supplementation of Trp in the diet increased plasma Trp and its metabolite Kyn. Moffett and Namboodiri (2003) commented that Trp metabolism along the Kyn pathway plays a large positive role in the immune response largely due to the enzyme 2,3 indoleamine dioxygenase (IDO). IDO is a key enzyme involved in the conversion of Trp to Kyn and is up regulated by pro-inflammatory cytokine interferon-gamma (INF-γ). The three major roles of IDO in immune function are thought to be first, that IDO is able to mediate antimicrobial effects; second, that cells activated by INF- γ and capable of producing IDO are able to inhibit pathogen growth; and third, IDO reduces the availability of Trp for pathogen growth (Trp depletion theory) (Le Floc'h and Seve 2007). The APP such as haptoglobin and fibrinogen are also known to contain high levels of Trp, thus under conditions of immune stress, particularly during the acute phase, the availability of Trp for protein deposition may be decreased with the additional production of these APP (de Ridder *et al.* 2012). The circulating Trp and Kyn levels found in the present study suggests that the dietary Trp is either being catabolised for increased protein deposition or to moderate the minimal immune response that occurred. However and unfortunately without measurements of INF- γ or IDO, this cannot be confirmed.

While the overall aim of this study was not achieved, mostly due to insufficient immune challenge, providing 0.24 SID Trp:Lys improved FCR and providing above 0.20 SID Trp:Lys suggests that protein utilisation efficiency was improved (as measured by PU). Furthermore, circulating levels of Trp and its metabolite Kyn indicated the additional Trp was being utilised. These data suggest that the optimum dietary ratio of SID Trp:Lys ratio likely lies between 0.20 and 0.24 for weaner pigs not supplied with AGP and regardless of infection with ETEC. It is suggested that a titration with five or more levels of Trp:Lys and validation in a commercial setting should be conducted to determine the optimum Trp:Lys ratio and it's commercial application.

Conflict of Interest

The authors declare they do not have any conflict interest

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Table 1. Composition of experimental diets [g/kg as fed basis]

SID Trp:Lys[#]	0.16	0.20	0.24
Ingredients [g/kg]			
Wheat	434.8	434.3	433.8
Barley	200.0	200.0	200.0
Soyabean meal, 48%	199.3	199.3	199.3
Whey powder	100.0	100.0	100.0
Canola oil	28.2	28.2	28.2
Dicalcium phosphate	13.4	13.4	13.4
Limestone	8.9	8.9	8.9
Salt	1.0	1.0	1.0
Choline chloride	0.2	0.2	0.2
Mineral Vitamin Premix*	2.0	2.0	2.0
L-Lys	5.51	5.51	5.51
L-Thr	2.52	2.52	2.52
DL-Met	2.38	2.38	2.38
L-Val	1.36	1.36	1.36
L-Ile	0.56	0.56	0.56
L-Trp, 98%	—	0.51	1.01
Calculated composition			
ME [MJ/kg]	14.13	14.13	14.13
NE[†] [MJ/kg]	10.40	10.41	10.41
Crude Protein [g/kg]	196	196	196
SID Amino Acids[#]			
Lys	12.4	12.4	12.4
Met	4.8	4.8	4.8
Met + Cys	7.8	7.8	7.8
Thr	8.2	8.2	8.2
Ile	7.2	7.2	7.2
Val	8.8	8.8	8.8
Leu	12.0	12.0	12.0
Arg	10.1	10.1	10.1
His	4.1	4.1	4.1
Phe	7.9	7.9	7.9
Trp	2.1	2.6	3.1

Note: [#]SID = standardised ileal digestible amino acid contents were calculated based on analysis of feed ingredients and tables from Sauvant *et al*. (2004). * BJ Grower (Biojohn Pty Ltd., WA, Australia) provided the following nutrients (per kg air dry diet) Vitamins: A 7000 IU, D3 1400 IU, E 20 mg, K 1 mg, thiamine 1 mg, riboflavin 3 mg, pyridoxine 1.5 mg, cyanocobalamin15 µg, calcium pantothenate 10.7 mg, folic acid 0.2 mg, niacin 12 mg, biotin 30 µg. Minerals: Co 0.2 mg (as cobalt sulphate), Cu 10 mg (as copper sulphate), iodine 0.5 mg (as potassium iodine), iron 60 mg (as ferrous sulphate), Mn 40 mg (as manganous oxide), Se 0.3 mg (as sodium selenite), Zn 100 mg (as zinc oxide). [†] Net energy values were calucalted as per (Sauvant *et al.* 2004)

Table 2. Analysed chemical composition [g/kg as fed basis]

SID Trp:Lys#	0.16	0.20	0.24
Gross energy [MJ/kg]	17.1	17.1	17.1
Crude protein [g/kg]	197	193	195
Amino acids, [g/kg]			
Met	5.0	4.8	4.8
Cys	3.6	3.6	3.6
Lys	13.8	13.1	13.1
The	9.5	9.1	9.2
Trp	2.4	2.8	3.3
Arg	10.9	10.5	10.9
Ile	7.9	7.6	8.1
Leu	13.9	13.4	13.9
Val	9.9	9.2	9.7
His	4.5	4.4	4.5
Phe	8.8	8.5	8.8
Gly	7.5	7.3	7.5
Ser	9.3	9.2	9.1
Pro	13.0	12.9	13.1
Ala	7.7	7.5	7.7
Asp	16.5	15.9	16.2
Glu	40.9	41.0	41.0
SID Trp:Lys#	0.16	0.20	0.24

Note: #SID = standardised ileal digestible amino acid contents were calculated based on analysis of feed ingredients and tables from Sauvant *et al*. (2004).

Table 3. Effect of the Trp:Lys ratio and infection on plasma levels of Trp, Kynurenine, acute phase proteins and plasma urea[#]

	Non-infected			Infected[‡]				*P*-value		
SID Trp:Lys (Trp)	0.16	0.20	0.24	0.16	0.20	0.24	SEM	Inf[*]	Trp[*]	Inf × Trp
Plasma Trp[†] [μmol/L]	49.1	80.7	89.5	42.6	86.5	80.6	4.677	0.399	<0.001	0.701
Plamsa Kynuerine[†] [μmol/L]	0.91	1.91	1.65	0.99	1.84	1.44	0.172	0.642	<0.001	0.701
Haptoglobin [mg/mL]										
d 3	2.13	2.23	2.34	2.13	2.28	1.98	0.169	0.466	0.735	0.438
d 11	1.68	1.77	1.60	1.82	1.64	1.87	0.166	0.495	0.962	0.474
d 19	1.40	1.92	1.44	1.68	1.74	1.41	0.254	0.906	0.260	0.645
Acid soluble protein [mg/mL]										
d 3	5.9	5.5	4.8	5.8	5.7	6.8	0.87	0.166	0.925	0.208
d 11	7.6	7.7	6.7	7.1	7.8	8.4	0.72	0.285	0.752	0.079
d 19	8.6	10.1	8.3	9.0	10.4	9.1	0.89	0.307	0.028	0.884
Plasma urea [mmol/L]										
d 3	3.27	3.87	3.68	3.68	4.43	2.36	0.448	0.748	0.046	0.073
d 11	4.85	3.75	3.87	4.46	3.09	4.16	0.375	0.405	0.007	0.432
d 19	3.03	2.73	2.88	3.21	2.77	3.02	0.290	0.614	0.455	0.971

Note: [#]Values are expressed as least square means with pooled standard error of mean (SEM). [‡] Pigs in the infected group were infected with *E. coli* at 72, 96 and 120 h after weaning. [*] Inf = Infection effect, Trp =SID Trp:Lys ratio effect . [†] Samples taken d 11.

Table 4. Effect of Trp:Lys ratio and infection on diarrhea index, faecal consistency and faecal ETEC shedding[#]

Infection (Inf)	Non-infected			Infected[‡]				*P*-value		
SID Trp:Lys (Trp)	0.16	0.20	0.24	0.16	0.20	0.24	SEM	Inf[*]	Trp[*]	Inf × Trp
Diarrhoea index,[†] [%]	0.0	0.0	0.0	4.2	1.2	1.2	1.27	0.039	0.405	0.405
Faecal Score[¶] [%]										
d 0-3	0.0	4.0	0.0	0.0	0.0	0.0	1.03	0.129	0.107	0.091
d 4-7	3.4	3.8	0.8	38.4	22.5	27.5	4.86	<0.001	0.212	0.254
d 8-10	0.9	1.0	4.0	13.8	15.6	14.7	3.82	<0.001	0.877	0.879
d 11-14	1.4	2.2	2.2	6.9	6.2	4.8	2.75	0.078	0.961	0.866
Faecal ETEC score[§]										
d 0	0.00	0.00	0.00	0.33	0.00	0.00	0.138	0.328	0.385	0.385
d 5	0.14	0.00	0.27	1.33	0.50	0.67	0.315	0.010	0.289	0.418
d 7	0.08	0.00	0.18	0.83	0.08	0.25	0.189	0.056	0.094	0.128
d 9	0.00	0.00	0.18	0.83	0.50	0.25	0.235	0.018	0.661	0.272
d 11	0.00[a]	0.00[a]	0.00[a]	0.08[a]	0.67[b]	0.00[a]	0.133	0.025	0.029	0.029
d 14	0.00	0.00	0.00	0.00	0.08	0.00	0.034	0.328	0.385	0.385

Note: [#]Values are expressed as least square means with pooled standard error of mean (SEM). [‡] Pigs in the infected group were infected with *E. coli* at 72, 96 and 120 h after weaning. [*]Inf = Infection effect, Trp =SID Trp:Lys ratio effect. [†] Percentage of days pigs had diarrhoea from d 0 to 14. [¶] Consistency was expressed as % cumulative score per day of pigs having more liquid faeces, higher values are associated with more liquid faeces. [§]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). [a,b] Means in the same row with different superscripts differ.

Table 5. Effect of the Trp:Lys ratio and infection with ETEC on pig production indices from weaning until 21 d after weaning[#]

Infection (Inf)	Non-infected			Infected[‡]			SEM	*P*-value		
SID Trp:Lys (Trp)	0.16	0.20	0.24	0.16	0.20	0.24		Inf[†]	Trp[†]	Inf × Trp
ADG[¶] [g/d]										
d 0 – 7	73	51	54	58	88	106	27.0	0.266	0.857	0.439
d 8 - 14	282	203	245	244	287	283	25.7	0.186	0.714	0.066
d 15 - 21	465	397	440	427	458	491	33.6	0.369	0.533	0.281
d 0 - 21	273	217	247	243	278	293	23.1	0.175	0.621	0.117
ADFI[¶] [g/d]										
d 0 - 14	241	190	205	216	269	250	26.6	0.139	0.997	0.151
d 15 - 21	676	550	591	632	701	662	50.4	0.156	0.816	0.157
d 0 - 21	386	310	334	355	413	387	32.1	0.120	0.946	0.117
FCR[§] [g/d]										
d 0 - 14	1.47	1.70	1.52	1.74	1.52	1.29	0.170	0.748	0.390	0.289
d 15 - 21	1.47	1.37	1.34	1.54	1.57	1.36	0.079	0.146	0.126	0.508
d 0 - 21	1.45	1.46	1.37	1.50	1.49	1.32	0.054	0.816	0.021	0.582

Notes: [#]Values are expressed as least square means with pooled standard error of the mean (SEM). [‡] Pigs in the infected group were experimentally challenged with *E. coli* are 72, 96 and 120 h after weaning. [†] Inf=Infection, Trp=Effect of Trp in the diet. [¶] All values were adjusted using initial body weight at weaning as a covariate. [§] Data were excluded from the data set for statistical analysis when negative feed conversion ration occurred for week 1 after weaning.