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Effects of dietary standardized ileal digestible tryptophan:lysine ratio on performance, plasma urea nitrogen, ileal histomorphology and immune responses in weaned pigs challenged with *Escherichia coli* K88☆

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ABSTRACT

A study was conducted to determine the optimal standardized ileal digestible (SID) tryptophan:lysine (Trp:Lys) ratio in piglets challenged with *Escherichia coli* K88 (*E.coli* K88) and fed antibiotic-free diets. Thirty individually housed mixed-sex pigs (Duroc × [Yorkshire × Landrace]) with an initial body weight (BW) of 6.4 ± 0.18 kg and weaned at 21 ± 1 day (d) were randomly assigned to 5 dietary treatments each with 6 pig replicates. Dietary treatments consisted of increasing levels of SID Trp:Lys ratios (16.1%, 18.6%, 20.3%, 22.9%, and 24.6%). Diets were corn-wheat-soybean meal-based with a constant SID Lys of 1.18% that was set to be the second limiting amino acid (AA) but adequate in other AA. Pigs had ad libitum access to feed and water for 13 d. After feeding the experimental diets for 6 d, pigs were orally challenged with 6 mL of *E. coli* K88 (2×10^{11} colony forming unit/mL) on d 7. Body weights and pen feed disappearance were recorded weekly to determine ADG, ADFI and G:F. On d 13, all pigs were euthanized and ileal tissue samples were collected to measure mRNA expression of tumour necrosis factor-α (TNF- α), interleukin-10 (IL-10) and interferon-γ (IFN-γ) using qRT-PCR. During the pre-challenge period (d 0–6), increasing dietary SID Trp:Lys ratio increased (linear, $P < 0.05$) ADG (157, 162, 173, 179 and 201 g/d) and G:F (0.71, 0.73, 0.74, 0.81 and 0.84). During the post-challenge period, increasing SID Trp:Lys ratios tended (linear, $P = 0.08$) to increase ADG (177, 180, 208, 210 and 213 g/d), whereas there was no effect on G:F (0.58, 0.63, 0.67, 0.66, 0.65) ($P > 0.10$). The optimal SID Trp:Lys ratio determined using the broken-line regression analysis for ADG and G:F in piglets subjected to *E.coli* K88 challenge was 21.7% and 20.1%, respectively. Expression of IL-10 mRNA, increased (linear and quadratic, $P < 0.01$) with increasing SID Trp:Lys ratio, however, expression of TNF- α and IFN- γ mRNAs were not affected. In conclusion, in antibiotic-free starter diets, an average optimal SID Trp:Lys ratio of 21% optimized performance of piglets under an *E. coli* K88 challenge.

1. Introduction

Tryptophan (Trp) is considered the second or third limiting amino acid (AA) in cereal-based diets for pigs (Guzik et al., 2005). The importance of Trp is not only for protein synthesis, but also for the control of immune response and health maintenance (Le Floc'h and Sève, 2007). The role of Trp in regulating the immune response is through the kynurenine pathway in which Trp is catabolised into kynurenine (Le Floc'h and Sève, 2007). Under inflammation or immune challenge conditions, there is an increase in Trp catabolism to kynurenine (Le Floc'h et al., 2008), which suggests that providing sufficient Trp is important to achieve optimal growth performance for pigs under stressful conditions.

Due to the ban on antimicrobial growth promoter (AGP) implemented by European Union, there is an insistence to completely eliminate or reduce the usage of AGP in livestock diets, which could be reflected in increased Trp:Lys requirements in young pigs. For young pigs (7 and 25 kg), NRC (2012), and BSAS (2003) recommend a diet with a standardized ileal digestible (SID) Trp:Lys ratio of 16% and 19%, respectively, but these values correspond to healthy pigs. Moreover, the Trp:Lys ratio requirement for piglets might vary when clinical or subclinical infections occur, specifically, when piglets are exposed to immunological challenge as is often the case under *Escherichia coli* K88 (*E. coli* K88) infection. Furthermore, *E. coli* K88 is one of the most common

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causes of diarrhoea in weaned piglets (Trevisi et al., 2009) characterized by watery faeces discharge during the post-weaning period and accompanied with the growth of *E. coli* K88 in the gut mucosa (Fairbrother et al., 2005).

Previous studies have shown that metabolism and requirement of Trp are modified in pigs during immune challenge conditions (Le Floc'h and Sève, 2007; Le Floc'h et al., 2009). Jayaraman et al. (2016) reported that the optimal SID Trp:Lys ratio for weaned pigs raised under unclean sanitary conditions was 4% units higher than for those raised under clean sanitary conditions. Furthermore, Trevisi et al. (2009) reported that piglets fed diets with an SID Trp:Lys ratio ranging from 20% to 28% and challenged with *E. coli* K88 had increased feed intake and maintained growth in *E. coli* K88 susceptible pigs, thereby partially compensating for immune system stimulation caused by *E. coli* K88 infection. Information about the Trp:Lys ratio requirement for piglets fed AGP-free diets and subjected to an immunological challenge is limited. We hypothesized that the optimal SID Trp:Lys ratio would be higher for weaned piglets subjected to immune challenge conditions. Thus, the aim of this experiment was to determine the optimal SID Trp:Lys ratio for weaned piglets subjected to an *E. coli* K88 challenge.

2. Materials and methods

The animal protocol for this study was reviewed and approved by the Animal Care Committee of the University of Manitoba and pigs were cared for in accordance with the guidelines of the Canadian Council on Animal Care (2009).

2.1. Animals and experimental design

Thirty piglets ((Duroc × [Yorkshire × Landrace]), females and castrated males) weighing

6.41 ± 0.18 kg (Mean ± SD) were obtained from Glenlea Research Station, Manitoba at weaning (21 ± 1 d of age). Diets were corn, wheat and soybean meal (SBM)-based with a constant SID Lys of 1.18% that was expected to be the second limiting AA (Table 1). Ingredients contributing AA (corn, wheat, SBM) were analyzed for AA contents and these values in combination with the respective SID of ingredients were used in diet formulation. The diets contained increasing levels of SID Trp:Lys ratio (16.1%, 18.6%, 20.3%, 22.9% and 24.6%) (Table 2). All other nutrients were provided in quantities meeting or exceeding the NRC (2012) recommendations for 6–10 kg pigs. Pigs had ad libitum access to feed and water. The experiment lasted for 13 d. Individual pig BW and pen feed disappearance were recorded during the pre-challenge and post-challenge periods to determine ADG, ADFI and G:F. On d 7, all pigs were orally inoculated with 6 mL of *E.coli* K88 culture (2×10^9 colony forming unit (cfu)/mL). Faecal consistency scoring (0 = normal, 1 = soft faeces, 2 = mild diarrhoea, and 3 = severe diarrhoea) was performed by 2 trained individuals in a treatment-blinded manner as described by Marquardt et al. (1999). Rectal temperature was measured in piglets before (24 h) and after (6 h, 24 h, 48 h) *E.coli* K88 challenge.

2.2. Escherichia coli K88 and culture condition

The *E. coli* K88 strain was obtained from Veterinary Diagnostic Services of Manitoba, (Winnipeg), Manitoba, Canada. From the frozen stock, *E. coli* K88 was streaked on brain heart infusion (BHI) agar and grown anaerobically at 37 °C overnight. Then a single colony was inoculated on two BHI plates (i.e. duplicate) and incubated anaerobically at 37 °C overnight.

Two tubes of 5 mL BHI broth (BD & Co., Franklin Lakes, New Jersey, USA) plus 2% casamino

acids (Fisher Scientific, Waltham, MA, USA) were inoculated from a single colony and grown overnight at 37 °C with shaking (200 *rpm*). The *E. coli* K88 identity was verified using an *E. coli* K88 fimbrex latex agglutination kit. Two flasks of 500 mL BHI broth plus 2% casamino acids were inoculated with 2 mL *E. coli* K88 from the 5 mL culture tube and then incubated anaerobically at 37 °C overnight with shaking (200 *rpm*). The two 500 mL flasks were combined and thoroughly mixed. With serial dilution of the culture 10-fold in PBS, 10^6 to 10^9 dilutions were plated on BHI plates to check that the culture was $> 1 \times 10^9$ cfu. Incubation was done anaerobically at 37 °C overnight. The colonies on the dilution plates were counted the following day to determine concentration and 6 mL of 2×10^{11} cfu/mL per piglet was used for inoculation.

Table 1
The composition and calculated nutrient contents of experimental diets.

Items	Standardized ileal digestible tryptophan:lysine, %				
Ingredient composition, g/kg	16.1	18.6	20.3	22.9	24.6
Corn	434.00	434.00	434.00	434.00	434.00
Wheat	210.00	210.00	210.00	210.00	210.00
Soybean meal	274.00	274.00	274.00	274.00	274.00
Vegetable oil	29.20	29.20	29.20	29.20	29.20
Corn starch	5.00	4.80	4.60	4.30	4.00
Limestone	10.90	10.90	10.90	10.90	10.90
Dicalcium phosphate	15.60	15.60	15.60	15.60	15.60
Iodized salt	3.00	3.00	3.00	3.00	3.00
Vitamin-mineral premix[a]	10.00	10.00	10.00	10.00	10.00
L-Lysine. HCl	4.40	4.40	4.40	4.40	4.40
DL-Methionine	1.70	1.70	1.70	1.70	1.70
L-Threonine	1.90	1.90	1.90	1.90	1.90
L-Tryptophan	0.00	0.24	0.48	0.72	0.96
L-Valine	0.60	0.60	0.60	0.60	0.60
Calculated net energy and nutrient content (g kg^{-1})					
NE (MJ kg^{-1})	14.00	14.00	14.00	14.00	14.00
CP	20.15	20.17	20.19	20.21	20.23
SID[b] Arg	10.4	10.4	10.4	10.4	10.4
SID His	4.4	4.4	4.4	4.4	4.4
SID Ile	6.6	6.6	6.6	6.6	6.6
SID Leu	13.7	13.7	13.7	13.7	13.7
SID Lys	11.8	11.8	11.8	11.8	11.8
SID Met	4.3	4.3	4.3	4.3	4.3
SID Met + Cys	7.1	7.1	7.1	7.1	7.1
SID Phe	7.9	7.9	7.9	7.9	7.9
SID Trp	1.9	2.2	2.4	2.7	2.9
SID Thr	7.6	7.6	7.6	7.6	7.6
SID Val	8.0	8.0	8.0	8.0	8.0

[a] Supplied the following per kilogram of diet:8250 IU of vitamin A, 835 IU of vitamin D3, 40 IU of vitamin E, 25 μg of vitamin B12, 4 mg of vitamin K, 25 μg of niacin, 600 mg of choline, 12 mg of riboflavin, 200 μg of biotin, 4.5 mg of pyridoxine, 4 mg of folic acid, 2 mg of thiamin, 50 mg of Mn, 150 mg of Zn, 120 mg of Fe, 25 mg of Cu, 0.35 mg of Se, 0.4 mg of I.
[b] Standardized ileal digestible.

Table 2
Analyzed AA contents of the experimental diets (as-fed basis, g kg^{-1}).

Items	Standardized ileal digestible tryptophan:lysine, %				
	16.1	18.6	20.3	22.9	24.6
CP	197.0	196.0	192.0	191.0	190.0
Lys	13.8	13.4	13.2	13.0	13.3
Trp	2.6	2.7	2.8	3.0	3.3
Met	4.5	4.8	4.9	4.9	4.8
Met + Cys	8.1	8.2	8.1	8.1	8.0
Thr	8.9	8.3	8.1	8.8	8.5
His	4.8	4.4	4.5	4.8	4.5
Ile	7.8	7.2	7.4	7.6	7.2
Leu	15.1	14.2	14.5	14.9	14.2
Phe	9.3	8.5	8.8	8.9	8.6
Val	9.5	9.1	9.1	9.2	9.1