

JOURNAL OF ANIMAL SCIENCE

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J ANIM SCI 2012, 90:191-193.
doi: 10.2527/jas.53900

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http://www.journalofanimalscience.org/content/90/Supplement_4/191



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An increased ratio of dietary tryptophan to lysine improves feed efficiency and elevates plasma tryptophan and kynurenine in the absence of antimicrobials and regardless of infection with enterotoxigenic *Escherichia coli* in weaned pigs

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ABSTRACT: This experiment examined if a higher ratio of dietary Trp:Lys in the absence of antimicrobials improves production indices and modulates diarrhea in weaned pigs infected with enterotoxigenic *Escherichia coli* (ETEC). Effects of the Trp:Lys ratio on plasma levels of Trp and its metabolite kynurenine (Kyn) were also examined. Individually housed mixed-sex pigs (n = 72) weaned at 21 d of age (Landrace × Large White; initial BW of 6.3 ± 0.32 kg) were stratified into 1 of 6 treatments (n = 12) according to a 2 × 3 factorial arrangement of (i) infection or without infection with ETEC and (ii) 3 dietary standardized ileal digestible (SID) Trp:Lys ratios of 0.17, 0.21, or 0.26 in a randomized complete block design. Pigs were fed diets (10.4 MJ NE; 1.24% SID Lys; 19.5% CP) ad libitum

for 3 wk after weaning. Pigs were infected with ETEC (O149:K98:K88) at 72, 96, and 120 h after weaning and then bled on day 11. A Trp:Lys ratio of 0.26 improved (P = 0.021) G:F over the study period compared to other ratios, without an infection effect (P > 0.05). Treatments did not affect ADG or ADFI (P > 0.05). Infection increased (P = 0.039) the diarrhea index and increased fecal consistency scores (P = 0.010). Plasma Trp and Kyn were lower (P < 0.001) in pigs fed 0.17 Trp:Lys than those fed ratios of 0.21 and 0.26 and were not affected (P > 0.05) by infection. In conclusion, in the absence of antimicrobials, increasing the dietary Trp:Lys ratio to 0.26 improved G:F after weaning and increased plasma levels of Trp and Kyn.

Key words: feed efficiency, kynurenine, pig, tryptophan, weaner

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J. Anim. Sci. 2012.90:191–193
doi:10.2527/jas53900

INTRODUCTION

Postweaning diarrhea is a multifactorial disease associated with proliferation of some strains of enterotoxigenic *Escherichia coli* (ETEC). For pigs susceptible to ETEC, additional Trp increased feed intake and maintained growth, thereby partially compensating for immune system (IS) stimulation caused by ETEC infection (Trevisi et al., 2009). Tryptophan is associated with many important physiological functions, and of particular interest is the degradation of Trp to kynurenine (Kyn). Plasma levels of Trp decrease and Kyn levels increase when the IS is stimulated and inflammation occurs (Le Floc'h et al., 2008). Pigs with inflammation and fed more Trp maintained plasma levels of Trp compared to unsupplemented pigs. These data indicate

an increased need for Trp during immune stress. This study tested the hypothesis that pigs challenged with an ETEC infection will have a greater requirement for Trp than pigs without IS stimulation as measured by production indices and plasma levels of Trp and its metabolite Kyn.

MATERIALS AND METHODS

The trial protocol was reviewed and approved by the Animal Ethics Committee of the Department of Agriculture and Food Western Australia. A total of 72 pigs were stratified into 1 of 6 treatments (n = 12) according to a 2 × 3 factorial arrangement of (i) infection or noninfection with ETEC and (ii) 3 levels of standardized ileal digestible (SID) Trp to Lys ratios of 0.17, 0.21, and 0.26 in a randomized complete block design. Pigs (Large white × Landrace, mixed

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sex) weighing 6.3 ± 0.32 (SE) kg were sourced from a commercial piggery at weaning (21 d of age). Pigs were housed individually and fed experimental diets for 3 wk after weaning. A basal diet was formulated to contain 10.4 MJ NE/kg based on wheat (*Triticum aestivum*), barley (*Hordeum vulgare*), whey, and soybean (*Glycine max*) meal. Formulation was based on analyzed AA content of ingredients and established SID coefficients to achieve sufficient levels of all AA, except Trp and Lys that were marginally limiting for these pigs (Rademacher et al., 2009). Two graded levels of L-Trp were added to basal diets to create 3 dietary levels of SID Trp:Lys (0.17, 0.21, and 0.26). Antimicrobial compounds were not included in the diets. Infection with 6, 8, and 10 mL of ETEC [2.16×10^8 cfu/mL; β -hemolytic serotype O149:K98:K88; heat-labile enterotoxin (LT) and heat stable enterotoxins 1 and 2 (ST1 and ST2)] occurred at 72, 96, and 120 h after weaning, respectively (McDonald et al., 2001). Fecal swabs were taken regularly after weaning to assess ETEC shedding and are reported as fecal ETEC score. The diarrhea index was calculated as percentage of days with diarrhea up to 14 d after weaning (Vicente et al., 2008). Fecal consistency was assessed for 14 d after weaning using a 4-point scale ranging from 1 to 4 with a score of 4 being considered diarrhea. Blood was collected on day 11 and analyzed for plasma Trp and Kyn using HPLC on a reverse-phase C-18 column (Laich et al., 2002). Plasma haptoglobin levels were analyzed using an Olympus AU400 analyzer based on the method by Makimura and Suzuki (1982). All statistical analyses were performed using SAS (version 9.3; SAS Institute Inc., Cary, NC) with pig as experimental unit. With significant treatment effects, means were separated using Fisher's protected LSD test. Statistical significance was accepted at $P < 0.05$ and $0.05 < P < 0.10$ was considered a trend.

RESULTS

Infection did not affect ($P > 0.05$; Table 1) growth performance variables and an interaction between SID Trp:Lys ratio and infection was not observed ($P > 0.05$). Gain:feed was greater ($P = 0.021$) for pigs fed the diet containing SID Trp:Lys ratio of 0.26 than for pigs fed the other ratios. The ADG or ADFI was not affected ($P > 0.05$) by Trp:Lys. Infection increased diarrhea index ($P = 0.039$). Infected pigs had higher ($P = 0.009$) fecal consistency scores on days 4 to 9 and on day 11 after weaning than noninfected pigs (data not shown). Fecal ETEC score was increased ($P = 0.010$) in infected pigs on day 5. Plasma levels of Trp and Kyn were higher ($P < 0.001$) in pigs fed SID Trp:Lys ratios of 0.21 and 0.26 than 0.17 (1.88 and 1.54 vs. 0.95). Plasma levels of Trp, Kyn, and haptoglobin were not affected ($P > 0.05$) by infection and an interaction between infection and SID Trp:Lys interaction ($P > 0.05$) was not observed.

DISCUSSION

This experiment examined whether the requirement for dietary Trp was higher in pigs under immune stimulation, using ETEC infection as challenge, compared to noninfected pigs. The lack of any interaction between Trp:Lys ratio and ETEC infection for any measurement indicates that whereas ETEC infection caused diarrhea, the magnitude of immune stimulation was insufficient to suitably test the hypothesis. Only 17% of infected pigs were classified as having diarrhea in this study. Nevertheless, feeding 0.26 SID Trp:Lys improved G:F, indicating enhanced efficiency of nutrient use. These data are similar to a recent meta-analysis indicating that Trp:Lys requirements of pigs from 7 to 30 kg were 0.22, 0.22, and 0.20 with ADG, ADFI, and G:F as response criteria, respectively (Simongiovanni et al. 2012). Others have reported beneficial effects of Trp above these ratios

Table 1. Effect of the Trp:Lys ratio and infection with enterotoxigenic *Escherichia coli* (ETEC) on pig production indices from weaning until 21 d after weaning, diarrhea index, fecal ETEC score on day 5, and plasma levels of Trp, kynurenine, and haptoglobin. Values are expressed as least square means with pooled SEM

Item	Noninfected SID ¹ Trp:Lys			Infected SID Trp:Lys			SEM	P-value ²		
	0.17	0.21	0.26	0.17	0.21	0.26		Inf	Trp	Inf × Trp
ADG ³ , g	273	217	247	243	278	293	23.1	0.175	0.621	0.117
ADFI ³ , g	386	310	334	355	413	387	32.1	0.120	0.946	0.117
G:F ³ , g/g	0.70	0.69	0.74	0.69	0.68	0.77	0.026	0.981	0.021	0.557
Diarrhea index ⁴ , %	0.0	0.0	0.0	4.2	1.2	1.2	1.27	0.039	0.405	0.405
Fecal ETEC score ⁵ , day 5	0.17	0.00	0.27	1.33	0.50	0.67	0.315	0.010	0.289	0.418
Kynurenine, μ mol/L	0.91	1.91	1.64	0.99	1.85	1.44	0.173	0.688	<0.001	0.701
Trp, μ mol/L	49.1	80.7	89.5	42.6	86.5	80.6	4.677	0.399	<0.001	0.233
Haptoglobin, mg/mL	1.68	1.77	1.60	1.82	1.64	1.87	0.166	0.495	0.962	0.474

¹SID = standardized ileal digestible.

²Inf = effect of infection; Trp = effect of SID Trp:Lys.

³All values adjusted using initial BW at weaning as the covariate.

⁴Diarrhea index: percentage of days with diarrhea up to 14 d after weaning (after Vicente et al., 2008).

⁵Fecal score was assessed from fecal swabs.

on performance (Jansman et al., 2010).

In contrast to the work by Le Floch et al. (2008) using a model of lung inflammation, pigs immune challenged with ETEC in the present study had the same plasma levels of Trp and Kyn as noninfected controls. The lack of decrease in plasma Trp levels or increase in plasma Kyn levels with ETEC infection or change in growth performance confirms that immune stimulation was likely insufficient. Le Floch et al. (2009) reported that low-grade inflammation reduced growth performance and plasma Trp concentrations, indicating that Trp availability for growth and other metabolic functions was affected; however, feeding more dietary Trp did not counteract the effects of inflammation. Furthermore, sanitary status or Trp inclusion level did not affect Kyn levels, but pigs housed under poor sanitary status had higher haptoglobin levels. In conclusion, in the absence of antimicrobials, a dietary SID Trp:Lys ratio to 0.26 improved G:F after weaning and feeding increasing SID Trp:Lys ratios from 0.17 to 0.21 or 0.26 increased plasma levels of Trp and Kyn.

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