

Feeding a diet with decreased protein content reduces indices of protein fermentation and the incidence of postweaning diarrhea in weaned pigs challenged with an enterotoxigenic strain of *Escherichia coli*¹

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ABSTRACT: This study evaluated the effect of feeding low protein (LP) diets for 7 or 14 d after weaning or a high protein (HP) diet for 14 d after weaning on postweaning diarrhea (PWD), indices of protein fermentation, and production in pigs infected or not infected per os with an enterotoxigenic strain of *Escherichia coli*. A total of 72 female pigs weaned at aged 21 d with initial BW of 5.9 ± 0.12 kg were used in a 3×2 factorial arrangement of treatments. The factors were 3 feeding regimens associated with different combinations of feeding duration and diet CP level: (i) HP diet (256 g of CP/kg) fed for 14 d after weaning, (ii) LP diet (175 g of CP/kg) fed for 7 d after weaning, and (iii) LP diet fed for 14 d after weaning; and infection or noninfection with an enterotoxigenic strain of *E. coli* (10^7 cfu/mL, serotype O149:K91:K88) at 72, 96, and 120 h after weaning. The LP diets were fortified with crystalline Ile and Val to achieve an ideal AA pattern. A second-stage diet (213 g of CP/kg) was fed to pigs at the conclusion of each feeding regimen, and the study finished 4 wk

after weaning. None of the diets contained antimicrobials. Feeding the LP diets decreased ($P < 0.001$) plasma urea nitrogen, fecal ammonia nitrogen concentrations, and the incidence of PWD, but increased ($P = 0.001$) fecal DM content compared with pigs fed HP in the 2-wk period after weaning. Infection increased shedding of β -hemolytic *E. coli* ($P < 0.001$), the incidence of PWD ($P < 0.001$), and fecal ammonia nitrogen concentrations ($P < 0.01$), but did not interact with feeding regimen, after weaning. Pigs challenged with *E. coli* grew more slowly ($P < 0.001$) and had decreased G:F ($P < 0.01$) compared with nonchallenged pigs in the 4-wk period after weaning. Feeding an LP diet for 7 or 14 d after weaning markedly reduced the incidence of PWD after infection with β -hemolytic *E. coli*. Infection was associated with decreased indices of protein fermentation in the distal gastrointestinal tract but did not compromise the growth of weaner pigs in the 4-wk period after weaning.

Key words: *Escherichia coli*, infection, low protein, pig, postweaning diarrhea, production

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INTRODUCTION

Postweaning diarrhea (PWD) is a multifactorial condition characterized by frequent discharge of watery feces that can cause a growth check, morbidity, and

mortality (Hampson, 1994; Madec et al., 2000). This condition is typically associated with fecal shedding of β -hemolytic enterotoxigenic strains of *Escherichia coli* (ETEC). These strains proliferate in the small intestine after they attach to epithelial receptors and release toxins (Hampson, 1994), although it is recognized that seemingly healthy pigs may also shed small numbers of these bacteria (Osek, 1999; Schierack et al., 2006).

Diets for weanling pigs generally contain 200 to 230 g of CP/kg to support maximum rates of lean tissue gain (NRC, 1998). However, not all dietary protein is available for metabolism. For example, Högberg and Lindberg (2004) reported that digestibility for CP at the terminal ileum was between 60 to 80% in weaned pigs

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aged 27 to 28 d fed a diet based on triticale and wheat. Undigested dietary protein plus proteins of endogenous origin pass into the distal gastrointestinal tract (**GIT**), which can encourage the growth of N-utilizing bacteria (Piva et al., 1996; Reid and Hillman, 1999) that ferment protein to produce potentially harmful end products such as branched-chain fatty acids (**BCFA**), indole, phenols, ammonia, and biogenic amines. In turn, these products have been implicated in the etiology of PWD (Gaskins, 2001; Pluske et al., 2002; Kim et al., 2008).

A recent study in our laboratory showed that feeding a decreased protein (173 g of CP/kg) diet after weaning reduced indices of protein fermentation with an associated decrease in diarrhea, and without adverse effects on production compared with feeding a greater protein (243 g of CP/kg) diet (Heo et al., 2008). This experiment was conducted in a facility having low infection pressure, which differs from commercial practice where piglets are exposed to greater levels of bacterial challenge. The responses to a low protein (**LP**) diet may therefore be different under conditions of greater bacterial pathogen load.

The hypothesis tested in this study was that feeding an LP diet supplemented with crystalline Ile and Val to maintain an ideal AA pattern would decrease the incidence of PWD without compromising growth. To elucidate whether bacterial infection pressure interacts with dietary protein level, an experimental ETEC infection model established in our laboratory was used to induce a moderate diarrhea after weaning (Montagne et al., 2004).

MATERIALS AND METHODS

The Murdoch University Animal Ethics Committee approved the practices and procedures used in this experiment.

Experimental Design

The experiment was a 3×2 factorial arrangement of treatments. The factors comprised 3 feeding regimens associated with different combinations of feeding duration and diet CP level, described as (i) high protein (**HP**) diet (256 g of CP/kg) fed for 14 d after weaning (**HP14**), (ii) LP diet (175 g of CP/kg) fed for 7 d after weaning (**LP7**), and (iii) LP diet fed for 14 d (**LP14**) after weaning; and infection or noninfection with ETEC at 72, 96, and 120 h after arrival, respectively (see below for further details). At the conclusion of each feeding regimen, all piglets then received a second-stage diet having an intermediate protein content (213 g of CP/kg) until the experiment finished 4 wk after weaning. However, because LP7 and LP14 were the same diets until d 7, all data (except data for performance) between d 1 and 7 were pooled and analyzed as HP or LP in the first week after weaning (see Statistical Analyses section). All diets were formulated to meet

the ideal pattern of ileal digestible AA according to the recommendations of Chung and Baker (1992). None of the diets contained antimicrobial compounds.

Animals, Housing, and Diets

A total of 72 female pigs (Large White \times Landrace) weaned at 21 d of age with initial BW of 5.9 ± 0.12 kg were used. Pigs were obtained from a commercial supplier (Wandalup Farms Ltd., Mandurah, Western Australia, Australia) at weaning and transported to an animal facility at Murdoch University. Pigs were allocated to their experimental treatments based on initial BW and block within room in the animal facility. This facility contains 3 rooms that allowed infected and noninfected pigs to be housed separately. The 36 infected pigs were housed in 1 room that contained 6 pens, with 6 pigs allocated to each pen (space allowance of 0.44 m² per pig and a feeder space allowance of 3.9 cm per pig). The 36 noninfected pigs were housed in another 2 rooms that also contained 6 pens each; in this case, each pen contained 3 pigs (space allowance of 0.88 m² per pig and a feeder space allowance of 7.8 cm per pig). These feeder and space allowances exceed those recommended for pigs of this BW (e.g., Wolter et al., 2002; Payne et al., 2006). In addition, the space allowance exceeded that recommended for nursery pigs by the Model Code of Practice for the Welfare of Animals: Pigs (CSIRO, 2008).

All infected pigs were housed in a single room to avoid any cross contamination of pigs from the different rooms associated with the disease challenge (after Ding et al., 2006) and to encourage proliferation of ETEC within a pen. We reasoned that housing more (i.e., $n = 6$) pigs per pen for the infection treatments would assist in exacerbating the disease due to the oral-fecal recycling of ETEC (Hampson, 1994). Pens contained plastic-slatted flooring with a nipple bowl drinker and a single-space feeder. A round feeding bowl was placed in each pen to encourage feed consumption in the immediate postweaning period. The ambient temperature was maintained at $29 \pm 1^\circ\text{C}$ for the initial week, and then gradually decreased by 2°C in wk 2 and 3. The pigs were offered their respective experimental diets ad libitum for 4 wk and had free access to water at all times. Cleaning and feeding schedules were implemented to ensure that movement between rooms was conducted in the order from noninfected to infected groups.

Diets were formulated to contain a similar DE content, but different protein levels (Tables 1 and 2), and were formulated using standardized ileal digestible AA contents (Sauvant et al., 2004). All diets were formulated to at least contain an ideal pattern of ileal digestible AA (Chung and Baker, 1992). Crystalline AA (Lys, Met, Trp, and Thr) were added to the HP and LP diets, with crystalline Ile and Val added to the LP diets to achieve the ideal pattern of essential AA. In this regard, to reflect a more expensive cost associated with fortifying diets with crystalline Ile and Val, LP

diets were fed for 7 or 14 d to examine whether such a diet could be fed for a shorter period after weaning and achieve the same effects, thereby reducing production costs. All diets were fed in mash form. Diet composition and nutrient contents of the experimental diets are presented in Tables 1 and 2. Pigs were weighed weekly for 4 wk.

Infection Procedures

A strain of enterotoxigenic β -hemolytic *E. coli*, serotype O149:K91:K88 [toxins heat labile toxins (**LT**), heat stable toxins (**ST**; variants STa and STb), Department of Natural Resources and Environment, Ben-

Table 1. Composition of the experimental diets (g/kg, as-fed basis)

Item	Diet ¹		
	HP	LP	Stage II
Ingredient, g/kg			
Barley	167.8	200.0	200.0
Wheat	355.6	424.0	406.4
Oat groats	50.0	86.8	50.0
Canola meal	100.0	100.0	100.0
Soybean meal	200.0	38.6	153.9
Fishmeal	52.7	21.7	20.0
Whey	50.0	81.1	30.0
Canola oil	5.0	5.0	5.0
Dicalcium phosphate	10.6	19.2	17.4
Limestone	6.2	11.9	11.2
Salt	1.0	1.0	1.0
Vitamin/mineral premix ²	1.0	1.0	1.0
L-Lys		6.0	3.0
DL-Met		1.0	0.5
L-Thr		1.8	0.7
L-Trp		0.3	
L-Ile		0.4	
L-Val		0.3	
Calculated composition			
CP, g/kg	240.0	180.0	205.0
DE, MJ/kg	14.00	13.80	13.80
SID ³ Lys, g/MJ of DE	0.81	0.80	0.78
Starch, g/kg	323.7	372.1	368.9
Total NSP, ⁴ g/kg	145	128	147
Insoluble NSP	106	94	108
Soluble NSP	39	34	39
SID AA, ⁵ g/kg			
Lys	11.3	11.0	10.8
Met	3.6	3.5	3.2
Thr	7.9	7.2	7.0
Trp	2.5	2.0	2.1
Ile	9.2	6.6	7.4
Leu	15.6	11.5	12.8
Val	10.2	7.5	8.4
Total AA, g/kg			
Arg	14.7	9.1	12.2
His	5.8	4.0	4.9
Ile	10.5	7.7	8.5
Leu	17.6	12.9	14.6
Lys	13.2	13.0	12.6
Met	4.0	4.0	3.6
Phe	10.9	7.4	9.2

Continued

Table 1 (Continued). Composition of the experimental diets (g/kg, as-fed basis)

Item	Diet ¹		
	HP	LP	Stage II
Thr	9.4	8.5	8.3
Trp	3.0	2.3	2.5
Val	12.0	8.9	10.0
Ala	10.6	7.2	8.5
Asp	21.0	12.3	16.7
Glu	48.4	37.3	42.9
Gly	11.0	7.6	8.9
Pro	15.1	12.8	13.7
Ser	11.1	7.7	9.3

¹HP = high protein diet; LP = low protein diet; stage II = second-phase diet.

²Provided the following nutrients (per kg of air-dried diet): vitamins: A, 7,000 IU; D₃, 1,400 IU; E, 20 mg; K, 1 mg; thiamine, 1 mg; riboflavin, 3 mg; pyridoxine, 1.5 mg; cyanocobalamin, 15 μ g; calcium pantothenate, 10.7 mg; folic acid, 0.2 mg; niacin, 12 mg; biotin, 30 μ g. Minerals: Co, 0.2 mg (as cobalt sulfate); Cu, 10 mg (as copper sulfate); iodine, 0.5 mg (as potassium iodine); iron, 60 mg (as ferrous sulfate); Mn, 40 mg (as manganous oxide); Se, 0.3 mg (as sodium selenite); Zn, 100 mg (as zinc oxide; BJ Grower 1, BioJohn Pty Ltd., WA, Australia).

³SID = standardized ileal digestible.

⁴Nonstarch polysaccharides (NSP) contents were calculated based on Bach Knudsen (1997).

⁵SID AA = standardized ileal digestible AA contents were calculated based on feed ingredient evaluation tables (Sauvant et al., 2004).

diglo, Victoria, Australia], was grown on sheep blood (50 mL/L) agar plates (Columbia base, Oxoid, Western Australia, Australia; McDonald et al., 2001), and incubated overnight at 37°C. A representative colony was then moved from the plate and seeded into 4 mL of sterile trypticase soy broth (Becton Dickinson, Franklin Lakes, NJ) in a McCartney bottle and incubated overnight at room temperature (20 to 25°C). It was then transferred to a larger volume of sterile broth (400 mL) and incubated at 37°C for 3 h. When the density reached 10⁷ cfu/mL, the solution was used to infect pigs by oral administration. Each infected pig received 3, 8, and 8 mL of freshly prepared broth at 72, 96, and 120 h after weaning, respectively. The inoculation procedure, which involved mild restraint of a pig and giving the broth per os with a 20-mL syringe, took 15 to 30 s to perform. We considered this very unlikely to elicit any stress response because, in calves, it was reported that restraint for less than 1 min followed by bleeding was insufficient to elicit a spike in cortisol concentrations (Stilwell et al., 2008).

Blood and Fecal Sampling

Four pigs per treatment closest to the median BW were selected for sampling at the start of the study, with the 4 heaviest and 4 lightest pigs in each treatment excluded. Blood samples (5 mL) were collected into vacutainer tubes coated with lithium heparin via puncture of an anterior vena cava on d 7 and 14. The blood samples were immediately placed on ice and then

centrifuged at $2,000 \times g$ for 10 min at 5°C. Plasma was stored at -20°C until analyzed for plasma urea nitrogen (PUN).

Between 0900 and 1200 h on d 7 and 14, feces from each pig that was observed to defecate were collected immediately from the floor. The number of defecation events varied per pig, but it was ensured that at least 1 fecal sample was collected from each pig during this time. Samples were immediately stored at -20°C for later analysis of VFA, BCFA, and fecal $\text{NH}_3\text{-N}$.

Incidence of PWD

Feces from each pig were visually examined each morning for 2 wk after weaning to determine the incidence of PWD and ascertain the health status of the pigs. Feces were assessed using the fecal consistency score according to Marquardt et al. (1999) using a subjective score on a 3-point scale ranging from 1 to 3, where 1 = well formed; 2 = sloppy; 3 = diarrhea. The incidence of PWD was expressed as the mean proportion of days that pigs had diarrhea with respect to total days (14 d) of observation (Mateos et al., 2006).

Shedding of β -Hemolytic *E. coli*

Fecal shedding of β -hemolytic *E. coli* was assessed at arrival and then again on d 5, 7, 10, 12, and 14 after weaning, by inserting a soft cotton bud into the anus. Swabs were used to inoculate sheep blood (50 mL/L) agar plates (Columbia base, Oxoid, Western Australia, Australia), and then assessed for β -hemolytic colonies displaying the characteristic morphology of *E. coli* after overnight incubation at 37°C in air (McDonald et al., 2001). The presence of β -hemolytic *E. coli* was then scored using a subjective score on a 6-point scale ranging from 0 to 5, where 0 = no growth and 5 = hemolytic *E. coli* present right out to the fifth section of the plate.

Chemical Analyses

To determine VFA concentrations, thawed fecal samples were diluted 1:1 (wt/vol) with distilled water, mixed, centrifuged at $13,000 \times g$ for 10 min at 4°C , and the supernatant fraction was analyzed chromatographically. The supernatant fraction (0.1 mL) was added to 1 mL of internal standard solution containing methyl-valeric acid before processing with capillary gas-chromatography. A working standard and a control (distilled water) were included in each run of the analysis, with the working standard containing acetic acid (60 mM), propionic acid (20 mM), isobutyric acid (6.67 mM), butyric acid (20 mM), isovaleric acid (10 mM), valeric acid (10 mM), and caproic acid (4 mM). The Hewlett Packard 5890A capillary gas-chromatography (Agilent Technologies, Forrest Hill, Victoria, Australia) was maintained at injector and detector FID settings of 260 and 265°C , respectively, and an initial and final

Table 2. Analyzed chemical composition (g/kg, as-fed basis) of the experimental diets

Item	Diet ¹		
	HP	LP	Stage II
Analyzed composition, g/kg			
CP	256.3	175.0	212.5
Crude fat	40.8	48.6	53.4
Crude fiber	30.1	34.5	29.6
ADF	41.5	48.9	43.4
NDF	98.2	98.1	99.5
Total AA, g/kg			
Arg	14.7	8.4	12.1
His	6.0	3.7	4.9
Ile	10.2	6.6	8.3
Leu	17.5	11.3	14.6
Lys	13.1	11.1	12.6
Met	2.6	2.5	2.3
Phe	10.9	6.8	9.2
Thr	9.3	7.6	8.4
Trp	3.2	2.0	2.3
Val	11.6	7.8	9.6
Ala	10.7	6.7	8.9
Asp	20.1	11.1	16.5
Glu	43.3	31.1	39.4
Gly	11.1	7.2	9.1
Pro	14.2	10.7	12.8
Ser	10.9	7.0	9.4

¹HP = high protein diet; LP = low protein diet; stage II = second-phase diet.

oven temperature of 120 and 240°C , respectively. The carrier gas flow rate was 5 mL/min, and the split-flow rate was 70 mL/min. The Hewlett Packard Chemstation integration system was used to calculate the VFA concentrations from the area of the peaks.

The AA contents in the diets were measured according to a method described by Cohen (2001). Briefly, a 200- to 300-mg sample was hydrolyzed with acid (6 M HCl) to convert protein-bound AA into free AA. The AA in the hydrolysate then underwent pre-column derivatization with 6-aminoquinolyl-*N*-hydroxysuccinimidyl carbamate. For Trp analysis, a separate 50- to 100-mg sample was hydrolyzed in an alkaline solution (5 M NaOH) and neutralized before pre-column derivatization. The AA derivatives were then separated and quantified by reversed-phase HPLC (ACQUITY UPLC system with UV detector, Waters Corporation, Milford, MA). For all analyses, a Waters AccQ-Tag Ultra column (BEH C18, 2.1×100 mm; $1.7 \mu\text{m}$) was used with column temperature at 55°C , detection at 260 nm, and flow rate of 0.7 mL/min.

The concentration of PUN was determined using an enzymatic (urease) kinetic method (Randox Laboratories Ltd., Crumlin, Co., Antrim, UK). Concentrations of fecal $\text{NH}_3\text{-N}$ in fresh fecal samples were measured according to a method described by Weatherburn (1967).

The DM content of samples was measured using the AOAC official method 930.15 (AOAC, 1997). The N content was determined with a Leco FP-428 (Leco Corp., St. Joseph, MI) N Analyzer using a combustion

method AOAC official method 990.03 (AOAC, 1997). The GE content was determined using a bomb calorimeter (SANYO Gallenkamp, Loughborough, Leicestershire, UK).

Statistical Analyses

Data were analyzed using the GLM procedure (SPSS Inc., Chicago, IL) with the main effects being feeding regimen treatments (HP14, LP7, or LP14) and infection or noninfection of pigs after weaning. Because LP7 and LP14 were the same diets until d 7, all data between d 1 and 7 were pooled and analyzed as HP or LP; data were then analyzed as 3 dietary treatments (i.e., LP7, LP14, HP14) from d 8 to 14. For the performance data, ADG, ADFI, and G:F were averaged over the 3 time periods (d 1 to 14, 15 to 28, and 1 to 28) and analyzed as the 3 dietary treatments. Block was used as a random factor in the model for all measured experimental variables. Least squares analysis and a 2-factor linear model with all interactions were used to evaluate responses. The ADG, ADFI, and G:F were assessed using the pen as the experimental unit. All other measurements were assessed using the individual pig as the experimental unit (Montagne et al., 2004), providing 12 replications per treatment, except PUN that provided 4 replications. Data for the incidence of PWD were expressed as the mean percentage of days with diarrhea relative to the first 14 d after weaning (Mateos et al., 2006). Initial BW was used as a covariate for growth data. Statistical significance was accepted at $P < 0.05$. Pair-wise means comparisons between the different feeding regimen treatments were made when appropriate using Fisher's-protected LSD.

RESULTS

General

Piglets remained healthy and performed well throughout the study, although 1 *E. coli*-infected pig in treatment LP14 died. A postmortem examination revealed that the death was caused by mild diffuse pulmonary edema. This pig was excluded from all experimental measurements in the current study.

Calculated total AA compositions and chemical compositions of the experimental diets were similar to the analyzed values as presented in Tables 1 and 2, except they had less Met content. The calculated Met:Lys ratio was 0.30, which is within the proposed ideal pattern, but the analyzed Met:Lys ratio was 0.20, indicating less Met content than in the formulation. The analyzed content of CP in the HP diet was in excess of that formulated (256 vs. 240 g/kg).

PUN, Fecal $\text{NH}_3\text{-N}$, Fecal DM, and *E. coli* Shedding

No significant interactions ($P > 0.05$) occurred between feeding regimen and ETEC infection for any of

the indices measured after weaning (Table 3). Pigs fed LP had decreased PUN concentrations compared with pigs fed HP at d 7 ($P = 0.002$), and at d 14, pigs fed only LP14 had less PUN than pigs fed HP14 after weaning ($P < 0.05$). The concentration of PUN was not affected ($P > 0.05$) by *E. coli* challenge at d 7 or 14. In contrast, infection with ETEC increased fecal $\text{NH}_3\text{-N}$ at d 7 ($P = 0.005$) and tended to increase fecal $\text{NH}_3\text{-N}$ at d 14 ($P = 0.060$). Feeding diet LP reduced fecal $\text{NH}_3\text{-N}$ concentrations compared with pigs fed HP at d 7 ($P < 0.001$), and at d 14, LP7 and LP14 reduced fecal $\text{NH}_3\text{-N}$ concentrations compared with pigs fed HP14 ($P < 0.05$).

There was a decrease ($P = 0.046$) in fecal DM contents associated with ETEC infection at d 7 (21.4 vs. 23.8%), but no effect ($P = 0.681$) was evident at d 14 (Table 3). There were main effects ($P < 0.001$) of feeding regimen on fecal DM content assessed on d 7 and 14. Pigs fed LP had drier feces than pigs fed HP at d 7 (26.0 vs. 20.5% DM). At d 14, pigs fed LP14 had more DM in their feces than pigs fed LP7 or HP14 (25.5% vs. 23.6 and 22.8%, respectively).

Fecal hemolytic *E. coli* was detected in some pigs on arrival, but there was no difference ($P > 0.05$) between experimental treatments. As anticipated, experimental ETEC infection increased the fecal hemolytic *E. coli* score assessed at d 5 and 7 and for the entire 2-wk period after weaning ($P < 0.001$). Although pigs fed LP tended to shed less ($P = 0.062$) hemolytic *E. coli* (mean score of 0.69) compared with pigs fed diet HP (mean score of 1.17) at d 7, no differences in feeding regimen were detected for the 2-wk period after weaning ($P = 0.360$; Table 3).

Incidence of PWD

There was no interaction ($P > 0.05$) between feeding regimen and ETEC infection in the overall 2-wk period after weaning ($P = 0.171$; Table 4). There was a main effect of feeding regimen ($P = 0.001$ to $P < 0.001$), and as anticipated, a significant main effect of infection ($P < 0.001$), on PWD during d 1 to 7, d 8 to 14, and overall from d 1 to 14. Feeding LP7 or LP14 decreased ($P = 0.001$) the incidence of PWD compared with pigs fed HP14 at d 14, but the incidence of PWD was the same ($P > 0.05$) between the 2 LP diets at d 8 to 14.

VFA Concentrations

Feeding diet LP decreased ($P = 0.005$) total VFA concentrations at d 7 compared with pigs fed HP (104 vs. 140 mmol/kg, respectively). At d 14, the main effect of *E. coli* infection caused decreased total VFA concentrations compared with noninfected pigs (131 vs. 96 mmol/kg respectively; $P = 0.002$). The molar proportions of BCFA were not affected ($P > 0.05$) by feeding regimen or *E. coli* infection. No interactions between feeding regimen and infection occurred for VFA or BCFA (Table 5).

Table 3. Effects of feeding regimen and experimental β -hemolytic enterotoxigenic strains of *Escherichia coli* (ETEC) infection after weaning on plasma urea nitrogen (PUN), fecal $\text{NH}_3\text{-N}$, fecal DM content, and fecal *E. coli* scores¹

Period	Feeding regimen ²						Pooled SEM	P-value		
	Noninfected			Infected				FR ⁴	I ⁵	FR \times I
	HP14	LP7	LP14 ³	HP14	LP7	LP14 ³				
PUN, mmol/L										
d 7	6.0	3.0		6.9	3.7		0.44	0.002	0.344	0.887
d 14	4.5 ^a	3.9 ^a	1.1 ^b	4.4 ^a	3.3 ^{ab}	1.9 ^b	0.45	<0.001	0.796	0.299
Fecal $\text{NH}_3\text{-N}$, mg/kg of DM										
d 7	360	287		409	309		5.7	<0.001	0.005	0.246
d 14	449 ^a	383 ^b	364 ^b	482 ^a	407 ^b	392 ^b	7.6	<0.001	0.060	0.970
Fecal DM, %										
d 7	22.7	25.1		18.2	24.6		0.52	<0.001	0.046	0.060
d 14	22.3 ^a	23.5 ^a	26.6 ^b	23.3 ^a	23.7 ^a	24.4 ^{ab}	0.33	0.001	0.681	0.130
Fecal <i>E. coli</i> score ^{6,7}										
d 5	0.5	0.2		1.1	1.5		0.12	0.867	<0.001	0.180
d 7	0.6	0.2		1.8	1.2		0.13	0.062	<0.001	0.684
d 1 to 14	0.3 ^a	0.1 ^a	0.1 ^a	0.6 ^b	0.5 ^b	0.6 ^b	0.11	0.360	<0.001	0.490

^{a,b}Means in the same row with different superscripts differ ($P < 0.05$).

¹Pigs in infected group were experimentally challenged at 72, 96, and 120 h after weaning. Values for fecal $\text{NH}_3\text{-N}$, DM, and *E. coli* are the mean of 12 replicates except LP14 in the infected group, which is the mean of 11 replicates. Values for PUN are the mean of 4 replicates.

²HP14 = high protein diet fed for 14 d; LP7 = low protein diet fed for 7 d; LP14 = low protein diet fed for 14 d. At the conclusion of each feeding regimen, all pigs then received a second-phase diet until 4 wk after weaning.

³Until d 7, pigs in feeding regimens LP7 and LP14 received the same diet; data were pooled and analyzed as a single diet (LP).

⁴Two feeding regimen effects (FR) were examined until d 7, and 3 FR were examined after d 7.

⁵I = infection effect.

⁶Agar plates were scored from 0 to 5 according to the number of streaked sections that had viable growth of hemolytic *E. coli*, where 0 = no growth, 1 = *E. coli* in first section, and so on (5 = heaviest growth of hemolytic *E. coli*).

⁷The *E. coli* scores were different between treatments only on d 5 and 7 in the 2-wk period after weaning.

Production

Feeding the LP7 or LP14 diet after weaning did not affect ADG, ADFI, or G:F compared with pigs fed diet HP14 in the 28 d after weaning ($P > 0.05$). However, ETEC infection decreased ADG by 31% ($P < 0.001$) and G:F by 26% ($P = 0.003$) in the 28-d period after weaning, without affecting ADFI ($P > 0.05$). The in-

teraction between ETEC infection and feeding regimen was not significant (Table 6).

DISCUSSION

Data from the present study supported our hypothesis that feeding a diet decreased in CP for 7 or 14 d can achieve the combined beneficial effects of a reduction

Table 4. The effect of feeding regimen and experimental β -hemolytic enterotoxigenic strains of *Escherichia coli* (ETEC) infection on the incidence of postweaning diarrhea (PWD) in the 2-wk period after weaning^{1,2}

Period	Feeding regimen ³						Pooled SEM	P-value		
	Noninfected			Infected				FR ⁵	I ⁶	FR \times I
	HP14	LP7	LP14 ⁴	HP14	LP7	LP14 ⁴				
d 1 to 7	16.7	10.1		48.8	29.7		1.85	0.001	<0.001	0.097
d 8 to 14	22.6 ^a	7.1 ^b	8.3 ^b	40.5 ^c	20.2 ^a	26.2 ^a	1.91	0.001	<0.001	0.842
d 1 to 14	19.6 ^a	9.5 ^b	8.3 ^b	44.6 ^c	21.4 ^a	31.5 ^d	1.52	<0.001	<0.001	0.171

^{a-d}Means in the same row with different superscripts differ ($P < 0.05$).

¹Pigs in infected group were experimentally challenged at 72, 96, and 120 h after weaning. The noninfection and infection pigs were housed in separated rooms with 3 or 6 pigs per pen, respectively. Values are mean of 12 replicates except LP14 in infected group, which is the mean of 11 replicates.

²Incidence of PWD observed during 14 d after weaning, providing 12 replicates per feeding regimen, is expressed as the mean percentage of days with diarrhea relative to the total 14 d after weaning (Mateos et al., 2006). Data are mean values per treatment combination assessed between d 1 to 7, 8 to 14, and 1 to 14.

³HP14 = high protein diet fed for 14 d; LP7 = low protein diet fed for 7 d; LP14 = low protein diet fed for 14 d, and at the conclusion of each feeding regimen, all pigs then received a second-phase diet until 4 wk after weaning.

⁴Until d 7, pigs in feeding regimens LP7 and LP14 received the same diet; data were pooled and analyzed as a single diet (LP).

⁵Two feeding regimen effects (FR) were examined until d 7, and 3 FR were examined after d 7.

⁶I = infection effect.

Table 5. Effect of feeding regimen and experimental β -hemolytic enterotoxigenic strains of *Escherichia coli* infection on fecal concentrations of VFA and molar proportions of branched-chain fatty acids (BCFA) in the 2-wk period after weaning¹

Period	Feeding regimen ²						Pooled SEM	P-value		
	Noninfected			Infected				FR ⁴	I ⁵	FR × I
	HP14	LP7	LP14 ³	HP14	LP7	LP14 ³				
Total VFA, mmol/kg of wet feces										
d 7	148	98		131	110		6.8	0.005	0.986	0.300
d 14	128 ^a	136 ^a	130 ^a	96 ^b	115 ^b	78 ^b	5.4	0.343	0.002	0.538
d 1 to 14	137	111	116	116	117	91	4.7	0.152	0.164	0.353
BCFA, ⁶ % total VFA										
d 7	4.9	6.0		5.4	5.1		0.33	0.375	0.666	0.296
d 14	5.3	6.2	4.2	6.1	5.9	5.8	0.22	0.079	0.166	0.223
d 1 to 14	5.3	6.2	5.2	5.7	5.4	5.6	0.22	0.625	0.980	0.411

^{a,b}Means in the same row with different superscripts differ ($P < 0.05$).

¹Pigs in infected group were experimentally challenged at 72, 96, and 120 h after weaning. The noninfection and infection pigs were housed in separated rooms with 3 or 6 pigs per pen, respectively. Values are mean of 12 replicates except LP14 in infected group, which is a mean of 11 replicates.

²HP14 = high protein diet fed for 14 d; LP7 = low protein diet fed for 7 d; LP14 = low protein diet fed for 14 d, and at the conclusion of each feeding regimen, all pigs then received a second-phase diet until 4 wk after weaning.

³Until d 7, pigs in feeding regimens LP7 and LP14 received the same diet; data were pooled and analyzed as a single diet (LP).

⁴Two feeding regimen effects (FR) were examined until d 7, and 3 FR were examined after d 7.

⁵I = infection effect.

⁶BCFA = branched-chain fatty acids: molar proportion of isobutyric and isovaleric acids with respect to the total VFA.

in PWD, in the absence of any dietary antimicrobial compounds, and not cause any loss in production in the postweaning period. Therefore, our data suggest that an LP diet fortified with crystalline Ile and Val can be fed for as little as 7 d after weaning to achieve these effects. Previous work has demonstrated that feeding an LP diet after weaning can compromise growth (Nyachoti et al., 2006; Wellock et al., 2006); however, when an LP diet is supplemented with Ile or Val, or both, to achieve

a proposed ideal AA pattern (Chung and Baker, 1992), growth was not compromised (Le Bellego and Noblet, 2002; Htoo et al., 2007; Heo et al., 2008). Mavromichalis et al. (1998) commented that apart from Lys, the first-limiting AA, Ile and Val should be considered as equally second limiting along with Met, Trp, and Thr for weaner pig diets. Furthermore, Lordelo et al. (2008) demonstrated that supplementation of Val alone, or in combination with Ile, to a decreased protein diet with

Table 6. The effect of feeding regimen and experimental β -hemolytic enterotoxigenic strains of *Escherichia coli* infection on growth performance in weaned pigs¹

Period	Feeding regimen ²						Pooled SEM	P-value ³		
	Noninfected			Infected				FR	I	FR × I
	HP14	LP7	LP14	HP14	LP7	LP14				
ADG, g										
d 1 to 14	129 ^a	113 ^a	150 ^a	55 ^b	63 ^b	63 ^b	7.0	0.304	<0.001	0.414
d 15 to 28	435 ^a	412 ^a	453 ^a	352 ^b	334 ^b	296 ^b	15.5	0.810	0.003	0.601
d 1 to 28	282 ^a	301 ^a	263 ^a	204 ^b	179 ^b	199 ^b	9.9	0.673	<0.001	0.524
ADFI, g										
d 1 to 14	192	186	235	196	206	189	9.4	0.374	0.692	0.321
d 15 to 28	562	461	581	501	486	418	33.8	0.598	0.391	0.464
d 1 to 28	377	408	324	349	304	346	25.9	0.598	0.354	0.379
G:F, g/g										
d 1 to 14	0.67 ^a	0.63 ^a	0.63 ^a	0.28 ^b	0.31 ^b	0.34 ^b	0.027	0.958	<0.001	0.775
d 15 to 28	0.79 ^a	0.83 ^a	0.79 ^a	0.70 ^b	0.71 ^b	0.70 ^b	0.020	0.408	0.023	0.269
d 1 to 28	0.76 ^a	0.85 ^a	0.74 ^a	0.58 ^b	0.58 ^b	0.59 ^b	0.030	0.518	0.003	0.677

^{a,b}Means in the same row with different superscripts differ ($P < 0.05$).

¹Pigs in infected group were experimentally challenged at 72, 96, and 120 h after weaning. Values are the mean of 4 replicates for noninfected treatments (2 rooms with 6 pens per room) and 2 replicates for infected treatments (1 room with 6 pens).

²HP14 = high protein diet fed for 14 d; LP7 = low protein diet fed for 7 d; LP14 = low protein diet fed for 14 d, and at the conclusion of each feeding regimen, all pigs then received a second-phase diet until 4 wk after weaning.

³FR = feeding regimen effect; I = infection effect.

adequate levels of Lys, Met, Thr, and Trp was necessary to achieve equivalent growth when dietary CP content was linearly reduced from 20 to 17%.

An ETEC challenge model that caused moderate diarrhea was chosen in this study because production responses in a commercial setting, where newly weaned pigs are exposed to a considerable biological challenge, are most likely different from those found in the cleaner environment of an experimental research facility. The presence of hemolytic *E. coli* in fecal samples increased significantly in the *E. coli*-challenged pigs compared with their nonchallenged counterparts. The significant main effect of infection on fecal *E. coli* score was evident only on d 5 and 7, and the score returned to the preinfection level on d 10. This short-term effect of experimental *E. coli* infection on fecal *E. coli* score is comparable with previous reports (Madec et al., 2000; Montagne et al., 2004; Wellock et al., 2008a) and demonstrated that induction of subclinical PWD was successfully achieved. Infection with ETEC significantly depressed ADG and G:F in the 28-d measurement period without reducing ADFI, which is in agreement with the work of Wellock et al. (2008b).

The PUN and fecal $\text{NH}_3\text{-N}$ levels were measured in plasma and feces, respectively, as markers of microbial protein fermentation or protein utilization efficiency (Chen et al., 1995; Coma et al., 1995), or both. Feeding decreased levels of dietary CP reduced concentrations of PUN and fecal $\text{NH}_3\text{-N}$ at 7 and 14 d after weaning, in accordance with other work (Hansen et al., 1993; Bikker et al., 2006; Nyachoti et al., 2006; Yue and Qiao, 2008). Greater concentrations of fecal $\text{NH}_3\text{-N}$ in the HP14-fed pigs presumably reflected the quantitatively greater entry of proteinaceous material into the distal GIT, given the lack of any difference in feed intake among treatments. The concentration of PUN can increase due to increased microbial production of $\text{NH}_3\text{-N}$ and its subsequent diffusion into the portal blood system and conversion to urinary N via the urea cycle in the liver (Younes et al., 1998), or to inefficiencies associated with an imbalance or excess of essential AA available for tissue protein synthesis, or to a combination of all factors. Absorbed AA in excess of those needed for biosynthesis cannot be stored and undergo inevitable catabolism with the production of urea (Moughan, 1999) and can be involved in various metabolic pathways such as gluconeogenesis (Linder, 1991). In addition, work by Jeaurond et al. (2008) demonstrated that pigs fed a diet containing greater levels of fermentable protein showed increased blood urea nitrogen concentrations compared with their counterparts (1.29 vs. 0.55% fermentable protein, respectively) despite no difference in the dietary protein level (21.9 vs. 20.2% CP, respectively).

It was not possible in our study to reconcile the contribution of microbially derived $\text{NH}_3\text{-N}$ vs. that derived from postabsorptive AA metabolism; however, the lack of any difference in G:F attributable to protein level and greater fecal $\text{NH}_3\text{-N}$ content in pigs fed an HP diet

suggests that greater PUN concentrations in HP14-fed pigs might be predominately of fermentative origin in the GIT.

In this light, PUN and $\text{NH}_3\text{-N}$ have been suggested as biomarkers for intestinal health in the postweaning period (Bikker et al., 2006; Nyachoti et al., 2006; Awati et al., 2007). Indeed, nitrogenous end products such as $\text{NH}_3\text{-N}$ can irritate mucosal surfaces of the colon (Visek, 1984; Lin and Visek, 1991) and small intestine (Nousiainen, 1991; Nabuurs et al., 1993). Fermentation of protein is most pronounced in the distal large GIT where carbohydrate can become a limiting factor for microbial fermentation and is accompanied by increased production of BCFA (predominately from Val, Leu, and Ile; Macfarlane et al., 1992), ammonia, indoles, phenols, amines, and sulfuric-containing compounds (Jensen, 2001). The concentrations of Val, Leu, and Ile were 33, 35, and 35% greater, respectively, in the HP diet compared with the LP diet; and hence, it was expected that feeding the HP diet would increase BCFA production. However, the BCFA content was not affected by protein level or infection. These data agree with those of Bikker et al. (2006), who reported that concentrations of BCFA in the ileum and colon of weanling pigs were not affected by diet protein level (217 vs. 153 g/kg). However, these data are in contrast to those of Nyachoti et al. (2006), who reported reduced BCFA abundance in the ileum as dietary CP decreased from 228 to 174 g/kg, and Htoo et al. (2007), who demonstrated a reduction in BCFA concentrations in the cecal digesta of early weaned pigs fed a diet with reduced CP (255 vs. 199 g/kg).

There are several possible reasons for this disparity among studies. First, the general malaise in the postweaning period commensurate with disturbed physiological conditions in the GIT will differ due to differences in weaning age and, hence, intestinal digestive and absorptive capacity, differences in feed intake, and differences in diet AA levels, any or all of which will influence BCFA production. Furthermore, because only fecal measures were made, it is difficult to reconcile these data considering that the concentration of BCFA in the feces simply reflects their relative rates of production and absorption more anterior in the large intestine. A second possible confounding factor when assessing acid production in the GIT is the contribution of fiber because it is known that VFA production is affected by many factors including the carbohydrate content and composition and retention time in the GIT (Pluske et al., 2001). In the present study, the decreased production of VFA in pigs fed the LP diets was most likely a consequence of reduced intake of non-starch polysaccharides compared with pigs fed the HP diet, which arose as a consequence of the difference in diet formulation to achieve the desired energy and AA levels. This difference disappeared by d 14, when it was observed that piglets challenged with *E. coli* produced less VFA than their nonchallenged counterparts. Pigs fed the HP diet had a greater incidence of PWD in

the 2 wk after weaning, and the flow of digesta into the large intestine presumably increased the amount of substrate available for fermentation that then increased VFA levels, compared with LP-fed pigs.

Infection with ETEC did not alter PUN concentrations, but increased the fecal $\text{NH}_3\text{-N}$ content. Infection with ETEC can reduce digestion and nutrient absorption in the small intestine by causing villous atrophy and, hence, reducing net absorption (Nabuurs, 1995). Therefore, a greater absolute amount of undigested N would presumably have been available for microbial fermentation in the large intestine attributable to ETEC infection. This notion is supported by the significantly decreased G:F ratio in ETEC-infected pigs, although part of the reduction could be due to the metabolic expense associated with immune stimulation (Stahly, 1996; van der Klis and Jansman, 2002).

In accordance with previous workers (e.g., Ball and Aherne, 1987; Heo et al., 2008; Yue and Qiao, 2008), feeding a diet decreased in CP reduced the incidence of PWD commensurate with increased fecal DM content in noninfected pigs, although effects were less consistent in the initial week after weaning because an interaction occurred between feeding regimen and ETEC infection. In this case, although feeding regimen had no effect in the noninfected pigs, feeding an LP diet significantly reduced PWD in their challenged counterparts. Piglets were infected per os on d 3, 4, and 5, and it generally takes 48 to 72 h for the GIT to become colonized with ETEC (Owusu-Asiedu et al., 2003). The period between 4 and 9 d after weaning is regarded as the time when maximum colonization of the small intestine occurs with ETEC; however, it was evident that pigs fed an LP diet had decreased PWD in the total 14 d after weaning compared with piglets fed an HP diet, irrespective of ETEC infection.

Feeding an LP diet did not reduce shedding compared with pigs fed other diets when this was calculated at d 5, 7 and also over the total 14-d period after weaning. In addition, and when all 71 pigs were used in a Pearson correlation analysis, significant but weak positive relationships were observed between first, the *E. coli* score and the incidence of PWD both averaged from d 1 to 7 after weaning ($r = 0.598$, $P < 0.001$), and second, the *E. coli* score and the incidence of PWD both averaged from d 1 to 14 after weaning ($r = 0.477$, $P < 0.001$). No significant correlation existed between these 2 measures from d 8 to 14 after weaning. Nevertheless, some workers have not been able to establish such relationships, suggesting that PWD after weaning is not always necessarily associated with an *E. coli* infection. Work by Callesen et al. (2007), for example, showed no correlation between the number of antibiotic treatments given for diarrhea and the degree of fecal shedding of *E. coli* by the same pigs. Adhesion of ETEC to receptors in the epithelium causes fluid and electrolyte secretion into the GIT and, coupled to a lack of ability to reabsorb the fluid and electrolytes (Nabuurs, 1998), could cause an osmotically active protein load

entering the large intestine to exacerbate the extent of PWD observed.

In conclusion, the present experiment demonstrated that with a moderate *E. coli* challenge, feeding an LP diet with added crystalline Val and Ile for 7 d only after weaning reduced the incidence of PWD commensurate with reduced indices of protein fermentation and did not compromise the growth performance of pigs. The LP diets fed in the present study did not contain any antimicrobial compounds and confirm the notion that such a strategy is warranted in situations in which feeding of antimicrobial compounds is restricted, not permitted, or ineffectual due to antimicrobial resistance.

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