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Efficacy of a reduced protein diet on clinical expression of post-weaning diarrhoea and life-time performance after experimental challenge with an enterotoxigenic strain of *Escherichia coli*

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Abbreviations: AA, amino acid(s); AMC, antimicrobial compounds; CEAA, crystalline essential amino acid; cfu, colony-forming units; CP, crude protein; DI, diarrhoea index; ETEC, enterotoxigenic *Escherichia coli*; PUN, plasma urea nitrogen; PWC, post-weaning colibacillosis; PWD, post-weaning diarrhoea; RP, reduced protein; SID, standardised ileal digestible.

Abstract

Previous experiments have shown that feeding a reduced protein diet within the first 2 weeks post-weaning reduces gastrointestinal protein fermentation and clinical expression of post-weaning diarrhoea (PWD). However, growth of young pigs receiving a reduced protein diet without crystalline essential amino acids (CEAA) supplementation is depressed after weaning. It has been argued that the short-term performance reduction caused by feeding a reduced protein diet would be compensated and the lifetime performance of pigs would not be affected. An experiment was therefore conducted to examine PWD and lifetime growth of pigs after feeding a reduced protein diet without and with CEAA supplementation for 2 weeks after weaning. Two hundred individually-housed pigs weaned at 21 d of age (Large White × Landrace, castrate:female ratio of 1:1, mean ± SEM body weight of 5.5 ± 0.05 kg) were stratified to one of four dietary treatments (n=50): High protein + antimicrobial compound diet (HP+AMC, 230 g crude protein (CP) with 2.5 g lincospectin and 3 g zinc oxide per kg feed), High protein diet (HP, 230g CP/kg), Reduced protein + amino acid supplemented diet (RP+AA, 185 g CP/kg with added CEAA up to HP level), and Reduced protein diet (RP, 185 g CP/kg without CEAA supplementation). Pigs were fed the experimental diet for 2 weeks and then all pigs were fed the same series of commercial diets until slaughter. All pigs were experimentally infected with an enterotoxigenic strain of *E. coli* (6 and 10 mL of 1.9 × 10^9 cfu/mL, serotype O149:K91:K88) at 72, and 96 h after weaning. Infection increased plasma haptoglobin levels ($P<0.01$) and faecal shedding of β-haemolytic *E. coli* on days 5, 7, and 9 after weaning ($P<0.001$). Pigs fed the HP diet showed an increased faecal score ($P<0.05$-0.001), diarrhoea index ($P<0.001$), and mean number of therapeutic antibiotic treatments ($P<0.001$) compared with pigs fed other diets. Pigs

fed the RP diet grew less (P<0.001), tended to eat less (P=0.063), and utilised the feed less efficiently (P<0.001) in the 2nd week post-weaning. When pigs fed an identical commercial diet on week 3, however, performance indices were not different between treatments. Lifetime performance was not affected by the dietary treatment (P>0.05). Carcass characteristics were not affected (P>0.05) by the treatments. The results indicate that although feeding a RP diet without CEAA supplementation decreased performance after weaning, it did not influence lifetime performance or carcass characteristics and reduced the clinical expression of PWD.

Keywords: *E. coli* challenge, Performance, Post-weaning diarrhoea, Reduced protein, Weaner pigs

1. Introduction

One of the most critical factors affecting the health of piglets experiencing post-weaning diarrhoea (PWD) is the damage to the intestinal epithelium, and hence weakened mucosal and cellular barrier functions, which are mainly caused by the change of diet, loss of passive immunity and other weaning-associated stressors (Pluske et al., 1997). Lack of active immunity and damage to gut integrity generally increase adhesion of pathogenic bacteria to the mucosal layer.

Previous research has demonstrated that feeding a reduced protein diet with crystalline essential amino acids (CEAA) fortification (180 g/kg vs. 230 g/kg) and devoid of antimicrobials markedly decreases the incidence of PWD following experimental infection with enterotoxigenic *E. coli (ETEC),* without influencing production indices (Heo et al., 2008, 2009). Other studies have shown that feeding a reduced protein diet without CEAA fortification generally constrains the growth of pigs after weaning as the amino acid content in the reduced protein diet is not

sufficient to meet the piglets' requirements (Nyachoti et al., 2006; Wellock et al., 2006). However, fortification of CEAA to a reduced protein (RP) diet is not practical in some countries because of registration issues and fortification also increases diet cost, which is unlikely to be acceptable as a feasible strategy by the commercial pig industry.

To address this issue, we hypothesised that feeding a RP diet without supplementing CEAA to the pigs' ideal amino acid (AA) requirements for 2 weeks after weaning would suppress PWD and, while it may result in an initial depression in growth rate, pigs would compensate in growth later such that they will not be disadvantaged by the time they reach slaughter weight (Fabian et al., 2002; 2004). If this hypothesis is proved to be correct, this nutritional management strategy would reduce the occurrence of PWD without using in-feed antimicrobials, and would also reduce diet cost without compromising the long-term growth of pigs.

2. Materials and methods

This study was reviewed and approved by the Western Australian Department of Agriculture and Food Animal Ethics Committee (5-09-30). Animals were handled according to the Australian code of practice for the care and use of animals for scientific purposes (NHMRC, 2004).

2.1 Experimental design and diets

A completely randomised block experiment was conducted to explore the impacts of feeding a number of diets differing in protein content and the use of in-feed antimicrobial compounds on the clinical expression of PWD and the subsequent lifetime performance of pigs. Two hundred individually-housed pigs weaned at 21

98 days of age (Large White × Landrace, castrate:female ratio of 1:1, mean ± SEM body
99 weight of 5.5 ± 0.05 kg) were stratified to one of four dietary treatments (n=50 per
100 treatment): (i) a high protein with antimicrobial compounds (HP+AMC, 230 g crude
101 protein (CP) with 2.5 g lincospectin and 3 g zinc oxide per kg feed); a high protein
102 diet without AMC (HP, 230g CP/kg); a reduced protein diet with CEAA fortification
103 (RP+AA, 185 g CP/kg with added CEAA up to HP level for lysine, methionine and
104 threonine and up to the ideal amino acid patterns for isoleucine, leucine and valine);
105 and a reduced protein diet without CEAA fortification (RP, 185 g CP/kg without
106 CEAA supplementation; 20% reduction in essential AA contents than the HP diet).
107 The levels of the essential amino aids other than lysine in all experimental diets were
108 formulated upon the ideal amino acid pattern (Chung and Baker, 1992). Pigs were fed
109 the experimental diet for 2 weeks after weaning and then all pigs were fed commercial
110 diets until slaughter (3-phase feeding). The wheat and soybean meal-based diet fed
111 after weaning was formulated to contain 14.8 MJ digestible energy (DE)/kg and 13.3
112 g standardised ileal digestible (SID) lysine/kg except for the RP diet, which was
113 formulated to contain 10.7 g SID lysine/kg diet. The composition of experimental
114 diets is presented in Table 1.
115
116 *2.2 Animals, housing, and feeding*
117 Pigs (200 castrate and female, 1:1) were acquired at weaning from a
118 commercial farm and transported to the Medina Research Station. Upon arrival, pigs
119 were weighed, ear tagged, housed individually and randomly stratified by gender, live
120 weight and position in the room to one of the 4 treatments. The pens were wire-mesh
121 floored crates having a space allowance of 0.4 m^2 per pig. Each pen was equipped
122 with a nipple bowl drinker and a metal trough. The ambient temperature was

maintained at 30 ± 1ºC for the initial week and then decreased by 2ºC every week. Piglets were fed their respective experimental diets on an *ad libitum* basis for 2 weeks after weaning and fed a commercial weaner pellet diet (14.5 MJ DE/kg and 10.2 g SID lysine/kg) in the third week. Pigs were weighed weekly and feed intake was measured on a weekly basis. After 3 weeks of study in the individual weaner pens, all pigs were moved to a barley straw-based deep-litter housing system and fed 3 commercial diets in a phase-feeding sequence. Diets were changed at approximately 20 kg (14.5 MJ DE/kg, 200 g CP/kg, 7 g SID/MJ DE), 50 kg (13.5 MJ DE/kg, 190 g CP/kg, 7 g SID/MJ DE) and 75 kg (12.4 MJ DE/kg, 158 g CP/kg, 5 g SID/MJ DE), respectively. Pigs were weighed monthly and carcass composition was assessed when the pigs were slaughtered at approximately 95 kg live weight.

2.3 Enterotoxigenic E. coli infection procedure

A strain of enterotoxigenic β-haemolytic *E. coli*, serotype O149; K91; K88 (toxins LT, STa, STb) was grown on sheep blood (50 mL/L) agar plates (Columbia base, Oxoid, WA, Australia) (McDonald et al., 2001), and incubated overnight at 37°C. This *E. coli* was isolated from a commercial farm with clinical case of post-weaning colibacillosis (PWC). The serotype and toxins were confirmed at the Department of Primary Industries (Bendigo, VIC, Australia). A representative colony was then removed from the plate and seeded into 20 mL of sterile Trypticase Soy Broth (Becton Dickinson, Sparks, MD, USA) in a McCartney bottle and incubated overnight at room temperature (20-25ºC). A volume of 4 mL was then aseptically transferred to a larger volume of sterile broth (400 mL) and incubated at 37°C for 3 to 4 hrs. When the density reached 10^8-10^9 cfu/mL, the solution was centrifuged at 3,000 x *g* for 15 min and the supernatant was discarded to remove toxins in the solution.

148 The *E. coli* pellet was then re-suspended to the 70% of the final volume (e. g., 400
149 mL × 0.7 = 280 mL) with sterile broth and transported to the Medina Research
150 Station within 30 min. in a refrigerated box. The broth was then mixed with 30% (of
151 the final volume) of a fruit-flavoured concentrate (sucrose- and citric-acid based
152 Raspberry Cordial, Cottees, Tullamarine, VIC., Australia) before infecting pigs, to
153 improve the broth's acceptability during the oral *E. coli* inoculation. Piglets were
154 trained to drink the fruit-flavoured concentrate during day 0 to 2 through an oral
155 drenching gun.

156 The survival of the *E. coli* when mixed with the fruit-flavoured concentrated
157 was tested by mixing 30% fruit-flavoured concentrate to the 70% soy broth *E. coli*
158 solution and incubating the solution for 2 h. The solution was then plated every 30
159 min.; survival rates of *E. coli* were 93% and 90% at 30 min and 2 h, respectively.
160 Also, the characteristic colony morphology of the β–haemolytic *E. coli* and final *E.*
161 *coli* concentrations were examined by plating the final solution onto sheep blood (50
162 mL/L) agar.

163 At approximately 72 and 96 h after weaning, all pigs received an oral ETEC
164 challenge (6 and 10 mL of 1.9×10^9 cfu of ETEC O149:K90:K88 per mL of broth
165 administered) to reproduce PWD.

166

167 *2.4 Measurements, sampling and chemical analysis*

168 Faecal score and the incidence of PWD were visually assessed daily for the
169 first 2 weeks of the experiment. Faecal consistency was assessed using a subjective
170 score on a four-point scale ranging from 1 to 4, where 1 = firm well formed, 2 = soft,
171 3 = loose and 4=diarrhoea. Pigs with faecal score 4 were considered as pigs having
172 diarrhoea and were used in the calculation of the diarrhoea index. Pigs having

diarrhoea (score 4) were treated with either Trisoprim-480 (trimethropin 80 mg/mL, sulfadiazine, 400 mg/mL, 0.05 mL/kg body weight, Troy Laboratories, Smithfield, NSW, Australia) or Betamox (150 mg/mL amoxicillin, Norbrook Lab Ltd, VIC, Australia) until considered healthy; the numbers of antibiotic treatments were recorded. The treatment was initiated when the faecal score was 4 and ceased when the faecal score was 3 (Kim et al., 2008). Data for faecal score were expressed as the mean faecal consistency value of pigs within a diet having score 1 (a value of 0%), score 2 (a value of 33%), score 3 (a value of 66%) or score 4 (a value of 100%) (Kim et al., 2008). Data for diarrhoea index (DI, %) were expressed as the mean proportion of days with diarrhoea with respect to 14 days after weaning (Mateos et al., 2006).

Shedding of β-haemolytic *E. coli* was determined from all pigs by inserting a soft cotton bud into the anus upon arrival, and then on days 3, 5, 7, 9, and 11 after weaning. Swabs were then cultured on sheep blood agar plates (McDonald et al., 2001) and plates were assessed for β-haemolytic colonies displaying morphology characteristic of *E. coli*, after overnight incubation at 37° C. The presence of haemolytic *E. coli* was scored using a six-point scale (0 = no growth, 1 = haemolytic *E. coli* in 1^{st} section, 2 = haemolytic *E. coli* in 2^{nd} section, 3 = haemolytic *E. coli* in 3^{rd} section, 4 = haemolytic *E. coli* in 4^{th} section, 5 = haemolytic *E. coli* in 5^{th} section).

Blood samples were collected from the anterior *vena cava* into a lithium heparin coated vacutainer from 8 randomly selected pigs per treatment at 2 days after weaning, and from 25 pigs per treatment at 8 days after weaning. Blood samples were centrifuged for 5 min at 2000 x *g* to harvest plasma samples. Plasma urea nitrogen (PUN) and haptoglobin contents were determined using an enzymatic (urease) kinetic method (Randox Laboratories, Crumlin, UK), and a commercial haptoglobin detection kit (Tridelta Development Ltd., Greystones, Ireland), respectively.

198 Dry matter (DM) in diet samples was measured using the AOAC official
199 method 930.15 (AOAC, 1997). The gross energy content was determined using a
200 ballistic bomb calorimeter (SANYO Gallenkamp, Loughborough, UK). Amino acid
201 contents in diets were measured according to a method described by Cohen (2001).
202 Briefly, a 200-300 mg sample was hydrolysed with 6M HCl to convert protein-bound
203 AA to free AA. The AA in the hydrolysate then underwent pre-column derivatisation
204 with 6-aminoquinolyl-*N*-hydroxysuccinimidyl carbamate. The AA derivatives were
205 then separated and quantified by reverse-phase HPLC (ACQUITY UPLC system with
206 UV detector, Waters Corporation, Milford, MA, USA). A Waters AccQ-Tag Ultra
207 column (BEH C18, 2.1 × 100 mm; 1.7 μm) was used for all analyses. Column
208 temperature employed was 55°C**,** detection was at 260 nm, and the flow rate was 0.7
209 mL/min.
210 Carcass weight and backfat depth at the P2 site (located 65 mm from the
211 midline of the carcass at the last thoracic rib) were determined on the hot carcass 45
212 min. after slaughter. Backfat depth was measured using the PorkScan Carcass
213 Classification System (Pork Scan Pty Ltd, Brisbane, QLD, Australia).
214
215 *2.5. Statistical Analysis*
216 Data were analysed using one-way analysis of variance (Genstat 12; VSN
217 International Ltd., Hemel Hempstead, UK). As there were no gender effects for any of
218 the measurements it was removed from the model. The pig was considered the
219 experimental unit for all measured variables. Faecal consistency score was considered
220 as repeated measurements on the same animal and analysed accordingly. Statistical
221 significance in plasma haptolobin, plasma urea nitrogen, and faecal shedding of β-
222 haemolytic *E. coli* between days after weaning was tested using paired-T tests. Where

223 the treatment effect was significant, the means were separated using Duncan's
224 multiple range tests. Treatment effects were considered significant at $P<0.05$,
225 considered as a trend at $P<0.10$.
226
227 **3. Results**
228 Experimental infection with ETEC on day 3 and 4 post-weaning was
229 successful as the infection increased plasma haptoglobin levels from 0.95 ± 0.048
230 mg/L to 1.58 ± 0.115 mg/L ($P<0.01$) at days 2 and 8 post-weaning, respectively. Also,
231 faecal shedding of β-haemolytic *E. coli* was increased on days 5 (0.7 ± 0.08), 7 (0.7 ±
232 0.08), and 9 (0.5 ± 0.06) after weaning compared with days 0 (0.1 ± 0.02), 3 (0.1 ±
233 0.04), and 11 (0.1 ± 0.04) ($P<0.001$).
234
235 *3.1 Faecal score, post-weaning diarrhoea, plasma haptoglobin and plasma urea*
236 *nitrogen*
237 The pigs fed a HP diet showed increased faecal scores following ETEC
238 infection on days 5, 6, 7, 9, 11, 12, and 13 after weaning ($P<0.05$-$P<0.001$, Figure 1),
239 while pigs fed diets RP+AA and RP maintained a consistent faecal score comparable
240 to the pigs fed a HP+AMC diet.
241 Approximately 50% of pigs fed diet HP had PWD (score 4) during the 2
242 weeks after weaning while only 16 and 18% of pigs fed the reduced protein diets
243 expressed PWD. Accordingly, the diarrhoea index (DI) was greater in HP-fed pigs ($P < 0.001$) and pigs fed this diet required more therapeutic antibiotic treatments
245 ($P<0.001$) in the 2-week period after weaning compared to pigs fed diets RP or
246 HP+AMC (Table 2).

Plasma haptoglobin level tended to be greater in the pigs fed the RP diet compared with pigs fed the HP+AMC or HP diets (P=0.067). Pigs fed the RP+AA diet showed intermediate levels of plasma haptoglobin on day 8 after weaning.

Levels of PUN were greater at day 2 after weaning compared with levels at day 8 (4.12 ± 0.21 mmol/L vs. 3.35 ± 0.14 mmol/L, respectively, P<0.01). On day 8 after weaning, pigs fed diet RP+AA showed reduced PUN levels compared with pigs fed the other diets (Table 2).

3.2 Post-weaning performance

Post-weaning growth performance is presented in Table 3. As 32% of the pigs (63/200 pigs) lost weight in the first week after ETEC infection, the data between 21-28 days of age were highly variable. Between 29 and 35 days of age, pigs fed the RP diet grew slower (P<0.001) compared with pigs fed the other diets, while pigs fed the RP+AA diet grew comparably to pigs fed the HP and HP+AMC diets. When pigs were fed the same commercial diet from 36-42 days of age, the difference in daily gain between treatments observed in the previous week disappeared (P>0.05). The early growth depression influenced overall daily gain between 21-42 days of age as pigs fed the RP diet without CEAA supplementation grew slower than pigs fed the other diets (P<0.05).

Pigs fed the RP diet tended to eat less feed than pigs fed the HP+AMC and RP+AA diets between 29 and 35 days of age (P=0.063), and this influenced overall post-weaning feed intake between 21 and 42 days of age (P=0.056).

Pigs fed the RP diet less efficiently used their feed compared with pigs fed the other diets between 29 and 35 days of age (P<0.001). However and when pigs were fed the same identical commercial diet from day 36, the difference disappeared.

3.3 Lifetime performance and carcass Ccharacteristics

Lifetime performance and carcass characteristics are presented in Table 4. Although pigs fed the RP diet were lighter than pigs fed the other diets at 42 days of age ($P<0.05$), the difference had disappeared by 70 days of age and there were no statistical differences in live weight between treatment groups through until slaughter at approximately 95 kg ($P>0.05$). Although it was not statistical significant, pigs fed the HP+AMC diet took 2.5, 2.5 and 3.8 days less to reach 90 kg ($P=0.138$) than pigs fed the HP, RP+AA and RP diets, respectively. There were no differences ($P>0.05$) in carcass characteristics between treatments.

4. **Discussion**

This study was conducted under conditions of experimental ETEC infection to mimic commercial nursery rearing conditions where greater pathogen loads are present. Experimental induction of PWD *per os* resulted in 32% of pigs expressing PWD (score 4) concomitant with increased plasma haptoglobin levels and greater excretion of β-haemolytic *E. coli*. Therefore, the results presented in this study represent the pigs' responses to the dietary intervention under significant pathogenic infection pressure.

Previous studies have consistently shown that feeding a RP diet decreases faecal score (firmer faeces) and the clinical expression of PWD compared with feeding a higher protein diet. It is considered that the reduction in intestinal protein fermentation (and production of epithelial irritants such as ammonia and amines) associated with reduced proliferation of nitrogen utilising pathogens such as *E. coli* is responsible for such effects (Wellock et al., 2006; Htoo et al., 2007; Heo et al., 2008,

297 2009). The consequences of feeding a HP diet in the present study were consistent
298 with previous publications, as the mean faecal score was significantly higher (looser
299 faeces), more pigs expressed PWD for a greater period of time, and more therapeutic
300 antibiotic treatments were required relative to pigs fed either the high protein diet with
301 AMC or the RP diet both with and without CEAA fortification (Table 2, Figure 1). It
302 was clearly demonstrated in this study that the quantity of dietary protein but not
303 CEAA fortification is associated with a greater incidence of PWD. Therefore, feeding
304 a RP diet without expensive CEAA fortification could be a short-term dietary
305 intervention in nursery units with a PWD problem where financial loss due to
306 pathogen-induced performance reduction is greater than that caused by performance
307 reduction caused by a short-term feeding of reduced protein diet.
308 Plasma haptoglobin level has been suggested as an index for pigs' responses
309 to stress (Pineiro et al., 2007), local inflammation (Eckersall et al., 1996), and
310 bacterial (Heegard, et al., 1998) and viral (Asai et al., 1999; Segales, et al., 2004)
311 infections. Haptoglobin is an interleukin (IL)-6-dependent acute-phase protein
312 (Gonzalez-Ramon et al., 2000) that is produced in the liver when a pig's immune
313 system detects pathogens in the system. Significantly elevated haptoglobin levels after
314 experimental ETEC infection (between days 2 and 8; Table 2) indicates that it is a
315 sensitive measure for the severity of ETEC infection. A similar or decreased plasma
316 haptoglobin level was expected when pigs were fed a RP diet compared with the level
317 in pigs fed a HP diet, as PWD was greater in pigs fed the high protein diet.
318 Surprisingly, pigs fed a RP diet without CEAA fortification showed higher plasma
319 haptoglobin levels while pigs fed diet RP+AA did not show increased plasma
320 haptoglobin levels compared with pigs fed diet HP+AMC. The reason behind this
321 observation is uncertain but CEAA supplementation up to the level of NRC

requirement was a contributing factor for the plasma haptoglobin level. Moreover, decreased feed intake in the pigs fed a RP diet may have contributed to the increased haptoglobin levels, as energy intake is positively associated with structure of intestinal epithelium (Pluske et al., 1996).

Plasma urea nitrogen content is a quantitative measure of mainly deaminated nitrogen either from excess dietary AA and/or mobilised muscle proteins in metabolic process such as gluconeogenesis, and ammonia from intestinal protein fermentation that diffuses into the portal blood circulation through the large intestinal epithelium (Zervas and Zijlstra, 2002). In the previous studies where a RP diet was used with CEAA fortification, increased PUN in pigs fed a HP diet was used as an index for intestinal protein fermentation (Nyachoti et al., 2006, Heo et al., 2008, 2009). However, the significant reduction in PUN content on day 8 in pigs fed diet RP+AA compared with pigs fed diet RP, HP, and HP+AA diets suggests that deamination of excess and unbalanced dietary AA rather than diffused ammonia from the intestinal protein fermentation is the major contributor for the PUN concentration in weaned pigs. Nevertheless, intestinal protein fermentation increased the intestinal ammonia content (Heo et al., 2008; 2009; Opapeju et al., 2008) and negatively affected intestinal architecture (Lin and Visek, 1991; Gu and Li, 2004; Opapeju et al., 2008). Protein fermentation in the intestine also decreased brush border disaccharidase activities in the small intestine (Yue and Qiao, 2008) and up-regulated IL-8, IL-12p40 and IL-18 mRNA expression in the ileum (Pie et al., 2007), although Opapeju et al. (2009) reported no influence of brush border disaccharidase and peptidase activities in the jejunum of pigs fed diets with 222 CP/kg vs. 173 g CP/kg.

Despite lower protein diets consistently reducing the clinical expression of PWD in the absence of AMC, significant reductions in growth rate and feed

efficiency have been reported when an ideal AA ratio is not adhered to in the formulation (Nyachoti et al., 2006; Wellock et al., 2006). Fortification of CEAA including isoleucine and valine is required up to the NRC recommended levels to maintain growth of pigs fed a reduced protein diet with around 170 g CP/kg (Heo et al., 2008; 2009; Norgaard and Fernandez, 2009). However and even with supplementation of isoleucine and valine, Opapeju et al. (2008) and Yue and Qiao (2008) found decreased performance at 170 g CP/kg while pigs maintained normal growth rate at 190 g CP/kg compared with 210 g CP/kg. Interestingly, the studies of Opapeju et al. (2008) and Yeu and Qiao (2008) used higher SID lysine levels (>13.0 g/kg) while maintaining ideal AA patterns, whereas other studies were formulated diets to contain about 11.5 g/kg standardised ileal lysine (Heo et al., 2008; 2009; Norgaard and Fernandez, 2009). The greater level of AA in the RP diet was achieved by reducing plant protein sources and extensively using animal protein sources such as whey, fish meal and plasma protein. As a result, the reduced protein diet used by Opapeju et al. (2008) and Yeu and Qiao (2008) contained minimal dietary fibre (75 g/kg) while the reduced protein diet in the other studies (Heo et al., 2008; 2009; Norgaard and Fernandez, 2009) contained approximately 140g non-starch polysaccharides (NSP)/kg. Although it cannot be concluded from the present data, differences in the fermentable fibre:fermentable protein ratio in the ileal digesta might have affected the proliferation of beneficial bacteria in the gastrointestinal tract (Kim et al., 2008) and may partly explain the differences in performance responses in pigs fed a RP diet.

Nevertheless, crystalline feed-grade isoleucine is not yet traded commercially and fortification of CEAA to a reduced protein diet to meet the NRC requirement (1998) significantly increases the cost of diet. In this regard, several authors

recommended feeding a RP diet without CEAA fortification, and hypothesised that pigs will compensate after this short-term post-weaning growth retardation once fed diets containing commercial levels of AA (i.e., 2 weeks post weaning) (Kil and Stein, 2010). Indeed Stein and Kil (2006) fed pigs diets with either 208 g CP/kg or 157 g CP/kg without CEAA fortification for 2 weeks after weaning, and observed a significant reduction in PWD and also average daily gain. When the pigs were returned to a diet containing 193 g CP/kg, the authors observed complete recovery after 3 weeks. Similarly, Wellock et al. (2009) fed diets with either 230 g CP/kg or 170 g CP/kg for 2 weeks after weaning and observed a reduction in performance at day 14. However, the difference in body weight vanished when pigs reached 33 kg body weight. Data from the current study agrees with these previous findings since pigs fed the RP diet without CEAA fortification grew slower in the post-weaning period but recovered by 70 days of age.

5. Conclusions

This finding reinforces the notion that feeding a reduced protein diet without CEAA fortification for 2 weeks after weaning is a potentially useful strategy to reduce PWD in the absence of in-feed AMC. Although there was no statistical significance (P=0.138), however, it was apparent that the omission of in-feed AMC immediately after weaning increased days to reach the same slaughter weight by 2.5 to 3.8 days, regardless of dietary protein content or CEAA fortification.

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519 Table 1

520 Composition of the experimental diets[a] (g/kg, as-fed basis)

Ingredients	HP+AMC	HP	RP+AA	RP
Barley	250	250	250	250
Wheat	348.7	358.8	485.6	483.1
Soybean meal	150	150		
Blood meal	20	20	20	20
Fish meal	70	68	81	90
Skim milk powder	100	100	95	100
Canola oil	37	34	40	40
$_{L}$-Lysine	1.2	1.3	5.2	1.2
$_{DL}$-Methionine	1.3	1.3	2.5	0.7
$_{L}$-Threonine	0.4	0.4	2.2	0.2
$_{L}$-Tryptophan			0.4	
$_{L}$-Isoleucine			1.4	
$_{L}$-Valine			0.4	
Vitamin/Mineral premix[b]	1.0	1.0	1.0	1.0
Limestone	7.1	7.2	7.2	7.1
Dicalcium phosphate	4.8	5.0	4.7	3.4
Salt	1.0	1.0	1.0	1.0
Zinc oxide	3.0			
Cholin chloride			0.4	0.3
Lincospectin[c]	2.5			
Digestibility marker[d]	2.0	2.0	2.0	2.0
Total	1000	1000	1000	1000
Calculated composition, g/kg[e]				
Digestible Energy, MJ/kg	14.8	14.8	14.8	14.8
Crude protein	230	230	185	185
Faecal digestible Phosphorus	4.5	4.5	4.5	4.5
Calcium	9	9	9	9
Neutral detergent fibre	106	107	111	111
Acid detergent fibre	33	33	30	30
Analysed composition, g/kg				
Dry Matter	913	913	912	917
Gross energy, MJ/kg	17.9	17.9	18.0	18.0
Lysine	15.5	15.3	15.5	11.9
Methionine	5.5	5.2	5.9	4.3
Threonine	9.8	9.7	9.3	7.5
Isoleucine	9.7	9.4	8.8	7.3
Leucine	19.5	18.9	15.3	15.3
Valine	12.6	12.3	10.5	10.1
Alanine	11.2	11.1	9.2	9.0
Arginine	13.5	13.1	9.1	9.2
Aspartic	21.0	20.5	14.1	14.0
Histidine	6.9	6.7	5.6	5.6
Glutamine	40.8	45.6	37.7	38.1

Glycine	7.9	10.4	8.6	8.4
Phenylalanine	11.6	11.3	8.8	8.8
Proline	13.8	16.1	14.3	14.4
Serine	9.5	11.2	8.6	8.5

Soybean meal and fish meal contained 480 g/kg and 650 g/kg crude proteins, respectively.

[a]HP+AMC: high protein with lincospectin and zinc oxide; HP: high protein without antimicrobial compounds; RP+AA: reduced protein with essential AA supplementation to the level in HP diet; RP: reduced protein diet without AA supplementation (20% lower than HP diet)

[b]Provided 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 μg; calcium pantothenate, 10.7 mg; folic acid, 0.2 mg; niacin, 12 mg; biotin, 30 μg. 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

[c]Lincospectin, 1 kg delivers 22g/t active lincomycin and 22 g/t of spectinomycin (Pfizer Australia, West Ryde, NSW, Australia).

[d]Titanium dioxide (Sigma Chemical Co., St Louis, MO, USA).

[e]INRA feed composition table was used for calculation of nutrient content (Sauvant et al., 2004).

Table 2

Effect of dietary treatments on post-weaning diarrhoea (PWD), the diarrhoea index, mean number of therapeutic antibiotic treatments, and the plasma haptoglobin and plasma urea nitrogen contents in pigs challenged with enterotoxigenic *E. coli* on days 3 and 4 after weaning

Treatment[a]	HP+AMC	HP	RP+AA	RP	SEM	P value
No. pigs with PWD[b]	8/50	26/50	8/50	9/50		
% pigs with PWD	16	52	16	18		
Diarrhoea Index, %[c]	1.1^{x}	8.1^{y}	1.7^{x}	2.0^{x}	0.95	0.001
Mean no. therapeutic antibiotic treatments per pig	0.2^{x}	1.1^{y}	0.2^{x}	0.3^{x}	0.07	0.001
Plasma haptoglobin, mg/mL						
Day 2 after weaning[d]	0.98	0.94	0.93	1.11	0.057	0.145
Day 8 after weaning[e]	1.39^{x}	1.30^{x}	1.57^{xy}	2.02^{y}	0.201	0.067
Plasma urea nitrogen, mmol/L						
Day 2 after wening[d]	4.5	4.0	3.7	3.8	0.26	0.142
Day 8 after weaning[e]	3.9^{x}	4.1^{x}	2.1^{y}	3.6^{x}	0.21	0.001

[xyz]Means in the same row with different superscripts differ (P<0.05)

[a]HP+AMC: high protein diet with antimicrobial compounds; HP: high protein diet without AMC; RP+AA: reduced protein diet with CEAA fortification; and RP: reduced protein diet without AA fortification

[b]Post-weaning diarrhoea was defined as pigs having faecal consistency score 4

553 [c]The mean proportion of days with diarrhoea with respect to 14 days after weaning

554 [d]Least significant mean from 8 observations

555 [e]Least significant mean from 25 observations

556

Table 3

Post-weaning performance of pigs fed four different dietary regimens and challenged with enterotoxigenic *E. coli* on days 3 and 4 after weaning[a]

Age of pigs/Treatment[b]	HP+AMC	HP	RP+AA	RP	SEM	P value
Body weight, kg						
Day 21 (weaning)	5.4	5.5	5.5	5.5	0.07	0.704
Day 28	5.7	5.7	5.7	5.5	0.10	0.455
Day 35	7.6^{x}	7.7^{x}	7.6^{x}	6.9^{y}	0.09	0.019
Day 42	10.0^{x}	9.9^{x}	9.9^{x}	9.0^{y}	0.30	0.062
Daily gain, g						
Day 21-28	42	20	27	9	10.3	0.167
Day 29-35	272^{x}	290^{x}	266^{x}	199^{y}	15.1	0.001
Day 36-42	332	313	336	293	20.2	0.419
Day 21-35	153^{x}	156^{x}	150^{x}	114^{y}	10.6	0.018
Day 21-42	215^{x}	207^{x}	210^{x}	169^{y}	12.4	0.035
Daily feed intake, g						
Day 21-28	102	91	106	88	8.0	0.346
Day 29-35	334^{x}	322^{xy}	339^{x}	272^{y}	19.5	0.063
Day 36-42	540	538	538	467	26.3	0.145
Day 21-35	218^{x}	206^{xy}	222^{x}	180^{y}	12.3	0.078
Day 21-42	326^{x}	317^{xy}	327^{x}	276^{y}	14.9	0.056
FCR, g/g						
Day 21-28	2.21	1.93	2.44	1.12	1.388	0.916
Day 29-35	1.24^{x}	1.13^{x}	1.28^{x}	1.46^{y}	0.056	0.001
Day 36-42	1.64	1.70	1.60	1.54	0.068	0.407
Day 21-35	1.56^{xy}	1.42^{x}	1.56^{xy}	1.73^{y}	0.069	0.024
Day 21-42	1.56	1.58	1.60	1.72	0.052	0.145

[xy]Means in the same row with different superscripts differ ($P<0.05$)

[a]All pigs received experimental diets for the first 2 weeks after weaning and were then fed a commercial weaner pellet diet in week 3

[b]HP+AMC: high protein with antimicrobial compounds; HP: high protein without AMC; RP+AA: reduced protein with CEAA supplementation to the level in HP diet; RP: reduced protein diet without AA supplementation (20% lower than HP diet)

Table 4

Impact of short-term feeding of high or reduced protein diets with and without essential amino acid fortification at weaning on lifetime performance and carcass characteristics.

Age of pigs/ Treatment[a]	HP+AMC	HP	RP+AA	RP	SEM	P value
Body weight, kg						
Day 70	24.9	24.8	24.8	23.7	0.47	0.228
Day 98	51.4	50.6	50.2	49.1	0.71	0.160
Day 126	80.4	79.3	78.8	78.3	0.89	0.379
Day 131	85.8	84.6	84.9	83.9	0.91	0.535
Daily gain, g						
Day 43-70	534	535	540	516	12.2	0.541
Day 71-98	946	924	912	909	15.3	0.306
Day 99-126	1042	1028	1019	1048	17.6	0.650
Day 127-131	1107	1081	1219	1109	49.1	0.202
Day 43-131	852	840	846	839	9.2	0.733
Days to 90 kg	133.9	136.4	136.4	137.7	1.14	0.138
Slaughter weight, kg	95.1	95.2	95.2	94.7	0.58	0.932
Carcass weight, kg[2]	63.8	63.8	63.9	63.7	0.24	0.907
Dressing %	65.7	67.2	67.2	67.1	0.75	0.391
P2 backfat, mm	12.0	12.2	12.0	11.3	0.39	0.386

[a]HP+AMC: high protein with antimicrobial compounds; HP: high protein without AMC; RP+AA: reduced protein with CEAA supplementation to the level in HP diet; RP: reduced protein diet without AA supplementation (20% lower than HP diet).

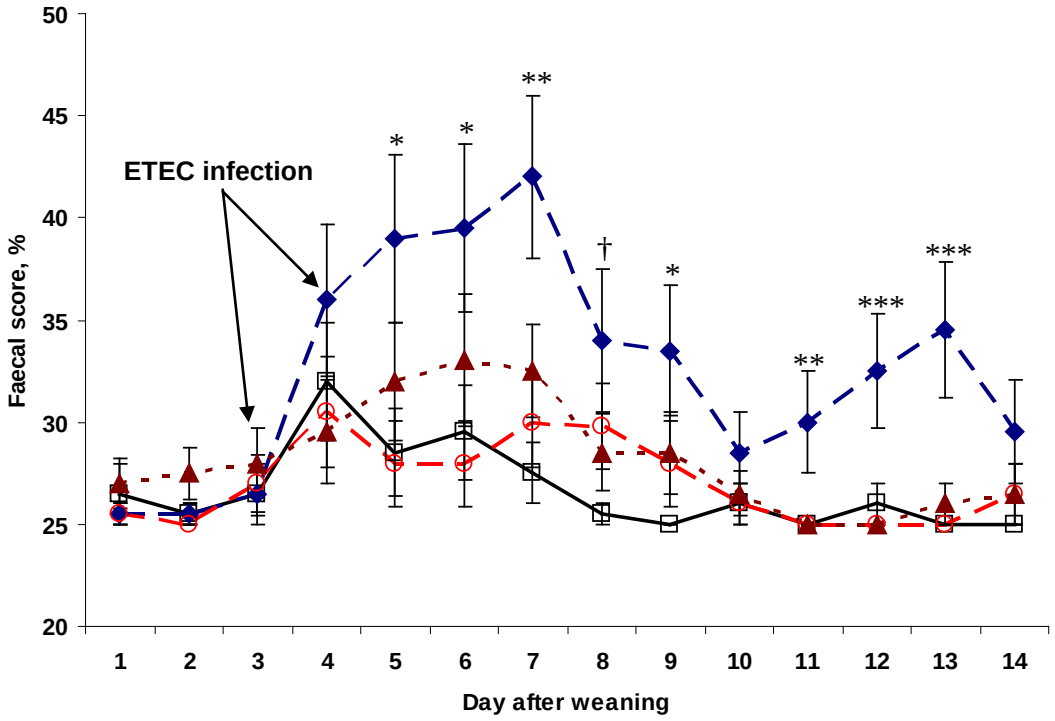

Figure 1

Faecal score measure for 14 days post-weaning under feeding a high protein diet with antimicrobial compounds (☐ HP+AMC), a high protein diet without AMC (◆ HP), a reduced protein diet with CEAA supplementation (○ LP+AA), and a reduced protein diet without AA supplementation (▲ RP). All pigs were experimentally infected with ETEC on days 3 and 4 after weaning. Significance: †: P<0.10, *: P < 0.05, **: P<0.01, ***: P<0.001.