Effect of diet composition on postweaning colibacillosis in piglets[1,2,3]

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ABSTRACT: The weaning of piglets is often associated with digestive disorders, particularly diarrhea—postweaning colibacillosis (PWC)—which is caused by infection with enterotoxigenic strains of *Escherichia coli*. It has been shown previously that a diet for newly weaned pigs based on cooked white rice and animal protein decreases the occurrence of PWC, whereas the addition of carboxymethylcellulose (CMC) to this diet enhances PWC. The aims of the current work were to 1) determine whether substitution of animal protein with plant proteins in the cooked-white-rice diet influenced its protective effects on PWC and 2) confirm that an increase in viscosity of the digesta by adding CMC to the diet favors the development of PWC—with (Exp. 1) or without (Exp. 2) experimental infection of piglets with *E. coli*. The diets were 1) cooked white rice and animal protein sources (RAP), 2) RAP + CMC added at 40 g of CMC/kg (air-dry basis) of diet, 3) cooked white rice and plant protein sources (RPP), and 4) wheat and plant protein sources (WPP). Experiments 1 and 2 were conducted using 32 and 24 piglets (eight and six per treatment), respectively. Piglets were weaned at 21 d (d 1), and fed ad libitum until slaughter on d 9. In Exp. 1, piglets were orally infected with enterotoxigenic *E. coli* on d 4, 5, 6, and 7. On d 8 of Exp. 1, the *E. coli* scores in feces of pigs fed RAP + CMC were higher than with RAP ($P < 0.01$). On d 9 after weaning, feces from pigs fed diet RAP were normal or moist, whereas feces from pigs fed RAP + CMC were wet to diarrheic. On d 7 of Exp. 2, pigs fed diets RAP + CMC and WPP had wetter feces than pigs fed diets RAP or RPP ($P < 0.05$). On d 8, the *E. coli* scores in feces were higher ($P < 0.01$) with pigs fed RAP + CMC than with all other diets. The *E. coli* scores in the digesta were also higher with pigs fed RAP + CMC, and to a lesser extent with diet WPP, than with pigs fed RAP or RPP ($P < 0.01$). The large intestine was heavier in pigs fed diets RPP and WPP, and the digesta were more acidic ($P < 0.05$). This study confirmed that diet RAP was protective against PWC, and that substitution of animal proteins with plant protein in a rice-based diet did not diminish its protective effects. The addition of CMC to cooked white rice increased digesta viscosity and enhanced PWC. Consequently, this diet represents a useful model for studying this condition.

Key Words: Diarrhea, Enterotoxigenic *E. coli*, Postweaning Colibacillosis, Rice, Weaning Piglet

 J. Anim. Sci. 2004. 82:2364–2374

Introduction

The weaning of piglets is associated with profound changes in the structure and functions of the gastrointestinal tract (Pluske et al., 1997). These perturbations can favor infection by enterotoxigenic strains of *E. coli* and lead to postweaning colibacillosis (**PWC**). Although PWC can be controlled by the use of in-feed antibiotics and minerals, such as zinc (Hampson, 1994), antimicrobial resistance is increasing (Barton, 2000), and concerns are being expressed about the routine use of antimicrobials in swine production.

Postweaning colibacillosis is a multifactorial disease, and its expression is influenced by the diet (Hampson, 1994). McDonald et al. (1999) demonstrated that a diet based on cooked white rice and animal protein sources was protective against the development of PWC after experimental challenge. However, there is increasing scrutiny associated with feeding animal protein to pigs, and such a diet would be more attractive if plant protein sources could be used. Plant protein sources, however,

[1]The authors thank B. Black, M. Boyce, J.-C. Kim for technical assistance; B. Mullan for formulating the diets; J. Quillet for gathering the literature; and B. Sève for advice with statistical analysis.

[2]This work was supported by a Special Research Grant from Murdoch University.

[3]Financial support received from the Région Bretagne (Contract No. B03925), INRA, and ENSAR from France, to which we are grateful.

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Received July 28, 2003.

Accepted May 11, 2004.

Table 1. Ingredients and chemical composition of the diets

Item	Diet[a] RAP	RAP + CMC	RPP	WPP
Ingredients, % air-dry diet				
White rice[b]	70.57	66.57	52.84	—
Wheat	—	—	—	53.26
Blood meal	3.00	3.00	—	—
Meat and bone meal	5.15	5.15	—	—
Fish meal	10.05	10.05	—	—
Dried whey	10.00	10.00	—	—
Australian sweet lupins	—	—	10.00	10.00
Canola meal	—	—	15.00	15.00
Full fat soybean meal	—	—	18.52	15.17
Canola oil	0.50	0.50	—	3.00
L-Lysine·HCl	0.07	0.07	0.39	0.51
DL-Methionine	0.04	0.04	0.11	0.15
L-Threonine	0.14	0.14	0.27	0.25
Tryptosine[c]	0.28	0.28	0.34	0.23
Choline chloride (60%)	0.04	0.04	0.04	0.04
Dicalcium phosphate	—	—	1.87	1.70
Limestone	—	—	0.45	0.52
NaCl	0.10	0.10	0.10	0.10
Vitamin and mineral mix[d]	0.07	0.07	0.07	0.07
Carboxymethylcellulose[e]	—	4.00	—	—
Calculated analysis, % air-dry diet or as specified				
DM	90.45	90.45	90.34	90.62
CP	20.00	19.84	20.00	21.47
Lysine	1.47	1.47	1.53	1.53
Threonine	0.96	0.96	1.02	1.02
Methionine + cysteine	0.77	0.77	0.82	0.87
Tryptophan	0.27	0.27	0.28	0.27
Fat	2.52	2.50	5.65	8.68
DE, MJ/kg	15.32	15.30	15.38	15.30

[a]RAP = diet based on rice and animal proteins; RAP + CMC = diet RAP containing carboxymethylcellulose; RPP = diet based on rice and plant proteins; WPP = diet based on wheat and plant proteins.

[b]Medium-grain white rice, Sunwhite Calrose (Leeton, NSW, Australia).

[c]Tryptophane + lysine.

[d]Provided the following amounts of vitamins and minerals (mg/kg of complete air-dried diet): retinyl acetate = 3.44, cholecalciferol = 0.065, α-tocopheryl acetate = 20, menadione = 4.4, riboflavin = 4, pyridoxine = 1.6, cyanocobalamin = 0.02, pantothenic acid = 14, nicotinic acid = 20, Co = 0.2 (sulfate), I = 0.6 (potassium iodide), Fe = 120 (sulfate), Mn = 60 (oxide), Zn = 100 (oxide), Cu = 10 (sulfate), and Se = 0.13 (selenium 4.5%, dust reduced).

[e]Low-viscosity carboxymethylcellulose (50 to 200 mPa·s for a solution of 40 g/L at 25°C), purchased from Sigma Aldrich, Catalogue No. C-5678.

generally have higher levels of dietary fiber, a lower digestibility, and the presence of antinutritional factors, and these factors could limit their effectiveness (Pluske et al., 1999).

Postweaning colibacillosis can be induced experimentally by inoculating piglets with an enterotoxigenic strain of *E. coli* after weaning. More recently, McDonald et al. (2001) observed that when carboxymethylcellulose (**CMC**)—a viscous, unfermentable polysaccharide—was added to a "protective" diet based on cooked white rice and animal proteins, PWC occurred. The use of CMC may therefore represent a novel method to disturb gut homeostasis without requiring experimental infection with *E. coli*.

The aims of the present work were to 1) determine whether animal protein sources in the cooked white rice diet could be replaced with plant protein sources without diminishing the protective effect of the diet on PWC and 2) confirm that an increase in viscosity of the digesta by adding CMC to the diet favors the development of PWC.

Materials and Methods

Diets and Feeding

The same four diets were tested in Exp. 1 and 2 (Table 1). The first diet contained medium-grain white rice (Sunwhite Calrose, Australian Ricegrowers Cooperative, Leeton, NSW, Australia) steam-cooked at a ratio of two volumes of water per volume of rice at 121°C in an autoclave for 15 min. This diet (**RAP**) was fortified with an animal protein supplement (fish meal, blood meal, meat and bone meal, and whey). The second diet (**RAP + CMC**) contained (at the expense of rice) low-viscosity CMC (40 g/L has viscosity of 50 to 200 mPa·s at 25°C; Sigma Aldrich C-5678) at 40 g/kg air-dry diet. The third diet (**RPP**) contained the same cooked white rice but was fortified with plant proteins, including Australian sweet lupins, canola meal, and full-fat soybean, which were substituted for the animal protein sources. The fourth diet (**WPP**) was a wheat-based diet fortified with the same plant proteins used in diet RPP. The four diets were formulated to meet requirements for growth of newly weaned pigs (NRC, 1988), and to be similar in crude protein (on average 205 g/kg DM) and DE (on average 15 MJ of DE/kg DM) contents. Diets contained neither antibiotics nor alternative antimicrobial substances.

Animals, Housing, and Experimental Design

The study was conducted with the approval of the Murdoch University Animal Ethics Committee (Ethics No. 897R-02). Both experiments were carried out with female piglets (Landrace × Large White) obtained from a commercial piggery, weaned at 21 d of age, and transported to the isolation animal house at Murdoch University.

Experiment 1 was an infection trial performed with 32 female pigs distributed into eight groups of four piglets of equal BW (average of 6.07 ± 0.10 kg). Two groups of four piglets each were randomly allocated to each of the four diets. Pigs were housed in two rooms, with each room accommodating four adjacent pens housing four piglets each. Each diet was tested in one pen in each room. Pens (2.5 × 2.5 m) were raised above the floor and had wire-mesh sides that allowed contact between the animals. Pens contained a covered, heated sleeping area; a feeding trough; and a nipple drinker. Pigs were raised in groups to encourage the oral-fecal transmission of the disease.

Experiment 2 was carried out with 24 pigs. They were distributed into four groups of equal BW (average of 5.67 ± 0.12 kg). Each group of pigs, representing a different diet, was randomly allocated to one of the four pens in a room. Six other female piglets of the same age and source were weaned, transported to Murdoch University, and immediately slaughtered to established baseline measurements of weights of gut contents and organs.

Experiments 1 and 2 were conducted using the same experimental design. Pigs were fed on an ad libitum basis from the day of weaning (d 1) until the time of slaughter (d 9). Estimates of daily food intake (DM basis) were calculated per pen, by weighing amounts offered each day and amounts recovered the next day. A sample of each diet was collected daily for calculation of DMI per pen per day. Water was available on an ad libitum basis. Pigs were monitored at least twice daily for signs of illness. On d 4 of Exp. 1, each piglet was orally infected with a broth containing enterotoxigenic *E. coli* (see inoculum section, which follows). Inoculation was repeated at a 24-h interval for three subsequent doses (d 5, 6, and 7). Fecal swabs were taken for culture on d 1, 3, 7, 8, and 9 after weaning. Wetness of feces was visually determined for each swab. The consistency of feces was scored from 1 to 4, where 1 corresponded to normal, 2 to moist, 3 to wet, and 4 to diarrheic feces. A cumulative score per diet and per day was then calculated.

Inoculum

A strain of enterotoxigenic hemolytic *E. coli* serotype O149;K91;K88 (toxins LT, STa, STb) isolated from a pig that died from a natural case of PWC was used to experimentally precipitate PWC in Exp. 1. Serotype was confirmed by slide coagglutination at the National *E. coli* Reference Laboratory (Bendigo, Victoria; after Hampson et al., 1993).

Preparation of inoculum in broth took approximately 36 h, and was performed using sterile techniques. The bacterium was transferred from a nutrient agar slope to an agar plate (Colombia base; Oxoid Ltd., Basingstoke, U.K.) supplemented with 5% (vol/vol) defibrinated ovine blood, and incubated at 37°C overnight. A representative colony was then removed from the plate and seeded into 20 mL of Trypticase soy broth (Becton, Dickinson, and Co., North Ryde, Australia). Broth cultures were checked for growth and lack of contamination by examination of aliquots under a phase contrast microscope. When the broth culture had reached mid-log phase, it was transferred to 400 mL of sterile broth, and reincubated for approximately 4 h until the cells were in active mid-log phase. Aliquots were removed, and cell numbers were estimated by microscopic examination in a counting chamber, before the culture was used for oral inoculation of the pigs (5 mL per pig on d 4, and 10 mL on d 5, 6, and 7).

Postmortem Procedure and Measurements

Pigs were weighed, killed by intravenous injection of a lethal dose of sodium pentobarbitone solution (300 mg/mL), and then exsanguinated. The gastrointestinal tract was removed and weighed, and then ligated and divided into four segments corresponding to the stomach, small intestine, cecum, and colon. Each segment was weighed full and empty of contents.

The pH value of digesta from the stomach, ileum, cecum, proximal colon, and feces was measured using a portable pH meter (Schindengen pH Boy-2; Schindengen Electric Mfg., Tokyo, Japan). Digesta from the ileum and proximal colon were collected and the viscosity measured. Digesta were centrifuged at 9,500 × *g* for 8 min (Sigma benchtop centrifuge 1-15; Quantum Scientific Pty. Ltd., Milton, Queensland, Australia) and the supernatant (0.5 mL) placed in a cone-plate rotational viscometer (Brookfield LVDVII+, CP40; Brookfield Engineering Laboratories Inc., Stoughton, MA), in which the viscosity was measured at a shear rate of 60/s at 37°C. The viscometer was rinsed with distilled water and wiped clean between samples. The viscosity

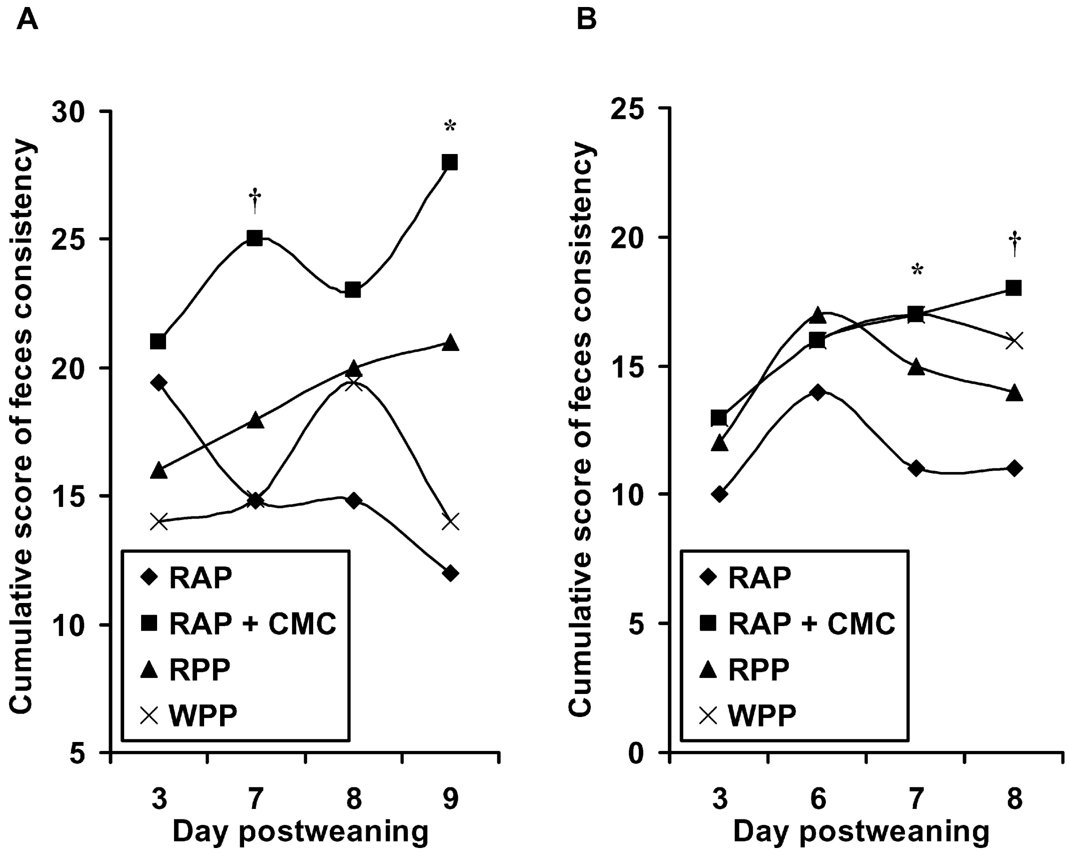

Figure 1. Consistency of feces after weaning in piglets fed a diet based on rice and animal proteins (RAP), rice and animal proteins plus 40 g/kg CMC (RAP + CMC), rice and plant proteins (RPP), or wheat and plant proteins (WPP), in Exp. 1 (experimental infection with enterotoxigenic *E. coli*; Panel A) and Exp. 2 (without experimental infection; Panel B). Fecal consistency is expressed as a cumulative score per day and per diet. Diets RAP, RAP + CMC, RPP, and WPP were tested on seven, eight, eight, and seven piglets in Exp. 1 and six piglets per group in Exp. 2. Higher values are associated with more-liquid feces. *P*-Values for a diet effect at each day were †$P < 0.10$ or *$P < 0.05$ as determined using the χ^2 differences to test the number of pigs having normal, wet, moist feces, or diarrhea for each diet and each day.

value was recorded as an apparent viscosity. In Exp. 1 and 2, 3 out of 60 samples and 8 out of 24 samples, respectively, from pigs fed diets RAP + CMC and WPP, were very thick, and a reliable viscosity estimate could not be recorded.

Bacteriology swabs were rolled in the intestinal contents and along the adjacent section of intestinal wall to be cultured for *E. coli*. Additional samples were taken from infected pigs from Exp. 1. Samples of mucosa from the middle 15 cm of the jejunum were scraped using a sterile scalpel, weighed, and placed in a bottle containing 9 mL of sterile PBS and kept on ice until dilutions could be made for subsequent *E. coli* counting.

Microbiology

Detection of Hemolytic E. coli. Fecal swabs and swabs of intestinal contents taken at slaughter were streaked onto blood agar plates before overnight incubation at 37°C. The plates were assessed for the presence of β-hemolytic colonies with a morphology characteristic of *E. coli*. The plates were scored from 0 to 5 according to the number of streaked sections that had viable hemolytic *E. coli*, where 0 corresponded to "no growth," 1 to "*E. coli* in the first section," 2 to "*E. coli* in the second section," and so on. This was referred to as the E. coli *score*.

E. coli *Counting*. Mucosal scrapings in PBS were mixed and serially diluted to 10^{-8}, using sterile PBS. Five 20-μL aliquots from each dilution were dropped equidistantly apart onto a blood agar plate that was incubated overnight at 37°C. The number of hemolytic colonies were counted and then expressed in log colony-forming units per gram of mucosal scraping.

Statistical Analyses

The variance homogeneity of the data was assessed using the Hartley test (Hartley, 1950) in Statview (version 5.0; SAS Inst., Inc., Cary, NC). Data for weight, average daily gains, and performances of piglets and for weight of the empty digestive segments had homoge-

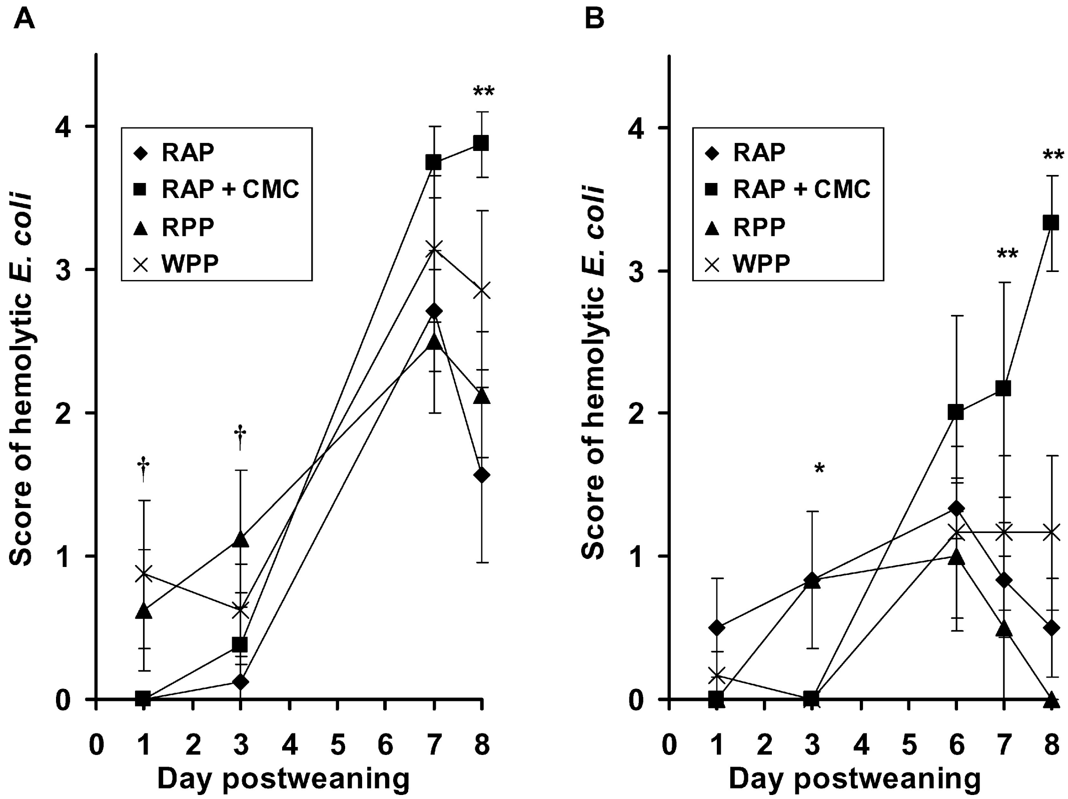

Figure 2. Fecal shedding of hemolytic *Escherichia coli* after weaning in piglets fed a diet based on rice and animal proteins (RAP), rice and animal proteins plus 40 g/kg CMC (RAP + CMC), rice and plant proteins (RPP), or wheat and plant proteins (WPP), in Exp. 1 (experimental infection with enterotoxigenic *E. coli*; Panel A) and Exp. 2 (without experimental infection; Panel B). Diets RAP, RAP + CMC, RPP, and WPP were tested on seven, eight, eight and seven piglets in Exp. 1 and six piglets per group in Exp. 2. Colonization is expressed as a score. Values are means with standard error. *P*-Values for a diet effect were †$P < 0.10$, *$P < 0.05$, or **$P < 0.01$ as determined by ANOVA using logarithmically transformed data.

neous variances. Data for colonization of the digestive tract by *E. coli*, for *E. coli* scoring in feces, and for viscosity of digesta were transformed using logarithmic function before statistical analysis.

Data from Exp. 1 and 2 were analyzed separately using the GLM procedure of SAS (SAS Inst. Inc.). Data for body weight, ADG, and performances of piglets; for weight of the empty digestive segments; and for colonization of the digestive tract by *E. coli* were analyzed for diet effects. Data for *E. coli* scoring in feces were analyzed for diets and time after weaning effects. Data for viscosity of digesta were analyzed for effects of diets and site along the gastrointestinal tract. The diet effects and diet × time, or diet × site effects, were further tested using the residual variations within pigs between diets as the error term. Differences between means were also analyzed using Student's *t*-test. For all these data means, pooled SEM are reported in the tables and figures.

Data for the weight of digesta in the gut segments and for pH values had heterogeneous variances, even after mathematical transformation. Median, minimum, and maximum values are reported for these data. Data from each experiment were analyzed separately for diet effects using Kruskal-Wallis nonparametric analysis of variance (Kruskal and Wallis, 1952). When the diet effect was significant, differences between diets were further identified using the multiple comparisons test, based on Kruskal and Wallis ranks, as described by Miller and Dunn (Hollander and Wolfe, 1973).

Data for fecal consistency (i.e., number of pigs having normal, moist, wet feces, or diarrhea) were analyzed for the effect of diet using the χ^2 test (Hollander and Wolfe, 1973). Data were reported as a cumulative score for all pigs on each day. Correlations between *E. coli* scores and fecal wetness scores were studied using the nonparametric Spearman test based on ranks (Hollander and Wolfe, 1973). All nonparametric analysis was performed using Statview. Differences were considered significant at an alpha level of $P < 0.05$.

Results

In Exp. 1, two piglets died soon after the first inoculation of *E. coli*. Therefore, the diets RAP, RAP + CMC, RPP, and WPP were tested with seven, eight, eight, and seven piglets, respectively. In Exp. 2, all six piglets

Table 2. Colonization of digestive segments by hemolytic *Escherichia coli*

Item	Exp. 1 (with experimental infection)						Exp. 2 (without experimental infection)					
	Diet[a]						Diet[a]					
	RAP	RAP + CMC	RPP	WPP	SEM	*P*-value[b]	RAP	RAP + CMC	RPP	WPP	SEM	*P*-value[b]
Swab score, % hemolytic *E. coli*												
Ileum	20.0	40.0	17.5	22.9	4.7	0.361	16.7^{y}	53.3^{x}	13.3^{y}	36.7^{xy}	4.3	0.012
Colon	68.6	65.0	52.5	57.1	4.8	0.495	16.7^{y}	70.0^{x}	23.3^{y}	63.3^{x}	4.3	0.001
Hemolytic *E coli*, log cfu/g mucosa												
Small intestine	0.94	1.66	0.68	2.07	2.55	0.328	Not determined					

[a]RAP = diet based on rice and animal proteins; RAP + CMC = diet RAP containing 40 g/kg CMC; RPP = diet based on rice and plant proteins; WPP = diet based on wheat and plant proteins. Diets RAP, RAP + CMC, RPP, and WPP were tested on seven, eight, eight, and seven piglets in Exp. 1 and six piglets per group in Exp. 2.
[b]Probability of a diet effect as determined by ANOVA.
[x,y]Within a row, in each experiment, means without a common superscript letter differ ($P < 0.05$).

allocated to each diet survived to the end of the experiment.

Proliferation of E. coli *and Incidence and Severity of Diarrhea*

Results for fecal consistency over the course of the experiments are summarized in Figure 1. In Exp. 1, diet affected feces consistency on d 9 after weaning (P = 0.048). Pigs fed diet RAP had mainly normal (three of seven pigs) or moist (three of seven pigs) feces, whereas pigs fed the diet containing CMC had mainly wet (four of eight pigs) or diarrheic (four of eight pigs) feces (Figure 1A). Replacement of animal proteins with plant proteins led to more liquid feces, with four of eight piglets fed diet RPP having wet feces. Finally, five of seven pigs fed diet WPP had normal or moist feces and two of seven had wet feces. The diet also tended to have an effect on d 7 after weaning (P = 0.059). Pigs fed the diet with CMC had wetter or more diarrheic feces (four and three of eight piglets, respectively) than pigs fed the other diets. In Exp. 2, diet also affected fecal consistency on d 7 after weaning (Figure 1B; P = 0.051). Feces from pigs fed diet RAP were less liquid than feces from pigs fed the three other diets. Diet RAP tended to lead to more normal feces than the other diets on d 9.

Results for fecal shedding of *E. coli* over the course of the experiments are summarized in Figure 2. A diet × time interaction was observed for the score of hemolytic *E. coli* from fecal swabs in Exp. 1 (Figure 2A; P = 0.048). On d 8, more hemolytic *E. coli* were found in the feces of pigs fed diet RAP + CMC than RAP (P = 0.004). A diet × time interaction (P = 0.001) was also observed in Exp. 2 (Figure 2B). On d 3, the hemolytic *E. coli* score in feces of pigs fed diet RAP + CMC was lower than scores observed with the diets RAP and RPP (P = 0.047). Conversely, on d 7, the *E. coli* score in feces of pigs fed diet RAP + CMC was higher than with diet RPP (P = 0.01) and, on d 8, higher than scores for all other diets. On d 8, more hemolytic *E. coli* were found in the feces of pigs fed diet WPP than in pigs fed diet RPP (P = 0.02).

No significant dietary effects were observed for the presence of hemolytic *E. coli* in the intestinal tract at slaughter in Exp. 1, but, in Exp. 2, swab scores in ileal and colonic digesta were higher in pigs fed both RAP + CMC and WPP than in pigs fed diets RAP or RPP (Table 2). Overall, hemolytic *E. coli* scores in feces were associated with more liquid feces in Exp. 1 (ρ of Spearman = 0.351; P = 0.001) and tended to be correlated in Exp. 2 (ρ = 0.172; P = 0.09). The correlation between hemolytic *E. coli* scores and feces consistency was most pronounced with the RAP + CMC diet (ρ = 0.359; P = 0.008; data not shown).

Whole Body and Intestinal Growth and Performance

Data for live BW, empty BW (live BW minus weight of total digesta), and empty gut BW (live BW minus weight of digestive tract and total digesta) at weaning and slaughter are presented in Table 3. The only diet effect was for empty-gut BW at slaughter in Exp. 1, where the weight for pigs fed WPP was less than the weights for pigs fed RAP + CMC and RPP (P = 0.011 and P = 0.019). There was also a trend (P = 0.101) for empty BW at slaughter to be lower in pigs fed WPP. No significant effects for ADG and BW gain were seen in Exp. 2, but in Exp. 1, the empty BW gain of pigs fed WPP was less than that of pigs fed RAP + CMC and RPP (P = 0.025 and P = 0.022, respectively), and the empty-gut BW gain by the pigs fed WPP was less (P = 0.032) than by those of the other three groups. No other significant differences were observed (Table 3).

The weights of the digesta in segments of the digestive tract are presented in Table 4. Significant effects were only seen in Exp. 2, in which they were recorded for the digesta weights for the entire tract, stomach, cecum, and colon. More digesta were found in the tract of piglets fed diet WPP than in piglets fed RAP + CMC (P = 0.03), and a similar effect was seen in the stomach. In the cecum and colon, digesta weights of pigs fed WPP were significantly greater than those fed RAP, whereas, in the colon, they were also greater than those fed RAP + CMC.

Table 3. Weights, average daily gains, and performances of piglets

	Exp. 1 (with experimental infection)						Exp. 2 (without experimental infection)					
	Diet[a]						Diet[a]					
Item	RAP	RAP + CMC	RPP	WPP	SEM	*P*-value[b]	RAP	RAP + CMC	RPP	WPP	SEM	*P*-value[b]
Live BW, kg												
Weaning[c]	6.09	6.09	6.00	6.10	0.10	0.988	5.68	5.57	5.78	5.66	0.12	0.946
Slaughter[d]	7.02	7.32	7.43	6.46	0.30	0.288	6.41	6.40	6.27	6.51	0.20	0.978
Empty BW, kg[e]												
Weaning	5.88	5.97	5.88	5.88	0.24	0.987	5.56	5.46	5.66	5.55	0.12	0.946
Slaughter	6.65	6.99	6.93	5.89	0.27	0.101	6.04	6.02	5.85	5.84	0.19	0.968
Empty gut BW, kg[f]												
Weaning	5.58	5.66	5.58	5.58	0.22	0.988	5.28	5.18	5.37	5.27	0.11	0.948
Slaughter	6.16[xy]	6.50[x]	6.40[x]	5.38[y]	0.24	0.049	5.58	5.54	5.40	5.30	0.17	0.935
ADG, g/d												
Live BW	127	153	178	58	21	0.238	83	97	56	98	13	0.663
Empty BW	96[xy]	127[x]	130[x]	1[y]	19	0.079	53	66	22	31	12	0.552
Empty-gut BW	72[x]	104[x]	103[x]	–25[y]	16	0.032	33	41	2	2	10	0.424
G:F, g/kg DM												
Live BW	376	548	475	231	92	0.656	398	374	263	545	65	0.511

[a]RAP = diet based on rice and animal proteins; RAP + CMC = diet RAP containing 40 g/kg CMC; RPP = diet based on rice and plant proteins; WPP = diet based on wheat and plant proteins. Diets RAP, RAP + CMC, RPP, and WPP were tested on seven, eight, eight, and seven piglets in Exp. 1 and six piglets per group in Exp. 2.
[b]Probability of a diet effect as determined by ANOVA.
[c]Piglets were weaned at 21 d on age.
[d]Piglets were slaughtered 9 d after weaning.
[e]Live BW minus weight of gut contents.
[f]Live BW minus weight of the gut organs and contents.
[x,y]Within a row, in each experiment, means without a common superscript letter differ ($P \leq 0.05$).

The weights of the empty portions of the intestinal tract are presented in Table 5. Significant effects ($P < 0.001$ to $P = 0.052$) were noted for the stomach and small intestine in both experiments, and for the colon in Exp. 2. In both studies, stomach tissue was heaviest in the pigs fed diets RPP and WPP, whereas the weights of the small intestines were greatest for the pigs fed RAP, and fed RAP + CMC in Exp. 2. In the latter experiment, colonic weights were greatest in the pigs fed WPP.

Digesta pH and Viscosity

Significant effects of diet on digesta pH were observed in the cecum and colon in both experiments, and in the ileum and feces in Exp. 2. In both experiments, pH

Table 4. Weight of digesta in different segments of the digestive tract[a]

	Exp. 1 (with experimental infection)					Exp. 2 (without experimental infection)				
	Diet[b]					Diet[b]				
Site	RAP	RAP + CMC	RPP	WPP	*P*-value[c]	RAP	RAP + CMC	RPP	WPP	*P*-value[c]
Digestive tract, g	392	316	465	568	0.113	360[xy]	348[y]	364[xy]	669[x]	0.010
	(207–553)	(129–650)	(355–699)	(63–1,301)		(223–625)	(245–568)	(262–615)	(513–852)	
Stomach, g	174	83	125	253	0.194	136[xy]	93[y]	119[xy]	241[x]	0.021
	(19–265)	(24–184)	(56–335)	(18–631)		(93–203)	(53–271)	(51–200)	(188–392)	
Small intestine, g	118	111	152	122	0.553	113	141	120	189	0.139
	(77–165)	(30–258)	(109–209)	(23–202)		(64–255)	(97–234)	(37–239)	(164–238)	
Cecum, g	13	21	61	38	0.159	19[y]	38[xy]	41[xy]	90[x]	0.016
	(3–38)	(4–80)	(5–77)	(6–90)		(7–77)	(24–50)	(25–52)	(30–97)	
Colon, g	93	86	132	95	0.214	65[y]	77[y]	116[xy]	136[x]	0.004
	(14–182)	(40–163)	(86–195)	(16–378)		(44–102)	(39–104)	(47–162)	(120–189)	

[a]Because data had heterogeneous variances, median values for each diet at each site are reported. The minimum and maximum values are shown in parentheses.
[b]RAP = diet based on rice and animal proteins; RAP + CMC = diet RAP containing 40 g/kg CMC; RPP = diet based on rice and plant proteins; WPP = diet based on wheat and plant proteins. Diets RAP, RAP + CMC, RPP, and WPP were tested on seven, eight, eight, and seven piglets in Exp. 1 and six piglets per group in Exp. 2.
[c]Probability of a diet effect as determined by Kruskal-Wallis test.
[x,y]Within a row, in each experiment, medians without a common superscript letter differ ($P < 0.05$).

Table 5. Weight of the empty digestive segments (g/100 g of empty digestive tract)

	Exp. 1 (with experimental infection)						Exp. 2 (without experimental infection)					
	Diet[a]						Diet[a]					
Site	RAP	RAP + CMC	RPP	WPP	SEM	*P*-value[b]	RAP	RAP + CMC	RPP	WPP	SEM	*P*-value[b]
Stomach	11.3^{yz}	10.3^{z}	12.6^{xy}	14.0^{x}	0.6	0.002	10.2^{y}	9.6^{y}	12.3^{x}	12.5^{x}	0.2	< 0.001
Small intestine	66.9^{x}	63.3^{xy}	63.7^{xy}	61.1^{y}	2.3	0.052	70.2^{x}	69.2^{x}	64.7^{y}	63.3^{y}	0.5	< 0.001
Cecum	3.8	4.3	4.4	4.8	0.2	0.198	4.2	3.8	4.5	4.7	0.1	0.107
Colon	18.0	22.1	19.3	20.0	0.9	0.129	15.4^{z}	17.4^{yz}	18.5^{xy}	19.5^{x}	0.4	0.004

[a]RAP = diet based on rice and animal proteins; RAP + CMC = diet RAP containing 40 g/kg CMC; RPP = diet based on rice and plant proteins; WPP = diet based on wheat and plant proteins. Diets RAP, RAP + CMC, RPP and WPP were tested on seven, eight, eight, and seven piglets in Exp. 1 and six piglets per group in Exp. 2.
[b]Probability of a diet effect as determined by ANOVA.
[x,y,z]Within a row, in each experiment, means without a common superscript letter differ ($P < 0.05$).

values in the cecum and colon were lower in pigs fed diets RPP and WPP than in pigs fed the other diets. The same trend was evident in the feces with respect to the RAP diet, but not the RAP + CMC diet. In the ileum in Exp. 2, the pH value was highest in the pigs fed RPP (Table 6).

The viscosity of the digesta is summarized in Figure 3. In Exp. 1, ileal digesta of pigs fed diet WPP were more viscous than digesta from pigs fed the RAP or RPP diets (Figure 3A). The viscosity of ileal digesta from pigs fed the diet RAP + CMC was intermediate and did not differ from that of the other diets. The cecal digesta of pigs fed diets RAP + CMC or WPP were more viscous than digesta from pigs fed the diets RAP or RPP. In pigs fed RAP + CMC, digesta from the cecum were more viscous than ileal digesta ($P = 0.05$). In Exp. 2, ileal and cecal digesta obtained from pigs fed the RAP + CMC and RPP diets were more viscous than digesta from pigs fed diet RAP (Figure 3B). Viscosity values obtained with diet WPP were intermediate and did not differ from other diets.

Discussion

After weaning, pigs need to be fed a diet that creates conditions in the gastrointestinal tract that stabilize the dynamic balance between the various components of the gut ecosystem, predominantly the mucosa and the microflora. If successful, this will minimize disturbance of the structure and functions of the gut and reduce the likelihood of enteric bacterial diseases such as PWC. In the current study, dietary RAP was protective against the development of PWC. Moreover, introducing plant proteins to a cooked white rice-based diet did not have significant consequences for proliferation of enterotoxigenic *E. coli* in the gastrointestinal tract.

Pigs fed diet RAP + CMC had more hemolytic *E. coli* in their feces and more liquid feces after infection, compared with pigs fed diet RAP, indicating that CMC exacerbated the experimental PWC. The increase in hemolytic *E. coli* in the feces and the greater fecal score of piglets fed diet RAP + CMC was also observed in Exp. 2 (without experimental challenge). In Exp. 2, and

Table 6. pH values of digesta[a]

	Exp. 1 (with experimental infection)					Exp. 2 (without experimental infection)				
	Diet[b]					Diet[b]				
Site	RAP	RAP + CMC	RPP	WPP	*P*-value[c]	RAP	RAP + CMC	RPP	WPP	*P*-value[c]
Stomach	3.4	3.1	2.5	2.9	0.876	3.0	2.0	2.4	2.8	0.619
	(1.9–3.8)	(2.2–3.8)	(1.7–4.4)	(1.6–4.3)		(2.2–3.4)	(1.6–4.1)	(1.4–3.2)	(1.4–4.7)	
Ileum	6.8	7.2	7.1	7.2	0.952	6.9^{xy}	6.8^{xy}	7.3^{x}	6.7^{y}	0.046
	(6.2–7.5)	(6.1–7.6)	(5.1–7.4)	(6.8–8.2)		(6.4–7.4)	(6.6–7.4)	(6.8–7.6)	(6.1–7.2)	
Cecum	6.4^{xy}	6.4^{x}	6.1^{y}	6.0^{xy}	0.048	6.2^{xy}	6.3^{x}	5.6^{z}	5.8^{yz}	0.001
	(6.0–6.6)	(6.3–6.9)	(5.4–6.8)	(5.6–7.1)		(5.9–6.6)	(5.9–6.8)	(5.3–5.8)	(5.5–5.9)	
Colon	6.5^{x}	6.5^{x}	6.2^{y}	6.1^{y}	0.008	6.3^{xy}	6.3^{x}	5.6^{z}	5.9^{yz}	0.001
	(6.1–6.7)	(6.3–6.7)	(5.3–6.4)	(5.5–6.8)		(6.1–6.6)	(6.1–6.8)	(5.1–6.2)	(5.8–6.1)	
Feces	7.4	6.5	6.7	6.7	0.076	7.3^{x}	6.1^{y}	7.0^{xy}	6.7^{xy}	0.005
	(6.4–8.0)	(6.2–6.9)	(5.6–7.10)	(5.9–8.2)		(7.2–8.0)	(6.1–6.6)	(5.8–7.6)	(6.2–7.0)	

[a]Because data had heterogeneous variances, median values for each diet at each site are reported. The minimum and maximum values are shown in parentheses.
[b]RAP = diet based on rice and animal proteins; RAP + CMC = diet RAP containing 40 g/kg CMC; RPP = diet based on rice and plant proteins; WPP = diet based on wheat and plant proteins. Diets RAP, RAP + CMC, RPP and WPP were tested on seven, eight, eight, and seven piglets in Exp. 1 and six piglets per group in Exp. 2.
[c]Probability of a diet effect as determined by Kruskal-Wallis test.
[x,y,z]Within a row, in each experiment, medians without a common superscript letter differ ($P < 0.05$).

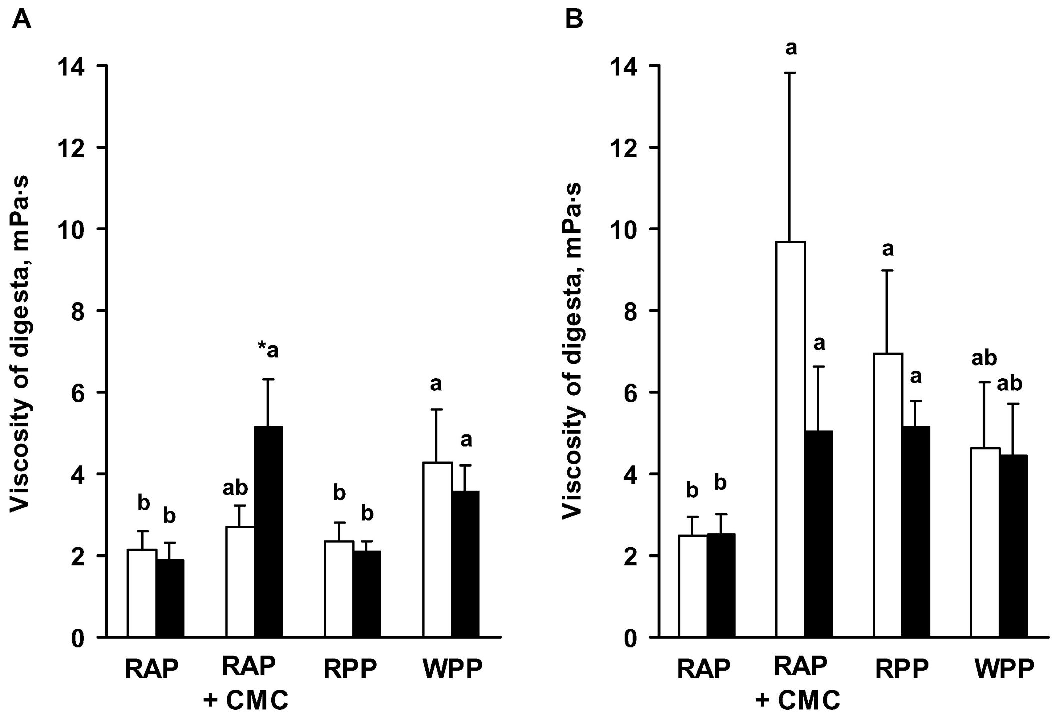

Figure 3. Viscosity of ileal (open bars) and cecal (solid bars) digesta after slaughter in piglets fed a diet based on rice and animal proteins (RAP), rice and animal proteins plus 40 g/kg CMC (RAP + CMC), rice and plant proteins (RPP), or a diet based on wheat and plant proteins (WPP), in Exp. 1 (experimental infection with enterotoxigenic *E. coli*; Panel A) and Exp. 2 (without experimental infection; Panel B). Values are means with standard error. Within a site (ileum or cecum), diets with different letters differ ($P < 0.05$), and an asterisk indicates that the ileal and cecal value differ for the indicated diet ($P < 0.05$).

especially on d 8, the fecal *E. coli* score of piglets fed diet RAP + CMC was similar to the score observed in the feces of experimentally infected piglets (Exp. 1). Feeding CMC increased the viscosity of the digesta in the gastrointestinal tract, and this possibly mediated the higher occurrence of PWC observed. As previously discussed (McDonald et al., 2001; Hopwood et al., 2002), the inclusion of CMC in a diet for newly weaned pigs based on cooked white rice might represent a novel method of perturbation of gut physiology that leads to proliferation of hemolytic *E. coli* in the gut without requiring experimental inoculation.

Consistent with previous observations made by McDonald et al. (1999), feeding piglets diet RAP decreased both colonization by *E. coli* and the incidence of diarrhea compared with pigs fed a wheat-based diet. A similar protective effect of RAP has been seen in pigs experimentally infected with the intestinal spirochete *Brachyspira hyodysenteriae*, the agent of swine dysentery (Pluske et al., 1996), and *B. pilosicoli*, the agent of porcine intestinal spirochetosis (Hampson et al., 2000). The protective effect of such a diet against bacterial infection has been attributed in part to the high digestibility of its protein and carbohydrates (Siba et al., 1996). In piglets, it is generally thought that diets containing less fiber and highly digestible ingredients—thereby limiting the amount of fermentable substrate entering the large intestine—are associated with a decrease in the incidence of PWC (Hampson, 1994; Montagne et al., 2003). Such diets may result in less accumulation of potential bacterial substrate in the upper small intestine, the primary site of proliferation of the pathogenic *E. coli* causing PWC.

One of the primary mechanisms by which toxin-producing bacteria, such as *E. coli* or *Salmonella*, initiate secretory diarrhea is the increase of water secretion by the small intestinal crypt cells, by a pathway involving cAMP (Kaunitz et al., 1995). In young pigs, the large intestine is incompletely developed and may not be capable of absorbing enough fluid to prevent clinical diarrhea and dehydration (Nabuurs, 1998). A component of boiled white rice recently identified and named the *rice factor* has been shown to block the secretory response of intestinal crypt cells to cAMP in guinea pigs (MacLeod et al., 1995; Mathews et al., 1999). A potential effect of this rice factor has not been demonstrated in other animal species; however, boiled rice has been used for many years in the treatment of diarrhea in humans, and is included in various oral rehydration products. Moreover, rice-based electrolyte solutions are though to be superior to standard glucose electrolyte solutions for human rehydration therapy (Bhan et al., 1994). As with other oral rehydration solutions, the main benefit is from nutrient-linked absorption. This, in addition to the high prececal digestibility of the RAP diet, might help explain its protective effect against PWC in piglets.

The main effects of introducing plant protein sources to the diet were an enhancement of fermentative activity in the large intestine, as indicated by the lower pH values of the digesta, and a greater weight of the cecum and colon in pigs fed RPP and WPP diets compared with pigs fed the RAP diet. The main factor influencing fermentative activity in the hindgut is the presence and type(s) of fermentable substrates (Williams et al., 2001). In the present study, this could have resulted from a higher concentration of soluble nonstarch polysaccharides (19.9 and 29.0 g/kg DM of the diet, respectively, vs. 2.1 g/kg DM for RAP), and/or a lower digestibility of plant proteins compared with animal proteins (McDonald, 2001).

The presence of fermentable carbohydrate in diets of newly weaned pigs may allow a microflora to develop that has a positive effect on piglet gut integrity (Williams et al., 2001), despite the fact that this type of diet reduces weight gain (Bolduan et al., 1988). Stable gut microflora participates in the maintenance of gut integrity by forming a barrier that prevents gut colonization by pathogenic bacteria, a phenomenon known as *colonization resistance* (Van der Waaij, 1989; Rolfe et al., 1997). Resident bacteria together with the intestinal immune system exert a protective function on the gut. Resident bacteria also produce the VFA and lactate that are responsible for an acidic pH (Bugaut and Bentéjac, 1993), which is generally decreased significantly after weaning (Pluske et al., 1996; Jensen, 1998). McDonald et al. (2001) observed higher VFA concentrations in the hindgut of pigs fed a diet based on raw wheat, or one containing Australian sweet lupins compared with a diet based on cooked white rice. Less acetate and more propionate and butyrate were recovered in the large intestinal digesta of pigs fed raw wheat compared with cooked white rice, indicating changes in the microbial activities resulting from likely changes in the microbial populations.

The VFA have trophic effects on the intestinal epithelium, thereby helping to maintain the mucosal defense barrier against invading organisms (Williams et al., 2001). In the large intestine, VFA stimulate the absorption of sodium and water (Argenzio and Whipp, 1979), thus limiting the risk of diarrhea. Moreover, in an acidic environment VFA are capable of inhibiting the growth of some intestinal pathogenic bacteria in pigs, such as *E. coli* and *Clostridium difficile* (Prohaska, 1986; May et al., 1994).

Another consequence of introducing plant proteins to a diet for newly weaned pigs observed in previous studies was a decrease in the villus length/crypt depth ratio (McDonald, 2001). This may have resulted in impaired hydrolysis and absorption in the small intestine and, consequently, an increase in the bulk of nutrients reaching the hindgut. Moreover, a decrease in the villus height/crypt depth ratio is also associated with an increase in water secretion through the crypt to the lumen, favoring diarrhea.

In the present study, because replacement of animal proteins by plant proteins did not have a significant effect on proliferation of *E. coli* and wetness of feces, we hypothesize that the positive effects of increased fermentation in the hindgut counterbalanced any likely negative effects of plant protein sources on gut function, as reported above.

Implications

Feeding a weaning diet based on cooked white rice supplemented with either animal or plant protein sources resulted in drier feces and lower *E. coli* scores compared with diets containing carboxymethylcellulose or based on wheat and plant proteins. Diets for newly weaned pigs based on suitably processed rice may be a useful option for swine herds in which postweaning colibacillosis is endemic and resistant strains of *E. coli* are present. Inclusion of 40 g/kg carboxymethylcellulose (air-dry basis) in the rice-based diet provides a useful experimental model of colibacillosis, without requiring experimental infectious challenge. Such a model could be used to test the efficiency of alternatives to in-feed antibiotics.

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