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Effects of pathogenic *E. coli* on diarrhea, growth performance, and blood profile of weaned pigs

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Abstract : The experiment was conducted to evaluate the effects of pathogenic *Escherichia coli* on diarrhea, growth performance, and blood profile of weaned pigs. A total of 48 pigs were used and housed in individual pens of disease containment chambers for 16 d (4 d before and 12 d after the first challenge [d0]). The treatments were with or without the pathogenic *E. coli* challenge (F-18 *E. coli* strain; heat-labile, heat-stable, and Shiga-like toxins). Pigs were orally inoculated with a dose of 10^{10}cfu *E. coli* per 3 mL PBS daily for 3 days. The common nursery diet and water were available at all times. The ADG, ADFI, G:F, diarrhea score, ratio of fecal β-hemolytic coliforms from total coliforms (RHT), and blood profile were measured. The pathogenic *E. coli* reduced ($P < 0.05$) ADG from d0 to 6 (117 vs. 297 g/d) and from d0 to 12 (377 vs. 238 g/d) compared with the control. Meanwhile, the pathogenic *E. coli* increased ($P < 0.05$) diarrhea score (average 3.4 vs. 1.4) and RHT (average 82 vs. 11%) on d3, 6, and 9 and the number of white blood cells (17.59 vs. 13.48 x10^3/μL) on d6 compared with the control. No differences were found on ADFI and others in the blood profile (total protein and hematocrit). In conclusion, pathogenic *E. coli* used in this experiment successfully caused mild diarrhea, increased number of white blood cells, and adversely affected growth rate of weaned pigs.

Key words **:** Diarrhea, Growth Performance, Pathogenic *Escherichia coli*, Weaned Pigs

I. Introduction

Colibacillosis is one of the main reasons of diarrhea and edema causing decline of growth rate and elevation of death rate for such weaning and suckling pigs (Wilson and Francis, 1986). According to the analysis (1999–2000) of the piglet diarrhea by the National Veterinary Research & Quarantine Service, the Colibacillocis showed 26.6% that was the highest percentage among the bacterial diarrhea. It is notable that Colibacillocis of the piglet diarrhea occurs year round, shows steady incidence, and has the highest sensitivity in the early stage of weaning (Francis, 2002).

Spread of pathogenic *E. coli* causing diarrhea is made through the mouth which is the common pathway of the gastrointestinal disease (Fairbrother and Gyles, 2006). It is mainly spread through sucking the nipple contaminated by the feces and through the intact environment that is contaminated like the floor. Stress and rapid growth of pathogenic *E. coli* cause the change of microorganism in intestines of piglet after post-weaning (Cho, 1990). In addition, feed transfer also causes piglet diarrhea and edema. Therefore, the objective of this experiment is to evaluate effects of pathogenic *E. coli* on diarrhea, growth performance, and blood profile of weaned pigs.

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II. Materials and methods

1. Animals, Housing, Diet, and Experimental Design

The experimental protocol was reviewed and approved

Table 1. Ingredient composition of experimental diet (as-fed basis).

Item	Diet
Ingredient, %	
Corn, ground	40.93
Dried whey	20.00
Soybean meal, 47%	10.00
Fishmeal	10.00
Lactose	7.22
Soy protein concentrate	5.00
Poultry byproduct meal	3.22
Soybean oil	2.92
Mineral premix[1]	0.35
Vitamin premix[2]	0.20
L-Lys HCl	0.06
DL-Met	0.05
L-Thr	0.03
L-Trp	0.02
Calculated energy and nutrient levels	
ME, kcal/kg	3480
CP, %	22.53
Fat, %	6.48
Ca, %	0.80
P, %	0.73
Available P, %	0.51
Lys, %	1.50
Lactose, %	21.00

[1]Provided as milligrams per kilogram of diet: 3,000 of NaCl; 100 of Zn from zinc oxide; 90 of Fe from iron sulfate; 20 of Mn from manganese oxide; 8 of Cu from copper sulfate; 0.35 of I from calcium iodide; 0.30 of Se from sodium selenite.
[2]Provided per kilogram of diet: 2,273 μg of retinyl acetate; 17 μg of cholecalciferol; 88 mg of DL-α-tocopheryl acetate; 4 mg of menadione from menadione sodium bisulfite complex; 33 mg of niacin; 24 mg of D-Ca-pantothenate; 9 mg of riboflavin; 35 μg of vitamin B12; 324 mg of choline chloride.

by the Institutional Animal Care and Use Committee at the University of Illinois. The same number of barrows and gilts (n=28; 6.8±0.8 kg BW) with similar weight were selected at weaning and assigned to treatments. Pigs were housed in individual pens of disease containment chambers for 16 d (4 d before [acclimation period] and 12 d after the first challenge [d0]). The treatments were with or without *E. coli* challenge treatment. The *E. coli* used for the challenge (isolate number UI-VDL 05-27242) was an F-18 fimbria+ *E. coli* strain that produced heat-labile toxin, heat-stable toxin, and Shiga-like toxin isolated from a field disease outbreak and provided at 10^{10} cfu/3 mL dose in phosphate-buffered saline to cause mild diarrhea. The unchallenged treatment was a 3 mL dose of PBS. Both the *E. coli* and sham inoculations were given orally to pigs daily for 3 consecutive days beginning 4 d after weaning (d0). The complex nursery control diet was formulated to meet or exceed NRC estimates of requirements of weanling pigs (Table 1).

2. Sample Collection, Analyses, and Measurements

Pigs and feeders were weighed on the day of weaning (d-4), the day of the first inoculation (d0), d6, and d12. Growth performance (ADG, ADFI, and G:F) was measured for each interval from d0 to 6, 6 to 12, and 0 to 12. Clinical observations (diarrhea and alertness scores) were recorded daily beginning on the first day of challenge (d0). Diarrhea score of each pig was assessed visually each day by 2 independent evaluators with a score from 1 to 5 (1 = normal feces, 2 = moist feces, 3 = mild diarrhea, 4 = severe diarrhea, and 5 = watery diarrhea). Frequency of diarrhea was calculated by counting pig days with diarrhea score of 3 or higher. Fecal samples were collected from the rectum of each pig on d0, 3, 6, 9, and 12 and kept on ice during transport to the laboratory. When it was not possible to get a bulk sample because of the absence of feces or watery diarrhea, a cotton swab was used to collect the sample. Samples were processed within 2 h after collection. Each sample was plated on blood agar to differentiate β-hemolytic coliforms (generally gray and shiny colonies; complete lysis of red blood cells surrounding colonies) from non-β-hemolytic coliforms. Growth on MacConkey agar was

compared to blood agar to support that hemolytic colonies on the blood agar were correctly identified as *E. coli* (generally flat pink colonies). Plates were incubated at 37°C and were read 24 h after plating. Populations of both total coliforms and β-hemolytic coliforms on blood agar were assessed visually, assigning a score from 0 through 8, where 0 corresponds to no growth and 8 to very heavy bacterial growth. The results were then expressed as a ratio of the β-hemolytic coliforms score to the total coliforms score, as an approximation of the proportion of *E. coli* that were β-hemolytic coliforms. Two blood samples (whole blood and serum) were collected from the jugular vein of each pig on d0, 6, and 12 to measure total and differential white blood cell counts, packed cell volume, and total protein by the Veterinary Clinical Pathology Laboratory at the Chungnam National University. Total and differential white blood cell counts by hematocrit concentration was analyzed on a multiparameter, automated hematology analyzer calibrated for porcine blood (CELL-DYN 3700, Abbott Laboratories, Abbott Park, IL). Serum total protein was analyzed on an automated biochemistry analyzer (HITACHI 917, Roche Diagnostics GmbH, Mannheim, Germany). TP was used as indicator for dehydration.

3. Statistical Analysis

Data were analyzed using the Proc GLM procedure of SAS (SAS Inst. Inc, Cary, NC). Pig was the experimental unit. The statistical model included effects of *E. coli* challenge as a fixed effect. There was a block with sex by BW. The α level of 0.05 was used for determination of significance among means.

III. Results and discussion

The pathogenic *E. coli* reduced ADG from d0 to 6 ($P < 0.05$), d7 to 12 ($P < 0.10$), and d0 to 12 ($P < 0.05$; Table 2) compared with control. In addition, the pathogenic *E. coli* tended to reduce ADFI from d0 to 6 ($P < 0.10$) and G:F from 0 to 12 ($P < 0.10$) compared with the control. The reduced growth performance may be due to the activation of immune system triggered by toxins secreting pathogenic *E. coli* and then energy may be used for the immune system

Table 2. Effects of pathogenic *E. coli* on growth performance of weaned pigs[1].

Item	Control	Challenge	SEM	P-value
Number of animals, n	14	14		
Initial body weight, kg	6.86	6.79	0.272	0.833
d0 to 6				
ADG, g	297	117	38	< 0.05
ADFI, g	646	577	23	< 0.10
G:F, g/g	0.463	0.204	0.056	< 0.05
d7 to 12				
ADG, g	454	350	36	< 0.10
ADFI, g	1051	945	67	0.185
G:F, g/g	0.433	0.372	0.067	0.749
d0 to 12				
ADG, g	377	238	39	< 0.05
ADFI, g	848	764	51	0.367
G:F, g/g	0.446	0.320	43	< 0.10

Control = unchallenged; Challenge = pathogenic *E. coli* challenged.

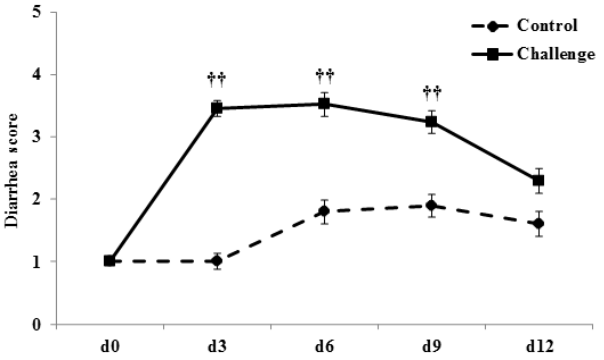

Fig. 1. Effects of pathogenic *E. coli* on diarrhea score of weaned pigs. Diarrhea score = 1, normal feces, 2, moist feces, 3, mild diarrhea, 4, severe diarrhea, 5, watery diarrhea. Control = unchallenged; Challenge = pathogenic *E. coli* challenged. Values are means ± SE. ††Significantly different between control and challenge, $P < 0.05$.

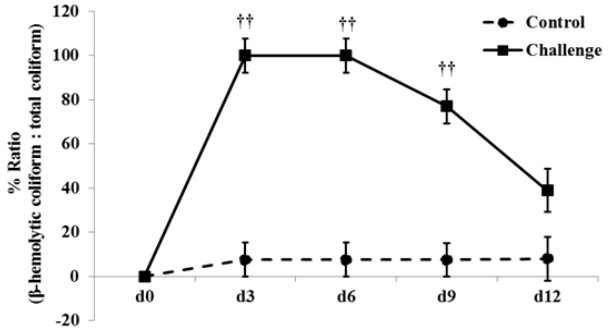

Fig. 2. Effects of pathogenic *E. coli* on ration between β -hemolytic coliform and total coliform in feces of weaned pigs. Control = unchallenged; Challenge = pathogenic *E. coli* challenged. Values are means ± SE. ††Significantly different between control and challenge, $P < 0.05$.

rather than growth of weaned pigs (Liu et al., 2013; Nagy and Fekete, 2005; Song et al., 2012).

Meanwhile, the pathogenic *E. coli* increased ($P < 0.05$) diarrhea score (Fig. 1), ratio of the β-hemolytic coliforms score to the total coliforms score (Fig. 2) compared with the control. Etiologically coliforms are the main causes of diarrhea of weaned pig resulting in high mortality (Moeser and Blikslager, 2007). Among such coliforms (K88, K99, 987P, F41 and F18), the F-18 is known to be the main pathogen causing diarrhea and mortality of weaned pigs. Therefore, the pathogenic *E. coli* used in this experiment successfully caused mild diarrhea (Dykstra et al., 1993; Gannon et al., 1989).

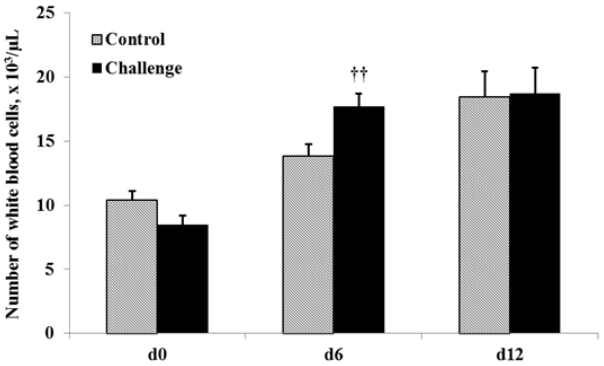

Fig. 3. Effects of pathogenic *E. coli* on the number of white blood cells of weaned pigs. Control = unchallenged; Challenge = pathogenic *E. coli* challenged. Values are means ± SE. †† Significantly different from control, $P < 0.05$.

The pathogenic *E. coli* increased ($P < 0.05$) number of white blood cells on d6 compared with the control (Fig. 3). However, no differences were found on blood profile (total protein and hematocrit; Table 3). The pathogenic *E. coli* can cause intestinal inflammation

Table 3. Effects of pathogenic *E. coli* on total protein and hematocrit of weaned pigs[1].

Item	Control	Challenge	SEM	*P*-value
Number of animals, n	14	14		
d0				
Total protein, g/dL	4.78	4.83	0.097	0.764
Hematocrit, %	33.44	32.34	1.079	0.556
d6				
Total protein, g/dL	4.48	4.68	0.108	0.270
Hematocrit, %	35.35	35.26	0.825	0.951
d12				
Total protein, g/dL	4.52	4.47	0.073	0.711
Hematocrit, %	37.85	36.91	1.448	0.298

Control = unchallenged; Challenge = pathogenic *E. coli* challenged.

locally and systemically resulting in increasing of number of white blood cells of weaned pigs (Liu et al., 2013; Song et al., 2012).

IV. Conclusion

The pathogenic *E. coli* used in this experiment successfully caused mild diarrhea, increased number of white blood cells, and adversely affected growth rate of weaned pigs.

Acknowledgements

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