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Original Research Article

# Effect of chicken egg anti-F4 antibodies on performance and diarrhea incidences in enterotoxigenic *Escherichia coli* K88$^+$-challenged piglets

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ABSTRACT

The aim was to evaluate the effects of dietary supplementation of spay-dried whole egg containing anti-F4 antibodies (SDWE) against recombinantly produced F4 antigens in enterotoxigenic *Escherichia coli* K88$^+$ (ETEC)-challenged piglets. Twenty-seven 21-d-old and individually housed piglets were randomly allotted to 3 treatments consisting of a wheat-soybean meal basal diet containing either 0 (control egg powder; CEP), 0.1% (SDWE1) or 0.4% (SDWE2) SDWE. After a 7-d adaptation period, blood samples were collected from all pigs, and pigs were weighed and orally challenged with an ETEC inoculum. Blood was sampled at 24 and 48 h post-challenge, and diarrhea incidences and scores were recorded. On d 14, all pigs were weighed and then euthanized to obtain intestinal tissue samples for histomorphology measurement. During the pre-challenge period, pigs fed the SDWE showed a linear improvement ($P < 0.05$) in average daily gain (ADG) and gain to feed ratio (G:F), but there were no differences among treatments in growth performance during the post-challenge period. Diarrhea incidences and scores, fecal shedding of ETEC, plasma urea nitrogen content and intestinal histomorphology were similar among treatments. The results show that 0.4% SDWE supported greater piglet performance before challenge although such benefits were not evident during the post-challenge period at either 0.1% or 0.4% supplementation.



## 1. Introduction

In pig production, post-weaning diarrhea (PWD) is a major health challenge with significant economic losses (Fairbrother et al., 2005; Daudelin et al., 2011) resulting from reduced growth performance (Boudry et al., 2002, 2004), compromised intestinal health (Moeser et al., 2007), increased susceptibility to diseases and high mortality rate (Madec et al., 2000). Infection with enterotoxigenic *Escherichia coli* expressing the F4 (K88$^+$) fimbriae (ETEC) is one of the most important causes of PWD in pigs (Fairbrother et al., 2005). It has been shown that colonization of the small intestine of the pig by ETEC adhering to the epithelium accounts for most gastrointestinal disorders in both neonatal and early-weaned piglets (Yokoyama et al., 1992; Marquardt et al., 1999). For more than 50 years, antimicrobials have been used in animal production for growth promotion (sub-therapeutic doses), disease prevention (prophylactic doses) and treatment (therapeutic doses; Diraviyam et al., 2014), and many reports have demonstrated the significant contributions of antimicrobials to the improved performance of animals (Turner et al., 2001; Cromwell, 2002). Fortifying starter diets with antimicrobial growth promoters is routinely used for controlling and mitigating effects of PWD in pigs (Pluske et al., 2002). However, there are public concerns about antimicrobial drug residues in food animals and the risk of the development of a reservoir of antibiotic resistant bacteria that cause disease in humans (Barton, 2000; Hulst et al., 2013; Diraviyam et al., 2014). Hence, much of the recent studies are focused on identifying effective and viable alternative therapies to antimicrobials (Owusu-Asiedu et al., 2003; Kiarie et al., 2009, 2011), and one such therapy

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is passive immunization of piglets using chicken egg antibodies against recombinantly produced F4 antigens. However, to our knowledge, the efficacy of anti-F4 antibodies (SDWE) against ETEC fimbrial antigens produced from laying hens hyper-immunized with recombinant F4 fimbrial antigens has not been studied in pigs.

Therefore, the objective of the present study was to determine growth performance and incidences of diarrhea in ETEC-challenged piglets fed diets containing chicken egg antibodies against recombinantly produced F4 antigens.

## 2. Materials and methods

All experimental procedures were reviewed and approved by the University of Manitoba Animal Care Committee, and pigs were cared for according to the guidelines of the Canadian Council on Animal Care (CCAC, 2009).

### 2.1. Animals, treatments and oral challenge

A total of 27 piglets ([Yorkshire × Landrace] × Duroc, 7.27 ± 0.47 kg initial body weight, BW) weaned at 21 ± 1 d from the University of Manitoba's Glenlea Swine Research Unit were used in this study. Pigs were individually housed in cages (0.76 m × 0.61 m × 0.38 m) within a room at the T. K. Cheung Centre for Animal Science Research, University of Manitoba, Winnipeg, Canada. Room temperature was maintained at 30 ± 1 °C and pigs had *ad libitum* access to feed and water throughout the experimental period. Piglets were randomly allotted to 3 dietary treatments ($n = 9$) consisting of a wheat–soybean meal basal diet containing either 0 (control egg powder; CEP), 0.1% sprayed dried whole powder containing SDWE (SDWE1) or 0.4% SDWE (SDWE2) for a 14-d study. The control egg powder and SDWE were added to the complete basal diet. The basal diet (Table 1) was formulated to meet the National Research Council (NRC, 2012) nutrient specifications for 5 to 10 kg BW pigs. The SDWE against recombinant F4 fimbrial antigens and CEP were supplied by Zyme Fast System Inc., Winnipeg, Manitoba, Canada. After the original isolation of F4 fimbrial antigens from wild type strain of ETEC for the immunization of laying hens, the antigen was produced recombinantly in a competent *E. coli* high expression system (Zyme Fast System Inc., Winnipeg, MB, Canada).

Body weights and feed intake were determined weekly and average daily BW gain (ADG), average daily feed intake (ADFI) and the ratio of BW gain to feed intake (G:F) were calculated. After a 7-d adaption, each piglet was bled (venipuncture through the jugular vein) to obtain blood samples and then orally challenged with 6 mL ($2 \times 10^9$ cfu/mL) of freshly grown ETEC inoculum at the back of oral cavity by using a polyethylene tube attached to a syringe as described previously (Kiarie et al., 2009). Fecal samples were also collected before the oral challenge for enumeration of fecal shedding of ETEC to ensure piglets were not infected with ETEC.

Pigs were monitored for 7 d post-challenge for incidences of diarrhea and general health conditions. Incidences and severity of diarrhea were assessed for each pig by 2 trained independent personnel (without prior knowledge of dietary treatment allotment) using a fecal consistency scoring system (0 = normal feces; 1 = soft feces; 2 = mild diarrhea; 3 = severe diarrhea [Marquardt et al., 1999]).

### 2.2. Enterotoxigenic *E. coli* K88$^+$ and culture condition

The ETEC strain was originally obtained from Veterinary Diagnostic Services of Manitoba, Winnipeg, Manitoba, Canada. To evaluate the proliferation of ETEC and to differentiate the inoculum from the indigenous strains, the pure ETEC was made resistant to ciprofloxacin in Mueller–Hilton broth (Becton Dickinson and Company, Sparks, MD, USA) as previously described by Opapeju et al. (2009).

**Table 1**
Ingredient and calculated nutrient composition of basal diet (as-fed basis).

| Item | Content |
|---|---|
| Ingredient, % | |
| Barley | 17.00 |
| Hard red winter wheat | 27.00 |
| Soybean meal | 24.16 |
| Fish meal | 5.00 |
| Dried whey | 19.00 |
| Vegetable oil | 5.00 |
| Limestone | 0.50 |
| $CaHPO_4$ | 0.40 |
| Iodized salt | 0.50 |
| Vitamin-mineral premix[1] | 1.00 |
| Lysine-HCl | 0.30 |
| DL-methionine | 0.07 |
| L-threonine | 0.07 |
| SDWE1[2] | 0.1 |
| SDWE2[3] | 0.4 |
| Calculated nutrient, % | |
| Metabolizable energy, kcal/kg | 3,375 |
| Crude protein | 22.46 |
| Lysine | 1.46 |
| Methionine | 0.40 |
| Methionine + Cysteine | 0.70 |
| Threonine | 0.82 |
| Tryptophan | 0.25 |
| Calcium | 0.89 |
| Available phosphorus | 0.45 |

[1] Vitamin-mineral premix per kg of diet: vitamin A 8,250 IU, vitamin $D_3$ 835 IU, vitamin E 40 IU, vitamin $K_3$ 4 mg, vitamin $B_{12}$ 0.025 mg, vitamin $B_1$ 2 mg, vitamin $B_2$ 12 mg, nicotinic acid 22.5 mg, folic acid 2 mg, pyridoxine 4.5 mg, biotin 0.2 mg, pantothenate 15 mg, choline 500 mg, Mn 50 mg, Fe 100 mg, I 0.4 mg, Cu 25 mg, Zn 150 mg, Se 0.3 mg.
[2] SDWE1 = basal diet plus 1 kg spay-dried whole egg containing anti-F4 antibodies (SDWE) and 3 kg control egg powder/t feed.
[3] SDWE2 = basal diet plus 4 kg SDWE/t feed.

### 2.3. Blood and fecal sample collections

Heparinized and non-heparinized blood samples to obtain plasma and serum for plasma urea nitrogen (PUN) and serum cytokine concentration analyses were collected from the jugular vein of each piglet on d 8 of study before ETEC oral inoculation, and on d 1, 2 and 7 post-challenge (i.e., d 9, 10 and 14 of study, respectively). Samples were immediately centrifuged at 2,000 × *g* for 10 min at 5 °C to harvest plasma and stored at −20 °C until required for PUN analysis. Also, sera from non-heparinized blood samples were harvested and stored at −20 °C until required for interleukin 6 (IL-6) and tumor necrosis factor-α (TNF-α) concentration analysis using ELISA (Quantikine ELISA, R&D Systems Inc., Minneapolis, Minnesota, USA) according to the manufacturer's instructions. Fecal samples (about 2 g) were collected from each piglet on d 8 pre-challenge and d 7 post-challenge and then stored at −80 °C until required for further analyses. Culture-based *E. coli* enumeration analysis was performed using 1 g of fecal sample from each piglet in 9 mL sterile 0.1% peptone water, vortexed for 60 s and a 10-fold dilution made in sterile peptone water. The ETEC in the serially diluted samples were quantified using Eosin Methyl Blue agar (Becton, Dickson and Company, New Jersey, USA) with ciprofloxacin (0.5 μg/mL). The plates were incubated aerobically at 37 °C for 24 h and then colonies were counted.

### 2.4. Digesta and intestinal tissue collections

Ileal tissue and digesta were collected from each piglet on d 7 post-ETEC challenge after being anesthetized by an intramuscular injection of ketamine and xylazine (20 and 2 mg/kg, respectively;

Bimeda-MTC Animal Health Inc., Cambridge, Ontario, Canada) and euthanized by an intracardiac injection of sodium pentobarbital (50 mg/kg of BW; Bimeda-MTC Animal Health Inc., Cambridge, Ontario, Canada). The digesta samples were collected from ileum 15 cm cranial to the ileocecal junction in sterile sample bags, preserved in ice pack and transferred to the laboratory for ETEC enumeration as previously described for fecal samples. Ileal tissue samples were stored in 10% formalin to fix the villus and crypt for subsequent histomorphometric measurement.

### 2.5. Intestinal histomorphology

Cross-sections from formalin-fixed samples were processed for histological examination using the standard Hematoxylin and Eosin method. Measurement of villus height (VH) and crypt depth (CD) was made on at least 10 well-oriented villi per specimen using a Zeiss photomicroscope equipped with a Sony 3 chip CCD color camera (Carl Zeiss, Oberkochen, Germany). Captured images were analyzed using NIH ImageJ software (NIH Image, Bethesda, Maryland, USA) with VH being measured from the tip to the villus-crypt junction and the CD from this junction to the base. The VH and CD for each piglet were obtained by averaging measurements of at least 10 well-oriented villi.

### 2.6. Statistical analysis

Data were analyzed using the Mixed Procedure of SAS version 9.4 (SAS Institute Inc., Cary, NC, USA). Cage was the random effect, and diets were the fixed effects. Bacterial enumeration data were transformed to $\log_{10}$ cfu/mL before statistical analysis. Orthogonal polynomial contrasts were used to determine linear and quadratic effects of increasing inclusion levels SDWE. Differences were considered significant when $P \leq 0.05$, and trends were noted when $0.05 < P < 0.10$.

## 3. Results

### 3.1. Growth performance

During the pre-challenge period, the ADG showed a linear improvement ($P = 0.003$) with increasing inclusion of SDWE (Table 2). Also, piglets in the SDWE groups grew faster with the G:F being higher (linear, $P = 0.004$) than that of the CEP group. However, there were no significant differences in ADFI among treatments. During the post-challenge period, growth performance was similar among dietary treatments.

**Table 2**
Effects of spray-dried whole egg powder containing anti-F4 antibodies (SDWE) on growth performance.

| Item[1] | Treatments[2] | | | SEM | *P*-value | |
|---|---|---|---|---|---|---|
| | CEP | SDWE1 | SDWE2 | | Linear | Quadratic |
| Initial BW, g | 7,300 | 7,250 | 7,230 | 163.7 | | |
| Final BW, g | 8,700 | 8,820 | 9,180 | 259.0 | | |
| ADG, g | | | | | | |
| d 0 to 7 | 47.50 | 76.67 | 110.7 | 13.89 | 0.003 | 0.882 |
| d 8 to 14 | 203.3 | 199.6 | 214.1 | 25.90 | 0.772 | 0.777 |
| ADFI, g | | | | | | |
| d 0 to 7 | 137.2 | 143.8 | 164.6 | 10.69 | 0.037 | 0.504 |
| d 8 to 14 | 261.1 | 255.6 | 285.3 | 21.72 | 0.438 | 0.515 |
| G:F, g/g | | | | | | |
| d 0 to 7 | 0.34 | 0.49 | 0.67 | 0.08 | 0.004 | 0.911 |
| d 8 to 14 | 0.78 | 0.75 | 0.72 | 0.05 | 0.457 | 0.986 |

CEP = control egg powder; SEM = standard error of the mean.
[1] d 0 to 7 = pre-challenge period; d 8 to 14 = post-challenge period; method of challenge = each piglet was orally challenged with 6 mL ($2 \times 10^9$ cfu/mL) of freshly grown *Escherichia coli*-K88 (ETEC) inoculum at the back of oral cavity by using a polyethylene tube attached to a syringe as described previously (Kiarie et al., 2009).
[2] CEP = basal diet plus 4 kg control egg powder/t feed (0); SDWE1 = basal diet plus 1 kg SDWE and 3 kg control egg powder/t feed (0.1%); SDWE2 = basal diet plus 4 kg SDWE/t feed (0.4%).

### 3.2. Plasma urea nitrogen and serum cytokines

The serum concentrations of IL-6 and TNF-α did not differ among treatments at any time point (Table 3). Also, the PUN content was not affected by dietary treatments during the pre-challenge period ($P > 0.05$) and at any of the sampling points during the post-challenge period except on d 7 where the PUN tended to differ quadratically ($P = 0.06$) with increasing inclusion of SDWE.

### 3.3. Diarrhea

As shown in Table 4, fecal consistency score did not vary among treatments for d 1, 2 and 3, post-challenge. However, fecal consistency score showed a tendency for reduction (quadratic, $P = 0.070$) during d 4 with increasing inclusion of SDWE.

### 3.4. ETEC enumeration and ileal histomorphometry

Culture-based ETEC isolation from fecal samples post-challenge tended to reduce (linear, $P = 0.100$) with increasing inclusion of

**Table 3**
Effects of spray-dried whole egg powder containing anti-F4 antibodies (SDWE) on plasma urea nitrogen (PUN) content and serum concentrations of interleukin-6 (IL-6) and tumor necrosis factor-alpha (TNF-α) in enterotoxigenic *Escherichia coli*-K88 (ETEC) challenged piglets.

| Item | Treatments[1] | | | SEM | *P*-value | |
|---|---|---|---|---|---|---|
| | CEP | SDWE1 | SDWE2 | | Linear | Quadratic |
| IL-6, pg/μL | | | | | | |
| d 0 | 147.82 | 90.85 | 94.69 | 28.72 | 0.203 | 0.396 |
| d 1 | 163.57 | 134.40 | 106.63 | 38.49 | 0.306 | 0.988 |
| d 2 | 134.15 | 172.79 | 135.06 | 41.90 | 0.988 | 0.464 |
| TNF-α, pg/μL | | | | | | |
| d 0 | 285.68 | 229.71 | 233.27 | 57.56 | 0.526 | 0.677 |
| d 1 | 168.64 | 163.41 | 232.57 | 42.86 | 0.302 | 0.486 |
| d 2 | 114.38 | 204.03 | 168.66 | 59.59 | 0.526 | 0.400 |
| PUN, mmol/L | | | | | | |
| d 0 | 5.06 | 5.24 | 4.51 | 0.43 | 0.382 | 0.392 |
| d 1 | 3.48 | 3.38 | 3.94 | 0.55 | 0.554 | 0.626 |
| d 2 | 4.22 | 3.83 | 4.98 | 0.59 | 0.373 | 0.298 |
| d 7 | 3.86 | 2.84 | 4.26 | 0.50 | 0.578 | 0.061 |

CEP = control egg powder; SEM = Standard error of the mean.
[1] CEP = basal diet plus 4 kg control egg powder/t feed (0); SDWE1 = basal diet plus 1 kg SDWE and 3 kg control egg powder/t feed (0.1%); SDWE2 = basal diet plus 4 kg SDWE/t feed (0.4%).

**Table 4**
Incidences of diarrhea in piglets fed sprayed dried whole egg powder containing anti-F4 antibodies (SDWE) in terms of fecal consistency score.[1]

| Post-challenge | Treatments[2] | | | SEM | *P*-value | |
|---|---|---|---|---|---|---|
| | CEP | SDWE1 | SDWE2 | | Linear | Quadratic |
| d 1 | 1.3 | 1.1 | 1.7 | 0.24 | 0.344 | 0.206 |
| d 2 | 0.7 | 0.3 | 0.8 | 0.27 | 0.772 | 0.248 |
| d 3 | 0.8 | 0.3 | 0.7 | 0.25 | 0.757 | 0.217 |
| d 4 | 0.7 | 0 | 0.3 | 0.22 | 0.284 | 0.070 |

CEP = control egg powder; SEM = Standard error of the mean.
[1] Fecal consistency score (average values for the treatment groups): 0, normal; 1, soft feces; 2, mild diarrhea; 3, severe diarrhea. ≤ 1 fecal consistency score means no diarrhea.
[2] CEP = basal diet plus 4 kg control egg powder/t feed (0); SDWE1 = basal diet plus 1 kg SDWE and 3 kg control egg powder/t feed (0.1%); SDWE2 = basal diet plus 4 kg SDWE/t feed (0.4%).

SDWE (Table 5). Moreover, the recovery of ETEC from ileal digesta increased quadratically ($P = 0.021$) with increasing SDWE inclusion in diet. Villus height, CD and ratio of VH to CD were not affected by dietary treatment (Table 5).

## 4. Discussion

The weaning transition is one of the most stressful events a piglet encounter in swine production (Xu et al., 2014) with consequent reductions in growth performance (Boudry et al., 2002, 2004; Heo et al., 2013), compromised intestinal health (Moeser et al., 2007), diarrhea (Heo et al., 2013) and high mortality rate (Madec et al., 2000) resulting in significant economic losses. Enterotoxigenic *E. coli* expressing F4 fimbrial antigens is an important etiology of PWD in pigs (Fairbrother et al., 2005). Therefore, we hypothesized that supplementing pig starter diet with SDWE against recombinantly produced F4 antigens would improve growth performance and reduce ETEC-induced diarrhea in early-weaned piglets.

The growth performance observed in the current study was similar to that reported for 21-d-old weaned piglets fed diets containing egg yolk antibodies (Marquardt et al., 1999; Owusu-Asiedu et al., 2002). Although there were no significant differences in the ADFI among treatment groups during the post-challenge period, the SDWE supplementation showed a linear improvement in ADFI during the pre-challenge period. This could, therefore, suggests that egg antibodies have appetite-enhancing effect that may be attributable to the presence of the specific antibodies and presumably, enabled antibody-fed piglets to consume more feed hence, better growth rate (Cook, 2004) as evidenced in the pre-ETEC challenge period. In pigs, several studies (e.g., Yokoyama et al., 1992; Imberechts et al., 1997; Zuniga et al., 1997; Marquardt et al., 1999; Liou et al., 2011) have demonstrated positive effect of egg anti-*E. coli* antibodies on the reduction of diarrhea and mortality whereas a few studies (Marquardt et al., 1999; Owusu-Asiedu et al., 2002; Heo et al., 2015) reported effects on growth performance response criteria with inconsistent results.

In the current study, although the growth-promoting effect of SDWE was evident during the pre-challenge period, such benefits were not observed after *E. coli* challenge contrary to observations reported by previous studies (Marquardt et al., 1999; Owusu-Asiedu et al., 2002) but partly in agreement to recent observations made by Heo et al. (2015). Heo et al. (2015) reported that egg antibodies did not significantly affect growth of 21-d-old piglets in the first phase (14-d period and unchallenged) of the investigation but increased the ADFI and tended to increase the ADG in the second phase (11-d period and unchallenged) when piglets were fed a common commercial diet. This may suggest a carry-over effect of supplementing piglet diets with egg antibodies during phase 1. Dosage, mode of administration (in-feed versus liquid), breed, age and study conditions (e.g., challenge vs. unchallenged) may partly explain differences between our results and previous observations. Hence, this warrants more investigations into the mechanisms underlying the growth performance-improving effects or otherwise of chicken egg antibodies.

The IL-6 is a potential marker for ongoing bacterial infections in pigs (Fossum et al., 1998) because of its upregulation particularly during ETEC infection (Zhang et al., 2010). In our study, there were neither linear nor quadratic effects of SDWE on serum IL-6 concentrations. This may partly be suggestive of the challenge model being insufficient enough to produce clinical PWD. The TNF-α has been shown to upregulate the expression of the immunoglobulin secretory component responsible for the transcytosis of newly synthesized IgA, which plays important roles in regulating eosinophil functions and enhancing local immune responses (Liu et al., 2007). These immune beneficial effects could not be suggested in our findings as the serum levels of TNF-α did not differ significantly among the dietary treatments.

The PUN content was not affected by dietary treatments at any time point during the study except on d 7 where the PUN tended to have quadratic effect. Although increased PUN level has been suggested to be an indication of body protein breakdown (catabolism) to generate energy and an inefficient utilization of dietary protein for protein synthesis as a result of deleterious effects of pathogenic microorganisms (Coma et al., 1995), our findings showed that SDWE had no significant effects on PUN.

Based on the results from the current study, the diarrhea-reducing effects of SDWE could not be substantiated. As shown in Table 4, supplementing piglet diets with SDWE did not have any effects on diarrhea incidences and severity, which may be partly attributable to the challenge model not being sufficient to induce clinical PWD. However, the diarrhea-reducing effects of chicken egg anti-ETEC antibodies are inconsistent and have been demonstrated and reported to be dose-dependent by others (Yokoyama et al., 1992; Erhard et al., 1996; Imberechts et al., 1997; Zuniga et al., 1997; Marquardt et al., 1999). Zuniga et al. (1997) reported protective effects of egg yolk antibodies against *E. coli*-induced diarrhea when each piglet was fed 5.5 g egg yolk powder per day but no effect with 3.5 g per pig per day whereas Marquardt et al. (1999) demonstrated positive effect with 1.5 g egg yolk powder antibodies per pig daily for 2 days post experimental infection. Also, Imberechts et al. (1997) used 30 g of egg yolk powder per pig daily to completely prevent experimentally induced PWD. In contrast, Chernysheva et al. (2004) reported no significant diarrhea-reducing effects when each piglet consumed daily approximately 13 g egg yolk powder containing SDWE. Chernysheva et al. (2004) therefore concluded that even at high inclusion rates, egg yolk antibodies may not be efficacious in 3 to 4 week-old-pigs. Gastric pH and digestive enzymes are among the reasons suggested by Chernysheva et al. (2004) to be likely responsible for no significant

**Table 5**
Viable counts of enterotoxigenic *Escherichia coli*-K88 (ETEC) in fecal and ileal digesta samples and ileal histomorphology of piglets fed control egg powder and sprayed dried whole egg powder containing anti-F4 antibodies (SDWE).

| Item | Treatments[1] | | | SEM | *P*-value | |
|---|---|---|---|---|---|---|
| | CEP | SDWE1 | SDWE2 | | Linear | Quadratic |
| d 8 pre-challenge, feces, lg cfu/mL | 6.8 | 6.1 | 5.8 | 0.68 | 0.348 | 0.216 |
| d 7 post-challenge, feces, lg cfu/mL | 7.5 | 7.3 | 5.9 | 0.73 | 0.100 | 0.497 |
| d 7 post-challenge, ileal digesta, lg cfu/mL | 7.8 | 6.3 | 8.2 | 0.51 | 0.662 | 0.021 |
| VH, μm | 325.1 | 317.0 | 309.8 | 16.80 | 0.526 | 0.983 |
| CD, μm | 263.4 | 264.6 | 276.0 | 14.43 | 0.541 | 0.773 |
| VH:CD | 1.25 | 1.21 | 1.16 | 0.09 | 0.450 | 0.944 |

VH = villus height; CD = crypt depth; VH:CD = villus height to crypt depth ratio; SEM = standard error of the mean.
[1] CEP = basal diet plus 4 kg control egg powder (CEP)/t feed (0); SDWE1 = basal diet plus 1 kg SDWE and 3 kg control egg powder/t feed (0.1%); SDWE2 = basal diet plus 4 kg SDWE/t feed (0.4%).

effects observed, since they break down the antibodies in the GIT thereby reducing the amount of egg antibodies available to effectively protect the pigs against *E. coli*.

In the present study, each piglet consumed less than 450 g (during post-challenge) of feed per day, meaning less than 1.8 g SDWE daily for the high-dose (0.4%, SDWE2) category and challenged with $2 \times 10^9$ cfu/mL ETEC inoculum which was less than the concentration used in above cited studies ($10^{10}$ to $10^{12}$ cfu/mL ETEC inoculum). The *E. coli* oral challenge model used in the present study was insufficiently sensitive to produce responses in challenged piglets similar to clinical cases of post-weaning colibacillosis. This may partly explain why no significant effects were seen in both growth and diarrhea incidences due to SDWE antibodies intake after ETEC inoculation. Also, for the assessment and evaluation of protective effects of orally administered egg antibodies, a certain nutritive effect of egg powder may be considered (Gurtler et al., 2004). O'Farrelly et al. (1992) demonstrated protective effect in rabbits against artificial infection by *E. coli* after administering egg yolk powder obtained from non-immunized laying hens. Therefore, the non-significant effects recorded in our study may be due to certain nutritive effects of egg powder from non-immunized laying hens (control egg powder), although small amounts were consumed. Further studies are required to substantiate any protective effects that egg powder from non-immunized or egg white from immunized laying hens might have against the diarrheal disease caused by ETEC.

During the pre-challenge period, there was no difference among treatment groups in the *E. coli* count in feces. The fecal shedding of ETEC on d 7 post-challenge showed a tendency for linear reduction, whereas ETEC recovery from ileal digesta increased quadratically with SDWE inclusion. Although severe diarrhea and mortalities were not observed in our study, lower recovered viable counts of ETEC in the feces and small intestines have been attributed to mild diarrhea and quick recovery observed in piglets fed egg antibodies (Yokoyama et al., 1992; Marquardt et al., 1999). These beneficial effects of egg antibodies could not be reasonably supported by our findings. Results of ileal histomorphology were similar among the 3 dietary treatments. Comparing data on intestinal morphology from different experiments is difficult because of differences in the diets, breed, age, experimental conditions as well as no known standards for the measurements of VH and CD (Heo et al., 2013). Nevertheless, within experiments of similar conditions, data may be compared, and some deductions made as previous studies (Hornich et al., 1973; Cera et al., 1988; Pluske et al., 1997; McCracken et al., 1999) have associated reduced VH and increased CD to reduced feed intake, post-weaning growth lag and diarrhea in early weaned pigs. But in the present study, the results of ileal morphology were similar among the 3 treatments correlating well with observed growth performance.

## 5. Conclusion

Under the conditions of the present study, it can be concluded that supplementation of piglet diets with egg antibodies produced against recombinantly derived F4 antigen did not support greater performance, reduce diarrhea incidences and severity during post-ETEC challenge period in 21-d-old piglets. Hence, further studies are needed to substantiate the beneficial effects of SDWE.

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