Animal Feed Science and Technology 263 (2020) 114447

Contents lists available at ScienceDirect

Animal Feed Science and Technology

journal homepage: www.elsevier.com/locate/anifeedsci

# Effects of copper hydroxychloride and dietary fiber on intestinal permeability, growth performance, and blood characteristics of nursery pigs

C.D. Espinosa[a], R.S. Fry[b], M.E. Kocher[b], H.H. Stein[a,*]

[a] *Department of Animal Sciences, University of Illinois, Urbana, 61801, United States*
[b] *Micronutrients, USA LLC, Indianapolis, IN 46241, United States*

## ARTICLE INFO

*Keywords:*
Copper
Copper hydroxychloride
Dietary fiber
Growth performance
Intestinal permeability
Pigs

## ABSTRACT

Two experiments were conducted to test the hypothesis that copper (Cu) hydroxychloride improves growth performance and blood characteristics, and reduces intestinal permeability of nursery pigs fed diets without or with inclusion of cereal co-products. In experiment 1, 32 pigs (13.53 ± 1.27 kg) were allotted to a 2 × 2 factorial arrangement with 2 types of diets (low-fiber or high-fiber) and 2 levels of Cu from Cu hydroxychloride (0 or 150 mg/kg). Pigs were adapted to diets for 5 days, followed by the oral administration of lactulose and mannitol on day 6. After administration, urine was collected during two 6 -h periods. Results indicated that pigs fed high-fiber diets tended to have greater ($P < 0.10$) urinary lactulose:mannitol ratio during the first 6 -h period, whereas dietary Cu concentrations did not affect lactulose:mannitol ratios of pigs. In experiment 2, 128 pigs (8.33 ± 1.32 kg) were allotted to the same dietary treatments as in experiment 1 and used in a 21-day growth assay. There were 4 pigs per pen and 8 replicate pens per diet. On day 7, day 14, and on day 21, blood samples were collected from 1 pig per pen and tumor necrosis factor-α, immunoglobulin G, interleukin-1 ß, interleukin-10 (IL-10), blood urea nitrogen (BUN), total protein, and albumin were analyzed. Results indicated that there were no interactions between diet type and Cu hydroxychloride for overall growth performance and blood characteristics of pigs. Overall average daily gain and gain:feed were greater ($P < 0.05$) for pigs fed diets containing Cu hydroxychloride compared with pigs fed diets without Cu hydroxychloride. The level of dietary fiber did not affect overall growth performance of pigs. However, BUN concentration tended to increase ($P < 0.10$), and a reduction ($P < 0.05$) in albumin and IL-10 concentrations on day 14 was observed for pigs fed high-fiber diets compared with pigs fed low-fiber diets. Supplementation of Cu hydroxychloride to diets positively influenced ($P < 0.05$) BUN, albumin, and cytokine concentrations of nursery pigs. In conclusion, supplementation of Cu hydroxychloride to low-fiber or high-fiber diets improved growth performance and some blood characteristics in nursery pigs.

---

*Abbreviations:* ADFI, average daily feed intake; ADG, average daily gain; BUN, blood urea nitrogen; Cu, copper; DDGS, distillers dried grains with solubles; G:F, gain to feed ratio; IgG, immunoglobulin G; IL-1ß, interleukin-1 ß; IL-10, interleukin-10; TNF-α, tumor necrosis factor-α

[*] Corresponding author.

*E-mail address:* hstein@illinois.edu (H.H. Stein).

https://doi.org/10.1016/j.anifeedsci.2020.114447
Received 28 October 2019; Received in revised form 2 February 2020; Accepted 10 February 2020

## 1. Introduction

Post weaning diarrhea is one of the contributing causes of reduction in growth performance and mortality in weanling pigs (Pluske et al., 1997). Weanling pigs are susceptible to infections, diseases, and villous atrophy in the gut, which indicates that the intestinal barrier function is disturbed after weaning (Wijtten et al., 2011). Intestinal permeability increases if pigs have diarrhea, and this may allow entry of toxins and pathogenic microorganisms through the epithelial cells (Zhang and Guo, 2009). Exposure of pigs to pathogenic or nonpathogenic antigens results in activation of the immune system and subsequent release of cytokines such as tumor necrosis factor α (TNF-α), interleukin-1 ß (IL-1ß), and interleukin-6. Cytokines exert physiological and pathological effects on intestinal tight junction barrier proteins, which results in further increasing intestinal permeability (Al-Sadi et al., 2009).

Most diets for weanling pigs contain highly digestible plant and animal proteins, but there is an increasing trend to include more fibrous co-products in diets for pigs to reduce diet costs (Li et al., 2018). However, feeding diets to weanling pigs with high concentrations of dietary fiber may reduce nutrient digestibility, induce intestinal inflammation, and subsequently depress growth performance (Tsai et al., 2017).

Copper sulfate and copper chloride are the most commonly used forms of supplemental Cu in animal feeding due to its availability and relatively low cost compared with other inorganic sources of Cu (Ma et al., 2006, 2007). However, using pharmacological concentrations of $CuSO_4$ in swine diets have resulted in environmental concerns due to high excretion of Cu in feces (Zhao et al., 2014). Therefore, other forms of inorganic Cu which are generally included in diets at a lower inclusion rate and are less reactive with other nutrients have been introduced into the feed market. One of these is Cu hydroxychloride which has low water solubility, but is highly soluble under acidic conditions (Spears et al., 2004). Addition of 100–200 mg Cu/kg from Cu hydroxychloride to diets also improves feed efficiency and reduces post weaning diarrhea in pigs (Cromwell et al., 1998; Fry et al., 2012; Espinosa et al., 2017). It is, therefore, possible that inclusion of Cu hydroxychloride may result in an improved intestinal barrier integrity and immune response, and thus, improved growth performance of pigs. However, there are at this point no data to demonstrate the effect of Cu hydroxychloride on intestinal barrier integrity of pigs fed low-fiber or high-fiber diets and it is not known if Cu hydroxychloride influences immune responses of pigs. Therefore, the objective of this work was to test the hypothesis that inclusion of 150 mg Cu/kg from Cu hydroxychloride reduces intestinal permeability and subsequently improves growth performance of pigs fed diets without or with high concentration of dietary fiber.

## 2. Materials and methods

Protocols for 2 experiments were submitted to the Institutional Animal Care and Use Committee at the University of Illinois and protocols were approved prior to initiation of the experiments. Pigs that were the offspring of Line 359 boars mated to Camborough females (Pig Improvement Company, Hendersonville, TN, USA) were used in both experiments.

### *2.1. Experimental design and sample collection*

#### *2.1.1. Experiment 1: Intestinal permeability*

Sixteen barrows and 16 gilts (13.53 ± 1.27 kg) that had been weaned for 28 days were used. Pigs were randomly allotted to a 2 × 2 factorial arrangement with 2 types of diets (low-fiber or high-fiber) and 2 levels of Cu from Cu hydroxychloride (0 or 150 mg/kg). For the low-fiber diets, two diets based on corn and soybean meal were formulated to meet the nutrient requirements for 11–25 kg pigs (Table 1; NRC, 2012). The only difference between the 2 diets was that one diet contained no Cu hydroxychloride, whereas the other diet contained 150 mg Cu/kg from Cu hydroxychloride (IntelliBond $C^{II}$; Micronutrients, Indianapolis, IN, USA). Two additional diets were formulated based on corn, soybean meal, wheat middlings, and distillers dried grains with solubles (DDGS) without or with Cu hydroxychloride. No antibiotic growth promoters or vaccines were administered to the pigs during the experimental period, and zinc was not included at pharmacological levels. Vitamins and minerals were included in all diets to meet or exceed current requirement estimates (NRC, 2012). A sample of each diet was collected at the time of diet mixing.

Pigs were placed in individual metabolism crates that were equipped with a self-feeder, a nipple waterer, a slatted floor, a screen floor, and a urine pan to allow for the total collection of urine without feces. Pigs were limit fed at 3 times the energy requirement for maintenance (i.e., 0.824 MJ/kg × body weight$^{0.60}$; (NRC, 2012), which was provided each day in 2 equal meals at 0800 and 1600 h. Throughout the study, pigs had ad libitum access to water. Pigs adapted to the diets for 5 days. On day 6, following an overnight fast, individual weights were recorded and intestinal permeability was assessed through the oral administration of lactulose (Sigma-Aldrich, St. Louis, MO, USA) and mannitol (Sigma-Aldrich, St. Louis, MO, USA) using a syringe with an extension tube to reach the back of the mouth. Prior to administration, the solution of lactulose (500 mg/kg body weight) and mannitol (50 mg/kg body weight) were mixed with 10 ml distilled water. After administration, urine was collected in urine buckets during two 6 -h periods. A preservative of 50 ml of 6*N* hydrochloric acid was added to the buckets, and the collected urine was stored at -20 °C immediately after collection.

#### *2.1.2. Experiment 2: Growth performance and blood characteristics*

A total of 128 pigs (8.33 ± 1.32 kg) that had been weaned for 2 weeks were randomly allotted to a 2 × 2 factorial arrangement using the same 4 diets as those used in experiment 1. Diets for experiments 1 and 2 were mixed in one batch. There were 4 pigs per pen (2 gilts and 2 barrows) with 8 replicate pens per treatment. No antibiotic growth promoters or vaccines were administered to the pigs during the experimental period.

**Table 1**

Ingredient composition of experimental diets (g/kg) used in experiments 1 and 2.

| | No added Cu | | 150 mg/kg Cu[1] | |
|---|---|---|---|---|
| Item | Low-fiber | High-fiber | Low-fiber | High-fiber |
| Ingredient | | | | |
| Ground corn | 529.9 | 387.1 | 529.62 | 386.82 |
| Soybean meal | 320.0 | 250.0 | 320.0 | 250.0 |
| Distillers dried grains with solubles | – | 200.0 | – | 200.0 |
| Wheat middlings | – | 100.0 | – | 100.0 |
| Dried whey | 100.0 | – | 100.0 | – |
| Soybean oil | 15.0 | 25.0 | 15.0 | 25.0 |
| Limestone | 13.0 | 15.8 | 13.0 | 15.8 |
| Dicalcium phosphate | 1.8 | – | 1.8 | – |
| Copper hydroxychloride, 54 % Cu | – | – | 0.28 | 0.28 |
| L-Lysine HCL | 2.6 | 4.5 | 2.6 | 4.5 |
| DL-Methionine | 0.7 | 0.4 | 0.7 | 0.4 |
| L-Threonine | 0.5 | 0.7 | 0.5 | 0.7 |
| Salt | 5.0 | 5.0 | 5.0 | 5.0 |
| Phytase premix[2] | 10.0 | 10.0 | 10.0 | 10.0 |
| Vitamin-mineral premix[3] | 1.5 | 1.5 | 1.5 | 1.5 |
| Calculated metabolizable energy, MJ/kg | 14.0 | 14.1 | 14.0 | 14.1 |

[1] Diets containing added Cu were fortified with 150 mg/kg of Cu from Cu hydroxychloride (IntelliBond C$^{II}$; Micronutrients, Indianapolis, IN, USA).

[2] The phytase premix contained phytase 5000 phytase units/g of Quantum Blue 5 G; AB Vista, Marlborough, United Kingdom) mixed with corn. The mixture was formulated to provide 500 units of phytase per kilogram of complete diet if included by 10 g/kg.

[3] Provided the following quantities of vitamins and micro-minerals per kilogram of complete diet: Vitamin A as retinyl acetate, 11,136 IU; vitamin D3 as cholecalciferol, 2,208 IU; vitamin E as DL-alpha tocopheryl acetate, 66 IU; vitamin K as menadione dimethylprimidinol bisulfite, 1.42 mg; thiamin as thiamine mononitrate, 0.24 mg; riboflavin, 6.59 mg; pyridoxine as pyridoxine hydrochloride, 0.24 mg; vitamin B12, 0.03 mg; D-pantothenic acid as D-calcium pantothenate, 23.5 mg; niacin, 44.1 mg; folic acid, 1.59 mg; biotin, 0.44 mg; Cu, 20 mg as copper chloride; Fe, 126 mg as ferrous sulfate; I, 1.26 mg as ethylenediamine dihydriodide; Mn, 60.2 mg as manganese hydroxychloride; Se, 0.3 mg as sodium selenite and selenium yeast; and Zn, 125.1 mg as zinc hydroxychloride.

Individual pig weights were recorded at the beginning of the experiment, on day 7, on day 14, and at the conclusion of the experiment on day 21. Feed addition was recorded daily and the weight of feed left in the feeders was recorded on day 7, day 14, and day 21. Data were summarized to calculate average daily feed intake (ADFI), average daily gain (ADG), and average gain:feed ratio (G:F) within each pen and treatment group. Data for growth performance were summarized for day 1–7, day 8–14, day 15–21, and for the entire experiment from day 1–21. On day 7, day 14, and on day 21, 2 blood samples were collected from 1 pig per pen via vena puncture. The same pigs were bled at each bleeding. These samples were collected in vacutainers that contained either heparin or ethylenediaminetetraacetic acid.

## *2.2. Chemical analyses*

### *2.2.1. Experiment 1: Intestinal permeability*

Diets were analyzed for dry matter (Method 930.15; (AOAC Int., 2007) and ash (Method 942.05; (AOAC Int., 2007). Crude protein was analyzed using the combustion procedure (Method 990.03; (AOAC Int., 2007) on a Leco FP628 protein analyzer (Leco Corporation, St. Joseph, MI, USA), and gross energy was determined using an isoperibol bomb calorimeter (Model 6400, Parr Instruments, Moline, IL, USA). Acid hydrolyzed ether extract was analyzed by acid hydrolysis using 3*N* hydrochloric acid (Ankom HCl Hydrolysis System, Ankom Technology, Macedon, NY, USA) followed by fat extraction (Ankom XT-15 Extractor, Ankom Technology, Macedon, NY, USA), and amino acids were analyzed (Method 982.30 E (a, b, c); (AOAC Int., 2007) on a Hitachi Amino Acid Analyzer (Model No. L8800; Hitachi High Technologies America, Inc., Pleasanton, CA, USA) using ninhydrin for postcolumn derivatization and norleucine as the internal standard. Minerals were analyzed by inductively coupled plasma optical emissions spectrometry using an internally validated method [(Method 985.01 A, B, and C; (AOAC Int., 2007)] after wet ash sample preparation [Method 975.03 B(b); (AOAC Int., 2007)]. Urinary lactulose and mannitol concentrations were determined by high-performance liquid chromatography using ion-exchange chromatography with pulsed amperometric detection (Generoso et al., 2003). The lactulose:mannitol ratio was calculated based on the percent recovery of lactulose and mannitol, and this was considered an index of intestinal permeability (Wijtten et al., 2011).

### *2.2.2. Experiment 2: Growth performance and blood characteristics*

Heparinized samples were frozen at -20 °C and were analyzed for blood urea nitrogen (BUN), total protein, and albumin using a Beckman Coulter Clinical Chemistry AU analyzer (Beckman Coulter, Inc., Brea, CA, USA). Heparinized samples were also analyzed for IL-1ß (R&D Systems, Inc., Minneapolis, MN, USA). Tumor necrosis factor-α and interleukin 10 (IL-10) were measured in plasma samples collected in the vacutainer with EDTA using ELISA kits according to the recommendations from the manufacturer (R&D

**Table 2**

Analyzed composition of experimental diets (as-fed basis) used in experiments 1 and 2.

| | No added Cu | | 150 mg/kg Cu[1] | |
|---|---|---|---|---|
| Item | Low-fiber | High-fiber | Low-fiber | High-fiber |
| Dry matter, g/kg | 883.9 | 882.2 | 883.5 | 878.9 |
| Ash, g/kg | 56.9 | 56.3 | 56.2 | 55.3 |
| Gross energy, MJ/kg | 16.6 | 17.5 | 16.5 | 17.3 |
| Crude protein, g/kg | 207.1 | 229.4 | 219.4 | 225.4 |
| Acid hydrolyzed ether extract, g/kg | 24.3 | 36.0 | 22.5 | 36.8 |
| Insoluble dietary fiber, g/kg | 97.0 | 168.0 | 98.0 | 174.0 |
| Soluble dietary fiber, g/kg | 7.0 | 12.0 | 5.0 | 9.0 |
| Total dietary fiber, g/kg | 104.0 | 180.0 | 103.0 | 183.0 |
| Minerals | | | | |
| Calcium, g/kg | 9.1 | 7.7 | 8.1 | 7.8 |
| Phosphorus, g/kg | 4.6 | 5.7 | 4.7 | 5.8 |
| Sodium, g/kg | 3.1 | 2.6 | 2.7 | 2.8 |
| Magnesium, g/kg | 1.6 | 2.2 | 1.6 | 2.2 |
| Potassium, g/kg | 11.2 | 10.6 | 11.4 | 10.8 |
| Sulfur, g/kg | 2.1 | 2.3 | 2.1 | 2.3 |
| Manganese, mg/kg | 76.20 | 85.20 | 76.50 | 81.10 |
| Iron, mg/kg | 210.00 | 181.00 | 241.00 | 207.00 |
| Zinc, mg/kg | 141.00 | 164.00 | 154.00 | 163.00 |
| Copper, mg/kg | 30.90 | 26.30 | 189.00 | 200.00 |
| Indispensable amino acids, g/kg | | | | |
| Arginine | 13.1 | 13.2 | 12.9 | 13.1 |
| Histidine | 5.3 | 5.6 | 5.3 | 5.7 |
| Isoleucine | 9.7 | 9.6 | 9.8 | 9.6 |
| Leucine | 18.1 | 19.4 | 18.2 | 19.3 |
| Lysine | 14.0 | 14.2 | 13.8 | 14.8 |
| Methionine | 3.6 | 3.5 | 3.2 | 3.7 |
| Methionine + Cysteine | 6.8 | 7.3 | 6.5 | 7.4 |
| Phenylalanine | 10.6 | 11.0 | 10.5 | 11.0 |
| Threonine | 8.3 | 8.5 | 9.2 | 10.0 |
| Tryptophan | 2.5 | 2.3 | 2.4 | 2.5 |
| Valine | 10.2 | 10.6 | 10.3 | 10.6 |
| Dispensable amino acids, g/kg | | | | |
| Alanine | 10.2 | 11.5 | 10.3 | 11.6 |
| Aspartate | 21.2 | 19.9 | 21.0 | 19.9 |
| Cysteine | 3.2 | 3.8 | 3.3 | 3.7 |
| Glutamate | 37.2 | 37.2 | 37.5 | 37.6 |
| Glycine | 8.6 | 9.5 | 8.6 | 9.6 |
| Serine | 8.1 | 8.6 | 8.1 | 8.9 |
| Tyrosine | 7.3 | 7.5 | 7.0 | 7.4 |

[1] Diets containing added Cu were fortified with 150 mg/kg of Cu from Cu hydroxychloride (IntelliBond C$^{II}$; Micronutrients, Indianapolis, IN, USA).

Systems, Inc., Minneapolis, MN, USA). Samples collected in the EDTA-containing tubes were also analyzed for immunoglobulin G (IgG; Bethyl Laboratories, Inc., Montgomery, TX, USA).

### 2.3. Statistical analyses

Data from both experiments were analyzed following a 2 × 2 factorial arrangement using the MIXED procedure of SAS (SAS Institute Inc., Cary, NC, USA) with pig and pen as the experimental unit in experiments 1 and 2, respectively. Fixed effects included Cu, diet type, and the interaction between Cu and diet type and the random effect was replicate. Least squares means were calculated for each independent variable. Results were considered significant at $P \leq 0.05$ and considered a trend at $P \leq 0.10$.

## 3. Results

Diet analyses indicate that the intended concentrations of total dietary fiber and Cu were present in all diets and concentrations of other nutrients were not affected by dietary treatment. (Table 2).

### 3.1. Experiment 1: Intestinal permeability

There were no diet type × Cu interactions observed for urinary lactulose:mannitol ratios of pigs (Table 3). Inclusion of DDGS and wheat middlings in diets tended to increase ($P < 0.10$) the lactulose:mannitol ratio during the initial 6 -h period. However, no

**Table 3**
Urinary lactulose:mannitol ratios (percent recovery basis) of pigs fed low fiber or high fiber diets containing 0 or 150 mg/kg Cu from Cu hydroxychloride[1].

| | No added Cu | | 150 mg/kg Cu[2] | | | *P*-value | | |
|---|---|---|---|---|---|---|---|---|
| Item | Low-fiber | High-fiber | Low-fiber | High-fiber | SEM | Fiber | Cu | Fiber × Cu |
| 0 to 6 h | | | | | | | | |
| Lactulose:mannitol | 0.105 | 0.148 | 0.077 | 0.141 | 0.03 | 0.062 | 0.530 | 0.993 |
| 6 to 12 h | | | | | | | | |
| Lactulose:mannitol | 0.232 | 0.375 | 0.217 | 0.300 | 0.08 | 0.175 | 0.580 | 0.711 |

[1] Data are least squares means of 8 observations per treatment.

[2] Diets containing added Cu were fortified with 150 mg/kg of Cu from Cu hydroxychloride (IntelliBond C$^{II}$; Micronutrients, Indianapolis, IN, USA).

difference was observed in the lactulose:mannitol ratio between pigs fed low-fiber diets and pigs fed high-fiber diets in the second 6 -h period. The level of dietary Cu did not affect urinary lactulose:mannitol ratios.

### *3.2. Experiment 2: Growth performance and blood characteristics*

No interactions between diet type and Cu were observed for the overall growth performance of pigs (Table 4). Greater ($P < 0.05$) ADG and G:F were observed from day 1–7 in pigs fed diets containing 150 mg/kg Cu from Cu hydroxychloride compared with pigs fed diets without Cu hydroxychloride. Pigs fed the Cu hydroxychloride diets also had greater ($P < 0.05$) G:F from day 8–14 compared with pigs fed diets without added Cu. For the overall experimental period, pigs fed diets containing Cu hydroxychloride had greater ($P < 0.05$) ADG and G:F compared with pigs fed diets without Cu hydroxychloride. The level of dietary fiber did not affect growth performance of pigs.

No interactions between diet type and Cu were observed for the overall blood characteristics of pigs (Table 5). Inclusion of Cu hydroxychloride to diets reduced ($P < 0.05$) the concentrations of BUN and TNF-$\alpha$ on day 7 and day 21, respectively. On day 14, pigs fed diets with 150 mg Cu/kg from Cu hydroxychloride had greater ($P < 0.05$) concentration of IL-10, and tended to have greater ($P < 0.10$) concentration of albumin compared with pigs fed diets without Cu hydroxychloride. Likewise, pigs fed the Cu hydroxychloride diets had increased ($P < 0.05$) albumin concentration compared with pigs fed diets without added Cu on day 21. Inclusion of DDGS and wheat middlings to diets resulted in a tendency for a reduction ($P < 0.10$) in the concentration of albumin on day 7. On day 14, pigs fed high-fiber diets had a tendency to have greater ($P < 0.10$) BUN concentration, and pigs fed high-fiber diets had reduced ($P < 0.05$) concentrations of albumin and IL-10 compared with pigs fed the low-fiber diets. Pigs fed high-fiber diets also had

**Table 4**
Growth performance for pigs fed low fiber or high fiber diets containing 0 or 150 mg/kg Cu from Cu hydroxychloride[1].

| | No added Cu | | 150 mg/kg Cu[2] | | | *P*-value | | |
|---|---|---|---|---|---|---|---|---|
| Item | Low-fiber | High-fiber | Low-fiber | High-fiber | SEM | Fiber | Cu | Fiber × Cu |
| d 1 to 7 | | | | | | | | |
| Initial body weight, kg | 8.314 | 8.318 | 8.334 | 8.353 | 0.475 | 0.981 | 0.955 | 0.988 |
| ADG[3], kg | 0.324 | 0.308 | 0.417 | 0.377 | 0.024 | 0.252 | 0.002 | 0.616 |
| ADFI[3], kg | 0.593 | 0.543 | 0.626 | 0.594 | 0.041 | 0.329 | 0.316 | 0.829 |
| G:F[3] | 0.549 | 0.570 | 0.670 | 0.634 | 0.028 | 0.946 | 0.003 | 0.307 |
| Final body weight, kg | 10.583 | 10.476 | 11.250 | 10.990 | 0.592 | 0.759 | 0.327 | 0.898 |
| d 8 to 14 | | | | | | | | |
| ADG, kg | 0.520 | 0.476 | 0.558 | 0.515 | 0.031 | 0.196 | 0.185 | 0.950 |
| ADFI, kg | 0.875 | 0.790 | 0.830 | 0.724 | 0.064 | 0.145 | 0.392 | 0.872 |
| G:F | 0.589 | 0.605 | 0.675 | 0.711 | 0.051 | 0.443 | 0.032 | 0.887 |
| Final body weight, kg | 14.240 | 13.806 | 15.156 | 14.598 | 0.745 | 0.511 | 0.261 | 0.934 |
| d 15 to 21 | | | | | | | | |
| ADG, kg | 0.558 | 0.560 | 0.609 | 0.577 | 0.033 | 0.639 | 0.307 | 0.601 |
| ADFI, kg | 0.988 | 0.911 | 0.975 | 0.967 | 0.046 | 0.366 | 0.642 | 0.464 |
| G:F | 0.571 | 0.620 | 0.629 | 0.594 | 0.031 | 0.785 | 0.645 | 0.178 |
| Final body weight, kg | 18.148 | 17.726 | 19.421 | 18.635 | 0.878 | 0.497 | 0.224 | 0.837 |
| day 1 to 21 | | | | | | | | |
| ADG, kg | 0.468 | 0.448 | 0.528 | 0.490 | 0.022 | 0.196 | 0.028 | 0.677 |
| ADFI, kg | 0.825 | 0.748 | 0.810 | 0.762 | 0.045 | 0.178 | 0.993 | 0.760 |
| G:F | 0.570 | 0.601 | 0.656 | 0.646 | 0.022 | 0.511 | 0.010 | 0.267 |

[1] Data are least squares means of 8 observations (pen as the experimental unit; 4 pigs per pen) per treatment.

[2] Diets containing added Cu were fortified with 150 mg/kg of Cu from Cu hydroxychloride (IntelliBond C$^{II}$; Micronutrients USA; Indianapolis, IN, USA).

[3] ADG = average daily gain; ADFI = average daily feed intake; G:F = gain:feed.

**Table 5**

Blood characteristics for pigs fed with low fiber or high fiber diets containing 0 or 150 mg/kg Cu from Cu hydroxychloride[1].

| | No Cu | | 150 mg/kg Cu[2] | | | *P*-value | | |
|---|---|---|---|---|---|---|---|---|
| Item | Low-fiber | High-fiber | Low-fiber | High-fiber | SEM | Fiber | Cu | Fiber × Cu |
| day 7 | | | | | | | | |
| BUN[3], mg/dL | 12.13 | 13.71 | 10.71 | 11.38 | 0.85 | 0.199 | 0.037 | 0.591 |
| Total protein, g/dL | 4.58 | 4.56 | 4.63 | 4.44 | 0.12 | 0.396 | 0.749 | 0.457 |
| Albumin, g/dL | 2.55 | 2.39 | 2.65 | 2.43 | 0.10 | 0.073 | 0.514 | 0.766 |
| TNF-α[3], pg/mL | 167.21 | 152.37 | 151.72 | 156.85 | 11.04 | 0.663 | 0.622 | 0.374 |
| IgG[3], mg/mL | 18.71 | 19.24 | 19.29 | 20.00 | 1.35 | 0.649 | 0.622 | 0.947 |
| Interleukin-1 beta, pg/mL | 34.27 | 34.48 | 34.58 | 34.89 | 0.70 | 0.687 | 0.584 | 0.942 |
| Interleukin-10, pg/mL | 20.69 | 19.74 | 22.20 | 20.92 | 1.29 | 0.398 | 0.307 | 0.901 |
| day 14 | | | | | | | | |
| BUN, mg/dL | 10.50 | 12.75 | 10.43 | 11.13 | 0.82 | 0.083 | 0.310 | 0.352 |
| Total protein, g/dL | 4.74 | 4.68 | 5.00 | 4.68 | 0.13 | 0.138 | 0.310 | 0.310 |
| Albumin, g/dL | 2.76 | 2.50 | 2.99 | 2.71 | 0.12 | 0.035 | 0.081 | 0.959 |
| TNF-α, pg/mL | 116.5 | 131.04 | 117.17 | 101.59 | 12.21 | 0.963 | 0.205 | 0.185 |
| IgG, mg/mL | 20.03 | 21.09 | 21.46 | 20.13 | 0.72 | 0.856 | 0.749 | 0.110 |
| Interleukin-1 beta, mg/mL | 18.58 | 17.92 | 18.39 | 18.30 | 0.24 | 0.119 | 0.675 | 0.222 |
| Interleukin-10, mg/mL | 16.19 | 14.34 | 18.96 | 15.25 | 1.15 | 0.003 | 0.039 | 0.280 |
| day 21 | | | | | | | | |
| BUN, mg/dL | 13.37 | 13.12 | 13.00 | 12.00 | 0.92 | 0.504 | 0.423 | 0.688 |
| Total protein, g/dL | 5.23 | 5.04 | 5.18 | 5.20 | 0.14 | 0.558 | 0.685 | 0.445 |
| Albumin, g/dL | 3.10 | 2.70 | 3.15 | 3.11 | 0.11 | 0.061 | 0.048 | 0.117 |
| TNF-α, pg/mL | 113.22 | 121.61 | 92.75 | 85.90 | 13.27 | 0.954 | 0.044 | 0.570 |
| IgG, mg/mL | 15.61 | 18.51 | 16.21 | 15.84 | 1.47 | 0.245 | 0.342 | 0.136 |
| Interleukin-1 ß, mg/mL | 15.16 | 15.58 | 15.71 | 15.50 | 0.38 | 0.780 | 0.538 | 0.419 |
| Interleukin-10, mg/mL | 10.97 | 10.33 | 11.41 | 11.15 | 0.89 | 0.623 | 0.490 | 0.837 |

[1] Data are least squares means of 8 observations (pen as the experimental unit; 4 pigs per pen) per treatment.

[2] Diets containing added Cu were fortified with 150 mg/kg of Cu from Cu hydroxychloride (IntelliBond C$^{II}$; Micronutrients, Indianapolis, IN, USA).

[3] BUN = blood urea nitrogen; TNF-α = tumor necrosis factor-α; IgG = immunoglobulin G.

reduced ($P < 0.05$) albumin concentration compared with pigs fed low-fiber diets on d 21. However, on day 21, concentrations of total protein, IgG, and IL-1ß were not affected by dietary Cu concentrations or inclusion of fibrous co-products in the diets.

## 4. Discussion

Distillers dried grains with solubles and wheat middlings are cereal co-products that are commonly included in diets for growing-finishing pigs. These co-products are usually less expensive than corn and soybean meal due to the high concentration of dietary fiber, which is resistant to enzymatic hydrolysis in the small intestine, and therefore, cannot be digested by pigs (Anguita et al., 2006; Bindelle et al., 2008). Growing-finishing pigs may obtain energy from dietary fiber via synthesis of volatile fatty acids as a result of microbial fermentation in the hindgut (Dierick et al., 1989; Macfarlane and Macfarlane, 2007), but younger growing pigs are less efficient than older pigs in utilizing dietary fiber, and dietary fiber may induce intestinal stress in young pigs (Yang et al., 2016).

### *4.1. Intestinal permeability*

The oral administration of lactulose and mannitol as markers to assess intestinal permeability in pigs is considered to be reliable and more advantageous than Ussing chambers because multiple measurements can be obtained from the same animal over time (Wijtten et al., 2011). Lactulose is a disacharride that transverses the intestinal epithelium through the paracellular route if gut barrier function is compromised, whereas mannitol is a monosaccharide that is absorbed by either paracellular or transcellular routes (Wijtten et al., 2011). These sugars are not metabolized in the body and the majority of the absorbed sugars is, therefore, excreted in the urine. The ratio between lactulose and mannitol recovered in the urine serves as an index for intestinal barrier function (Bjarnason et al., 1995). The observed tendency for an increased lactulose:mannitol ratio during the initial 6 -h period after administration of sugars of pigs fed high-fiber diets indicates that the intestinal barrier function of these pigs was disturbed compared with pigs fed the low-fiber diets. Pigs experience a variety of stresses after weaning such as physiological, nutritional, environmental, and social challenges (Campbell et al., 2013). Inclusion of fibrous co-products in diets, which are less digestible and palatable than some low-fiber ingredients, may induce stress (Yang et al., 2016) and result in compromised intestinal health and subsequently increase intestinal permeability. The lack of differences in the lactulose:mannitol ratio of pigs fed diets containing 0 or 150 mg Cu/kg from Cu hydroxychloride indicates that high concentration of dietary Cu did not impact intestinal permeability of pigs. This is in contrast with data indicating that addition of 1500 mg/kg of Cu-exchanged montmorillonite to diets reduced intestinal permeability of pigs, with plasma diamine oxidase and D-lactate used as markers for intestinal barrier integrity (Song et al., 2013).

### 4.2. Growth performance and blood characteristics

The observation that inclusion of fibrous co-products to diets did not affect overall growth performance of nursery pigs indicates that under the conditions of this experiment, weanling pigs from approximately 8–18 kg tolerated diets containing 200 g/kg DDGS and 100 g/kg wheat middlings without apparent negative effects on growth performance. All diets were formulated to contain similar concentrations of metabolizable energy and standardized ileal digestible amino acids, which likely contributed to the lack of differences in growth performance (Gutierrez et al., 2013; Jaworski et al., 2014). However, these results are in contrast with some previous data indicating that inclusion of 300 g/kg DDGS to diets resulted in a reduction in nutrient digestibility and reduced ADG of nursery pigs (Tsai et al., 2017). In contrast, Jones et al. (2010) reported that inclusion of high concentration of DDGS to diets did not influence overall ADG, ADFI, or G:F of nursery pigs. Thus, pigs appear to react differently to inclusion of DDGS and wheat middlings in the diets, which possibly is an effect of differences in the nutritional quality of the sources of DDGS and wheat middlings that are used.

The observation that supplementation of Cu hydroxychloride to diets resulted in an improvement in ADG and G:F of pigs is in agreement with previous data (Cromwell et al., 1989, 1998; Espinosa et al., 2017). Inclusion of 125 mg Cu/kg from copper sulfate increased the mRNA abundance of growth hormone-releasing hormone and also suppressed the mRNA abundance of somatostatin in the hypothalamus of pigs (Zhou et al., 1994). Therefore, it is possible that the observed improvement in growth performance of pigs upon supplementation with Cu hydroxychloride to the diets is due to the effect of Cu on stimulating growth-promoting regulatory peptides such as growth hormone-releasing hormone (LaBella et al., 1973; Zhou et al., 1994). It is also possible that Cu stimulates post-translational modification of regulatory peptides, which may also contribute to improved growth performance (Eipper and Mains, 1988). Improved ADG and G:F in pigs fed diets containing Cu hydroxychloride may also be a result of the beneficial effect of Cu on intestinal health of pigs, increased villus height, or changed microbiota profile in the gastointestinal tract of pigs (Namkung et al., 2006; Zhao et al., 2007). Dietary Cu may also improve growth performance of pigs by upregulating mRNA abundance of proteins involved in the uptake and utilization of fatty acids. Addition of 45 mg/kg of Cu to diets for rabbits improved body mass gain by upregulating mRNA transcription of fatty acid binding proteins and fatty acid transport proteins (Lei et al., 2017), indicating an increase in cellular uptake of fatty acids (Chen et al., 2016).

Concentrations of BUN, total protein, and albumin that were observed in experiment 2 were within the normal physiological ranges (Tumbleson and Kalish, 1972) and were in agreement with previous data (Casas and Stein, 2016; Espinosa et al., 2017). The observed reduction in the concentration of albumin on days 7, 14, and 21 in pigs fed the high-fiber diets is possibly due to a reduced absorption of nutrients from the small intestine caused by an increased concentration of dietary fiber. Inclusion of fibrous co-products results in a reduction in the apparent ileal digestibility and apparent total tract digestibility of organic matter (Graham et al., 1986; Bach Knudsen and Hansen, 2007), and energy from fiber is absorbed following fermentation in the large intestine rather than absorption of glucose from the small intestine. Albumin constitutes approximately 60 % of total plasma protein and one of its main functions is to bind and transport nutrients including fatty acids, glucose, amino acids, and metal ions such as zinc and Cu (Quinlan et al., 2005; Francis, 2010). Therefore, if inclusion of fibrous co-products results in reduced glucose absorption, pigs fed high-fiber diets may have a reduced need for albumin to transport nutrients from the liver to extrahepatic tissues (Ramos et al., 2016).

Blood urea nitrogen is often used as an index for the efficiency of amino acid utilization and nitrogen excretion in pigs (Coma et al., 1995; Russek-Cohen et al., 2005). The observed tendency for an increase in the concentration of BUN on day 14 in pigs fed high-fiber diets indicates that high concentrations of dietary fiber reduces the efficiency of nitrogen utilization of pigs. Inclusion of high concentration of dietary fiber in diets often results in a reduction in digestibility of crude protein (Zhang et al., 2013), and this may result in a reduction in the efficiency of amino acid utilization with a subsequent increase in the concentration of BUN.

Interleukin-10 is an anti-inflammatory cytokine, which inhibits activation and effector function of T cells, monocytes, and macrophages (Moore et al., 2001). The reduction in the concentration of IL-10 on day 14 in pigs fed the high-fiber diets is in contrast with data indicating that inclusion of 75 g/kg DDGS in diets for weanling pigs increased mRNA abundance of IL-10 in ileal tissue of pigs (Weber et al., 2008). However, the observed reduction in the concentration of IL-10 is in agreement with the observed increase in the intestinal permeability of pigs. Anti-inflammatory agents, which include IL-10, contribute to protecting the intestinal barrier integrity from effects of interferon-ϒ and TNF-α in inducing barrier disruption (Al-Sadi et al., 2009). Effects of dietary fiber on cytokine concentrations of pigs may vary and may depend on the age of the animal, as well as fermentability and viscosity of the fiber, and inclusion level of cereal co-products in the diet (Ferrandis Vila et al., 2018).

The increase in the concentration of albumin that was observed on days 14 and 21 in pigs fed the Cu hydroxychloride diets may be due to an increased absorption of Cu. Most of the absorbed $Cu^{2+}$ in the hepatic portal vein needs to be bound to albumin (Linder, 1991) for transport to the liver, where it is taken up by hepatocytes as $Cu^{+}$ using Cu reductase. The increased albumin concentration in pigs fed Cu hydroxychloride may also result in an increased efficiency of transporting other nutrients to the liver and from the liver to peripheral tissues (Ramos et al., 2016). The reduction in the concentration of BUN that was observed on day 7 in pigs fed diets containing Cu hydroxychloride indicates that Cu hydroxychloride improves the efficiency of amino acid utilization by pigs (Whang and Easter, 2000), and this may also result in an increased availability of amino acids for protein synthesis and skeletal muscle growth.

The reduction in the concentration of TNF-α on day 21 that was observed when diets were supplemented with Cu hydroxychloride is in agreement with previous data (Song et al., 2013). Tumor necrosis factor-α is a cytokine produced by macrophages, lymphocyte cell lines, and monocytes in response to diseases and infections caused by parasites and pathogens (Pauli, 1995). Therefore, the observed reduction in the concentration of TNF-α upon Cu supplementation may be a result of the impact of dietary Cu on improving the intestinal health and immune response of pigs. Dietary Cu may reduce bacterial populations in the intestinal tract of pigs (Højberg et al., 2005), and this may result in a reduced inflammation caused by pathogens. The observed increase in IL-10 concentration of pigs fed diets containing Cu hydroxychloride may also contribute to an increased protection and an improved ability to combat diseases and infection, and thereby improve growth performance of pigs.

## 5. Conclusion

Inclusion of 200 g/kg DDGS and 100 g/kg wheat middlings in diets for nursery pigs did not affect overall growth performance. However, negative changes were observed in intestinal permeability and BUN concentration, as well as in concentrations of albumin and IL-10, in pigs fed diets containing DDGS and wheat middlings. Supplementation of Cu hydroxychloride to low-fiber or high-fiber diets improved growth performance of pigs and Cu hydroxychloride positively influenced BUN, albumin, and cytokine concentrations in pigs.

## Author statement

CDE, RSF and HHS conceptualized the experiment, CDE conducted the experiment, analyzed data, and wrote the first draft of the manuscript, MEK, RSF and HHS contributed with data interpretation, HHS supervised the project and edited the final version of the manuscript.

## Declaration of Competing Interest

The authors have no conflicts of interest.

## Acknowledgements

The authors appreciate the financial support for this research from Micronutrients, USA LLC, Indianapolis, USA, and Agrispecialist Inc., Laguna, Philippines.

## References

Al-Sadi, R., Boivin, M., Ma, T., 2009. Mechanism of cytokine modulation of epithelial tight junction barrier. Front. Biosci. 14, 2765–2778.

Anguita, M., Pérez, J.F., Jensen, B.B., Canibe, N., 2006. Influence of the amount of dietary fiber on the available energy from hindgut fermentation in growing pigs: Use of cannulated pigs and in vitro fermentation. J. Anim. Sci. 84, 2766–2778.

AOAC Int, 2007. Official Methods of Analysis of AOAC International. AOAC international, Gaithersburg, MD.

Bach Knudsen, K.E., Hansen, I., 2007. Gastrointestinal implications in pigs of wheat and oat fractions: 1. Digestibility and bulking properties of polysaccharides and other major constituents. Br. J. Nutr. 65, 217–232.

Bindelle, J., Leterme, P., Buldgen, A., 2008. Nutritional and environmental consequences of dietary fibre in pig nutrition: A review. Biotechnol. Agron. Soc. Environ. 12, 69–80.

Bjarnason, I., Macpherson, A., Hollander, D., 1995. Intestinal permeability: an overview. Gastroenterol. 108, 1566–1581.

Campbell, J.M., Crenshaw, J.D., Polo, J., 2013. The biological stress of early weaned piglets. J. Anim. Sci. Biotechnol. 4, 19–22.

Casas, G.A., Stein, H.H., 2016. Effects of full fat or defatted rice bran on growth performance and blood characteristics of weanling pigs. J. Anim. Sci. 94, 4179–4187.

Chen, F., Luo, Z., Chen, G.H., Shi, X., Liu, X., Song, Y.F., Pan, Y.X., 2016. Effects of waterborne copper exposure on intestinal copper transport and lipid metabolism of *Synechogobius hasta*. Aquat. Toxicol. 178, 171–181.

Coma, J., Carrion, D., Zimmerman, D.R., 1995. Use of plasma urea nitrogen as a rapid response criterion to determine the lysine requirement of pigs. J. Anim. Sci. 73, 472–481.

Cromwell, G.L., Stahly, T.S., Monegue, H.J., 1989. Effects of source and level of copper on performance and liver copper stores in weanling pigs. J. Anim. Sci. 67, 2996–3002.

Cromwell, G.L., Lindemann, M.D., Monegue, H.J., Hall, D.D., Orr, J.D.E., 1998. Tribasic copper chloride and copper sulfate as copper sources for weanling pigs. J. Anim. Sci. 76, 118–123.

Dierick, N.A., Vervaeke, I.J., Demeyer, D.I., Decuypere, J.A., 1989. Approach to the energetic importance of fibre digestion in pigs. I. Importance of fermentation in the overall energy supply. Anim. Feed Sci. Technol. 23, 141–167.

Eipper, B.A., Mains, R.E., 1988. Peptide α-Amidation. Annu. Rev. Physiol. 50, 333–344.

Espinosa, C.D., Fry, R.S., Usry, J.L., Stein, H.H., 2017. Copper hydroxychloride improves growth performance and reduces diarrhea frequency of weanling pigs fed a corn–soybean meal diet but does not change apparent total tract digestibility of energy and acid hydrolyzed ether extract. J. Anim. Sci. 95, 5447–5454.

Ferrandis Vila, M., Trudeau, M.P., Hung, Y.T., Zeng, Z., Urriola, P.E., Shurson, G.C., Saqui-Salces, M., 2018. Dietary fiber sources and non-starch polysaccharide-degrading enzymes modify mucin expression and the immune profile of the swine ileum. PLoS One 13, e0207196.

Francis, G.L., 2010. Albumin and mammalian cell culture: implications for biotechnology applications. Cytotechnology 62, 1–16.

Fry, R.S., Ashwell, M.S., Lloyd, K.E., O'Nan, A.T., Flowers, W.L., Stewart, K.R., Spears, J.W., 2012. Amount and source of dietary copper affects small intestine morphology, duodenal lipid peroxidation, hepatic oxidative stress,and mRNA expression of hepatic copper regulatory proteins in weanling pigs. J. Anim. Sci. 90, 3112–3119.

Generoso, M., De Rosa, M., De Rosa, R., De Magistris, L., Secondulfo, M., Fiandra, R., Carratù, R., Cartenì, M., 2003. Cellobiose and lactulose coupled with mannitol and determined using ion-exchange chromatography with pulsed amperometric detection, are reliable probes for investigation of intestinal permeability. J. Chromatogr. B Analyt. Technol. Biomed. Life Sci. 783, 349–357.

Graham, H., Hesselman, K., Åman, P., 1986. The influence of wheat bran and sugar-beet pulp on the digestibility of dietary components in a cereal-based pig diet. J. Nutr. 116, 242–251.

Gutierrez, N.A., Kerr, B.J., Patience, J.F., 2013. Effect of insoluble-low fermentable fiber from corn-ethanol distillation origin on energy, fiber, and amino acid digestibility, hindgut degradability of fiber, and growth performance of pigs. J. Anim. Sci. 91, 5314–5325.

Højberg, O., Canibe, N., Poulsen, H.D., Hedemann, M.S., 2005. Influence of dietary zinc oxide and copper sulfate on the gastrointestinal ecosystem in newly weaned pigs. Appl. Environ. Microbiol. 71, 2267–2277.

Jaworski, N.W., Shoulders, J., González-Vega, J.C., Stein, H.H., 2014. Effects of using copra meal, palm kernel expellers, or palm kernel meal in diets for weanling pigs. Prof. Anim. Sci. 30, 243–251.

Jones, C.K., Nelssen, J.L., DeRouchey, J.M., Bergstrom, J.R., Tokach, M.D., Goodband, R.D., Dritz, S.S., 2010. Efficacy of commercial enzymes in diets containing various concentrations and sources of dried distillers grains with solubles for nursery pigs. J. Anim. Sci. 88, 2084–2091.

LaBella, F., Dular, R., Vivian, S., Queen, G., 1973. Pituitary hormone releasing or inhibiting activity of metal ions present in hypothalamic extracts. Biochem. Biophys. Res. Commun. 52, 786–791.

Lei, L., Xiaoyi, S., Fuchang, L., 2017. Effect of dietary copper addition on lipid metabolism in rabbits. Food Nutr. Res. 61, 1348866.

Li, Q., Gabler, N.K., Gould, S.A., Patience, J.F., Loving, C.L., 2018. A dietary carbohydrase blend improved intestinal barrier function and growth rate in weaned pigs fed higher fiber diets. J. Anim. Sci. 96, 5233–5243.

Linder, M.C., 1991. Biochemistry of copper. Plenum Press, New York, New York.

Ma, Y.L., Xu, Z.R., Guo, T., 2006. Effect of inorganic copper/montmorillonite nanomaterial on growth performance, intestinal microbial flora and bacterial enzyme activities in broilers. Chin. J. Anim. Sci. 42, 28–31.

Ma, Y.L., Guo, T., Xu, Z.R., 2007. Effect of Cu (II)-exchange montmorillonite on diarrhea incidence, intestinal microflora and mucosa morphology of weaning pigs. Chin. J. Vet. Sci. 27, 279–283.

Macfarlane, S., Macfarlane, G.T., 2007. Regulation of short-chain fatty acid production. Proc. Nutr. Soc. 62, 67–72.

Moore, K.W., Malefyt, Rd.W., Coffman, R.L., O'Garra, A., 2001. Interleukin-10 and the interleukin-10 receptor. Annu. Rev. Immunol. 19, 683–765.

Namkung, H., Gong, J., Yu, H., de Lange, C.F.M., 2006. Effect of pharmacological intakes of zinc and copper on growth performance, circulating cytokines and gut microbiota of newly weaned piglets challenged with coliform lipopolysaccharides. Can. J. Anim. Sci. 86, 511–522.

NRC, 2012. Nutrient Requirements of Swine, 11th rev. ed. Natl. Acad. Press, Washington, D.C.

Pauli, U., 1995. Porcine TNF: a review. Vet. Immunol. Immunopathol. 47, 187–201.

Pluske, J.R., Hampson, D.J., Williams, I.H., 1997. Factors influencing the structure and function of the small intestine in the weaned pig: a review. Livest. Prod. Sci. 51, 215–236.

Quinlan, G.J., Martin, G.S., Evans, T.W., 2005. Albumin: biochemical properties and therapeutic potential. Hepatol. Commun. 41, 1211–1219.

Ramos, D., Mar, D., Ishida, M., Vargas, R., Gaite, M., Montgomery, A., Linder, M.C., 2016. Mechanism of copper uptake from blood plasma ceruloplasmin by mammalian cells. PLoS One 11, e0149516.

Russek-Cohen, E., Dinneen, M.M., Kohn, R.A., 2005. Using blood urea nitrogen to predict nitrogen excretion and efficiency of nitrogen utilization in cattle, sheep, goats, horses, pigs, and rats. J. Anim. Sci. 83, 879–889.

Song, J., Li, Y.L., Hu, C.H., 2013. Effects of copper-exchanged montmorillonite, as alternative to antibiotic, on diarrhea, intestinal permeability and proinflammatory cytokine of weanling pigs. Appl. Clay Sci. 77-78, 52–55.

Spears, J.W., Kegley, E.B., Mullis, L.A., 2004. Bioavailability of copper from tribasic copper chloride and copper sulfate in growing cattle. Anim. Feed Sci. Technol. 116, 1–13.

Tsai, T., Dove, C.R., Cline, P.M., Owusu-Asiedu, A., Walsh, M.C., Azain, M., 2017. The effect of adding xylanase or glucanase to diets with corn distillers dried grains with solubles (CDDGS) on growth performance and nutrient digestibility in nursery pigs. Livest. Sci. 197, 46–52.

Tumbleson, M.E., Kalish, P.R., 1972. Serum biochemical and hematological parameters in crossbred swine from birth through eight weeks of age. Can. J. Comp. Med. 36, 202–209.

Weber, T.E., Kerr, B.J., Ziemer, C.J., 2008. Effects of adding fibrous feedstuffs to the diet of young pigs on growth performance, intestinal cytokines, and circulating acute-phase proteins. J. Anim. Sci. 86, 871–881.

Whang, K.Y., Easter, R.A., 2000. Blood urea nitrogen as an index of feed efficiency and lean growth potential in growing-finishing swine. Asian-Australas. J. Anim. Sci. 13, 811–816.

Wijtten, P.J.A., Meulen, J.V.D., Verstegen, M.W.A., 2011. Intestinal barrier function and absorption in pigs after weaning: A review. Br. J. Nutr. 105, 967–981.

Yang, H., Xiong, X., Wang, X., Li, T., Yin, Y., 2016. Effects of weaning on intestinal crypt epithelial cells in piglets. Sci. Rep. 6, 36939–36949.

Zhang, B., Guo, Y., 2009. Supplemental zinc reduced intestinal permeability by enhancing occludin and zonula occludens protein-1 (ZO-1) expression in weaning piglets. Br. J. Nutr. 102, 687–693.

Zhang, W., Li, D., Liu, L., Zang, J., Duan, Q., Yang, W., Zhang, L., 2013. The effects of dietary fiber level on nutrient digestibility in growing pigs. J. Anim. Sci. Biotechnol. 4 17-17.

Zhao, J., Harper, A.F., Estienne, M.J., Webb, K.E., McElroy, A.P., Denbow, D.M., 2007. Growth performance and intestinal morphology responses in early weaned pigs to supplementation of antibiotic-free diets with an organic copper complex and spray-dried plasma protein in sanitary and nonsanitary environments. J. Anim. Sci. 85, 1302–1310.

Zhao, J., Allee, G., Gerlemann, G., Ma, L., Gracia, M.I., Parker, D., Vazquez-Anon, M., Harrell, R.J., 2014. Effects of a chelated copper as growth promoter on performance and carcass traits in pigs. Asian. Austral. J. Anim. 27, 965–973.

Zhou, W., Kornegay, E.T., van Laar, H., Swinkels, J.W., Wong, E.A., Lindemann, M.D., 1994. The role of feed consumption and feed efficiency in copper-stimulated growth. J. Anim. Sci. 72, 2385–2394.