Animal Feed Science and Technology 256 (2019) 114257

Contents lists available at ScienceDirect

Animal Feed Science and Technology

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

Effects of copper hydroxychloride and choice white grease on growth performance and blood characteristics of weanling pigs kept at normal ambient temperature or under heat stress

C.D. Espinosa[a], R.S. Fry[b], J.L. Usry[b], H.H. Stein[a,*]

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

A R T I C L E I N F O

Keywords:
Copper
Copper hydroxychloride
Growth performance
Heat stress
Pigs

A B S T R A C T

An experiment was conducted to test the hypothesis that copper (Cu) hydroxychloride improves growth performance and blood characteristics, and reduces diarrhea incidence in weanling pigs without or with exposure to heat stress. One hundred sixty pigs (6.14 ± 0.90 kg) were allotted to a 2 × 2 factorial arrangement with 2 levels of choice white grease (CWG; 0 or 50 g/kg) and 2 levels of Cu from Cu hydroxychloride (0 or 100 mg/kg). There were 5 pigs per pen and 8 pen replicates per diet. Fecal scores were visually assessed using a score from 1 to 5 (1 = normal feces to 5 = watery feces). On day 40 until the end of the experiment, ambient temperature was increased from 24 °C to 32 °C to create a mild heat stress. On day 14, day 28, day 40, and on day 44, blood samples were collected from 1 pig per pen and tumor necrosis factor-α (TNF-α), peptide YY, immunoglobulin G, blood urea nitrogen, total protein, and albumin were analyzed. Results indicated that there were no interactions between CWG and Cu hydroxychloride for overall growth performance. Greater ($P < 0.05$) gain:feed was observed from day 29 to 40 and from day 41 to 44 for pigs fed diets with 50 g/kg CWG compared with pigs fed diets without supplemental fat. Average daily gain was greater ($P < 0.05$) from day 15 to 28 and also during exposure to heat stress, and fecal scores were reduced over the entire period ($P < 0.05$) for pigs fed diets with Cu hydroxychloride compared with pigs fed diets without Cu hydroxychloride. There was also an increase ($P < 0.05$) in concentration of peptide YY and a reduction ($P < 0.05$) in TNF-α concentration on day 14 for pigs fed Cu hydroxychloride diets compared with pigs fed diets without Cu hydroxychloride. This may be attributed to the effect of Cu in enhancing the expression of hypothalamic appetite regulators and its bacteriostatic property in reducing inflammation caused by pathogens. In conclusion, supplementation of Cu hydroxychloride to diets fed to weanling pigs without or with addition of CWG reduces diarrhea incidence and improves growth performance and some blood characteristics.

Abbreviations: ADF, acid detergent fiber; ADFI, average daily feed intake; ADG, average daily gain; AEE, acid hydrolyzed ether extract; Ca, calcium; BUN, blood urea nitrogen; Cu, copper; CWG, choice white grease; DDGS, distillers dried grains with solubles; EDTA, ethylenediaminetetraacetic acid; Fe, iron; G:F, gain to feed ratio; IgG, immunoglobulin G; Mn, manganese; NDF, neutral detergent fiber; P, phosphorus; TNF-α, tumor necrosis factor-α; Zn, zinc

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

https://doi.org/10.1016/j.anifeedsci.2019.114257
Received 14 October 2018; Received in revised form 12 August 2019; Accepted 13 August 2019

1. Introduction

Heat stress is one of the contributing causes of reduction in growth performance and occurrence of diseases in pigs (Le Dividich, 1981; Hicks et al., 1998). Heat stress may affect behavior, endocrine responses, and immune responses of pigs, and may increase concentrations of serum cortisol and tumor necrosis factor-α (TNF-α) in pigs upon exposure (Hicks et al., 1998; Carroll et al., 2012). Heat stress also induces increased gut permeability and inflammation, which may affect growth performance and intestinal function of pigs (Lee et al., 2016).

Copper (Cu) is a trace mineral that has bacteriostatic and bactericidal properties (Stahly et al., 1980). Dietary Cu may result in reduced bacterial populations in the intestine and affect growth and community structure of microorganisms in the cecum and colon, and also contribute to improved intestinal health (Højberg et al., 2005). Copper hydroxychloride (IntelliBond C^{II}; Micronutrients, USA LLC, Indianapolis, IN 46241, USA) is one of the sources of Cu that may be used in diets for pigs, and Cu hydroxychloride has low water solubility, but is highly soluble under acidic conditions (Spears et al., 2004). Copper hydroxychloride also improves growth rate and feed efficiency in pigs (Cromwell et al., 1998; Fry et al., 2012; Espinosa et al., 2017). It is, therefore, possible that dietary Cu hydroxychloride will result in an improved immune response of pigs and contribute to prevention of diseases, and thus, improve growth performance of pigs exposed to heat stress. Pigs under heat stress often respond by reducing average daily feed intake (ADFI), but addition of fat to the diet may help pigs maintain energy intake (Kellner et al., 2016). However, there is no information about the effects of adding both Cu and fat to diets during heat stress. Therefore, an experiment was conducted to test the hypothesis that inclusion of 100 mg/kg of supplemental Cu from Cu hydroxychloride in diets fed to pigs without or with addition of choice white grease (CWG) will improve growth performance and blood concentrations of indicators for protein utilization, inflammatory responses, and hormonal effects if pigs are kept under normal temperature and if they are exposed to heat stress.

2. Materials and methods

The protocol for the experiment was submitted to the Institutional Animal Care and Use Committee at the University of Illinois and was approved prior to initiation of the experiment. Pigs that were the offspring of Line 359 boars mated to Camborough females (Pig Improvement Company, Hendersonville, TN, USA) were used. No antibiotic growth promoters were included in the diets, and pharmacological levels of Zn were also not used.

Table 1
Fatty acid profile (g/kg of total fat) of choice white grease (CWG) used in the experiment.[a]

Item	Choice white grease
Crude fat, g/kg	> 985.0
C14:0	15.9
C14:1	1.2
C15:0	1.4
C16:0	220.8
C16:1	24.7
C17:0	5.0
C17:1	3.6
C18:0	110.2
C18:1	419.7
C18:2	133.3
C18:3	6.2
C20:0	1.8
C20:1	8.5
C20:2	6.2
C20:3	1.2
C20:4	4.7
C21:0	1.6
C22:0	0.5
C23:0	1.2
Total saturated fatty acids	358.5
Total monounsaturated fatty acids	458.0
Total polyunsaturated fatty acids	151.6

[a] C15:1, C20:4, C20:5, C20:3, C21:5, C22:1, C22:5, C24:0, and C24:1 were analyzed, but concentrations were below 0.3 g/kg in all samples. Total saturated fatty acids = C14:0 + C15:0 + C16:0 + C17:0 + C18:0 + C20:0 + C21:0 + C22:0 + C23:0 + C24:0. Total monounsaturated fatty acids = C14:1 + C15:1 + C16:1 + C17:1 + C18:1 + C20:1 + C22:1 + C24:1. Total polyunsaturated fatty acids = C18:2 + C18:3 + C20:2 + C20:3 + C20:4.

2.1. Diets and feeding

All diets from day 1 to 14, day 15 to 28, and day 29 to 44 were formulated to meet current estimates for nutrient requirements for 6 to 8, 8 to 12, and 12 to 25 kg pigs, respectively (NRC, 2012). Diets were based on corn and soybean meal. Within each period, pigs were fed a control diet, the control diet plus 50 g/kg CWG (Darling Ingredients, Mason City, IL, USA; Table 1), the control diet plus 100 mg/kg Cu from Cu hydroxychloride (IntelliBond C^{II}; 540 g Cu/kg; Micronutrients, USA LLC, Indianapolis, IN, USA), or the control diet plus 50 g/kg CWG and 100 mg/kg Cu from Cu hydroxychloride. The control diet contained 20 mg of Cu from CuCl that was provided by the vitamin-mineral premix. All diets were fed to pigs in mash form.

2.2. Animals and housing

A total of 160 pigs weaned at 3 weeks of age (6.14 ± 0.90 kg) were allotted to a 2 × 2 factorial arrangement with 2 levels of CWG (0 or 50 g/kg) and 2 levels of Cu from Cu hydroxychloride (0 or 100 mg/kg). Weanling pigs were allotted by initial body weight in a randomized complete block design. Pens were 1.20 × 1.35 m and provided 0.40 m^2 per pig, and each pen was equipped with a feeder and a nipple drinker. There were 5 pigs per pen (3 gilts and 2 barrows) and 8 replicate pens per treatment.

2.3. Method of collection

Individual pig weights were recorded at the beginning of the experiment, on day 14, day 28, day 40, and on day 44. Feed addition was recorded daily and weight of feed left in the feeder was recorded on days 14, 28, 40, and 44. Fecal scores were assessed visually per pen every other day using a score from 1 to 5 (1 = normal feces; 2 = moist feces; 3 = mild watery feces; 4 = medium watery feces; and 5 = watery feces). Frequency of high fecal score was obtained by totaling the number of pen days with fecal scores ≥3 divided by the total number of pen days multiplied by 100. At the conclusion of the experiment, data were summarized to calculate ADFI, average daily gain (ADG), and gain:feed ratio (G:F) within each treatment group. Data were summarized for day 1 to 14, day 15 to 28, day 29 to 40, day 41 to 44, day 1 to 40, and for the entire experiment.

On day 14, day 28, day 40, and on day 44, 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 (EDTA). Heparinized samples were frozen at -20 °C and were analyzed for blood urea nitrogen, total protein, and albumin using a Beckman Coulter Clinical Chemistry AU analyzer (Beckman Coulter, Inc., Brea, CA, USA). Tumor necrosis factor-α, immunoglobulin G (IgG), and peptide YY were measured in plasma samples collected in the vacutainer with EDTA using ELISA kits according to the recommendations from the manufacturer (R&D Systems, Inc., Minneapolis, MN, USA; Bethyl Laboratories, Inc., Montgomery, TX, USA; and Phoenix Pharmaceuticals, Inc., Burlingame, CA, USA, respectively).

The temperature from day 1 to 40 followed normal farm practices (i.e., approximately 28 °C from day 1 to 7, 26 °C from day 7 to 14, and 24 °C from day 18 to 39). However, from day 40 until the end of the experiment, the temperature in the barn was increased from 24 °C to 32 °C to create a mild heat stress for the pigs. Pigs were allowed ad libitum access to feed and water during the entire experiment.

2.4. Chemical analyses

Crude fat was determined in CWG by ether extraction (Method 920.39 (A); AOAC Int., 2007). Methyl esters of fatty acids were extracted from CWG (Method Ce-266; AOCS, 2017), and the concentration of fatty acids in CWG was measured using a capillary gas liquid chromatography (Method 996.06; AOAC Int., 2007). All diet samples were ground through a 1-mm screen in a Wiley mill (model 4; Thomas Scientific, Swedesboro, NJ, USA) prior to chemical analysis. Diets were analyzed for dry matter (Method 930.15; AOAC Int., 2007), ash (Method 942.05; AOAC Int., 2007), and gross energy using bomb calorimetry (Model 6400; Parr Instruments, Moline, IL, USA). Diets were also analyzed for neutral detergent fiber inclusive of residual ash (NDF) and acid detergent fiber inclusive of residual ash (ADF) using fiber bags ($Ankom^{2000}$ fiber analyzer; Ankom Technology, Macedon, NY, USA) followed by an adaptation of the procedure described by Van Soest et al. (1991). Acid hydrolyzed ether extract was analyzed by acid hydrolysis using 3*N* HCl (Ankom HCl Hydrolysis System, Ankom Technology, Macedon, NY, USA) followed by fat extraction (Ankom XT-15 Extractor, Ankom Technology, Macedon, NY, USA), and crude protein was analyzed using the combustion procedure (Method 990.03; AOAC Int., 2007). Amino acids were analyzed on a Hitachi AA Analyzer (Model No. L8800; Hitachi High Technologies America, Inc., Pleasanton, CA, USA) using ninhydrin for postcolum derivatization and norleucine as the internal standard (Method 982.30 E (a, b, c); (AOAC Int., 2007)). Minerals including Cu were analyzed by inductively coupled plasma optical emission spectrometry using an internally validated method (Method 985.01 A, B, and C; AOAC, 2007) after wet ash sample preparation (Method 975.03 B[b]; AOAC, 2007).

2.5. Statistical analysis

Data and diarrhea scores were analyzed following a 2 × 2 factorial arrangement using the MIXED procedure of SAS (SAS Institute Inc., 2013) with the pen as the experimental unit. The model included CWG, Cu, and CWG × Cu as fixed effects, and replicate as a random effect. Least square means were calculated for each independent variable and means were separated using the PDIFF option. The chi-squared test was used to analyze the frequency of diarrhea among treatments. Results were considered significant at

Table 2
Composition of experimental diets without or with choice white grease (CWG) and without or with copper from copper hydroxychloride.

	day 1 to 14 Diets				day 15 to 28 Diets				day 29 to 44 Diets			
	No Cu[a]		100 mg/kg Cu		No Cu		100 mg/kg Cu		No Cu		100 mg/kg Cu	
Item	-CWG	+ CWG	- CWG	+ CWG	-CWG	+ CWG	- CWG	+ CWG	- CWG	+ CWG	- CWG	+ CWG
Ingredient, g/kg												
Ground corn	501.3	450.9	501.3	450.9	447.1	396.7	447.1	396.7	449.7	399.2	449.7	399.2
Soybean meal	265.0	265.0	265.0	265.0	270.0	270.0	270.0	270.0	220.0	220.0	220.0	220.0
Whey, dried	150.0	150.0	150.0	150.0	100.0	100.0	100.0	100.0	–	–	–	–
Corn DDGS[a]	–	–	–	–	150.0	150.0	150.0	150.0	300.0	300.0	300.0	300.0
Fish meal	60.0	60.0	60.0	60.0	–	–	–	–	–	–	–	–
Choice white grease	–	50.0	–	50.0	–	50.0	–	50.0	–	50.0	–	50.0
Limestone	10.5	10.5	10.5	10.5	14.4	14.4	14.4	14.4	15.5	15.3	15.5	15.3
Dicalcium phosphate	–	–	–	–	3.8	3.8	3.8	3.8	0.6	1.0	0.6	1.0
Cornstarch	1.0	1.0	0.815	0.815	1.0	1.0	0.815	0.815	1.0	1.0	0.815	0.815
Copper hydroxychloride, 540 g/kg Cu	–	–	0.185	0.185	–	–	0.185	0.185	–	–	0.185	0.185
L-Lysine	3.5	3.6	3.5	3.6	5.1	5.2	5.1	5.2	5.5	5.6	5.5	5.6
DL -Methionine	1.2	1.4	1.2	1.4	1.0	1.2	1.0	1.2	0.3	0.4	0.3	0.4
L -Threonine	0.9	1.0	0.9	1.0	1.0	1.1	1.0	1.1	0.8	0.9	0.8	0.9
Phytase premix[b]	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Salt	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0
Vitamin-mineral premix[c]	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5
Analyzed composition												
Dry matter, g/kg	859.4	870.2	861.8	878.7	861.0	872.9	863.3	870.9	853.8	862.0	857.6	864.4
Ash, g/kg	63.6	61.7	54.0	62.8	63.7	57.6	58.5	59.6	48.5	52.4	54.8	44.7
Gross energy, MJ/kg	16.0	17.3	16.0	17.3	16.2	17.5	16.1	17.4	16.7	18.1	16.7	18.1
Crude protein, g/kg	171.5	168.6	187.0	175.3	187.3	172.9	184.7	171.5	197.6	172.7	191.9	177.7
AEE, g/kg	34.0	82.0	29.8	75.3	34.9	80.1	34.3	84.2	48.9	106.5	45.0	96.8
ADF, g/kg	65.4	58.0	65.9	53.9	73.9	68.0	81.8	76.2	88.2	82.3	84.5	81.2
NDF, g/kg	73.3	72.0	74.3	70.5	118.2	111.0	118.7	109.6	188.2	182.1	182.3	179.8
Ca, g/kg	8.8	8.9	8.9	8.0	8.2	7.7	7.2	8.2	6.5	6.7	6.8	6.4
P, g/kg	5.8	5.9	6.0	5.7	5.8	5.7	5.7	5.9	5.6	5.4	5.5	5.5
Mn, mg/kg	84.8	73.6	65.0	67.5	72.6	53.6	74.8	66.9	66.4	68.6	60.8	76.2
Fe, mg/kg	335	197	195	296	198	163	303	177	243	171	290	166
Zn, mg/kg	175	168	156	153	171	125	148	152	190	178	152	193
Cu, mg/kg	30	26	121	121	27	19	108	125	25	25	102	126
Amino acids, g/kg												
Arginine	12.3	12.8	12.6	12.5	11.9	13.3	12.6	12.6	12.3	11.7	12.4	12.2
Histidine	5.2	5.3	5.2	5.1	5.3	5.7	5.5	5.5	5.5	5.4	5.6	5.5
Isoleucine	9.4	9.7	9.7	9.4	9.1	9.8	9.8	9.7	9.0	9.0	9.1	9.0
Leucine	17.0	17.1	17.3	16.8	18.3	19.2	19.0	18.8	19.3	19.4	19.3	19.2
Lysine	15.1	15.8	15.8	15.4	14.2	15.6	15.2	15.1	13.3	14.9	14.2	14.1
Methionine	4.4	4.6	4.6	4.4	3.9	4.6	4.0	3.9	3.5	3.9	3.5	3.5
Methionine + Cysteine	7.4	7.6	7.6	7.4	6.9	7.6	7.0	6.9	6.5	6.9	6.5	6.5
Phenylalanine	9.6	9.9	9.8	9.6	10.0	10.8	10.5	10.4	10.4	10.4	10.6	10.3
Threonine	8.8	9.4	9.2	9.1	9.0	9.6	9.0	9.4	8.7	8.5	8.5	8.6
Tryptophan	2.5	2.2	2.5	2.5	2.3	2.4	2.4	2.5	2.1	2.1	2.1	2.2
Valine	10.2	10.5	10.5	10.2	10.1	10.8	10.8	10.7	10.6	10.5	10.7	10.5

[a] Cu as Cu hydroxychloride (IntelliBond C^{II}; Micronutrients, USA LLC, Indianapolis, IN, USA); DDGS = distillers dried grains with solubles. AEE = acid hydrolyzed ether extract; ADF = acid detergent fiber inclusive of ash; NDF = neutral detergent fiber inclusive of ash.

[b] Quantum Blue 5000 (5000 units of phytase per gram); AB Vista, Marlborough, United Kingdom. At 0.1 g/kg inclusion, the premix was calculated to provide 500 units of phytase per kg complete diet.

[c] Provided the following quantities of vitamins and micro-minerals per kilogram of complete diet: Vitamin A as retinyl acetate, 11,150 IU; vitamin D_3 as cholecalciferol, 2210 IU; vitamin E as selenium yeast, 66 IU; vitamin K as menadione nicotinamide bisulfate, 1.42 mg; thiamin as thiamine mononitrate, 1.10 mg; riboflavin, 6.59 mg; pyridoxine as pyridoxine hydrochloride, 1.00 mg; vitamin B_{12}, 0.03 mg; D-pantothenic acid as D-calcium pantothenate, 23.6 mg; niacin, 44.1 mg; folic acid, 1.59 mg; biotin, 0.44 mg; Cu, 20 mg as copper chloride; Fe, 125 mg as iron sulfate; I, 1.26 mg as ethylenediamine dihydriodide; Mn, 60.2 mg as manganese hydroxychloride; Se, 0.30 mg as sodium selenite and selenium yeast; and Zn, 125.1 mg as zinc hydroxychloride.

$P < 0.05$ and trends at $0.05 \leq P < 0.10$.

3. Results

Diet analyses indicate that the intended concentrations of acid hydrolyzed ether extract and Cu were present in all diets and concentrations of other nutrients were not affected by dietary treatment. Likewise, no differences in amino acid composition among diets were observed (Table 2).

Table 3

Growth performance for pigs fed diets containing 0 or 50 g/kg choice white grease (CWG) and 0 or 100 mg/kg Cu from Cu hydroxychloride.[a]

	No Cu		100 mg/kg Cu			*P*-value		
Item	CWG	+ CWG	CWG	+ CWG	SEM	Cu	CWG	CWG × Cu
day 1 to 14								
Initial body weight, kg	6.12	6.14	6.14	6.14	0.056	0.801	0.810	0.868
Final body weight, kg	7.54	8.04	8.31	8.07	0.342	0.111	0.607	0.139
ADG[b], g	101	136	155	138	0.017	0.112	0.627	0.137
ADFI, g	179	206	239	212	0.017	0.013	0.997	0.039
G:F	0.562	0.664	0.646	0.647	0.095	0.468	0.323	0.335
day 15 to 28								
ADG, g	412	402	455	450	0.023	0.048	0.901	0.756
ADFI, g	714	679	821	769	0.035	0.001	0.168	0.980
G:F	0.580	0.594	0.551	0.589	0.050	0.264	0.212	0.359
Final body weight, kg	13.30	13.67	14.68	14.37	0.686	0.014	0.422	0.472
day 29 to 40								
ADG, g	639	641	629	634	0.024	0.616	0.820	0.929
ADFI, g	1,073	999	1,085	1,053	0.053	0.400	0.184	0.597
G:F	0.599	0.643	0.582	0.608	0.019	0.071	0.020	0.519
Final body weight, kg	20.97	21.36	22.23	21.98	0.536	0.213	0.739	0.882
day 41 to 44[c]								
ADG, g	538	601	637	685	0.054	0.041	0.206	0.859
ADFI, g	1,289	1,235	1,309	1,222	0.062	0.936	0.128	0.715
G:F	0.420	0.487	0.489	0.564	0.034	0.042	0.048	0.901
Final body weight, kg	23.12	23.76	24.78	24.72	0.841	0.077	0.496	0.846
day 1 to 40								
ADG, g	371	380	400	396	0.018	0.204	0.905	0.309
ADFI, g	617	607	680	633	0.023	0.013	0.093	0.269
G:F	0.599	0.622	0.588	0.621	0.032	0.818	0.314	0.693
day 1 to 44								
ADG, g	386	401	424	422	0.017	0.081	0.874	0.264
ADFI, g	756	743	817	764	0.023	0.023	0.064	0.249
G:F	0.515	0.542	0.522	0.553	0.027	0.716	0.126	0.709

[a] Data are least square means of 8 observations (pen as the experimental unit; 5 pigs per pen) for all treatments.
[b] ADG = average daily gain; ADFI = average daily feed intake; G:F = gain:feed ratio.
[c] Day 41 to 44 = Heat stress period.

3.1. Growth performance and fecal scores

An interaction between CWG and Cu hydroxychloride was observed for ADFI of pigs from day 1 to 14 (Table 3). Addition of 50 g/kg CWG to the control diet resulted in increased ADFI of pigs, whereas addition of 50 g/kg CWG to the diet supplemented with Cu hydroxychloride did not increase ADFI of pigs. Greater ($P < 0.05$) ADFI was observed by pigs fed diets with 100 mg/kg Cu from Cu hydroxychloride compared with pigs fed diets without Cu hydroxychloride from day 15 to 28, day 1 to 40, and during the overall experimental period. Greater ($P < 0.05$) ADG and final body weight were observed from day 15 to 28 for pigs fed the Cu hydroxychloride diets compared with pigs fed diets without Cu hydroxychloride. Pigs fed diets with 50 g/kg CWG had greater G:F ($P < 0.05$) compared with pigs fed diets without supplemental fat. When heat stress was induced, no interactions between CWG and Cu hydroxychloride were observed. However, pigs fed diets with Cu hydroxychloride had greater ($P < 0.05$) ADG compared with pigs fed diets without Cu hydroxychloride. An improvement ($P < 0.05$) in G:F was also observed during the heat stress period when pigs were fed diets with 50 g/kg CWG compared with pigs fed diets without CWG.

Pigs fed diets containing Cu hydroxychloride had reduced ($P < 0.05$) improved fecal scores compared with pigs fed diets without Cu hydroxychloride from day 1 to 14, day 15 to 28, day 1 to 40, and over the entire experimental period (Table 4). Pigs fed diets with 50 g/kg CWG had improved ($P < 0.05$) fecal scores compared with pigs fed diets without supplemental fat from day 29 to 40. A reduction ($P < 0.05$) in frequency of feces with high score was also observed for pigs fed the Cu hydroxychloride diets compared with pigs fed diets without supplemental Cu from day 15 to 28, day 1 to 40, and for the overall experimental period.

3.2. Blood characteristics

On day 14, pigs fed diets with 100 mg/kg of Cu from Cu hydroxychloride had increased ($P < 0.05$) albumin concentration and reduced ($P < 0.05$) concentration of TNF-α compared with pigs fed diets without Cu hydroxychloride (Table 5). An interaction between CWG and Cu was observed for the concentration of albumin on day 44 because addition of 50 g/kg CWG to the control diet resulted in increased albumin concentration in plasma of pigs, whereas no difference was observed when 50 g/kg CWG was added to the diet supplemented with Cu hydroxychloride.

Table 4
Fecal score and frequency of high fecal score for pigs fed diets containing 0 or 50 g/kg choice white grease (CWG) and 0 or 100 mg/kg Cu from Cu hydroxychloride.[a]

	No Cu		100 mg/kg Cu			*P*-value		
Item	CWG	+ CWG	CWG	+ CWG	SEM	Cu	CWG	CWG × Cu
Fecal score[b]								
day 1 to 14	2.00	2.18	1.84	1.73	0.088	0.002	0.689	0.117
day 15 to 28	2.29	2.25	1.84	1.79	0.112	< 0.001	0.694	0.937
day 29 to 40	1.69	1.56	1.60	1.35	0.086	0.101	0.038	0.473
day 41 to 44	1.25	1.25	1.25	1.13	0.192	0.667	0.667	0.667
day 1 to 40	1.99	2.00	1.76	1.62	0.067	< 0.001	0.327	0.289
day 1 to 44	1.81	1.81	1.63	1.50	0.062	< 0.001	0.266	0.235
Frequency of high fecal score								
day 1 to 14								
Pen days[c]	56	56	56	56				
Frequency (%)[d]	28.57	33.93	23.21	17.86	–	0.242		
day 15 to 28								
Pen days	56	56	56	56				
Frequency (%)	25.00	32.14	8.93	14.29	–	0.009		
day 29 to 40								
Pen days	48	48	48	48				
Frequency (%)	2.08	4.17	2.08	0.00	–	0.564		
day 41 to 44								
Pen days	16	16	16	16	–			
Frequency (%)	0.00	0.00	0.00	0.00		< 0.999		
day 1 to 40								
Pen days	160	160	160	160	–			
Frequency (%)	19.38	24.38	11.88	11.25		0.003		
day 1 to 44								
Pen days	176	176	176	176	–			
Frequency (%)	17.61	22.16	10.80	10.23		0.004		

[a] Data are least square means of 8 observations for all treatments.
[b] Fecal score = 1, normal feces; 2, moist feces; 3, mild watery feces; 4, medium watery feces; 5, watery feces.
[c] Pen days = number of pens × the number of days assessing diarrhea scores.
[d] Frequency of high fecal score = (number of pen days with fecal scores ≥ 3/pen days) * 100.

4. Discussion

Diets fed from day 1 to 14, day 15 to 28, and day 29 to 44 were formulated to meet estimates for nutrient requirements for 6 to 8, 8 to 12, and 12 to 25 kg pigs, respectively (NRC, 2012).

4.1. Growth performance and fecal scores

Pigs undergo heat stress when environmental temperature is greater than their thermoneutral zone, and in this condition, pigs reduce their metabolic function by altering behavioral and physiological activity (Rauw et al., 2017). Under heat stress, pigs suppress feed intake as a mechanism to reduce heat production, and as a result, ADG of heat-stressed pigs is reduced compared with pigs kept under normal temperature (Kellner et al., 2016). Within 2–6 h of exposure to heat stress, a reduction in feed intake with a subsequent reduction in intestinal function and gut integrity may be observed in pigs (Pearce et al., 2013; Gabler et al., 2018). Pigs also become hyperglycemic due to an altered digestive capacity and changed post-absorptive metabolism within 24 h of exposure to heat stress (Pearce et al., 2012). To ameliorate these negative effects, ingredients with low heat increment such as crystalline amino acids and dietary fat are often used during periods where pigs may observe heat stress. However, it is not known if supplementation of Cu as Cu hydroxychloride without or with addition of CWG to diets can have beneficial effects in pigs exposed to heat stress.

Dietary fat increases energy concentration of diets (Kerr et al., 2015), and the reduction in ADFI that was observed in pigs fed diets with supplemental fat over the entire experimental period is likely due to the greater energy density in these diets. This observation is in agreement with data indicating that ADFI of pigs linearly decreased as the concentration of CWG increased in the diets (De la Llata et al., 2001). The observed improvement in G:F of pigs fed diets containing 50 g/kg CWG without or with heat stress is in agreement with results indicating that addition of 40 g/kg soybean oil improved G:F of pigs kept at 20 °C or 30 °C (Hsia and Lu, 2004). The reason no change in G:F was observed from day 1 to 14 and from day 15 to 28 may be that pigs do not utilize added dietary animal fat efficiently before they have a body weight close to 15 kg (Cera et al., 1990; Reinhart et al., 1992; Adeola et al., 2013).

The observation that ADG and ADFI were greater for pigs fed diets containing Cu hydroxychloride than for pigs fed the control diet under normal temperature is in agreement with previous data (Cromwell et al., 1998; Espinosa et al., 2017). The improved fecal score observed in pigs fed diets with supplemental Cu are also in agreement with previous data (Rutkowska-Pejsak et al., 1998;

Table 5
Blood characteristics for pigs fed diets containing 0 or 50 g/kg choice white grease (CWG) and 0 or 100 mg/kg Cu from Cu hydroxychloride.[a]

	No Cu		100 mg/kg Cu			*P*-value		
Item	- CWG	+ CWG	- CWG	+ CWG	SEM	Cu	CWG	CWG × Cu
day 14								
BUN[b], mg/dL	10.50	7.86	8.63	8.50	1.66	0.485	0.243	0.275
Total protein, g/dL	5.00	4.77	4.97	5.09	0.22	0.424	0.763	0.312
Albumin, g/dL	2.48	2.60	2.71	2.69	0.11	0.048	0.460	0.371
TNF-α, pg/mL	181.44	168.54	129.06	144.13	18.17	0.039	0.952	0.436
IgG, mg/mL	20.75	21.10	17.99	18.67	3.44	0.457	0.882	0.963
Peptide YY, ng/mL	1.33	1.63	1.57	1.99	0.20	0.082	0.038	0.732
day 28								
BUN, mg/dL	8.00	9.63	7.25	7.25	0.95	0.111	0.398	0.398
Total protein, g/dL	4.88	4.80	4.76	4.79	0.13	0.636	0.850	0.705
Albumin, g/dL	2.63	2.69	2.75	2.70	0.09	0.345	0.931	0.439
TNF-α, pg/mL	216.91	202.44	188.41	190.62	19.35	0.341	0.770	0.691
IgG, mg/mL	18.96	18.88	16.04	18.70	5.29	0.809	0.773	0.798
Peptide YY, ng/mL	1.33	1.13	1.32	1.35	0.15	0.416	0.513	0.337
day 40								
BUN, mg/dL	10.92	12.04	11.67	10.79	0.83	0.696	0.845	0.126
Total protein, g/dL	5.17	5.08	5.07	5.02	0.16	0.557	0.619	0.892
Albumin, g/dL	2.86	2.99	3.08	3.00	0.09	0.090	0.647	0.127
TNF-α, pg/mL	175.88	156.89	168.36	171.75	8.70	0.681	0.386	0.217
IgG, mg/mL	16.73	12.94	16.32	14.74	2.95	0.795	0.322	0.682
Peptide YY, ng/mL	1.41	1.39	1.28	1.26	0.11	0.115	0.832	0.975
day 44								
BUN, mg/dL	12.13	11.50	11.63	10.88	0.70	0.293	0.201	0.906
Total protein, g/dL	5.23	5.13	5.16	4.98	0.15	0.471	0.331	0.768
Albumin, g/dL	2.84	3.14	3.02	2.94	0.07	0.110	0.856	0.010
TNF-α, pg/mL	114.09	117.40	110.63	114.34	7.99	0.692	0.670	0.981
IgG, mg/mL	6.39	4.87	4.98	5.24	0.66	0.449	0.354	0.193
Peptide YY, ng/mL	1.19	0.98	0.95	0.96	0.13	0.266	0.360	0.333

[a] Data are least square means of 8 observations for all treatments.
[b] BUN = blood urea nitrogen. TNF-α = tumor necrosis factor-α.

Espinosa et al., 2017). It is possible that the observed increase in ADG and ADFI may be attributed to the effect of Cu in stimulating the secretion of growth-promoting regulatory peptides such as growth hormone-releasing hormone (LaBella et al., 1973; Zhou et al., 1994) and its involvement in post-translational modification of regulatory peptides (Eipper and Mains, 1988).

The beneficial effect of adding high concentrations of Cu to diets is well established; however, to our knowledge, there are no published data indicating the effect of supplementing Cu hydroxychloride to diets fed to pigs exposed to heat stress. The observation that ADG and G:F were greater for pigs fed diets containing Cu hydroxychloride than for pigs fed diets without supplemental Cu during heat stress is possibly a result of the beneficial effects of Cu on gastrointestinal health and immune function (Zhao et al., 2007). Dietary Cu is also believed to have bacteriostatic properties because it may reduce clostridium, salmonella, and coliform populations in the small intestine, as well as in the cecum and colon of pigs (Ma et al., 2006, 2007). During heat stress, a decrease in intestinal integrity and immune competence can be observed, which may result in disease and sudden reduction in nutrient absorption (Pearce et al., 2015). Therefore, addition of Cu hydroxychloride to diets may have prevented these negative effects, which subsequently resulted in improved growth performance.

4.2. Blood characteristics

Concentrations of blood urea nitrogen, total protein, and albumin were within the normal physiological ranges (Tumbleson and Kalish, 1972) and were in agreement with previous data (Casas and Stein, 2016). The lack of differences in blood urea nitrogen and total protein concentration among treatments indicate that dietary Cu concentrations and fat supplementation have no effect on the efficiency of nitrogen utilization of pigs. However, the increase in the concentration of albumin that was observed on day 14 and day 40 in pigs fed the Cu hydroxychloride diets may be due to an increased absorption of Cu. Albumin constitutes approximately 60% of the total plasma protein and is involved in the binding and transport of fatty acids, amino acids, and metal ions such as Zn and Cu (Quinlan et al., 2005; Francis, 2010). Therefore, the increased absorption of Cu and increased ADFI in pigs fed diets containing Cu hydroxychloride may have increased the need for albumin to transport nutrients to the liver and from the liver to extrahepatic tissues (Ramos et al., 2016).

The reduction in the concentration of TNF-α on day 14 that was observed when diets were supplemented with Cu hydroxychloride may be a result of the impact of Cu hydroxychloride on improving the immune status of pigs. It is possible that this effect also is due to the bacteriostatic property of Cu in reducing inflammation caused by pathogens. The observed increase in the concentration of PYY on day 14 by pigs fed the Cu hydroxychloride diets may be a consequence of Cu enhancing the expression of hypothalamic appetite regulators (Zhu et al., 2011). Peptide YY is a gastrointestinal hormone, which belongs to the pancreatic polypeptide family together with neuropeptide Y (Batterham and Bloom, 2003). Peptide YY is the most potent stimulator of food intake; therefore, the increase in peptide YY that was observed on day 14 may have contributed to the observed increase in ADFI of pigs fed the Cu hydroxychloride diets. The increased concentration of peptide YY in pigs fed diets with CWG on day 14 may also be a result of the increased energy intake of these pigs (Batterham et al., 2006) because the concentration of peptide YY depends on the type of diet ingested by the animal, with fat being the most potent nutrient in terms of stimulating the release of peptide YY (Batterham and Bloom, 2003).

5. Conclusion

In conclusion, supplementation of diets for weanling pigs with choice white grease resulted in improved gain to feed ratio of pigs under both normal and heat-stressed conditions, which is likely due to the high energy density and low heat increment of dietary fat. Supplementation of copper hydroxychloride without or with addition of choice white grease also improved growth performance both if pigs were kept under normal temperature and if they were exposed to heat stress, and there were positive changes in concentrations of tumor necrosis factor-α, albumin, and peptide YY on day 14 in pigs fed diets containing copper hydroxychloride. These measurable changes may be a result of the bacteriostatic property of copper hydroxychloride and its effect on enhancing the secretion and expression of growth-promoting regulatory peptides and hypothalamic appetite regulators, but further research is needed to verify these hypotheses.

Declaration of Competing Interest

The authors declare that they 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

Adeola, O., Mahan, D.C., Azain, M.J., Baidoo, S.K., Cromwell, G.L., Hill, G.M., Pettigrew, J.E., Maxwell, C.V., Shannon, M.C., 2013. Dietary lipid sources and levels for weanling pigs. J. Anim. Sci. 91, 4216–4225.

AOAC Int, 2007. Official Methods of Analysis of AOAC Int. AOAC Int., Gaithersburg, MD, USA.

AOCS, 2017. Official Methods and Recommended Practices of the AOCS, 7th ed. Am. Oil Chem. Soc., Champaign, IL, USA.

Batterham, R.L., Bloom, S.R., 2003. The gut hormone peptide YY regulates appetite. Ann. N.Y. Acad. Sci. 994, 162–168.

Batterham, R.L., Heffron, H., Kapoor, S., Chivers, J.E., Chandarana, K., Herzog, H., Le Roux, C.W., Thomas, E.L., Bell, J.D., Withers, D.J., 2006. Critical role for peptide YY in protein-mediated satiation and body-weight regulation. Cell Metab. 4, 223–233.

Carroll, J.A., Burdick, N.C., Chase, C.C., Coleman, S.W., Spiers, D.E., 2012. Influence of environmental temperature on the physiological, endocrine, and immune responses in livestock exposed to a provocative immune challenge. Domest. Anim. Endocrinol. 43, 146–153.

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.

Cera, K.R., Mahan, D.C., Reinhart, G.A., 1990. Effect of weaning, week postweaning and diet composition on pancreatic and small intestinal luminal lipase response in young swine. J. Anim. Sci. 68, 384–391.

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.

De la Llata, M., Dritz, S.S., Tokach, M.D., Goodband, R.D., Nelssen, J.L., Loughin, T.M., 2001. Effects of dietary fat on growth performance and carcass characteristics of growing-finishing pigs reared in a commercial environment. J. Anim. Sci. 79, 2643–2650.

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.

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.

Gabler, N.K., Koltes, D., Schaumberger, S., Murugesan, G.R., Reisinger, N., 2018. Diurnal heat stress reduces pig intestinal integrity and increases endotoxin translocation. Transl. Anim. Sci. 2, 1–10.

Hicks, T.A., McGlone, J.J., Whisnant, C.S., Kattesh, H.G., Norman, R.L., 1998. Behavioral, endocrine, immune, and performance measures for pigs exposed to acute stress. J. Anim. Sci. 76, 474–483.

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.

Hsia, L.C., Lu, G.H., 2004. The effect of high environmental temperature and nutrient density on pig performance, conformation and carcass characteristics under restricted feeding system. Asian-Australas. J. Anim. Sci. 17, 250–258.

Kellner, T.A., Baumgard, L.H., Prusa, K.J., Gabler, N.K., Patience, J.F., 2016. Does heat stress alter the pig's response to dietary fat? J. Anim. Sci. 94, 4688–4703.

Kerr, B.J., Kellner, T.A., Shurson, G.C., 2015. Characteristics of lipids and their feeding value in swine diets. J. Anim. Sci. Biotechnol. 6, 30–53.

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.

Le Dividich, J., 1981. Effects of environmental temperature on the growth rates of early-weaned piglets. Livest. Prod. Sci. 8, 75–86.

Lee, I.K., Kye, Y.C., Kim, G., Kim, H.W., Gu, M.J., Umboh, J., Maaruf, K., Kim, S.W., Yun, C.H., 2016. Stress, nutrition, and intestinal immune responses in pigs—a review. Asian-Australas. J. Anim. Sci. 29, 1075–1082.

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.
NRC, 2012. Nutrient Requirements of Swine, 11th rev. ed. Natl. Acad. Press, Washington, D.C., USA.
Pearce, S.C., Mani, V., Boddicker, R.L., Johnson, J.S., Weber, T.E., Ross, J.W., Baumgard, L.H., Gabler, N.K., 2012. Heat stress reduces barrier function and alters intestinal metabolism in growing pigs. J. Anim. Sci. 90, 257–259.
Pearce, S.C., Mani, V., Weber, T.E., Rhoads, R.P., Patience, J.F., Baumgard, L.H., Gabler, N.K., 2013. Heat stress and reduced plane of nutrition decreases intestinal integrity and function in pigs. J. Anim. Sci. 91, 5183–5193.
Pearce, S.C., Sanz Fernandez, M.V., Torrison, J., Wilson, M.E., Baumgard, L.H., Gabler, N.K., 2015. Dietary organic zinc attenuates heat stress–induced changes in pig intestinal integrity and metabolism. J. Anim. Sci. 93, 4702–4713.
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.
Rauw, W.M., Mayorga, E.J., Lei, S.M., Dekkers, J.C.M., Patience, J.F., Gabler, N.K., Lonergan, S.M., Baumgard, L.H., 2017. Effects of diet and genetics on growth performance of pigs in response to repeated exposure to heat stress. Front. Genet. 8, 1–18.
Reinhart, G.A., Simmen, F.A., Mahan, D.C., Simmen, R.C.M., White, M.E., Trulzsch, D.V., 1992. Perinatal ontogeny of fatty acid binding protein activity in porcine small intestine. Nutr. Res. 12, 1345–1356.
Rutkowska-Pejsak, B., Mokrzycka, A., Szkoda, J., 1998. Influence of zinc oxide in feed on the health status of weaned pigs. Med. Weter. 54, 194–200.
SAS Institute Inc, 2013. User's Guide: Statistics Version 9.4. SAS Institute Inc., Cary, NC, USA.
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.
Stahly, T.S., Cromwell, G.L., Monegue, H.J., 1980. Effects of the dietary inclusion of copper and(or) antibiotics on the performance of weanling pigs. J. Anim. Sci. 51, 1347–1351.
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.
Van Soest, P.J., Robertson, J.B., Lewis, B.A., 1991. Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. J. Dairy Sci. 74, 3583–3597.
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.
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.
Zhu, D., Yu, B., Ju, C., Mei, S., Chen, D., 2011. Effect of high dietary copper on the expression of hypothalamic appetite regulators in weanling pigs. J. Anim. Feed Sci. 20, 60–70.