

Effects of reducing dietary crude protein and metabolic energy in weaned piglets

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Abstract

The objective of this experiment was to determine the effects of a pure reduction in the dietary crude protein (CP) and metabolic energy (ME) contents on growth performance, nutrient digestibility, blood profile, faecal microflora and odour gas emission in weaned pigs. A total of 80 weaned piglets ((Landrace × Yorkshire) × Duroc) with a mean initial bodyweight (BW) of 6.8 ± 0.5 kg were randomly allotted to four treatments with four replicate pens of five piglets per pen (based on average BW) for 45 days. The dietary treatments consisted of i) CON: basal diet; ii) LME: reduction of 10% of ME in basal diet; iii) LCP: reduction of 10% of CP in basal diet; iv) MECP: reduction of 10% of CP and 10% of ME in basal diet. During the experimental period, average daily feed intake (ADFI) improved in piglets fed the LME and LCP diets, compared with those fed the CON diets. Average daily gain (ADG) and gain to feed (G : F) ratio decreased in piglets fed LCP, LME, and MECP diet, when compared with those fed the CON diet. However, during the total experimental period, ADG and G : F ratio were not affected by dietary treatment. With regard to nutrient digestibility, apparent total tract digestibility (ATTD) of CP was not affected by experimental diets. The concentration of blood urea nitrogen (BUN) in blood decreased more in piglets fed LME, LCP, and MECP than those fed the CON diet. The emissions of ammonia (NH₃), hydrogen sulfide (H₂S), and volatile fatty acids (VFAs) were lower in piglets fed LME, LCP, and MECP diet than those fed the CON diet. In conclusion, these results indicate that reduction in dietary CP and ME content did not decrease growth performance and nutrient digestibility, and they increased positive effects such as BUN and gas emission reduction.

Keywords: Blood profile, digestibility, growth performance, odour emission, pig

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Introduction

Dietary energy and protein are important for growth in pigs. Supplementation of fat and increasing nutrient concentration in diets has been reported to improve ADG and G : F ratio (Song *et al.*, 2003; Hastad *et al.*, 2005). Yan *et al.* (2010) found that growing-finishing pigs fed high energy density diets had greater ADG and G : F ratio, associated with improved feed intake and digestibility, than pigs fed low energy density diets. An increase in nutrient density, such as protein and energy, is expected to raise nutrient intake and weight gain. Additionally, dietary adjustment was aimed at maximizing production performance without regard to nutrient oversupply, namely protein and amino acids (Lee *et al.*, 2001). However, several studies found that energy levels in the diets did not affect growth performance in weaned piglets (Tokach *et al.*, 1995; Smith *et al.*, 1999). Digestibility of CP at the terminal ileum was from 60% to 80% in weaning pigs fed a diet based on triticale and wheat (Hogberg & Lindberg, 2004). That means there is much more protein and energy that cannot be digested in diets for piglets than expected. Undigested protein causes a rapid increase in pathogenic bacteria and diarrhoea through fermentation gas (VFA, ammonia, amine, indoles, phenols and branched-chain fatty acids) in the gastrointestinal tract (Dong *et al.*, 1996; Bolduan *et al.*, 1988; Aumaitre *et al.*, 1995; Anderson *et al.*, 1999; Gaskins, 2001; Pluske *et al.*, 2002; Houdijk *et al.*, 2007). The components of mixed ammonia, VFAs, alcohols and sulfides through fermentation in the intestine causes odour and environmental pollution (Burnett, 1969; Miner, 1977; Ritter 1989; Hartung & Phillips, 1994; Zahn *et al.* 1997).

Therefore, the current study was conducted to investigate the effects of reducing CP and ME levels in diet on growth performance, nutrient digestibility, blood profile, faecal microflora, and odour gas emission in weaned piglets.

Materials and methods

The experimental protocol used in this study was approved by the Animal Care and Use Committee of Chungbuk National University. A total of eighty weaned piglets ((Landrace × Yorkshire) × Duroc) with mean initial BW of 6.8 ± 0.5 kg were used in 45 experimental days. These piglets were allotted to one of four experimental diets based on average BW at weaning in a complete block design and four replicates with five pigs per pen in each replication. Dietary treatments consisted of i) CON: basal diet; ii) LME: reduction of 10% of ME in basal diet; iii) LCP: reduction of 10% of CP in basal diet; iv) MECP: reduction of 10% of CP and 10% of ME in basal diet. The experiment included two phases: phase 1 provided the diet in days 0 to 14 and phase 2 provided with diet in days 15 to 42. Ingredients and nutrient composition of basal diet are provided in Table 1. All diets for weaned piglets were formulated to meet or exceed the recommendations of NRC (1998). All pigs were allowed to consume feed and water *ad libitum* from a feeder and water nipple in an environmentally controlled nursery room. This room was provided with artificial light for 24 hours a day and maintained at a temperature between 27 °C and 30 °C.

Table 1 Ingredient and nutrient composition of experimental diets fed to weaning pigs

Items	Phase 1 (days 0–14)				Phase 2 (days 15–42)			
	CON	LME	LCP	MECP	CON	LME	LCP	MECP
Ingredients, %								
Corn	41.67	47.17	45.01	50.31	56.84	61.85	63.55	67.45
Soybean meal, 48%	30.00	29.00	30.00	29.00	30.00	29.00	25.00	23.00
Lactose	10.00	10.00	10.00	10.00	0.00	0.00	0.00	0.00
Wheat bran	0.00	0.00	0.00	0.00	5.00	5.00	3.00	5.00
Whey dried	5.00	5.00	5.00	5.00	0.00	0.00	0.00	0.00
Fish meal	5.00	5.00	1.00	0.00	0.00	0.00	0.00	0.00
(Menhaden)	5.00	1.00	5.00	1.00	5.00	1.00	5.00	1.00
Lard	0.21	0.21	0.39	0.39	0.21	0.20	0.30	0.33
L-Lysine-HCl	0.04	0.04	0.04	0.60	0.04	0.04	0.04	0.04
DL-Methionine	0.03	0.03	0.11	0.15	0.03	0.03	0.03	0.10
L-Threonine	1.00	1.00	1.40	1.50	1.00	1.00	1.20	1.20
Dicalcium phosphate	1.25	1.25	1.25	1.25	1.25	1.25	1.25	1.25
Limestone	0.50	0.00	0.50	0.50	0.50	0.50	0.50	0.50
Salt	0.10	0.10	0.10	0.10	0.08	0.05	0.05	0.05
Vitamin premix ³	0.20	0.20	0.20	0.20	0.05	0.08	0.08	0.08
Calculated composition, g/kg								
ME, Kcal/kg	3508	3344	3487	3283	3459	3289	3484	3277
Crude Protein	217	217	197	196	200	199	179	177
Calcium	10.8	10.8	9.6	9.3	8.0	7.9	8.2	8.2
Lys	14.6	14.5	14.2	13.6	12.5	12.3	11.7	11.6
Met	4.1	4.1	3.4	3.4	3.5	3.5	3.2	3.2

CON: basal diet; LME: reduction of 10% of ME in basal diet; LCP: reduction of 10% of CP in basal diet; MECP: reduction of 10% of CP and 10% of ME in basal diet.

¹Standard levels of metabolic energy and crude protein; ²Reducing metabolic energy and crude protein levels

³Provided per kilogram of diet: 4,500 mg of vitamin A, 93.75 mg of vitamin D3, 37.5 mg of vitamin E, 2.55 mg of vitamin K3, 3 mg of thiamin, 7.5 mg of riboflavin, 4.5 mg of vitamin B6, 24 µg of vitamin B12, 51 mg of niacin, 1.5 mg of folic acid, 0.2 mg of biotin, and 13.5 mg of pantothenic acid; 37.5 mg of Zn, 37.5 mg of Mn, 37.5 mg of Fe, 3.75 mg of Cu, 0.83 mg of I, 62.5 mg of S, and 0.23 mg of Se

After the feed and faecal samples were mixed with a blender, they were analysed for dry matter (DM) (method 930.15) (AOAC, 2005) and CP (method 990.03) (AOAC, 2005) concentrations. Nitrogen (N) was determined with a Kjeltac 2300 nitrogen analyser (Foss Tecator AB, Hoeganaes Sweden). CP was calculated as $N \times 6.25$. Gross energy (GE) was analysed by the heat of combustion in the sample with a Parr 6100 oxygen bomb calorimeter (Parr Instrument Co., Moline, IL).

During the experiment, individual BWs of piglets and feed consumption were weighed at days 0, 14, 42 of the experimental period and recorded to calculate ADG, ADFI and G : F ratio. All piglets were fed diets to which 0.20% chromic oxide (Cr_2O_3) was added as an indigestible marker on day 38 to determine the ATTD of DM, CP and energy (Ball & Aherne, 1987).

Faecal samples were collected from all piglets by rectal massage and stored at $-20\text{ }^\circ\text{C}$ in the refrigerator. Additionally, frozen faecal samples were mixed in a blender for 15 minutes and then analysed with the above methods (AOAC, 2005). Chromium was determined via ultraviolet absorption spectrophotometry (UV-1201, Shimadzu, Kyoto, Japan), according to the method of Williams *et al.* (1962). The ATTD of DM and N were calculated with indirect-ratio methods using the following formula:

$$\text{Coefficient of apparent total tract digestibility} = \{1 - [(N_f \times C_d) / (N_d \times C_f)]\} \times 100$$

where: N_f = nutrient concentration in faeces (% DM)
 N_d = nutrient concentration in diet (% DM)
 C_f = chromium concentration in faeces (% DM)
 C_d = chromium concentration in diets (% DM)

To measure the blood profile, all piglets from each pen were used to collect blood samples. These samples were collected via vacuum tubes (Becton Dickinson & Co., Franklin Lakes, NJ) on day 41. They were transferred and pre-processed as described in Hossain *et al.* (2016). The concentration of red blood cells (RBC) and white blood cells (WBC) and lymphocyte percentage were analysed with an automatic blood analyser (ADVIA, Bayer, Tarrytown, NY). The concentration of immunoglobulin G (IgG) and BUN were determined with a nephelometer (Dade Behring, Marburg, Germany) and the concentrations of BUN and glucose were determined with an automatic biochemistry analyser (Hitachi 747, Hitachi Ltd., Tokyo, Japan).

To measure odour gas emission, faeces and urine were collected from all piglets of each pen at day 42 of the experimental period. Faecal and urine (100 g + 100 g) samples were mixed and stored in a plastic box (Size: 4.2 L) at room temperature ($28\text{ }^\circ\text{C}$) and allowed to ferment from days 42 to 45. The plastic boxes were punctured with a roasting drill and headspace air was sampled approximately 2.0 cm above the samples (faeces and urine) at a rate of 100 mL/min. The concentrations of NH_3 , H_2S and VFAs were measured using a gas detector tube of Gastec Corp (scope of 1.0–30.0 ppm; No. 3L for NH_3 , scope of 2.5–60.0 ppm; No. 4LL for H_2S and scope of 0.25–10.0ppm; No. 81L for VFA).

After measuring odour gas emission, the rest of the faeces was used to analyse microbiota and stored until further analyses in the laboratory refrigerator. The dilution and counting for measuring microbiota in the faeces were carried out in the same way as Zhao *et al.* 2013 had recorded.

All the data concerning growth performance, nutrient digestibility, blood profile, odour gas emission and faecal microbiota were analysed by ANOVA using the general linear model (GLM) procedure of SAS (SAS Institute, 2008), with the pen being defined as the experimental unit. Differences among treatments were separated by Duncan's multiple range test. The results were expressed as the least square mean and SEM. Probability values less than 0.05 were considered significant.

Results

Growth performances of piglets fed experimental dietary treatments are presented in Table 2. The final BW of weaned piglets fed the CON diet was greater ($P < 0.05$) by 1.0 kg and by 0.64 kg, respectively, compared with weaned piglets fed LCP and MECP diet. The ADFI decreased ($P < 0.05$) in piglets fed the CON diet for days 0 to 14, compared with those fed the other treatment diets. During days 14 to 42 and for the whole experimental period, ADFI improved ($P < 0.05$) in piglets fed the LME and LCP diets compared with those fed the CON diets. ADG and G : F ratio decreased ($P < 0.05$) in piglets fed the LCP, LME and MECP diets, compared with those fed the CON diet. However, during the total experimental period, ADG and G : F ratio were not affected ($P > 0.05$) by dietary treatment.

Table 2 Effects of low metabolic energy and crude protein in diets on growth performance in weaning pigs¹

Items ²	CON	LME	LCP	MECP	SE ³	P-value
Bodyweight, kg						
d 0	6.83	6.81	6.78	6.79		
d 14	15.05	15.02	15.12	15.14	0.13	0.90
d 42	28.30 ^a	27.80 ^{ab}	27.30 ^b	27.36 ^b	0.28	0.04
ADG, g						
d 0–14	391.6	391.2	397.2	397.7	7.0	0.86
d 14–42	630.8 ^a	608.3 ^{ab}	579.7 ^b	581.9 ^b	13.4	0.02
d 0–42	511.1	499.8	488.4	489.9	7.0	0.08
ADFI, g						
d 0–14	621.0 ^c	627.8 ^{ab}	626.0 ^b	631.5 ^a	1.8	0.01
d 14–42	865.8 ^a	858.5 ^b	860.3 ^b	866.3 ^a	1.2	0.01
d 0–42	687.5 ^b	686.5 ^b	686.0 ^b	691.0 ^a	0.9	0.01
G : F						
d 0–14	0.631	0.623	0.635	0.630	0.011	0.90
d 14–42	0.729 ^a	0.709 ^{ab}	0.674 ^b	0.673 ^b	0.015	0.03
d 0–42	0.744	0.729	0.712	0.709	0.010	0.07

¹CON: basal diet; LME: reduction of 10% of ME in basal diet; LCP: reduction of 10% of CP in basal diet; MECP: reduction of 10% of CP and 10% of ME in basal diet

²ADG: average daily gain; ADFI: average daily feed intake; G : F: gain to feed ratio

³Standard error

^{ab}Means in the same row with different superscripts differ ($P < 0.05$)

The ATTD of DM, CP and GE of piglets fed experimental dietary treatment are presented in Table 3. The ATTD of DM and GE decreased ($P < 0.05$) in piglets fed LME, LCP and MECP diets compared with those fed the CON diet. However, there were no significant differences ($P > 0.05$) in ATTD of CP among CON, LME and LCP treatments.

Table 3 Effects of low metabolic energy and crude protein in diets on nutrient digestibility in weaning pigs¹

Items, %	CON	LME	LCP	MECP	SE ²	P-value
Dry matter	84.78 ^a	81.01 ^c	82.69 ^b	78.07 ^d	0.50	0.01
Crude protein	80.08 ^a	78.64 ^{ab}	76.90 ^{ab}	71.94 ^b	0.83	0.01
Gross energy	84.18 ^a	81.15 ^b	79.45 ^b	76.29 ^c	0.55	0.01

¹CON: basal diet; LME: reduction of 10% of ME in basal diet; LCP: reduction of 10% of CP in basal diet; MECP: reduction of 10% of CP and 10% of ME in basal diet

²Standard error

^{ab}Means in the same row with different superscripts differ ($P < 0.05$)

Blood profiles of piglets fed the experimental dietary treatments are presented in Table 4. The concentrations of glucose, IgG, WBC, RBC and lymphocyte percentage were not affected ($P > 0.05$) by dietary treatment. However, the concentration of BUN decreased ($P < 0.05$) in piglets fed LME, LCP and MECP compared with those fed the CON diet.

Emissions of NH₃, H₂S and VFAs of piglets fed the experimental dietary treatment are presented in Table 5. Odour emissions such as H₂S and VFAs of piglets fed LME, LCP and MECP diets and emission of NH₃ of piglets fed LCP and MECP diet decreased ($P < 0.05$) compared with the CON diet.

Table 4 Effects of low metabolic energy and crude protein in diets on blood profile in weaning pigs¹

Items ²	CON	LME	LCP	MECP	SE ³	P-value
BUN, mg/dL	10.4 ^a	7.5 ^b	6.2 ^b	7.8 ^b	0.8	0.01
Glucose, mg/dL	104	105	103	102	10	0.10
IgG, mg/dL	228	240	216	220	17	0.70
WBC, 10 ³ /μℓ	20.0	19.1	19.4	16.8	1.6	0.51
RBC, 10 ⁶ /μℓ	7.00	6.26	6.81	6.84	0.22	0.13
Lymphocyte, %	77.8	76.0	80.8	77.5	1.7	0.34

¹CON: basal diet; LME: reduction of 10% of ME in basal diet; LCP: reduction of 10% of CP in basal diet; MECP: reduction of 10% of CP and 10% of ME in basal diet

²BUN: blood urea nitrogen; IgG: immunoglobulin G; WBC: white blood cell; RBC: red blood cell

³Standard error

^{ab}Means in the same row with different superscripts differ ($P < 0.05$).

Table 5 Effects of low metabolic energy and crude protein in diets on odour gas emission in weaning pigs¹

Items, ppm ²	CON	LME	LCP	MECP	SE ³	P-value
NH ₃	3.64 ^a	3.35 ^a	1.69 ^b	1.31 ^b	0.46	0.0001
H ₂ S	17.25 ^a	13.00 ^b	10.88 ^b	10.75 ^b	2.02	0.0004
VFAs	3.00 ^a	0.75 ^b	1.38 ^b	1.00 ^b	0.42	<.0001

¹CON: basal diet; LME: reduction of 10% of ME in basal diet; LCP: reduction of 10% of CP in basal diet; MECP: reduction of 10% of CP and 10% of ME in basal diet

²NH₃: ammonia; H₂S: hydrogen sulfide; VFAs: volatile fatty acids

³Standard error

^{ab}Means in the same row with different superscripts differ ($P < 0.05$).

Microbiota in faeces of piglets fed the experimental dietary treatments are presented in Table 6. There were no significant differences ($P > 0.05$) between treatments in the faecal *E. coli* counts. However, *Lactobacillus* counts in piglets fed the LCP diet decreased ($P < 0.05$) compared with those fed other diets.

Table 6 Effects of low metabolic energy and crude protein in diets on faecal microbiota in weaning pigs¹

Items, log ₁₀ /g	CON	LME	LCP	MECP	SE ²	P-value
<i>Lactobacillus</i>	7.83 ^a	7.50 ^{ab}	7.31 ^b	7.54 ^{ab}	0.16	0.01
<i>E. coli</i>	5.07	4.67	5.20	4.50	0.34	0.55

¹CON: basal diet; LME: reduction of 10% of ME in basal diet; LCP: reduction of 10% of CP in basal diet; MECP: reduction of 10% of CP and 10% of ME in basal diet

²Standard error

^{ab}Means in the same row with different superscripts differ ($P < 0.05$).

Discussion

Previous studies in which reducing 4% dietary CP with supplementation of amino acid (AA) during the nursery phase have shown that growth performance could be maintained compared with a control diet formulated to meet or exceed NRC (1998) recommendations (Hansen *et al.*, 1993; Kerr *et al.*, 1995). Reducing dietary CP with supplementation of AA indicated positive effects such as a reduction in the amount

of N excreted, improving or maintaining performance, and carcass composition against control diet (Wahlstrom & Libal, 1974; Easter & Baker, 1980; Kerr & Easter, 1995; Kendall, 2000; Figueroa *et al.*, 2002). However, previous studies did not conduct experiments but merely reduced CP in diets, and growth performance and carcass characteristics of pigs fed reduced CP diets varied (Dourmad *et al.*, 1993; Kerr *et al.*, 1995; Figueroa *et al.*, 2002). Therefore, this experiment was performed to investigate the effects of pure reduction of CP and ME content in diets on growth performance, nutrient digestibility, blood profile, faecal microflora and odour gas emission in weaned piglets.

Supplementation of dietary protein and energy in the weaning phase is closely related to growth performance. Kephart & Sherrit (1990) and Shriver *et al.* (2003) reported that pigs fed a low CP amino acid-supplemented diet had slower growth rates than those fed the CON diet. Additionally, Russell *et al.* (1983) reported that growth performance was not affected by reducing CP by 4% with the addition of lysine, tryptophan and threonine. In the current study, it was shown that pigs fed three treatment diets (LME, LCP, and MECP) decreased ($P < 0.05$) in ADG and G : F ratio compared with pigs that were fed control diet on days 14 to 42, although there were no significant differences ($P > 0.05$) between the CON and treatment diets on days 0 to 42. On days 0 to 14, pigs fed the three treatment diets increased in ADFI compared with those that were fed the control diet. However, there were no significant difference ($P > 0.05$) on days 14 to 42 and on days 0 to 42 between the CON, LME and LCP treatments. According to the results of growth performance, final bodyweight was significantly decreased by LCP, MECP diets at day 42. However, since this is the result from a limited period, further research should be conducted from new-born to shipment.

In the current experiment, the results showed that pigs fed the control diet increased ($P < 0.05$) in digestibility of DM and GE compared with pigs fed the three treatment diets. Stahly (1984) reported that the supplementation of fat in diets increased nutrient digestibility. These results mean that fat is digested more efficiently, which promotes more energy being available, since less heat is being in fermentation and nutrient metabolism. Therefore, the digestibility of pig fed the control diet was higher than pigs fed the treatments. Bikker *et al.* (2006) reported that the apparent ileal digestibility (AID) of CP in weanling pigs was decreased by reducing the dietary CP content from 21.7% to 15.3% by replacing soybean meal in a high-CP diet with wheat in the low CP diet. Also, numerous reports indicated an increase in nitrogen digestibility as CP levels rose (Just, 1982a; Just, 1982b; Noblet *et al.*, 1987; Kephart & Sherritt, 1990). However, results of the digestibility of CP from the current study are different from those performed by Noblet *et al.* (1987), Kephart & Sherritt. (1990) and Bikker *et al.* (2006). Our results showed that pigs fed LCP diet in the digestibility of CP was similar to pigs fed CON diet.

Results from the current experiment showed that BUN of pigs fed the control diet increased ($P < 0.05$) compared with pigs fed the treatment diets. However, there were no significant differences ($P > 0.05$) among the experimental diets on glucose, IgG, WBC, RBC and lymphocyte. Whittemore *et al.* (1978) and Whang *et al.* (2003) found that the BUN levels or N breakdown and excretion were lower in the pigs fed a protein-deficient diet than those that were fed a normal diet. For unknown reasons, results from the current experiment showed similar results to those reported by Whittemore *et al.* (1978) and Whang *et al.* (2003).

Compounds that cause odour may be classified in four groups: i) sulfurous compounds; ii) indolic and phenolic compounds; iii) VFAs; and iv) ammonia and amines (Bonnin *et al.*, 1990). The production of microbial metabolites (ammonia and VFAs) in digesta were used as indicators of intestinal health and microbial activity (Nyachoti *et al.*, 2006). In the current study, it was shown that NH_3 , H_2S , VFAs emissions in pigs fed LCP and LME were lower ($P < 0.05$) than in pigs fed the control diet. Further, the odour emission (H_2S and VFAs) of pigs fed the MECP diet was similar to pigs fed the LME and LCP diets. Reduced odour gases such as NH_3 , H_2S , VFAs in weaned piglets fed low protein and low metabolic energy diets agrees with those reported by Aarnink *et al.* (1993), Turner *et al.* (1996), and Nyachoti *et al.* (2006). However, there was no study to investigate mechanisms to reduce odour gas emission. Therefore, further research is needed to understand the mechanism.

Wellock *et al.* (2008) reported that decreasing dietary protein supply might help to reduce enterotoxigenic *Escherichia coli* (ETEC) proliferation and faecal ETEC shedding. High protein in the diets encourages the proliferation of pathogenic bacteria by increasing microbial fermentation of undigested protein (Ball & Aherne, 1987). However, the current experiment showed that pigs fed LCP tended ($P < 0.055$) to increase the count of *E. coli* in faeces compared with those that were fed the CON. In addition, when pigs were fed the LCP diet, *Lactobacillus* of beneficial bacteria was lower ($P < 0.05$) than in those fed the control diet (CON). The component of intestinal microbiota is known to have a great deal of volatility such as in temperature and use of probiotics (Penders *et al.*, 2006). Therefore, further research is needed about a relationship between microflora and dietary nutrient density.

Conclusion

These results indicated that reducing dietary CP and ME could decrease BUN concentration in the

blood and NH₃, H₂S, VFAs emissions in faeces without affecting growth performance in weaned piglets.

Furthermore, these results indicated that reducing dietary CP and ME decreased BUN concentration in blood and NH₃, H₂S, VFAs emission in faeces. However, growth performance was not affected by reducing dietary CP and ME.

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Authors' Contributions

JHC and MHS oversaw the project design. WY supervised the course of the study. CHL, WGK, HJO oversaw sample collecting and participated in results and statistics. JHL and HBK wrote the manuscript and revised it.

Conflict of Interest Declaration

The authors declare that they have no conflict of interest.

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