Journal of Animal Science, 2022, **100**, 1–13
https://doi.org/10.1093/jas/skac080
Advance access publication 15 March 2022
Non Ruminant Nutrition

Full-fat field cricket (*Gryllus bimaculatus*) as a substitute for fish meal and soybean meal for weaning piglets: effects on growth performance, intestinal health, and redox status

Waewaree Boontiam,[†,1] Jinsu Hong,[‡] Sumetee Kitipongpysan,[§] and Suchat Wattanachai[¶]

[†]Division of Animal Science, Faculty of Agriculture, Khon Kaen University, Khon Kaen 40002, Thailand
[‡]Department of Animal Science, South Dakota State University, Brookings, SD 57007, U083SA, USA
[§]Division of Agriculture, Faculty of Agriculture and Life Science, Chandrakasem Rajabhat University, Bangkok 10900, Thailand
[¶]Division of Surgery and Theriogenology, Faculty of Veterinary Medicine, Khon Kaen University, Khon Kaen 40002, Thailand
[1]Corresponding author: waewbo@kku.ac.th

Abstract

Full-fat field cricket meal (**FCP**) is an alternative protein ingredient in livestock production; however, the effects of replacing conventional protein sources with FCP in nursery diets have not been determined. In this study, the effects of the partial replacement of either fish meal or soybean meal with FCP on weaning pigs were evaluated, including the analyses of growth performance, nutrient utilization, intestinal morphology, immunity, oxidative stress, and fecal microbial counts. A total of 100 crossbred weaning pigs [(Landrace × Large White) × Duroc] were allotted to one of the following five treatments with five replicates (four pigs/pen) and fed for 28 d postweaning. Treatments were 1) a corn-soybean meal (**SBM**)-based diet with 5% fish meal (Positive control; PC), 2) a corn-SBM-based diet without fish meal (Negative control; NC), 3) field crickets replacing fishmeal on a total Lys basis (**FCP1**), 4) field crickets replacing fishmeal on a kg/kg basis (**FCP2**), and 5) field crickets replacing fish meal and soybean meal (**FCP3**). The piglets on FCP1 had a higher body weight on days 14 and 28, and an increased average daily gain over the experimental period than NC ($P < 0.05$); FCP2 and FCP3 were similar to the FCP1 treatment. The incidence of diarrhea was lower under an FCP-supplemented diet than under the NC diet throughout the study ($P < 0.05$). Pigs fed FCP1 and FCP2 had a higher digestibility of crude protein ($P = 0.041$), and all FCP groups increased crude fat digestibility ($P = 0.024$). FCP1 and FCP2 also increased jejunal villus height ($P = 0.009$), whereas the increase in jejunal villus-to-crypt ratios ($P = 0.019$) was greater in pigs fed the FCP2 diet than those fed the NC diet. Furthermore, FCP2 supplementation increased serum immunoglobulin A levels on days 14 and 28, including reduced serum interleukin-6 and tumor necrosis factor alpha levels ($P < 0.05$). Pigs fed an FCP2 diet had reduced malondialdehyde levels than those fed a PC diet, while pigs fed an FCP2 diet had higher superoxide dismutase and glutathione peroxidase levels, and more fecal *Lactobacillus* spp. than those fed an NC diet ($P < 0.05$). These results support the use of FCP as an alternative protein ingredient with beneficial effects on growth performance, intestinal morphology, antioxidant capacity, and intestinal microbiota. In particular, FCP can be used as a partial substitute for fish meal and soybean meal without detrimental effects on weaning pigs.

Lay Summary

Crickets are a promising, widely available protein source for pigs; however, their practical utilization requires detailed analyses of their effects in comparison with those of traditional protein sources. In this study, we demonstrate that the partial replacement of either fish meal or soybean meal with full-fat field cricket meal promotes growth and reduces the rate of diarrhea in weaning pigs. These beneficial effects may be mediated by the effects of FCP on intestinal morphology, serum immunoglobulin A concentration, proinflammatory and antioxidant enzyme activity, and fecal microbiota. Our findings provide a basis for the expanded use of field cricket powder as a replacement for fish meal and soybean meal in livestock feed.

Key words: full-fat cricket, high protein ingredient, weaning pig, gut integrity, redox status, growth performance

Abbreviations: ADG, average daily gain; ADFI, average daily feed intake; BW, body weight; CD, crypt depth; FCP, field cricket powder; ELISA, enzyme-linked immunosorbent assay; FM, fish meal; G:F, gain-to-feed ratio; GPx, glutathione peroxidase; IgA, immunoglobulin A; IL1, interleukine-1β; IL6, interleukine-6; MDA, malondialdehyde; ROS, reactive oxygen species; SBM, soybean meal; SFA, saturated fatty acids; SOD, superoxide dismutase; TNFα, tumor necrosis factor alpha; TMB, 3,3,5,5′-tetramethylbenzidine; VH, villus height; VH:CD, villus height/crypt depth

Introduction

The global population is projected to increase over 9.6 billion in 2050, and global food demand is expected to rise by 56%, with a concomitant increase in hunger (United Nation, 2013; Dijk et al., 2021). In order to overcome a global food crisis with regard to the increasing global population, suppling adequate animal-based meat for consumers is an urgent issue. Future livestock production needs to find an effective approach towards sustainability in potential feedstuffs which are greater in quantity, reasonably priced, and provide nutrients comparable to conventional feed ingredients. It is well established that fish meal (**FM**) and soybean meal (**SBM**) are conventional protein ingredients which are commonly used in poultry and swine diets owing to their balance of

Received November 21, 2021 Accepted March 11, 2022.

amino acids, fatty acids, vitamins, and minerals (NRC, 2012). However, these ingredients are relatively expensive; moreover, FM contains pathogens and several biogenic amines, whereas SBM represents antinutritional factors which consequently cause transient hypersensitivity (Li et al., 1990; NRC, 2012; Jones et al., 2018). This subsequently causes an abnormality of the gastrointestinal tract via decreased villus height (**VH**) and increased crypt hypertrophy, which can impair animal performance (Li et al., 1990). Recently, alternative feed derived from insects or insect meal has been introduced in swine diets to substitute FM or SBM (Veldkamp and Vernooij, 2021). Insects represent approximately three-fourths of the total species found in nature (Pedigo, 2002). They may be amenable to mass production under controlled environments, with approximately 10 to 11 generations per year, making them an attractive novel protein ingredient in animal nutrition.

The field cricket (*Gryllus bimaculatus*) is commonly found in Asia, Africa, and Southern Europe and can be easily harvested in considerable quantities. It can be used as an alternative protein source for monogastric animals (Ibitoye et al., 2019; Permatahati et al., 2019). Rats fed field cricket powder (**FCP**) did not show any toxicological responses (Lee et al., 2016). Wang et al. (2005) demonstrated that FCP contains a high level of crude protein, ranging from 55% to 73%, as well as sufficient essential amino acids. Furthermore, the true amino acid digestibility of FCP is higher than that of fish meal (92.9% vs. 91.3%). It can be included at 15% or 8% as a partial or full replacement for FM in the diet of broiler chickens, without any detrimental effects on growth performance, feed efficiency, and nutrient digestibility (Wang et al., 2005; Kovitvadhi et al., 2019). A previous study of weaning pigs has shown that supplementation of the diet with 17.8% whole cricket meal and 18.5% body cricket meal results in greater weight gain after 10 d of feeding and greater nutrient digestibility than in pigs fed FM (Miech et al., 2017). However, studies of the use of FCP to replace protein sources in the diet of weaning pigs are lacking. We hypothesize that dietary FCP would provide adequate nutrients and result in similar or superior gut health and performance to conventional protein sources. In this study, the effects of full-fat field cricket as a sustainable substitute for either FM or SBM on growth performance, nutrient utilization, intestinal morphology, immunity, redox status, and microbial counts were evaluated in weaning piglets.

Materials and Methods

The procedure (authorization no. IACUC-KKU 22/64) was approved by the Institutional Animal Care and Use Committee of Khon Kaen University (Khon Kaen, Thailand).

Cricket preparation

Adult field crickets (50 ± 5 d) were harvested, washed with tap water, and freeze-dried at -40 °C (Santor, Namphong, Khon Kaen, Thailand). One kilogram of crickets was heated at 100 °C for 30 min, oven-dried at 65 °C for 48 h, and ground to an 80-mesh particle size. Moisture, ash, crude protein, ether extract, crude fiber, nitrogen-free extract, calcium, phosphorus, and gross energy were analyzed following the guideline by AOAC (2000). The ground field cricket samples (300 g each) were transferred to the laboratory (Central Laboratory, Muang, Khon Kaen, Thailand) for quantitative amino acid and fatty acid profiling following standard procedures, as described by Sarwar et al. (1988) and the AOAC (2000), respectively.

Animals, management, and treatments

One hundred (50 barrows and 50 gilts) crossbred piglets [(Landrace × Large White) × Duroc] were weaned at 28 ± 2 d of age (7.33 ± 1.43 kg). The weaning pigs were housed in pens with slatted concrete floors (20 slats, 1.6 × 2.1 m) at a stocking density of 0.84 m^2 per pig. Each pen was equipped with a low-pressure nipple drinker, stainless self-feeder, and heating lamp. Bedding materials of rice straw and gunny bags were provided and changed daily at 0600 and 1900 for 2 wk after weaning as per the European instructions (EU Directive 2010/63/EC for animal experiment). The housing temperature was controlled at 32 ± 1 °C during the first week and was decremented by 1 °C per week for a 28-d trial. The vaccination programs for transmissible gastroenteritis, Aujeszky's disease, salmonellosis, and porcine respiratory syndrome were administered to the weaning pigs following the farm practice.

A total of 100 healthy weaning pigs were assigned to five dietary treatments with five replicates and four pigs each (two barrows and two gilts) in a randomized complete block design. The five treatments were as follows: corn-SBM based diet with 5% fish meal (positive control; **PC**), corn-SBM based diet without animal protein (negative control; **NC**), field cricket replacing fishmeal on a total Lys basis (**FCP1**), field cricket replacing fishmeal on a kg/kg basis (**FCP2**), and field cricket replacing fish meal and soybean meal (**FCP3**). Feed in two phases [Phase I (days 1 to 14 postweaning) and Phase II (days 15 to 28 postweaning)] was provided thrice daily (0600, 1200, and 1700) based on consumption on the previous day. The nutrient composition of the experimental diets was calculated to meet or exceed the recommendations for weaning pigs (Table 1; NRC, 2012). Mash feed and clean water were provided ad libitum throughout the entire period.

Growth performance and diarrhea rate

Body weight (**BW**) and feed consumption were recorded on days 0, 14, and 28 and used to calculate the average daily gain (**ADG**), average daily feed intake (**ADFI**), and feed efficiency (gain-to-feed ratio, **G:F**).

A visual assessment of diarrhea occurrence was performed every morning at 0800. The four consistency categories were: score 0 = firm and shaped, score 1 = soft and shaped, score 2 = loose and score, and 3 = watery, where scores of 0 and 1 indicate normal feces and scores of 2 and 3 indicate diarrhea. The diarrheal rate (%) was calculated as: number of piglets with diarrheal/(total number of piglets × diarrhea days) × 100.

Apparent total tract digestibility

A total of 15 crossbred barrows [10.46 ± 0.32 kg of initial BW; [(Landrace × Large White) × Duroc] were placed in individual pens for 5 d for adaptation and 3 d for fecal sample collection. During the experimental period, all pigs were adjusted to a daily allowance of three times the estimated maintenance requirement for energy (i.e., 106 kcal for metabolizable energy per kg of $BW^{0.75}$; NRC, 2012). Daily feed allowance was provided in two equal proportions at 0700 and 1900 h. Fecal collection was performed using the marker-to-marker approach. Chromic oxide was mixed in each experimental diet in the first meal, and ferric oxide was included in the last meal after the 3-d fecal collection. Both were included in the diet at the same amount (5 g/kg feed, as-fed basis; Adeola, 2001;

Table 1. Ingredient and nutrient composition of the experimental diet (% as fed basis)[1,2]

Ingredient	Phase I (days 1 to 14)					Phase II (days 15 to 28)				
	PC	NC	FCP1	FCP2	FCP3	PC	NC	FCP1	FCP2	FCP3
Corn	47.12	41.43	47.51	51.89	52.51	70.13	60.90	70.51	69.98	71.97
Dehull soybean meal	20.00	30.00	20.00	20.00	13.00	6.00	20.00	6.00	6.00	3.00
Fish meal	5.00	-	-	-	-	5.00	-	-	-	-
Field cricket meal	-	-	4.20	5.00	6.00	-	-	4.20	5.00	6.00
Broken rice	10.00	10.00	10.00	5.00	10.00	5.00	5.00	5.00	5.00	5.00
Sweet whey powder	15.00	15.00	15.00	15.00	15.00	10.00	10.00	10.00	10.00	10.00
Soybean oil	0.07	0.47	0.10	-	0.09	0.18	0.38	0.22	0.05	-
Dicalcium phosphate	0.60	1.03	1.08	1.06	1.18	0.97	1.30	1.47	1.45	1.50
Limestone	0.59	0.62	0.51	0.50	0.40	0.48	0.58	0.38	0.38	0.32
L-Lysine HCl (78%)	0.57	0.46	0.57	0.52	0.71	0.94	0.69	0.94	0.89	0.94
DL-Methionine (99%)	0.02	0.03	0.01	-	0.01	0.08	0.07	0.07	0.06	0.05
L-Threonine (99%)	0.23	0.16	0.22	0.23	0.30	0.42	0.28	0.41	0.39	0.42
Sodium chloride	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30
Vitamin-mineral premix	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Total	100	100	100	100	100	100	100	100	100	100
Calculated values, %										
Metabolizable energy, kcal/kg	3,400.17	3,400.12	3,400.10	3,400.16	3,400.10	3,360.20	3,360.38	3,360.06	3,360.13	3,360.62
Crude protein	19.48	21.01	19.20	19.60	17.43	14.15	17.15	13.86	14.22	13.56
Lysine	1.53	1.53	1.53	1.53	1.53	1.40	1.40	1.40	1.40	1.40
Methionine	0.44	0.44	0.44	0.44	0.44	0.40	0.40	0.40	0.40	0.40
Threonine	0.95	0.95	0.95	0.95	0.95	0.87	0.87	0.87	0.87	0.87
Calcium	0.80	0.80	0.80	0.80	0.80	0.80	0.80	0.80	0.80	0.80
Phosphorus	0.60	0.60	0.60	0.60	0.60	0.60	0.60	0.60	0.60	0.60
Chitin	0.00	0.00	0.20	0.24	0.29	0.00	0.00	0.20	0.24	0.29
Fat	2.89	2.32	3.49	3.82	4.04	3.51	2.86	4.11	4.29	4.58

[1]Provided (per kg of diet): 8,000 IU vitamin A; 1,600 IU vitamin D3; 34 IU vitamin E; 64 g d-biotin; 3.4 mg riboflavin; 8 mg calcium pantothenic acid; 16 mg niacin; 12 g vitamin B12; 2.4 mg vitamin K; 0.1 mg Se as Na_2SeO_3; 0.32 mg I as KI; 25.2 mg Mn as $MnSO_4$; 53.9 mg Cu as CuSO4; 127.3 mg Fe as FeSO4; 83.46 mg Zn as ZnSO4, and 0.28 mg Co as $CoSO_4$.
[2]Calculated values of ingredient composition data were obtained from feed manufacturing (Bangkok Animal Research Center, Co., Ltd., Thailand) and NRC (2012).

Wang and Adeola, 2018). The fecal output was collected when the appearance of marker-colored feces started and ceased when the marker-colored feces stopped. Feces were collected daily at 1900 h and weighed using a digital scale. Representative fecal sample were kept in sealed plastic bags, and feed samples in each dietary group were collected after mixing homogenously, and then dried for 72 h at 60 °C to a constant weight, after which they were finely ground with a centrifugal mill to a particle size of 0.88 mm. All ground, feed, and fecal samples were analyzed for dry matter (#procedure 930.15, forced dried oven at 135 °C for 2 h), crude protein (**CP**, #procedure 984.13; N x 6.25), ash (#procedure 942.15, muffle furnace at 600 °C for 2 h), and crude fat (#procedure 920.39, Soxhlet apparatus), following the protocol of the AOAC (2000). The apparent total tract digestibility was calculated as previously described by Adeola (2001).

Intestinal morphology

At the end of the feeding trial, four pigs per treatment (two barrows and two gilts) were selected based on the average pen body weight for slaughter at a local abattoir (Kasetsombun, Chaiyaphum, Thailand). Segments of the duodenum (at 30 cm caudal to the pyloric region of the stomach), jejunum (about two-fifths of the remaining segment of the small intestine below the duodenum), and ileum (at 50 cm above the ileocecal junction) were cut longitudinally (approximately 5 cm), gently flushed with phosphate saline solution, and washed with neutral buffer (pH 7.0) and formalin solution (10% vol/vol) for 72 h. Then, samples were dehydrated with ethanol, diaphanized with xylol, and rinsed in decreasing ethanol concentrations prior to embedding in liquid paraffin at 60 ± 5 °C using a rotary microtome. The embedded samples were sectioned transversely to a thickness of 4 to 5 µm, placed on glass slides, and stained with hematoxylin and eosin (H&E staining) at room temperature. A total of 10 intact, well-oriented villi were examined per slide, and mean parameter values for each weaning pig were obtained. Villus height (**VH**) was measured between the crypt tips and the villus-crypt junction, and the associated crypt depth (**CD**) was measured between the villus-crypt junction and submucosa using an optical light microscope (Olympus Biological Model CX31, Shinjuku, Tokyo, Japan) at 10× magnification. The villus height/crypt depth (**VH: CD**) was calculated.

Immunity and cytokine responses

On days 14 and 28, five healthy pigs per treatment (n = 25, two gilts and three barrows) were randomly chosen for blood collection (10 mL) by jugular vein puncture using a sterilized

syringe with a needle and the samples were transferred into a serum-coated tube with silica (BD Vacutainer, Plymouth, UK). The blood samples were placed at room temperature for 60 min and centrifuged at 13,000 × *g* for 5 min at 4 °C prior to storage at −20 °C. To quantify immunoglobulin A (**IgA**), each reagent was prepared according to the manufacturer's guideline prior to assays. Briefly, 100 µL of the standard solution (1/100 dilution) was added, and serum (10 µL) was supplemented with buffered saline solution to obtain a 1/20,000 dilution. The microplate was incubated at 37 °C for 45 min, and 100 µL of HRP-conjugated IgA was added to each well and placed at room temperature for 15 min. After washing with wash buffer (1/20 dilution with deionized water), a chromogenic substrate (100 µL, 3,3,5,5′-tetramethylbenzidine, **TMB**) was added. The stop solution (100 µL) was added after incubation for 10 min, followed by detection at 450 nm. Interleukine-1 (**IL1**) and Interleukine-6 (**IL6**) concentrations were determined using enzyme-linked immunosorbent assay (**ELISA**) Porcine Reagent Kits (Abcam, Cambridge, UK). Briefly, 100 µL of the standard and sample were added to appropriate wells and incubated for 2.5 h at 37 °C with gentle mixing. The discarded solution was washed four times with wash solution (300 µL), and 100 µL of biotinylated IL1 or biotinylated IL6 detection antibody was added, followed by incubation for 60 min. The HRP-Streptavidin solution (320-fold dilution; 100 µL) was pipetted into each well plate after washing, followed by incubation at room temperature for 45 min with gentle mixing. Then, TMB (100 µL) was added and incubated for 30 min, followed by the addition of stop solution (100 µL, 2 mol/L sulfuric acid). Tumor necrosis factor alpha (**TNFα**) was analyzed using the ELISA Porcine Immunoassay Kit (Abcam). One hundred microliters of sample were added to each plate coated with biotinylated TNF antibody reagent. Detection was performed using 3,3′,5,5′ tetramethylbenzidine and stop solution (2 mol/L H_2SO_4). All measurements were read at an absorbance of λ = 450 nm in triplicate, with a total of 5 samples per treatment (n = 25).

Antioxidant enzyme activity and malondialdehyde concentration

Serum antioxidant enzyme activity of superoxide dismutase (**SOD**) and glutathione peroxidase (**GPx**), including malondialdehyde (**MDA**) concentration were quantified using a commercial test kit (Sigma–Aldrich, St. Louis, MO) according to the manufacturer's protocol. To determine SOD, 200 µL of R1 reagent (WST solution diluted 20-fold with buffer) was incubated at 37 °C for 3 min, followed by the addition of 20 µL of sample. Twenty microliters of R2 reagent (enzyme solution diluted 167-fold with buffer) were pipetted into each microplate to start the reaction, followed by incubation at 37 °C for 72 s prior to detection. To assay GPx, the GPx buffer (900 µL, 50 mM Tris HCl, 0.5 mM EDTA) was pipetted into a quartz cuvette (1 mL), followed by the addition of 50 µL of a 0.25 mM solution of NADPH reagent. Then, 50 µL of sample was added to the cuvette and mixed by inversion to obtain a homogenous mixture. Then, 10 µL of 30 mM *tert*-butyl hydroperoxide solution (70% aqueous solution) was added and mixed. Similar to MDA, 600 µL of the thiobarbituric acid solution (reconstituted with 7.5 mL of glacial acetic acid) was injected into a 96-well plate with the subsequent addition of 200 µL of MDA-TBA. Serum (20 µL) containing a preparation of 300 µL of 1-butanol and 100 µL of 5 M NaCl was added prior to the colorimetric assay. SOD, GPx, and MDA were detected at λ = 450, 340, and 532 nm, respectively.

Microbial count

At the termination of the feeding trial, approximately 20 g of fresh fecal samples (five samples/treatment, n = 25) were collected via a rectal palpation. For the enumeration of *Escherichia coli, Salmonella* spp., and *Lactobacillus* spp., MacConkey, Salmonella-Shigella, and Lactobacillus medium II, respectively (Difco Laboratories, Detroit, MI), were prepared by suspending 50 g of the powder agar in 1,000 mL of deionized water, gentle mixing, boiling for 60 s until completely dissolved, and then sterilizing for 15 min in an autoclave at 121 °C. After cooling, 20 mL of each medium was poured on aseptic Petri dishes before inoculation. The composite of fecal samples (one gram) was diluted with 9 mL of peptone broth (Becton Dickinson, Franklin Lakes, NJ) and thoroughly mixed for 10 min using a vortex mixer. One milliliter of diluted feces was inoculated by directly streaking on the agar surface and inoculated aerobically at 35 °C for 24 h. The visible spotted colonies of each bacterium were determined based on morphology and color (pinkish-red for *E. coli*, colonies with a black center for *Salmonella* spp., and light tan colonies for *Lactobacillus* spp.). Values are presented as the logarithm of colony-forming units per gram of feces.

Statistical analysis

Data were analyzed using the general linear model procedure of SAS (version 9.4, SAS Inst, Inc., Cary, NC) in accordance with a randomized complete block design. Each pen served as the experimental unit for analysis for growth performance and diarrhea rate, whereas an individual pig served as the experimental unit for nutrient digestibility, intestinal morphology, blood analyses, and fecal microbial counts. The statistical model was analyzed according to the following mixed model:

$$\Upsilon_{ij} = \mu + \alpha_i + \beta_j + \varepsilon_{ij}$$

where Y_{ij} is the observed value for the experimental unit within the ith group of the FCP treatment of the jth block for the ith pen; μ is the population mean; α_i is the fixed effect of the ith group of FCP (i = 1 to 5); and β_j is the random effect of the jth block (j = 1 to 5); and ε_{ij} is the associated variance as presented by the model for Y_{ij}; assuming $\beta_j \sim N(0, I\sigma^2_{\beta j})$, and $\varepsilon_{ijk} \sim N(0, I\sigma^2_{\varepsilon})$, where I is the identity matrix. Tukey's multiple range test was employed for comparisons of parameter values among dietary treatments at $P < 0.05$. Results are presented as the least square means and standard error of the mean for all measurements.

Results

Nutritional composition

The nutritional composition of FCP is summarized in Table 2. The FCP powder was enriched in crude protein (52.70%), crude fat (25.50%), and crude fiber (7.39%). The total essential amino acid content of FCP (17.77%) was comparable to that of FM and SBM (21.95% and 21.31%, respectively). Lysine was the most common essential amino acid, followed by tyrosine and leucine, accounting for 4.58%, 2.62%, and 2.61%, respectively, which is higher than in FM and

Table 2. Nutrient composition of field cricket powder (*Gryllus bimaculatus*, dry matter basis)[1]

Nutrients	Content, g/100 g			Nutrients	Field cricket, g/100 g
	Field cricket	Fish meal	SBM[2]		
				Saturated fatty acids (SFA)	
Dry matter	95.43	93.70	88.64	Butyric acid (C4:0)	ND[8]
Ash	5.27	15.07	—	Caproic acid (C6:0)	ND
Crude protein	52.70	59.78	51.60	Caprylic acid (C8:0)	0.11
Ether extract	25.50	8.69	1.70	Capric acid (C10:0)	ND
Crude fiber	7.39	0.76	5.63	Undecanoic acid (C11:0)	ND
Nitrogen-free extract	4.57	—	—	Lauric acid (C12:0)	1.93
Calcium	0.58	4.05	0.34	Tridecanoic acid (C13:0)	ND
Phosphorus	0.69	2.64	0.14	Myristic acid (C14:0)	0.05
GE, kcal/kg[3]	5,907.74	4,563	4,834	Pentadecanoic acid (C15:0)	0.01
ME, kcal/kg[4,5]	4,323	3,821	3,344	Palmitic acid (C16:0)	0.02
Essential amino acids[6]				Heptadecanoic acid (C17:0)	0.02
Isoleucine	1.59	1.91	2.39	Steric acid (C18:0)	0.83
Leucine	2.61	3.81	4.00	Unsaturated fatty acids (USFA)	
Lysine	4.58	3.98	3.14	Palmitoleic acid (C16:1n7)	0.09
Methionine	0.33	1.44	0.70	cis-10-heptadecanoic acid (C17:1n10)	0.02
Phenylalanine	1.99	2.55	2.67	cis-9-Oleic acid (C18:1n9)	2.02
Threonine	0.70	2.17	2.04	cis-9,12-linoleic acid (C18:2n6)	2.99
Tryptophan	0.21	0.54	0.64	α-Linolenic acid (C18:3n3)	0.11
Tyrosine	2.62	1.88	1.91	γ-Linolenic acid (C18:3n6)	0.03
Histidine	1.34	1.34	1.36	cis-11,14-Eicosadienoic acid (C20:2)	ND
Valine	1.80	2.33	2.46	cis-11,14,17-Eicosatrienoic acid (C20:3n3)	ND
Total	17.77	21.95	21.31	cis-8,11,14-Eicosatrienoic acid (C20:3n6)	ND
EAA recovery, %	33.72	36.72	41.30		
Nonessential amino acids[5,6]				Arachidonic acid (C20:4n6)	ND
Alanine	2.06	3.93	2.07	Eicosapentaenoic acid (C20:5n3)	ND
Arginine	1.09	3.05	3.77	Erucic acid (C22:1n9)	ND
Aspartic acid	1.51	5.41	5.66	Docosadienoic acid (C22:2)	ND
Cystine	0.82	0.61	2.11	Docosahexaenoic acid (C22:6n3)	ND
Glutamic acid	2.50	7.88	8.94	Nervonic acid (C24:1n9)	0.05
Glycine	1.04	4.71	2.11	Total SFA	2.97
Serine	0.86	2.43	2.24	Total USFA	5.31
Proline	1.47	2.89	2.47		
Total	11.35	30.91	29.37		
NEAA recovery[7]	21.54	51.71	56.92		
Total amino acid recovery, %[1]	55.26	88.42	98.22		

[1]Amino acid recovery = (total amino acid/% crude protein) × 100.
[2]SBM, soybean meal.
[3]GE, gross energy.
[4]ME, metabolizable energy.
[5]NRC (2012).
[6]Data were obtained from Bangkok Animal Research Center, Co., Ltd.
[7]NEAA, nonessential amino acids.
[8]ND, not detected.

SBM (with the exception of leucine). Among the total saturated and unsaturated fatty acids, lauric acid (C12:0) and linoleic acid (C18:2n6) levels were the highest, accounting for approximately 1.93% and 2.99% of the total fatty acids, respectively.

Growth performance and diarrhea incidence

The growth performance and occurrence of diarrhea in weaning pigs fed FCP as a replacement for SBM and fish meal are presented in Table 3. No significant differences in BW, ADG, ADFI, G:F ratio, and diarrheal rate were observed in any periods between PC and NC, and PC and FCP-supplemented diets ($P > 0.05$). However, BW of weaning pigs fed FCP1 was higher on days 14 and 28 by 11.80% ($P = 0.19$) and 8.63% ($P = 0.027$), respectively, compared to those fed the NC diet. Supplementation with FCP1 and FCP3 significantly improved ADG in the overall period in comparison to NC ($P = 0.007$). In addition, diarrhea occurrence was lower in piglets fed the FCP1 and FCP2 diets

during phase I and II ($P = 0.013$ and $P = 0.005$, respectively) than in those fed the NC diet but comparable to PC exception for the overall period. The FCP-supplemented diets also decreased the diarrhea rate in weaning pigs for the overall period ($P = 0.007$).

Apparent total tract digestibility

The apparent total tract digestibility of weaning pigs in each group is summarized in Table 4. The apparent total tract digestibility of nutrients did not differ between the PC and NC diets ($P > 0.05$). Furthermore, the digestibility of DM, CP and ash was comparable for PC and FCP-supplemented diets ($P > 0.05$), with the exception of crude fat digestibility, which was lower in pigs fed a FCP1 diet ($P = 0.024$). Pigs fed the FCP1 and FCP2-supplemented diets had greater fat digestibility than pigs fed the NC diet ($P = 0.041$).

Intestinal morphology

No significant difference in intestinal morphology was observed between the PC and NC, and PC and FCP-supplemented diets ($P > 0.05$; Table 5). However, jejunal VH in pigs fed the FCP1 and FCP2 diets was greater than that in pigs fed the NC diet ($P = 0.009$). FCP2 also increased jejunal VH:CD compared to the NC treatment ($P = 0.019$). However, CD show no effect of FCP supplementation in any segment of the small intestine ($P > 0.05$).

Table 3. Growth performance and diarrheal incidence in weaning pigs fed field cricket powder[1]

Item	Treatment[1]					SEM	*P*-value
	PC	NC	FCP1	FCP2	FCP3		
BW, kg[2]							
Day 0	7.32	7.32	7.35	7.36	7.29	0.239	0.999
Day 14	10.42^{ab}	10.17^{b}	11.37^{a}	10.87^{ab}	10.64^{ab}	0.228	0.019
Day 28	15.63^{ab}	15.18^{b}	16.49^{a}	16.11^{ab}	16.27^{ab}	0.277	0.027
ADG, g/d							
Days 1 to 14	221.82	203.68	287.25	250.25	239.14	18.371	0.053
Days 15 to 28	371.96	357.57	365.86	374.54	402.21	17.270	0.460
Overall	296.89^{ab}	280.63^{b}	326.55^{a}	312.39^{ab}	320.68^{a}	8.229	0.007
ADFI, g/d							
Days 1 to 14	344	336	328	344	343	20.066	0.969
Days 15 to 28	662	658	648	652	657	17.750	0.980
Overall	541	534	563	529	549	12.193	0.348
G:F ratio							
Days 1 to 14	0.649	0.634	0.888	0.761	0.698	0.088	0.279
Days 15 to 28	0.566	0.544	0.562	0.575	0.617	0.032	0.591
Overall	0.555	0.527	0.582	0.591	0.588	0.018	0.119
Diarrhea rate, %							
Days 1 to 14	6.29^{ab}	8.81^{a}	3.06^{b}	4.18^{b}	5.91^{ab}	1.040	0.013
Days 15 to 28	2.36^{ab}	3.62^{a}	0.97^{b}	0.85^{b}	2.09^{b}	0.479	0.005
Overall	8.25^{ab}	11.91^{a}	3.35^{c}	3.83^{c}	7.29^{bc}	1.341	0.007

[1]PC, corn-soybean meal diet with special protein product (fish meal); NC, corn-soybean meal diet without special protein product; FCP1, field cricket replacing fishmeal on a total Lys basis; FCP2, field cricket replacing fishmeal on a kg/kg basis; and FCP3, field cricket inclusion at the expense of soybean meal and fish meal.
[2]Values shows means of five replicates (pen) per treatment ($n = 25$).
[a-c]Values with in rows without a common superscript are differ significantly ($P < 0.05$).

Table 4 Apparent total tract digestibility of nutrients in weaning pigs fed field cricket powder[1]

Item	Treatment[1]					SEM	*P*-value
	PC	NC	FCP1	FCP2	FCP3		
Apparent total tract digestibility, %[2]							
Dry matter	86.52	88.27	87.61	87.06	87.80	0.547	0.276
Crude protein	86.11^{ab}	84.69^{b}	87.97^{a}	88.12^{a}	86.86^{ab}	0.693	0.041
Ash	55.56	61.87	58.02	56.86	57.35	2.485	0.499
Crude fat	76.19^{bc}	73.98^{c}	81.87^{a}	81.41^{ab}	80.11^{ab}	1.539	0.024

[1]PC, corn-soybean meal diet with special protein product (fish meal); NC, corn-soybean meal diet without special protein product; FCP1, field cricket replacing fishmeal on a total Lys basis; FCP2, field cricket replacing fishmeal on a kg/kg basis; and FCP3, field cricket inclusion at the expense of soybean meal and fish meal.
[2]Values shows mean of three pigs per treatment (average BW 10.46 ± 0.32 kg).
[a-c]Values with in rows without a common superscript are differ significantly ($P < 0.05$).

Table 5. Intestinal morphology in weaning pigs fed field cricket powder[1]

Item	Treatment[1]					SEM	*P*-value
	PC	NC	FCP1	FCP2	FCP3		
VH, μm[2]							
Duodenum	391.79	389.03	460.39	449.93	381.53	0.038	0.147
Jejunum	383.81[ab]	333.94[b]	441.53[a]	436.98[a]	396.38[ab]	0.051	0.009
Ileum	361.33	294.44	362.72	359.03	376.09	0.023	0.714
CD, μm							
Duodenum	235.39	236.17	211.81	229.23	205.87	0.039	0.878
Jejunum	236.24	230.58	206.07	202.43	248.82	0.052	0.391
Ileum	209.32	195.58	179.87	198.39	208.76	0.057	0.752
VH:CD							
Duodenum	1.76	1.67	2.22	2.18	1.85	3.931	0.455
Jejunum	1.65[ab]	1.47[b]	2.14[ab]	2.20[a]	1.69[ab]	6.210	0.019
Ileum	1.72	1.52	2.07	1.88	1.81	4.084	0.612

[1]PC, corn-soybean meal diet with special protein product (fish meal); NC, corn-soybean meal diet without special protein product; FCP1, field cricket replacing fishmeal on a total Lys basis; FCP2, field cricket replacing fishmeal on a kg/kg basis; and FCP3, field cricket inclusion at the expense of soybean meal and fish meal.
[2]Mean values from four replicate pens with one pig per replicate pen (n = 20).
[a,b]Values with in rows without a common superscript are differ significantly ($P < 0.05$).

Immunity and cytokine responses

Concentrations of immune-related factors and proinflammatory cytokines in weaning piglets in all groups are summarized in Table 6. The inclusion of the PC diet did not affect serum IgA, IL1, IL6, and TNFα concentrations in both periods, compared with NC- and FCP-supplemented diets ($P > 0.05$), except for lower serum IgA in the FCP1 and FCP2-supplemented diets on days 28 ($P < 0.05$). Pigs fed FCP2 and FCP3 diets had higher serum IgA concentrations than those in pigs fed the NC diet on day 14 ($P = 0.044$); however, pigs fed FCP1 and FCP2-supplemented diets had greater serum IgA concentrations than those in pigs fed the NC and PC diets on day 28 ($P = 0.030$). On day 14, TNFα decreased with FCP supplementation compared to NC but was comparable to PC. For IL-6, pigs fed the FCP2 diet had lower levels than those in pigs fed the NC diet ($P < 0.05$). No significant difference in serum IL-2 secretion was observed among treatments at any point.

Antioxidant enzyme activity and malondialdehyde concentration

The PC diet did not affect serum concentrations of SOD, GPx, and MDA compared to the NC diet ($P > 0.05$; Table 7). However, serum GPx was lower in pigs fed the PC diet compared to that in pigs fed the FCP1 and FCP2 diets ($P = 0.031$). Indeed, FCP2 more effectively maintained the redox status in weaning pigs through the activation of serum SOD ($P = 0.014$) secretion than the NC diet, and had greater GPx concentration than both control treatments ($P < 0.05$). However, the inclusion of FCP3 did not cause any change in redox status of the weaning pigs in the control or FCP diets ($P > 0.05$).

Microbial counts

Fecal microbial counts on day 28 after weaning are summarized in Table 8. Fecal microbial count were not observed between PC and NC, and PC and FCP diets ($P > 0.05$). However, the weaning pigs that consumed the FCP-supplemented diet showed higher abundances of *Lactobacillus* spp. than those in pigs fed the NC diet ($P = 0.014$). Furthermore, lower abundance of *Escherichia coli* was detected in pigs that consumed FCP2-supplemented diets compared to those fed the NC-supplemented diet ($P = 0.021$). However, fecal counts of *Salmonella* spp. did not differ among groups.

Discussion

Full-fat FCP is considered an alternative feed ingredient not only as a protein source but also as an energy source. Furthermore, the CP content of FCP is comparable to that of FM and slightly higher than that of SBM. This makes it a valuable feed ingredient in the nursery diet of weaning pigs as a substitute for conventional protein ingredients.

Growth performance and diarrhea rate

Fish meal and SBM are normally used as an excellent protein source in swine diets. They contain relatively high amounts of limiting amino acids, particularly lysine, threonine and tryptophan, which have greater digestibility than other conventional cereal grains (NRC, 2012). Our study did not detect significant differences in growth performance and diarrheal occurrence between the PC and NC diets. This may possibly due to the presence of biogenic amine contaminants in fish meal and/or the majority of phosphorus being bound to phytic acid in the controls (Kim et al., 2008; NRC, 2012). This result was consistent with that of Jones et al. (2010), who observed that the growth performance of pigs fed 5% FM was similar to those fed a standard SBM-based diet without the addition of specialty sources. Our approach to substituting fish meal with FCP and decreasing the quantity of SBM in the diet affected growth performance to a degree comparable to that of PC and greater than that of NC, especially in pigs fed the FCP1-supplemented diet. A report by Nyachoti et al. (2004) showed that the growth rate of pigs is typically increased by activating voluntary feed intake or nutrient intake. This is consistent with the increase in BW and ADG in the FCP-supplemented diet.

Table 6. Immunoglobulin and proinflammatory cytokines in weaning piglets fed field cricket powder[1]

Item	Treatment[1]					SEM	*P*-value
	PC	NC	FCP1	FCP2	FCP3		
Days 14[2]							
IgA, mg/mL	0.68[ab]	0.56[b]	0.77[ab]	0.83[a]	0.91[a]	0.078	0.044
IL1, pg/mL	54.57	65.98	42.54	44.44	46.01	8.601	0.322
IL6, pg/mL	96.87[ab]	102.99[a]	87.44[ab]	78.43[b]	97.22[a]	3.593	0.002
TNFα, pg/mL	18.51[ab]	21.83[a]	17.32[b]	16.97[b]	16.68[b]	1.194	0.044
Days 28							
IgA, mg/mL	0.63[b]	0.62[b]	0.94[a]	0.89[a]	0.82[ab]	0.079	0.030
IL1, pg/mL	57.74	61.59	62.49	63.09	60.08	9.941	0.996
IL6, pg/mL	87.01	104.26	80.82	77.33	79.44	7.725	0.142
TNFα, pg/mL	10.02	9.52	8.78	7.93	8.04	0.886	0.407

[1]PC, corn-soybean meal diet with special protein product (fish meal); NC, corn-soybean meal diet without special protein product; FCP1, field cricket replacing fishmeal on a total Lys basis; FCP2, field cricket replacing fishmeal on a kg/kg basis; and FCP3, field cricket inclusion at the expense of soybean meal and fish meal.
[2]Mean values from five replicate pens with one pig per replicate pen (n = 25).
[a,b]Values with in rows without a common superscript are differ significantly ($P < 0.05$).

Table 7. The serum concentrations of antioxidant enzyme activity and malondialdehyde concentration in weaning pigs fed field cricket powder[1]

Item	Treatment[1]					SEM	*P*-value
	PC	NC	FCP1	FCP2	FCP3		
SOD (U/mL)[2]	143.29[ab]	130.03[b]	152.35[ab]	166.77[a]	156.31[ab]	6.638	0.014
GPx (U/mL)	666.31[b]	674.24[b]	867.28[a]	896.34[a]	832.99[ab]	58.544	0.031
MDA(nmol/mL)	4.43[a]	3.92[ab]	3.04[ab]	2.71[b]	3.28[ab]	0.356	0.023

[1]PC, corn-soybean meal diet with special protein product (fish meal); NC, corn-soybean meal diet without special protein product; FCP1, field cricket replacing fishmeal on a total Lys basis; FCP2, field cricket replacing fishmeal on a kg/kg basis; and FCP3, field cricket inclusion at the expense of soybean meal and fish meal.
[2]Mean values from five replicate pens with one pig per replicate pen (n = 25).
[a,b]Values with in rows without a common superscript are differ significantly ($P < 0.05$).

One possible reason is that the FCP can provide adequate lysine and has a comparable essential amino acid recovery to SBM (33.72% vs. 41.30%). This can help utilize protein efficiently, with less undigestible protein excretion in the FCP-formulated diets with the CP recommendation of the NRC (2012), except for digestible lysine. Furthermore, the ether extract content in FCP was higher than that in FM and SBM (25.50% vs. 8.69% and 1.70%, respectively), including the ME value (4,323 vs. 3,821 and 3,344 kcal/kg, respectively); the saturated fatty acids (**SFA**) and (2.97%) and USFA (5.31%) composition may positively affect their performance. Another underlying mechanism is that cricket chitin can activate growth hormone and insulin-like growth factor 1 secretions, leading to heavier BW in weaning pigs and broilers, at 0.2% and 0.5% chitin, respectively (Xu et al., 2013; Ibitoye et al., 2019). It appears that the chitin level was approximately 0.3% higher than in pigs fed the FCP1 diet, which might limit chitinase function (Kawasaki et al., 2021). Previous reports have shown that pigs fed 0.1% to 0.2% chitin show a significant increase in growth performance and lower diarrheal occurrence via the attached site on type 1 fimbriae of *E. coli* (Tran et al., 2011; Xu et al., 2013). However, several antinutritional factors in SBM such as proteinase inhibitors, lectins, and allergenic agents (glycinin, and β-conglycinin) can cause adverse effects of trypsin and chymotrypsin function, cause agglutination of red blood cells, and disturb epithelial cell development, thereby hindering nutrient uptake and growth performance of young animals (Friesen et al., 1993; Wang et al., 2020). This is consistent with the impairment of growth performance during Phase I in the NC group. Additionally, in the heating process, stachyose and raffinose contents in SBM were approximately 5% of DM and would negatively induce diarrhea in piglets (Chen et al., 2013). However, high levels of toxins and other contaminants were not observed in a previous study on field cricket supplementation (Fernandez-Cassi et al., 2020). In addition, lower production of toxic metabolites (ammonia, amine, phenolic, and indolic compounds) originating from undigestible protein are further implicated in lower post-weaning diarrhea (Gilbert et al., 2018). This supports our observation that the rate of diarrhea was lower in pigs fed the FCP-supplemented diet. Based on these results, the positive effects of FCP supplementation on growth and the rate of diarrhea may improve protein digestibility, thereby reducing protein fermentation in the hindgut. These findings indicate that the FCP is a high-quality protein source and is an alternative to vegetable-animal protein ingredients to maximize postweaning performance in pigs.

Apparent total tract digestibility

Dietary FCP could be provided as an alternative protein ingredient in livestock feed to enhance nutrient utilization and ultimately promote growth. Our findings indicate that the inclusion of FCP retains high digestibility of dry matter (greater

Table 8. Microbial counts (cfu/g feces) in weaning piglets fed field cricket powder[1]

Item	Treatment[1]					SEM	*P*-value
	PC	NC	FCP1	FCP2	FCP3		
Lactobacillus spp.[2]	6.59[ab]	4.86[b]	7.57[a]	8.13[a]	6.88[a]	0.592	0.014
Escherichia coli	8.38[ab]	9.92[a]	7.07[ab]	5.59[b]	7.14[ab]	0.818	0.021
Salmonella spp.	5.27	6.67	5.39	5.88	5.67	0.759	0.714

[1]PC, corn-soybean meal diet with special protein product (fish meal); NC, corn-soybean meal diet without special protein product; FCP1, field cricket relacing fishmeal on a Lys basis; FCP2, field cricket relacing fishmeal on a kg/kg basis; and FCP3, field cricket inclusion at the expense of soybean meal and fish meal.
[2]Mean values from five replicate pens with one pig per replicate pen ($n = 25$).
[a,b]Values with in rows without a common superscript are differ significantly ($P < 0.05$).

than 87% in the current study), which is in accordance with the findings of Bosch et al. (2014), who demonstrated that dogs and cats fed diets containing crickets had higher nutrient digestibility than those fed animal protein-based diets. This was in contrast to Kilburn et al. (2020), who demonstrated the adverse effects on the digestibility of CP and crude fat when the cricket content was 8%, because of the high chitin content. Although the FCP consisted of 43 g/kg DM chitin, upon inclusion, approximately 0.2% did not have a detrimental effect on nutrient availability (Xu et al., 2014). This may be the case because piglets from the 14- to 56-d stage can secrete chitin-degrading enzymes in stomach tissues (Kawasaki et al., 2021). It is possible that chitin is partially digested in the stomach, and other edible parts of the protein components are utilized by the intestinal proteinase of the small intestine. Indeed, the modulation effect of chitin on nutrient utilization may be attributed to the indirect action of microbial abundance from hindgut fermentation which degrades chitin (Beier and Brtilsson, 2013). Recent reports show that chitin and it derivatives cause an increase in brush-border digestive enzyme activity (lactase, maltase, sucrose, and proteinase) in the small intestinal by altering intestinal morphology (Woodring et al., 2007; Wan et al., 2017), therefore improving the digestion and absorption of nutrients (Wang et al., 2020). Interestingly, FCP inclusion in place of SBM or FM, with concomitant differences in CP levels, especially in the NC diet, resulted in a greater utilization of CP and crude fat than that for pigs fed the two conventional protein sources. The explanation for increased CP digestibility may be related to the fact that FCP contains higher CP of a higher digestibility than other conventional cereal grains (NRC, 2012). Although the CP level was reduced in the experimental diet, the remaining non-limiting lysine content provided amino acids to meet the requirements (NRC, 2012), thereby reducing nutrient need from body reserves. The increase in ether extract digestibility may be influenced by the increased fat level from full-fat FCP supplementation, which was approximately 4.11% to 4.58% higher than that of control groups (2.86% to 3.51%). Other factors that could impair nutrient digestibility are poor quality of FM and antinutritional factors presence in SBM of the control groups (Jeong et al., 2016; Wang et al., 2020). It is possible that the positive effects of the FCP diet on jejunal development could increase nutrient digestibility and ultimately improve growth performance in the weaning pigs.

Intestinal morphology

There is clear evidence that weaning stress causes tremendous changes in the morphology of the intestinal mucosa, including villus atrophy and crypt hyperplasia (Smith et al., 2010). Shortened villi decrease the mucosal surface of the small intestine, leading to impaired digestive and absorptive capacities and growth retardation. Additionally, crypts are located in the base of villi and contain epithelial stem cells required for the repopulation of villi. A longer crypt is associated with rapid turnover of the intestinal epithelium, resulting in cell migration during normal proliferation, inflammation, and sloughing of the mucosal segment from infectious diarrhea (Parker et al., 2017). The villus-to-crypt ratio is typically used to determine intestinal function. Our morphometric analysis of the increased jejunal VH and VH:CD in pigs fed an FCP-supplemented diet indicates that FCP supplementation may promote enterocyte maturation and enzyme activity. One possible reason is that the brush-border membrane of the small intestine has several digestive enzymes, including membrane-bound peptidases, which ultimately utilize CP, amino acids, and other nutrients in an FCP-supplemented diet more efficiently (Wan et al., 2017). This ensures the availability of nutrients for undifferentiated cells and their maturation into mature enterocytes, with an increased surface area of epithelial cells (Han et al., 2012; Wan et al., 2017). Another underlying mechanism is that chitin and its derivatives have a positive effect on enterocyte proliferation and the prevention of villus atrophy (Han et al., 2012). According to Wan et al. (2017), weaning pigs fed a chitin-derivative had significantly increased VH via an increased activity of digestive enzyme (maltase, lactase and surcease) in the small intestine. However, the modulatory effect of FCP on digestive enzyme activity is still unknown and further investigation is needed to elucidate these effects. Based on this finding, FCP could be used to replace fish meal in the nursery diet to promote intestinal development, initially influencing jejunal morphology and consequently affecting weaning pig performance by increasing the absorptive capacity of nutrients. Although the FCP used in the current study contained a high amount of chitin (43 g/kg DM), the inclusion of nearly 0.2% did not have a detrimental effect on nutrient availability (Xu et al., 2014). A recent report by Kawasaki et al. (2021) confirmed that the piglets from 14 to 56 d can secrete chitin-degrading enzymes in stomach tissues. It is possible that chitin is partially digested in the stomach and other edible parts of protein components are utilized by the intestinal proteinase of the small intestine, probably explaining the greater digestion and absorption than those for pigs fed both conventional protein sources. Indeed, the modulating effect of chitin on nutrient utilization may be attributed to the indirect action of microbial abundance from hindgut fermentation which, in turn, degrades chitin (Beier and Brtilsson, 2013). Therefore, FCP addition in nursery diets may positively enhance enterocyte proliferation and prevent villus atrophy in intestinal structures.

Immunity and cytokine responses

Disturbances in mucosal permeability cause inflammation by the activation of proinflammatory cytokines, including IL1,

IL6, and TNFα (DeGroot et al., 2021). It has been shown that the consumption of either a soy-based or milk-based diet modulates the intestinal inflammatory response in piglets (Park et al., 2020). This is in agreement with our results, in which concentrations of IL6 and TNFα were higher for pigs fed a soybean meal-based diet than for pigs fed diets including FCP in Phase I. In general, piglets are particularly susceptible to stressors during the 14-d postweaning period, contributing to mucosal inflammation and changes in VH and CD. However, these detrimental effects are attenuated with increasing age (Campbell et al., 2013). The SBM diet-induced proinflammatory response can be attributed to the presence of allergic proteins, namely glycinin approximately 14.3%) and β-conglycinin (approximately 10%; Park et al., 2020). These allergens disrupt the functions of tight junction proteins, which play essential roles in intestinal nutrient absorption, epithelial cell homeostasis, and protection against pathogens. The lower expression of these specific proteins increases the risks of *E. coli*-induced diarrhea and intestinal damage, contributing to increased serum concentrations of proinflammatory cytokines in weaning pigs (Wu et al., 2016; Xun et al., 2018; Park et al., 2020). However, peptidoglycan in the insect skeleton is an immunomodulator that can inhibit inflammation via attachment to specific binding sites of the pathogen receptors (Wolf and Underhill, 2018). Peptidoglycans then promotes the development of immunity by the secretion of IgA into the intestinal lumen, which subsequently inhibits the colonization of bacteria and prevents the penetration of the pathogen via the epithelial layer (Ha et al., 2006). In addition, a positive effect of an insect-enriched protein diet on the regulation of cytokine production has been reported for medium-chain fatty acids, chitin, and its derivatives by providing acidic conditions, adhesion to bacterial cells, and the promotion of host prebiotic microorganisms (Hu et al., 2018; Jackman et al., 2020), consistent with our results. Our results showed that the field cricket has a high lauric acid (C12:0) content of over 50% of the total saturated fatty acids. Lauric acid has anti-inflammatory and immunostimulatory properties, which may alleviate intestinal disorders caused by post-weaning stress (Jackman et al., 2020). Interestingly, the IgA concentration was lower for the SBM-based diet and FM treatments than for the FCP-supplemented diet. This can be explained by the defense mechanism in mesenteric lymph nodes, which activates the synthesis of IgA-expressing cells of the host, thereby preventing pathogen invasion and allergen absorption. Additionally, relatively high levels of polyunsaturated fatty acids in fish meal and allergenic proteins in SBM contribute to oxidative stress, especially lipid peroxidation (Rao, 2008). The disruption of cellular redox homeostasis causes the excessive secretion of proinflammatory cytokines and impaired intestinal function (Rosero et al., 2015). Our results suggest that FCP can protect against inflammation, contributing to nutrient absorption and subsequent growth performance, making it an attractive protein source for weaning pigs.

Antioxidant enzyme activity and malondialdehyde concentration

Under stressed circumstance, piglets exhibit insufficient removal of reactive oxygen species (**ROS**) which subsequently induces a cellular redox imbalance (DeGroot et al., 2020). ROS can damage DNA, proteins, and lipids via oxidative or radical-mediated reactions, affecting cell functionality (Bacou et al., 2021). Furthermore, this detrimental effect severely impacts pigs fed diets high in unsaturated fatty acids (USFA; Shurson et al., 2015) which subsequently alter the lipid peroxide byproduct MDA (Silva-Guillen et al., 2020). Previous reviews have shown that the composition of USFA is 45.38% in SBM and 52.43% in FM (Cho and Kim, 2011; NRC, 2012). This amount is negligible in the two conventional ingredients, thereby no scavenging effect on ROS was observed in this study. However, obtaining FCP products from a local cricket farm with a lower USFA content (~5.31%) and freeze-storage control (-80 °C) prior to the addition to the diet could reduce cellular damage induced by ROS more efficiently. Moreover, Ahn et al. (2020) revealed that field crickets are an excellent source of the antioxidant compound glycosaminoglycan (3.4 g/kg), which contributes to the antioxidant capacity during oxidative stress. Its chemical structure includes a carboxylic group for binding to positively charged transitional metal ions (Fe^{2+}, Cu^{2+}, or Zn^{2+}) involved in the initial step of the Fenton reaction, consequently increasing hydroxyl radicals from hydrogen peroxide. In this process, metal chelators play an important role as cofactors (Cu, Zn-SOD) of SOD, whereas iron acts as a cofactor of catalase in scavenging superoxide anions and hydrogen peroxide (Sabuncuoglu et al., 2015). It also activates various antioxidant enzymes, such as GPx, thereby lowering protein carbonyl as an effective indicator of protein oxidative stress in animals (Ahn et al., 2020). Additionally, chitin derivatives with low molecular weights (20 to 30 kDa) tend to increase the total antioxidant capacity for defense against free radicals (Hu et al., 2018). The chitin content in crickets is expected to be a key factor in improving antioxidant status through increased SOD and GPx and lowered MDA concentrations in weaning pigs fed the FCP-supplemented diet. One such mechanism is that chitin and its derivatives can initiate the inhibition of ROS by lipid molecules via the action of nitrogen on the C2 position, which generally has a non-binding pair of electrons (Park et al., 2004). It can further attach to the hydrogen ion to form a stable molecule of ammonium (NH_3^+) that could eliminate the chain reaction of ROS molecules (Xia et al., 2011). Based on this finding, it can be implied that the ability to protect superoxide radicals and hydrogen peroxide formation before attachment to cellular tissues can preserve the cellular membrane integrity in weaning piglets fed an FCP-supplemented diet.

Microbial counts

Previous review studies on pigs showed that an increased CP level (greater than 19%) in an isonitrogenous diet induced the production of harmful microbial metabolites in the hindgut, which directly changed hindgut pH for proper enumeration of harmful bacteria (Jha and Berrocoso, 2016). The disciplinary result between the PC (19% CP) and NC (21% CP) groups could be influenced by the fact that older pigs have a greater ability to defend against the infection of harmful bacteria. However, dietary FCP consists of chitin, which is a linear long chain β-1,4-linked polymer of *N*-acetyl-D-glucosamine. Chitin has the ability to combine with glucan (chitin-glucan complex), an effective prebiotic. It plays an important role as a fiber substrate that is used selectively by host microorganisms to retain more nitrogen for their own growth; it adheres to the colon and shifts the gut microbial community towards certain

beneficial bacteria (Lopez-Santamarina et al., 2020). The chitin-glucan complex shows strongly inhibitory effects against the proliferation of gram-positive bacteria, particularly *E. coli*, and promotes *Lactobacillus* spp. (Wan et al., 2017; Selenius et al., 2018). In addition, its chitosan derivative has the potential to reduce the severity of diarrhea in *E. coli* K88-inoculated weaning pigs (Qian et al., 2016). The positive charge of chitosan (NH_3^+) can interact with the negative charge of pathogens on the cell-wall surface and rapidly penetrate the microbial cell membrane to exert bacteriostatic and bactericidal effects (Guan et al., 2019). It also has an indirect impact on modulating the beneficial microbial growth of *Bifidobacterium*, *Methanobrevibacter*, *Faecalibacterium*, and *Lactobacillus* (Kong et al., 2014), consistent with our findings. However, there was no evidence of a beneficial effect on the fecal microbiota in this study, and further research is required to elucidate this effect. Another possible mechanism may be associated with lauric acid composition which has a low critical micelle concentration (CMC, approximately 900 µmol/L at pH 7.4), with a greater ability to disrupt phospholipid membranes of pathogens (Valle-González et al., 2018). Furthermore, FCP contained approximate 0.11% total medium-chain fatty acids (caprylic acid), with a CMC value of approximately 60 µmol/L and a high pKa value (pKa approximately 14), thus remaining nonionic in acidic pH conditions (stomach) for greater inhibitory effects than those of C_{12} fatty acids (Valle-González et al., 2018; Jackman et al., 2020). Interestingly, no significant differences were observed in pigs fed the highest chitin content in the FCP3 diet. The defining mechanism for this is unknown, suggesting further exploration of this detrimental effect or consideration of the ratio of protein to carbohydrate in the hindgut as it influences carbohydrate fermentation while inhibiting protein fermentation. Based on the enrichment of commensal *Lactobacillus* spp., it may activate growth inhibitory effects or compete with *E. coli*.

Conclusions

FCP is an effective protein ingredient that could fully replace fish meal on either a Lys basis or a kg/kg basis and can partially replace soybean meal, without impairing the growth performance and health status of weaning pigs. Furthermore, FCP improves nutrient utilization, intestinal morphology, serum immunoglobulin, antioxidant status, and *Lactobacillus* spp. growth, while reducing proinflammatory cytokine secretion and *E. coli* growth. Accordingly, the inclusion of FCP in dietary formulations as an alternative to SBM or FM during the post-weaning period is a promising strategy.

Acknowledgments

Funding was provided (grant no. 224/2564) by the National Research Council of Thailand (NRCT). The authors would also like to thank anonymous reviewers for their valuable suggestions.

Conflict of interest statement

The authors declare that there are no competing interest.

Literature Cited

Adeola, O. 2001. *Digestion and balance techniques in pigs*. Washington (DC): CRC Press.

Ahn, M. Y., B. J. Kim, H. J. Kim, J. M. Jin, H. J. Joon, J. S. Hwang, and B. M. Lee. 2020. Anti-diabetic activity of field cricket glycosaminoglycan by ameliorating oxidative stress. *BMC Complement. Med. Ther*. 20:232. doi:10.1186/s12906-020-03027-x

AOAC. 2000. *Official methods of analysis of AOAC International*. 17th ed. Gaithersburg (MD): Official Analytical Chemists.

Bacou, E., C. Walk, S. Rider, G. Litta, and E. Perez-Calvo. 2021. Dietary oxidative stress: a review of nutritional challenges as models for poultry, swine and fish. *Antioxidants*. 10:525. doi:10.3390/antiox10040525

Beier, S., and S. Brtilsson. 2013. Bacterial chitin degradation-mechanisms and ecophysiological strategies. *Front. Microbiol*. 4:149. doi:10.3389/fmicb.2013.00149

Bosch, G., S. Zhang, D. G. Oonincx, and W. H. Hendriks. 2014. Protein quality of insects as potential ingredients for dog and cat foods. *J. Nutr. Sci*. 3:e29. doi:10.1017/jns.2014.23

Campbell, J. M., J. D. Crenshaw, and J. Polo. 2013. The biological stress of early weaning piglets. *J. Anim. Sci. Biotechnol*. 4:19. doi:10.1186/2049-1891-4-19

Chen, X., C. M. Parson, and N. Bajjalieh. 2013. Nutritional evaluation of new reduced oligosaccharides soybean meal in poultry. *Poult. Sci*. 92:1830–1836. doi:10.3382/ps.2012-02856

Cho, J. H., and I. H. Kim. 2011. Fish meal-nutritive value. *J. Anim. Physiol. Anim. Nutr*. 95:685–692. doi:10.1111/j.1439-0396.2010.01109.x

DeGroot, J., H. Vergauwen, W. Wang, C. V. Ginneken, S. D. Smet, and J. Michiels. 2020. Changes of the glutathione redox system during the weaning transition in piglets, in relation to small intestinal morphology and barrier function. *J. Anim. Sci. Biotechnol*. 11:45. doi:10.1186/s40104-020-00440-7

Dijk, M., T. Morley, M. L. Rau, and Y. Saghai. 2021. A meta-analysis of projected global food demand and population at risk of hunger for the period 2010-2050. *Nat. Food*. 2:494–501. doi:10.1038/s43016-021-00322-9

Fernandez-Cassi, X., K. Söderqvist, A. Bakeeva, M. Vaga, J. Dicksved, I. Vagsholm, A. Jansson, and S. Boqvist. 2020. Microbial communities and food safety aspects of crickets (*Acheta domestica*) reared under controlled conditions. *J. Insects as Food Feed*. 60:429–440. doi:10.3920/JIFF2019.0048

Friesen, K. G., R. D. Goodband, J. L. Nelssen, F. Blecha, D. N. Reddy, P. G. Reddy, and L. J. Kats. 1993. The effect of pre- and postweaning exposure to soybean meal on growth performance and on the immune response in the early-weaning pig. *J. Anim. Sci*. 71: 2089–2098. doi:10.2527/1993.7182089x

Gilbert, M., N. Ijssennagger, A. K. Kies, and S. W. C. van Mil. 2018. Protein fermentation in the gut: implications for intestinal dysfunction in human, pigs, and poultry. *Am. J. Physiol. Gastrointest. Liver Physiol*. 315:159–170. doi:10.1152/ajpgi.00319.2017

Guan, G., A. K. Azad, Y. Lin, S. W. Kim, Y. Tian, G. Liu, and H. Wang. 2019. Biological effects and applications of chitosan and chito-oligosaccharides. *Front. Physiol*. 10:516. doi:10.3389/fphys.2019.00516

Ha, S. A., M. Tsuji, K. Suzuki, B. Meek, N. Yasuda, T. Kaisho, and S. Fagarasan. 2006. Regulation of B1 cell migration by signal through toll-like receptors. *J. Exp. Med*. 203:2541–2550. doi:10.1084/jem.20061041

Han, X. Y., W. L. Do, Q. C. Huang, Z. R. Xu, and Y. Z. Wang. 2012. Changes in small intestinal morphology and digestive enzyme activity with oral administration of copper-loaded chitosan nanoparticles in rats. *Biol. Trace Elem. Res*. 145:355–360. doi:10.1007/s12011-011-9191-x

Hu, S., Y. Wang, X. Wen, L. Wang, Z. Jiang, and C. Zheng. 2018. Effect of low-molecular-weight chitosan on the growth performance, intestinal morphology, barrier function, cytokine expression, and antioxidant system of weaning piglets. *BMC Vet. Res*. 14:215. doi:10.1186/s12917-018-1543-8

Ibitoye, E. B., I. H. Lokman, M. N. M. Hezmee, Y. M. Goh, A. B. Z. Zuki, A. A. Jimoh, A. Danmaigoro, and N. P. Nicholas. 2019. Gut health and serum growth hormone levels of broiler chickens fed dietary chitin and chitosan from cricket and shrimp. *Poult. Sci.* 98:745–752. doi:10.3382/ps/pey419

Jackman, J. A., R. D. Boyd, and C. C. Elrod. 2020. Medium chain fatty acids and monoglycerides as additives for pig production: towards gut health improvement and feed pathogen mitigation. *J. Anim. Sci. Biotechnol.* 11:44. doi:10.1186/s40101-020-00446-1

Jeong, J. S., J. W. Park, S. I. Lee, and I. H. Kim. 2016. Apparent ileal digestibility of nutrients and amino acids in soybean meal, fish meal, spray-dried plasma protein and fermented soybean meal to weaning pigs. *Anim. Sci. J.* 87:697–702. doi:10.1111/asj.12483

Jha, R., and J. F. D. Berrocoso. 2016. Dietary fiber and protein fermentation in the intestine of swine and their interactive effects on gut health and on the environment: a review. *Anim. Feed Sci. Technol.* 212:18–26. doi: 10.1016/j.anifeedsci.2015.12.002

Jones, C. K., J. M. DeRouchey, J. L. Nelssen, M. D. Tokach, S. S. Dritz, and R. D. Goodband. 2010. Effects of fermented soybean meal and specialty animal protein sources on nursery pig performance. *J. Anim. Sci.* 88:1725–1732. doi: 10.2527/jas.2009-2110

Jones, A. M., F. Wu, J. C. Woodworth, M. D. Tokach, R. D. Goodband, J. M. DeRouchey, and S. S. Dritz. 2018. Evaluating the effects of fish meal source and level on growth performance of nursery pigs. *Trans. Anim. Sci.* 2:144–155. doi:10.1093/tas/txy010

Kawasaki, K., T. Osafune, S. Tameira, and K. Yano. 2021. Piglets can secrete acidic mammalian chitinase from the pre weaning stage. *Sci. Rep.* 11:1297. doi:10.1038/s41598-020-80368-0

Kilburn, L. R., A. T. Carlson, W. Lewis, and M. C. R. Serao. 2020. Cricket (*Gryllodes sigillatus*) meal fed to healthy adult dogs does not affect general health and minimally impacts apparent total tract digestibility. *J. Anim. Sci.* 98:1–8. doi:10.1093/jas/skaa083

Kim, J. C., B. P. Mullan, D. J. Hampson, and J. R. Pluske. 2008. Addition of oat hull to an extruded rice-based diet for weaner pigs ameliorates the incidence of diarrhoea and reduces indices of protein fermentation in the gastrointestinal tract. *Br. J. Nutr.* 99: 1217–1225. doi:10.1017/S0007114507868462

Kong, X. F., X. L. Zhou, G. Q. Lian, F. Blachier, G. Liu, B. E. Tan, C. M. Nyachoti, and Y. L. Yin. 2014. Dietary supplementation with chitooligosacharides alters gut microbiota and modifies intestinal ruminal metabolites in weaned huanjiang mini-piglets. *Livest. Sci.* 160:97–101. doi:10.1016/j.livsci.2013.11.023

Kovitvadhi, A., P. Chundang, J. Luapan, P. Amarapitak, P. Sriyaphai, R. Buahom, T. Cham-iam, K. Leelehapongsathon, C. Tirawattanawanich, and K. Thongprajukaew. 2019. Cricket powder as an alternative protein source for broiler based on in vitro digestibility. *J. Insects Food Feed.* 5:185–191. doi:10.3920/JIFF2018.0043

Lee, S., K. S. Ahn, H. Y. Ryu, H. J. Kim, J. K. Lee, M. H. Cho, M. Y. Ahn, and K. S. Song. 2016. Safety evaluation of cricket (*Gryllus bimaculatus*) extract in Sprague-Dawley rats. *Int. J. Indust. Entomol.* 32:12–25. doi:10.7852/ijie.2016.32.1.12

Li, D. F., J. L. Nelssen, P. G. Reddy, F. Blecha, J. D. Hancook, G. L. Alle, R. D. Goodband, and R. D. Klemm. 1990. Transient hypersensitivity to soybean meal in the early-weaning pigs. *J. Anim. Sci.* 68:1790–1799. doi:10.2527/1990.6861790x

Lopez-Santamarina, A., A. C. Mondragon, A. Lamas, J. M. Miranda, C. M. Franco, and A. Cepeda. 2020. Animal-origin prebiotics based on chitin: a n alternative for the future? a critical review. *Foods.* 9:782. doi:10.3390/foods9060782

Miech, P., J. E. Lindberg, A. Berggren, T. T. Chhay, and A. Jansson. 2017. Apparent faecal digestibility and nitrogen retention in piglets fed whole and peeled Cambodian field cricket meal. *J. Insect Food Feed.* 3:279–287. doi:10.3920/JIFF2017.0019

NRC. 2012. *Nutrient requirement of swine*. 11th rev. ed. Washington (DC): National Academies Press.

Nyachoti, C. M., R. T. Zijlstra, C. de Lange, and J. Patience. 2004. Voluntary feed intake in growing-finishing pigs: a review of the main determining factors and potential approaches for accurate predictions. *Can. J. Anim. Sci.* 84:579–566. doi:10.4141/A04-001

Park, P. J., J. Y. Je, and S. K. Kim. 2004. Free radical scavenging activities of differently deacetylated chitosans using an ESR spectrometer. *Carbohydr. Polym.* 55:17–22. doi:10.1016/j.carbpol.2003.05.002

Park, S., J. W. Lee, A. J. Cowieon, G. Pappenberger, and T. A. Woyengo. 2020. Soybean meal allergenic protein degradation and gut health of piglets fed protenase-supplemented diets. *J. Anim. Sci.* 98:10. doi:10.1093/jas/skaa308

Parker, A., O. J. Maclaren, A. G. Fletcher, D. Muraro, P. A. Kreuzaler, H. M. Byrne, P. K. Maini, A. J. M. Watson, and C. Pin. 2017. Cell proliferation within small intestine crypts is the principal driving force for cell migration on villi. *FASEB J.* 31:636–649. doi:10.1096/fj.201601002

Pedigo, L. P. 2002. *Entomology and pest management*. New York (NY): Prentice Hall.

Permatahati, D., R. Mutia, and D. A. Astuti. 2019. Effect of cricket meal (*Gryllus bimaculatus*) on production and physical quality of Japanese quail egg. *Trop. Anim. Sci. J.* 42:53–58. doi:10.5398/tasj.2019.42.1.53

Qian, L., X. Yue, L. Hu, Y. Ma, and X. Han. 2016. Changes in diarrhea, nutrient apparent digestibility, digestive enzyme activities of weaning piglets in response to chitosan-zinc chelate. *Anim. Sci. J.* 87:564–569. doi:10.1111/asj.12460

Rao, R. 2008. Oxidative stress-induced disruption of epithelial and endothelial tight junctions. *Front. Biosci.* 13:7210–7226. doi:10.2741/3223

Rosero, D. S., J. Odle, A. J. Moeser, R. D. Boyd, and E. van Heugten. 2015. Peroxidised dietary lipids impair intestinal function and morphology of the small intestine villi of nursery pigs in a dose-dependent manner. *Br. J. Nutr.* 114:1985–1992. doi:10.1017/S000711451500392X

Sabuncuoglu, S., A. Eken, A. Aydin, H. Ozgunes, and H. Orhan. 2015. Cofactor metals and antioxidant enzymes in cisplatin-treated rats: effect of antioxidant intervention. *Drug Chem. Toxicol.* 38:375–382. doi:10.3109/01480545.2014.974107

Sarwar, G., H. G. Botting, and R. W. Peace. 1988. Complete amino acid hydrolysis in hydrolysates of foods and feces by liquid chromatography of precolumn phenylisothiocyanate derivatives. *J. Assoc. Off. Anal. Chem.* 71:1172–1175.

Selenius, O., J. Korpela, S. Salminen, and C. G. Gallego. 2018. Effect of chitin and chitooligosaccharide on in vitro growth of lactobacillus rhamnosus GC and *Escherichia coli* TG. Appl. *Food Biotechnol.* 5:163–172. doi:10.22037/afb.v5i3.20468

Shurson, G. C., B. J. Kerr, and A. R. Hanson. 2015. Evaluating the quality of feed fats and oils and their effects on pig growth performance. *J. Anim. Sci. Biotechnol.* 6:10. doi:10.1186/s40104-015-0005-4

Silva-Guillen, Y. V., C. Arelano, R. D. Boyd, G. Martinez, and E. van Heugten. 2020. Growth performance, oxidative stress and immune status of newly weaning pigs fed peroxidized lipids with or without supplemental vitamin E or polyphenol. *J. Anim. Sci. Biotechnol.* 11:22. doi:10.1186/s40104-020-0431-9

Smith, F., J. E. Clark, B. L. Overman, C. C. Tozel, J. H. Huang, J. E. F. Rivier, A. T. Blisklager, and A. J. Moeser. 2010. Early weaning stress impairs development of mucosal barrier function in porcine intestine. *Am. J. Physiol. Gastrointest. Liver Physiol.* 298:352–363. doi:10.1152/ajpgi.00081.2009

Tran, H. T., N. Barnich, and E. Mizoguchi. 2011. Potential role of chitinases and chitin-binding proteins in host-microbial interactions during the development of intestinal inflammation. *Histol. Histopathol.* 26:1453–1464. doi:10.14670/HH-26.1453

United Nation. 2013. *World population prospects: The 2012 Revision*. New York (NY): United Nation.

Valle-González, E. R., J. A. Jackman, B. K. Yoon, S. Park, T. N. Sut, and N. J. Cho. 2018. Characterizing how acidic pH conditions affect the membrane-disruptive activities of lauric acid and glycerol monolaurate. *Langmuir.* 34:13745–13753. doi:10.1021/acs.langmuir.8b02536

Veldkamp, T., and A. G. Vernooij. 2021. Use of insect products in pig diets. *J. Insects as Food Feed.* 7:781–793. doi:10.3920/jiff2020.0091

Wan, J., F. Jiang, Q. Xu, D. Chen, B. Yu, Z. Huang, X. Mao, J. Yu, and J. He. 2017. New insights into the role of chitosan oligosaccharide

in enhancing growth performance, antioxidant capacity, immunity and intestinal development of weaning pigs. *RSC Adv*. 7:9669–9679. doi:10.1039/C7RA00142H

Wang, T., and O. Adeola. 2018. Digestibility index marker type, but not inclusion level affects apparent digestibility of energy and nitrogen and marker recovery in growing pigs regardless of added oat bran. *J. Anim. Sci*. 96:2817–2825. doi:10.1093/jas/sky153

Wang, W., Y. Wang, X. Hao, Y. Duan, Z. Meng, X. An, and J. Qi. 2020. Dietary fermented soybean meal replacement alleviates diarrhea in weaning piglets challenged with enterotoxigenic *Escherichia coli* K88 by modulating inflammatory cytokine, levels and cecal microbiota composition. *BMC Vet. Res*. 16:245. doi:10.1186/s12917-020-02466-5

Wang, M., C. Yang, Q. Y. Wang, J. Z. Li, Y. L. Li, X. Q. Ding, Y. Yin, H. S. Yang, and Y. L. Yin. 2020. The growth performance, intestinal digestive and absorptive capacities in piglets with different lengths of small intestines. *Animal*. 14:1196–1203. doi:10.1017/S175173111900288X

Wang, D., S. W. Zhai, C. X. Zhang, Y. Y. Bai, S. H. An, and Y. N. Xu. 2005. Evaluation on nutritional value of field crickets as a poultry feedstuff. *Asian-Aust. J. Anim. Sci*. 18:667–670. doi:10.5713/ajas.2005.667

Wolf, A. J., and D. M. Underhill. 2018. Peptidoglycan recognition by the innate immune system. *Nat. Rev. Immunol*. 18:243–254. doi:10.1038/nri.2017.136

Woodring, J., K. H. Hoffmann, and M. W. Lorenz. 2007. Activity, release and flow of digestive enzymes in the cricket, Gryllus bimaculatus. *Physiol. Entomol*. 32:56–63. doi:10.1111/j.1365-3032.2006.00541.x

Wu, J. J., Y. Zhang, J. H. Dong, C. M. Cao, B. Li, S. B. Feng, H. Y. Ding, L. Y. Ma, X. C. Wang, and Y. Li. 2016. Allergens and intestinal damage induced by soybean antigen proteins in weaning piglets. *Ital. J. Anim. Sci*. 15:437–445. doi:10.1080/1828051X.2016.1200441

Xia, W., P. Lin, J. Zhang, and J. Chen. 2011. Biological activities of chitosan and chitooligosaccharides. *Food Hydrocoll*. 25:170–179. doi:10.1016/j.foodhyd.2010.03.003

Xu, Y., B. Shi, S. Yan, T. Li, Y. Guo, and J. Li. 2013. Effects of chitosan on body weight gain, growth hormone and intestinal morphology in weaning pigs. *Asian-Australas. J. Anim. Sci*. 26:1484–1489. doi:10.5713/ajas.2013.13085

Xu, Y., B. Shi, S. Yan, J. Li, T. Li, Y. Guo, and X. Guo. 2014. Effects of chitosan supplementation on the growth performance, nutrient digestibility, and digestive enzyme activity in weaning pigs. Czech. *J. Anim. Sci*. 59:156–163. doi:10.17221/7339-cjas

Xun, W., L. Shi, H. Zhou, G. Hou, and T. Cao. 2018. Effect of weaning age on intestinal mucosal morphology, permeability, gene expression of tight junction proteins, cytokines and secretory IgA in wuzhishan mini piglets. *Ital. J. Anim. Sci*. 17:976–983. doi:10.1080/1828051X.2018.1426397