RESEARCH ARTICLE

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Effect of insect protein and protease on growth performance, blood profiles, fecal microflora and gas emission in growing pig

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Abstract

Two experiments were conducted to determine the effect of *Hermetia illucens* larvae (HIL) as protein and protease on growth performance, blood profiles, fecal microflora, and gas emission in growing pig. In experiment 1, the seventy-two crossbred growing pigs ([Landrace × Yorkshire] × Duroc) with an initial body weight (BW) of 27.98 ± 2.95 kg were randomly allotted to one of four dietary treatments (3 pigs per pen and 6 replicates pen per treatments). The experimental design was a 2 × 2 factorial arrangement of treatments evaluating two diets (Poultry offal diets and HIL diets) without or with supplementing protease. The poultry offal in basal diet has been replaced by HIL. In experiment 2, the four crossbred growing pigs ([Landrace × Yorkshire] × Duroc) with an initial BW of 28.2 ± 0.1 kg were individually accepted in stainless steel metabolism cages. The dietary treatments included: 1) PO- (PO-; poultry offal diet), 2) PO+ (PO- + 0.05% protease), 3) HIL- (3% PO of PO- diet was replacement 3% HIL), 4) HIL+ (HIL- + 0.05% protease). In experiment 1, From weeks 0 to 2, average daily gain (ADG) and feed efficiency (G:F) were significantly increased in the PO diet group compared with the HIL group. From weeks 2 to 4, ADG and G:F were higher for protease group than for non-protease group. At weeks 2 and 4, the PO diet group had lower blood urea nitrogen (BUN) levels than HIL diet group. In experiment 2, crude protein (CP) and nitrogen (N) retention were decreased by HIL diet at weeks 2 and 4. The fecal microflora and gas emission were not affected by HIL and protease. The HIL diet showed lower CP digestibility than PO diet and total essential amino acids digestibility tended to higher in PO diet than HIL diet. In summary, the present study revealed that replacement of the PO protein with the HIL protein and the additive of protease in growing pig diets during the overall experimental period had no negative effect.

Keywords: *Hermetia illucens* larvae, Protease, Growth performance, Nutrient digestibility, Amino acids

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Availability of data and material
Upon reasonable request, the datasets of this study can be available from the corresponding author.

Authors' contributions
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INTRODUCTION

Pig industry is the fastest growing livestock industry in the world. Feed costs account for the largest portion when rearing pigs [1]. Protein feed for pigs accounts for 60%–70% of the total feed cost. Fishmeal and soybean meal are mainly used as protein ingredients. However, they are expensive. Furthermore, the use of substances such as fishmeal, fish oil, soybean meal, and grain is growing in both human and animal feed. Food source insecurity has driven up animal feedstock prices in recent years [2,3]

Insects are highly nutritious and good sources of protein. They are considered promising high-quality feed components [4, 5]. Many experiments are being conducted by replacing some proteins in feed with insect feed. In addition, replacing 0%–6% of soybean meal with mealworm (*Tenebro molitor*) can linearly improve average daily gain (ADG) and average daily feed intake (ADFI) [6,7]. *Hermetia illucens* larvae (HIL) has a fat content of 15% to 49% based on dry matter (DM) [8]. HIL is also high-quality sources of protein as they contain about 40%–44% crude proteins (CP) with an amino acid profile similar or better than soybean meal [9]. Also Yu et al. [9] reported that 4% HIL in the diet of finishing pigs can significantly increase the ADG and decrease the gain:feed (G:F). Although insect diet has advantages, one study showed that the nutrient digestibility of HIL is lower than that of other protein sources because the outer wall of HIL is made of chitin [10]. Sánchez-Muros et al. [11] reported that chitin can negatively affect protein digestibility. Kim et al. [12] reported protease as part of an enzyme cocktail can increase the hydrolysis of proteins in the small intestine, making it profitable for pigs to absorb amino acids and peptides [13]. A diet supplemented with protease can affect the apparent ileal digestibility of gross energy (GE) [14] and improve the apparent ileal digestibility of protein in pigs [15]. We hypothesized that 1) poultry offal (PO) can be completely replaced with HIL. 2) If replacement with HIL is not effective, protease can assist.

A complete replacement of HIL would be possible and that if replacement of HIL does not have a positive effect on the growth performance and nutrient digestibility of growing pigs, it would be assisted using proteases. Therefore, the aim of the present study was to evaluate the effect of HIL as a protein resource added with protease on growth performance, digestibility, blood profiles, fecal microflora, and gas emission in growing pigs.

MATERIALS AND METHODS

Preparation diet and protease

PO diet and *Hermetia illucens* larvae (HIL) diet were provided by Cherrybro (Okcheon, Korea). The 3% PO of basal diet was replaced with 3% HIL of insect diet. Table 1 shows nutrient compositions of the diets. The chemical compositions and amino acid profiles of HIL are reported in Table 2. The PT125TM (an alkaline serine endopeptidase produced by *streptomyces* spp.) was provided and used by Eugene-Bio (Suwon, Korea).

Animals and experiment design

Experiment 1

Seventy-two crossbred growing pigs ([Landrace × Yorkshire] × Duroc) with an initial body weight (BW) of 27.98 ± 2.95kg were randomly allotted to one of four dietary treatments (3 pigs per pen and 6 replicates pen per treatments) based on BW. The experiment was conducted for 4 weeks. The dietary treatments were arranged in a 2 × 2 factorial design with two types of feedstuffs (3% PO, 3% HIL) and two levels of protease (0%, 0.05%). The dietary treatments included: 1) PO– (PO–;

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Table 1. Nutrient composition of the basal diet (%, as fed basis)

Items	PO	HIL
Ingredient (%)		
Corn (medium)	47.32	44.66
Corn (fine)	7.28	7.28
Wheat (fine)	5.00	5.00
Rice pollards	4.00	4.00
Soybean oil meal (45%)	24.06	26.20
Palm kernel	2.00	2.00
DDGS (28%)	3.00	3.00
Poultry offal	3.00	-
Hermetia illucens larvae	-	3.00
Animal fats	2.23	2.86
L-Lysine-SO_4	0.49	0.42
DL-Methionine	0.15	0.18
L-Threonine	0.15	0.14
L-Tryptophan	0.19	0.25
Salt	0.30	0.30
Limestone	0.37	0.25
MDCP	0.25	0.25
Mineral premix[1)]	0.12	0.12
Vitamin premix[2)]	0.10	0.10
Calculated value		
Metabolizable energy (kcal/kg)	3,001.86	2,927.04
Crude protein, (%)	19.5	19.5
Fat (%)	6.07	6.56
Ash (%)	4.41	4.65
Calcium (%)	0.40	0.50
Total phosphorous (%)	0.51	0.49
Available phosphorous (%)	0.24	0.22
Lys (%)	1.23	1.21
Met + Cys (%)	0.80	0.78

[1)]Provided per kg of complete diet: copper (as $CuSO_4 \cdot 5H_2O$), 12 mg; zinc (as $ZnSO_4$), 85 mg; manganese (as MnO_2), 8 mg; iodine (as KI), 0.28 mg; and selenium (as $Na_2SeO_3 \cdot 5H_2O$), 0.15 mg.

[2)]Provided per kg of complete diet: vitamin A, 11,025 IU; vitamin D_3, 1,103 IU; vitamin E, 44 IU; vitamin K, 4.4 mg; riboflavin, 8.3mg; niacin, 50 mg; thiamine, 4 mg; D-pantothenic, 29 mg; choline, 166 mg; and vitamin B_{12}, 33 μg.

PO, poultry offal; HIL, *Hermetia illucens* larvae; DDGS, Distiller's dired grains with solubels; MDCP, monodicalcium phophate; Lys, lysine; Met, methionine.

poultry offal diet), 2) PO+ (PO− + 0.05% protease), 3) HIL− (3% PO of PO− diet was replacement 3% HIL), 4) HIL+ (HIL− + 0.05% protease). The pigs had *ad libitum* access to feed and water. Each pen was equipped with a single-sided stainless steel automatic feeder and nipple drinker. The daily feed allowance was arranged to 2.7 times the requirement to maintain digestible energy (DE, 2.7 × 110 kcal of DE/kg $BW^{0.75}$).

Experiment 2

A total of four crossbred growing pigs ([Landrace × Yorkshire] × Duroc) with an initial BW of 28.2 ± 0.1kg were individually accepted in 1.2 m × 0.7 m × 0.96 m stainless steel metabolism cages. Pigs

Table 2. Amino acid profile and chemical composition of *Hermetia illucens* larvae

Chemical composition	%
CP	44.67
EE	12.19
CF	7.75
Ash	18.87
Ca	5.83
P	0.87
Amino acid profile	
Aspartic acid	4.08
Threonine	1.78
Serine	1.83
Glutamic acid	4.69
Glycine	2.21
Alanine	2.79
Valine	2.48
Isoleucine	1.74
Leucine	2.79
Tyrosine	2.48
Phenylalanine	1.75
Lysine	2.56
Histidine	1.06
Arginine	2.03
Cystine	0.37
Methionine	0.66
Proline	2.55

CP, crude protein; EE, ether extract; CF, crude fiber; Ca, calcium; P, phosphorus.

were randomly fed on four diets for four weeks (4 × 4 Latin square design). The dietary treatments included: 1) PO– (PO–; poultry offal diet), 2) PO+ (PO– + 0.05% protease), 3) HIL– (3% PO of PO- diet was replacement 3% HIL), 4) HIL+ (HIL– + 0.05% protease). The diets were provided at 08:00 and 17:00 every day. Water was provided freely for pigs to drink during the experiment. The experimental environment was maintained a relative humidity of 83 ± 2.3%, temperature of 23 ± 1.5℃ and a wind speed of 0.25 ± 0.03m/s.

Measurements and sampling

Nutrient digestibility

In experiment 1, fecal samples were collected for each treatment group at 2 and 4 weeks, and then immediately analyzed Chromic oxide (0.2%) as an indigestible marker was added in pigs' diet to determine the apparent total tract digestibility (ATTD) of DM, CP, and GE. Cr_2O_3 was measured by an acid digestion method using a Spectrophotometer (Model V-550, Jasco, Tokyo, Japan). The ATTD was calculated using the following formula: digestibility (%) = [1 – {(Nf × Cd)/(Nd × Cf)}] × 100, where Nf = nutrient concentration in feces (% DM), Nd = nutrient concentration in diet (% DM), Cd = chromium concentration in diet (% DM), and Cf = chromium concentration in feces (% DM).

In experiment 2, fecal was collected by total collection method and immediately after collection, fecal was placed in plastic bag and stored in a –21℃ freezer. The urine samples were taken daily and

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placed in buckets with 50 mL of 6 mol/L HCl underneath the metabolic cages. The whole amount of collected urine was weighed and kept at 20°C. Feces and urine collection were performed according to the method described in Kim et al. [12]. Fresh excreta samples were randomly collected every week and stored at 20℃ until analysis. The collected samples were combined with chromic oxide three days before sampling. The fecal, urine, and feed samples were thawed and dried at 60℃ for 72 hours before being crushed on a 1-mm screen and thoroughly pooled. Sub-samples were then collected for chemical analysis. The GE was calculated using a calorimeter bomb to measure the samples' heat of combustion (Parr 6100, Parr Instrument, Moline, IL, USA). In accordance with the approach outlined in AOAC [16], analyses of DM and CP were performed, and analyses of AAs were performed using High Performance Liquid Chromatography (HPLC) (SHIMADZU Model LC-10AT, Shimadzu, Kyoto, Japan) techniques. A Kjeltec 2300 Analyzer was used to determine the N content (Foss Tecator AB, Hoeganaes, Sweden).

Growth performance

On day 0, week 2, and week 4 growing pigs BW and feed intake were measured, and ADG, ADFI and G:F were calculated.

Fecal microflora and gas emissions

At week 2 and week 4, fresh fecal samples were collected from 2 pigs of each pen (Experiment 1) or each treatment (Experiment 2) by rectal massage. The collected feces were placed in an icebox for analysis and taken to the laboratory. Bacterial colonies were counted by the pour plate method. In order to measure the number of *Lactobacillus* and *Escherichia coli* (*E. coli*), de Man, Rogosa and Sharpe (MRS) agar for *Lactobacillus*, and MacConkey agar for *E. coli* were used, and *E. coli* was cultured at 37℃ for 24 h, and *Lactobacillus* was cultured for 48 h. The feces (300 g) collected per treatment were placed in a plastic box with small holes and the holes were sealed with plaster. The feces in the plastic box were fermented for 24 hours and 48 hours in room temperature (25℃) to ferment. NH_3 and H_2S concentrations were determined in the ranges of 50.0 to 100.0 ppm (No. 3La, detection tube, Gastec, Kanagawa, Japan).

Blood profiles

Blood samples were collected from anterior vena cava of 6 pigs each treatment at 2 and 4 weeks for analyzing concentration of white blood cells (WBCs), red blood cell (RBC), total protein and blood urea nitrogen (BUN) in whole blood and serum. After collection, serum samples were centrifuged (3,000 g) for 15 min at 4℃. The WBC, RBC, total protein and BUN were determined using an automatic blood analyzer (ADVIA 120, Bayer, Leverkusen, Germany).

Statistical analysis

Data for effects of two types of feedstuffs (3% PO, 3% HIL) added with different levels of protease on growth performance, digestibility of DM, CP, GE, blood profiles, gas emissions and fecal microflora of growing pigs were subjected to two-way ANOVA, with different feed, protease addition level, and their interactions as main effects and litter as a covariate. The data were statistically analyzed by the generalized linear model (GLM) procedure in IBM SPSS statistics v.25 (SPSS, Chicago, IL, USA). Each cage was used for each experimental unit. Differences between treatment groups was determined using Tukey's honest significant difference (HSD) test with a p-value of < 0.05 indicating significance and $0.05 < p$-value < 0.10 indicating a tendency.

RESULT

Experiment 1

Growth performance

From weeks 0 to 2, ADG and G:F were significantly increased ($p < 0.05$; $p < 0.05$ respectively) in the PO diet group compared with the HIL diet group (Table 3). From weeks 2 to 4, ADG and G:F were higher ($p < 0.001$; $p < 0.006$ respectively) for the protease group than for the non-protease group. From weeks 0 to 4, G:F were higher ($p < 0.05$) for the PO diet group than the HIL diet group of pigs.

Nutrient digestibility

The ATTD of DM and GE was not significantly ($p > 0.05$) affected by diet and protease (Table 4). CP was decreased ($p < 0.033$) by the HIL diet group at week 2 and 4. The HIL diet group showed lower CP digestibility than the PO diet group.

Blood profiles

From weeks 2 and 4, the PO diet group had lower BUN levels than the HIL diet group ($p < 0.004$ and $p < 0.005$, respectively; Table 5). Diet and protease had no effect ($p > 0.050$) on WBC, RBC, Lymphocyte, and total protein at weeks 2 and 4.

Fecal microflora

E. coli and *Lactobacillus* were not affected ($p > 0.05$) by diet and protease (Table 6).

Table 3. The effect of replacement dietary of animal protein with insect meal (*Hermetia illucens* larvae) and additive the protease on the growth performance in growing pigs (Exp1)

Items	Protein source		Protease		SE	*p*-value		
	PO	HIL	−	+		D	P	D × P
BW (kg)								
Initial	28.1	27.9	28.0	28.0	0.3	0.880	0.997	0.985
2 wk	38.9	37.8	38.4	38.3	0.4	0.255	0.800	0.404
4 wk	50.4	49.4	49.3	50.4	0.4	0.390	0.252	0.383
ADG (g)								
0 to 2 wk	776	706	746	737	15	0.036	0.608	0.079
2 to 4 wk	818	820	778	861	13	0.634	0.001	0.742
0 to 4 wk	797	765	764	798	9	0.164	0.056	0.059
ADFI (g)								
0 to 2 wk	1,710	1,752	1,740	1,722	24	0.339	0.740	0.059
2 to 4 wk	1,915	2,043	2,006	1,953	33	0.075	0.506	0.448
0 to 4 wk	1,814	1,903	1,877	1,840	31	0.098	0.413	0.413
G:F (g/g)								
0 to 2 wk	0.458	0.406	0.434	0.429	0.010	0.011	0.580	0.642
2 to 4 wk	0.434	0.413	0.397	0.450	0.009	0.499	0.006	0.823
0 to 4 wk	0.447	0.412	0.416	0.443	0.008	0.042	0.075	0.646

PO, poultry offal diet; HIL, *Hermetia illucens* larvae diet; D, diet; P, protease; BW, body weight; ADG, average daily gain; ADFI, average daily feed intake; G:F, gain:feed.

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Table 4. The effect of replacement dietary of animal protein with insect meal (*Hermetia illucens* larvae) and additive the protease on the nutrient digestibility of in growing pigs (Exp1)

Items (%)	Protein source		Protease		SE	*p*-value		
	PO	HIL	−	+		D	P	D × P
2 wk								
DM	81.38	81.03	81.26	81.15	0.17	0.290	0.761	0.527
CP	75.09	73.97	74.40	74.66	0.26	0.033	0.618	0.462
GE	72.67	73.07	72.73	73.01	0.36	0.588	0.707	0.868
4 wk								
DM	77.57	78.45	77.50	78.52	0.37	0.229	0.167	0.523
CP	73.82	72.05	72.85	73.02	0.41	0.033	0.832	0.891
GE	72.17	72.10	72.11	72.16	0.38	0.935	0.941	0.835

PO, poultry offal diet; HIL, *Hermetia illucens* larvae diet; D, diet; P, protease; DM, dry matter; CP, crude protein; GE, gross energy.

Table 5. The effect of replacement dietary of animal protein with insect meal (*Hermetia illucens* larvae) and additive the protease on the blood profile in growing pigs (Exp1)

Items	Protein source		Protease		SE	*p*-value		
	PO	HIL	−	+		D	P	D × P
2 wk								
WBC (10^3/μL)	22.56	20.17	20.85	21.88	1.06	0.285	0.641	0.442
RBC (10^3/μL)	7.11	6.89	7.27	6.73	0.18	0.528	0.148	0.876
Lymphocyte (%)	55.70	57.17	56.11	56.76	1.23	0.580	0.806	0.806
Total protein (mg/dL)	5.67	5.85	5.73	5.79	0.06	0.176	0.616	1.000
BUN (mg/dL)	9.00	10.67	10.25	9.42	0.31	0.004	0.124	0.751
4 wk								
WBC (10^3/μL)	19.40	20.91	19.84	20.47	0.51	0.156	0.548	0.767
RBC (10^3/μL)	7.02	7.42	7.27	7.16	0.21	0.345	0.797	0.309
Lymphocyte (%)	56.50	54.52	55.11	55.91	1.22	0.447	0.758	0.547
Total protein (mg/dL)	5.80	5.91	5.92	5.79	0.09	0.547	0.487	0.096
BUN (mg/dL)	10.33	12.84	12.17	11.00	0.47	0.005	0.153	0.834

PO, poultry offal diet; HIL, *Hermetia illucens* larvae diet; D, diet; P, protease; WBC, white blood cell; RBC, red blood cell; BUN, blood urea nitrogen.

Gas emission

NH_3 and H_2S gas emissions were not affected ($p > 0.05$) by diet and protease (Table 7).

Experiment 2

Nutrient digestibility

Protease did not affect ($p > 0.05$) the ATTD of DM, CP, and GE (Table 8). The HIL diet group showed lower ($p < 0.05$) CP than the PO diet group. The ATTD of arginine, histidine, lysin and tryptophan were higher ($p < 0.05$) in the PO diet group than the HIL diet group. The ATTD of threonine was higher ($p < 0.05$) in the protease group than the non-protease group. The ATTD of total essential AAs tended to be higher ($p = 0.05$) in the PO diet group than the HIL diet group. The ATTD of cysteine was higher ($p < 0.05$) in the protease group than the non-protease group. The ATTD of glycine was higher ($p < 0.05$) in the PO diet group than the HIL diet group. There was no interaction between diet and protease in nutrient digestibility.

Table 6. The effect of replacement dietary of animal protein with insect meal (*Hermetia illucens* larvae) and additive the protease on the fecal microflora in growing pigs (Exp1)

Items (log_{10}CFU/g)	Protein source		Protease		SE	*p*-value		
	PO	HIL	−	+		D	P	D × P
2 wk								
E. coli	5.725	5.808	5.885	5.648	0.26	0.577	0.136	0.600
Lactobacillus	8.982	9.016	9.077	8.920	0.16	0.725	0.136	0.733
4 wk								
E. coli	5.818	5.743	5.849	5.712	0.19	0.518	0.262	0.509
Lactobacillus	9.162	9.061	9.108	9.115	0.13	0.222	0.931	0.182

PO, poultry offal diet; HIL, *Hermetia illucens* larvae diet; D, diet; P, protease; *E. coli, Escherichia coli*.

Table 7. The effect of replacement dietary of animal protein with insect meal (*Hermetia illucens* larvae) and additive the protease on the fecal gas emission in growing pigs (Exp1)

Items (ppm)	Protein source		Protease		SE	*p*-value		
	PO	HIL	−	+		D	P	D × P
NH_3								
24 h	12.3	13.0	12.8	12.5	0.9	0.156	0.632	0.213
48 h	20.4	20.4	22.8	18.0	2.6	0.671	0.366	0.608
H_2S								
24 h	4.1	5.3	4.9	4.4	0.7	0.082	0.431	0.749
48 h	9.7	11.1	11.1	9.7	1.1	0.236	0.247	0.804

PO, poultry offal diet; HIL, *Hermetia illucens* larvae diet; D, diet; P, protease; SE, standard error; NH_3, ammonia; H_2S, hydrogen sulfide.

Fecal microflora

E. coli and *Lactobacillus* were not affected ($p > 0.05$) by diet and protease (Table 9).

Nitrogen utilization

The HIL diet group increased ($p < 0.05$) the fecal N excretion, urine N excretion, total N excretion, and decreased ($p < 0.05$) N retention (Table 10). The protease group decreased ($p < 0.05$) the urine N excretion and total N excretion and increased the N retention. There was no interaction between diet and protease in N utilization.

DISCUSSION

Experiment 1

Driemeyer [17] reported that partially replacing fishmeal with HIL (3.5%) does not affect the growth performance of pigs. Biasato et al. [18] showed no significant difference in the effect of replacing 0%, 5%, or 10% soybean meal with HIL on the growth performance of piglets. However, many researchers suggested that replacing protein sources in animal feed with insect protein could improve growth performance and nutrient digestibility [19]. In addition, Ma et al. [20] showed that ADG is increased but feed conversion ratio is decreased when a diet is supplemented with 4% HIL than a diet supplemented with 8% HIL in growing pigs. However, in the present study, replacing PO with HIL decreased growth performance. This reduction was caused by an anti-nutritional factor (i.e., chitin) included in HIL. When the component of chitin in the exoskeleton of HIL is not digested, it can bind to proteins and reduce digestibility [21,22]. Barragan et al. [3] reported that HIL should only be partially replaced because the performance is degraded by factors such

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Table 8. The effect of replacement dietary of animal protein with insect meal (*Hermetia illucens* larvae) on digestibility of nutrient and amino acids in growing pigs (Exp 2)

Items (%)	Protein source		Protease		SE	*p*-value		
	PO	HIL	−	+		D	P	D × P
Apparent total digestibility								
DM	81.23	80.43	80.45	81.21	0.27	0.149	0.165	0.545
CP	81.14	79.21	79.71	80.64	0.37	0.005	0.128	0.856
GE	80.44	79.16	79.34	80.26	0.36	0.071	0.177	0.360
Essential amino acids								
Arginine	92.53	90.58	91.46	91.65	0.36	0.004	0.738	0.272
Histidine	90.06	85.53	87.11	88.49	0.77	0.001	0.208	0.867
Isoleucine	81.18	79.75	80.04	80.88	1.08	0.554	0.727	0.665
Leucine	82.67	81.37	81.58	82.47	0.56	0.280	0.452	0.616
Lysine	85.77	82.84	83.74	84.87	0.59	0.009	0.254	0.601
Methionine	84.47	81.57	81.49	84.55	0.89	0.091	0.076	0.980
Phenylalanine	83.65	82.95	82.51	84.09	0.56	0.542	0.186	0.553
Threonine	81.59	80.44	79.49	82.54	0.57	0.190	0.003	0.347
Valine	80.29	78.80	78.69	80.40	0.60	0.223	0.164	0.764
Tryptophan	83.82	70.87	77.34	77.36	2.00	0.000	0.994	0.988
Total	84.70	82.75	83.14	84.31	0.50	0.050	0.221	0.626
Non-essential amino acids								
Alanine	75.53	74.03	74.00	75.55	0.65	0.267	0.254	0.464
Aspartic acid	82.15	81.67	81.11	82.70	0.43	0.576	0.081	0.691
Cysteine	84.56	81.96	81.39	85.13	0.91	0.101	0.025	0.237
Glycine	81.09	76.81	79.52	78.39	0.75	0.000	0.224	0.031
Glutamic acid	86.77	86.69	86.41	87.05	0.27	0.885	0.266	0.247
Proline	84.88	84.03	84.62	84.29	0.43	0.376	0.713	0.416
Serine	84.08	83.56	84.02	83.62	0.36	0.506	0.604	0.265
Tyrosine	80.66	79.48	79.36	80.78	0.82	0.512	0.430	0.587
Total	83.46	82.64	82.67	83.43	0.30	0.180	0.210	0.428
Total amino acids	84.04	82.70	82.89	83.85	0.39	0.086	0.208	0.514

PO, poultry offal diet; HIL, *Hermetia illucens* larvae diet; D, diet; P, protease; DM, dry matter; CP, crude protein; GE, gross energy.

Table 9. The effect of replacement dietary of animal protein with insect meal (*Hermetia illucens* larvae) and additive the protease on the fecal microflora in growing pigs (Exp2)

Items (log_{10}CFU/g)	Protein source		Protease		SE	*p*-value		
	PO	HIL	−	+		D	P	D × P
2 wk								
E.coli	5.497	5.488	5.449	5.536	0.030	0.896	0.187	0.846
Lactobacillus	8.894	8.894	8.916	8.872	0.037	0.928	0.369	0.894
4 wk								
E.coli	5.519	5.526	5.486	5.559	0.015	0.990	0.179	0.997
Lactobacillus	8.977	8.973	8.995	8.954	0.023	0.934	0.422	0.845

PO, poultry offal diet; HIL, *Hermetia illucens* larvae diet; D, diet; P, protease; *E. coli, Escherichia coli*.

Table 10. The effect of replacement dietary of animal protein with insect meal (*Hermetia illucens* larvae) and additive the protease on nitrogen utilization in growing pigs (Exp2)

Items (g/d)	Protein source		Protease		SE	*p*-value		
	PO	HIL	−	+		D	P	D × P
Daily balance of N								
N intake	42.4	41.7	41.9	42.1	0.8	0.648	0.923	0.853
Fecal N excreted	8.0	8.6	8.5	8.1	0.1	0.026	0.167	0.964
Urine N excreted	4.7	6.0	6.4	4.3	0.4	0.037	0.002	0.134
Retained N	29.8	27.1	27.1	29.8	0.9	0.115	0.117	0.452
Total N excreted	12.6	14.6	14.9	12.3	0.4	0.003	0.001	0.142
N retention rate	69.7	64.7	64.0	70.4	1.2	0.018	0.003	0.185

PO, poultry offal diet; HIL, *Hermetia illucens* larvae diet; D, diet; P, protease; N, nitrogen.

as high fat content and ash in insect meal. In the present study, pigs fed diets added with 0.05% protease had better ADG and G:F than pigs fed diets without protease. These results agreed with the results of Zuo et al. [23] and Park et al. [24] showing that supplementation of 200 mg and 0.2% protease in the swine diet, respectively, could improve growth performance. Min et al. [25] reported that the positive effect on growth performance could be explained by increased protein hydrolysis caused by the addition of dietary protease, thereby improving nutrient digestion and utilization.

The BUN concentration is commonly used to determine nitrogen utilization and excretion rates. It is also used to find renal damage in animals and humans [26,27]. BUN is decreased when nitrogen is efficiently used in protein synthesis. It is increased spontaneously as the kidney filtration rate decreases [28,29]. In the current study, the HIL resulted in higher BUN concentrations. Fang et al. [30] reported that BUN levels are negatively correlated with ADG and G:F ratios. Chitin supplementation may decrease ammonia levels, in part by affecting BUN levels [31], consistent with growth performance and BUN results of the current experiment. The BUN level is considered to be increased in this experiment due to low growth performance with the HIL diet compared to the PO diet.

The possibility of improving intestinal health by using insect replacement diet has been suggested based on immune system stimulating properties of chitin, antimicrobial peptides (AMPs), and antimicrobial activity of lauric acid [32–34]. A previous study reported that the antibacterial peptide of HIL shows antibacterial activity against pathogenic bacteria such as *E. coli* [35]. However, it had no significant effect on fecal microbiota.

Major components of fecal gas that cause air pollution are NH_3 and H_2S [36]. In general, fecal gas emission is related to dietary digestibility. Enhanced digestion, which may reduce microbial fermentation substrates in the large intestine, can lower gas emissions [37]. However, The HIL and protease showed no significant effect on NH_3 or H_2S emission in the overall experiment.

Experiment 2

Many previous studies reported that insect protein sources such as house fly, black soldier fly, HIL, and domestic larvae can be suitable ingredients in the diets of animals [38–41]. They explained that insect ingredients have similar or superior amino acid composition and higher protein content than conventional protein sources. Tan et al. [41] reported that amino acid content in insect or amino acid digestibility of supplement insect meal are similar to soybean meal or fish meal in growing pigs. However, in this experiment, the digestibility of essential AAs including lysine was significantly decreased in the HIL group. CP digestibility of the HIL group also decreased compared to that of the PO group. Bovera et al. [5] reported that digestibility of DM and CP is decreased when

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Tenebrio molitor is added to broiler diet. The low nutrient digestibility of the HIL group is caused by the mechanisms of chitin in HIL. Endogenous enzymes do not breakdown β1–4 links between N-acetylglucosamine that compose chitin [42]. Therefore, any protein encapsulated by chitin would not be digested or absorbed by the pig's small intestine [43]. Mammals express acidic mammalian chitinase (AMCase). Pig AMCase is stable in the presence of other digestive proteases. It functions as chitinolytic enzyme under gastrointestinal conditions [44]. Zuo et al. [23] reported that supplement of protease can increase the growth performance when pigs are fed with a low digestible protein source. Protease is a proteolytic active enzyme. It can improve protein digestibility in several experiments [23,45,46]. Therefore, we tried to weaken the antinutrient action of chitin by adding protease. However, the addition of protease showed no significant difference compared to the control without such addition.

CONCLUSION

In summary, the present study revealed that the replacement of PO with HIL and additive of the protease in growing pig diets during the overall experimental period (week 0–4) had no negative effect. The supplementation of protease in the HIL diet increased the ADG and AAs digestibility. But, trends in interactive effects between diet and protease were not observed in this experiment. More research is needed.

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