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Research article

Nutritional values and nutrient digestibility of ground perilla cake (*Perilla frutescens*) in growing pig diets

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Abstract

This study determined the nutritional values of ground perilla cake (GPC) and the potential for dietary supplementation in growing pigs based on *in vitro* ileal digestibility (IVID) and apparent total tract digestibility (ATTD). The IVID evaluated at four dietary supplementation levels: 0, 5, 10, and 20%. The ATTD was measured by using twenty-four grower crossbred pigs. Pigs were randomly assigned to three dietary supplementation levels (0, 5, and 10%). From these analytical results, GPC raw material contained crude protein (CP) content (31.54%). That total essential amino acid (EAA) was 138.34 mg/g, mainly leucine (28.87 mg/g), and contained notably limiting amino acids for pigs, such as lysine (19.52 mg/g) and methionine (10.94 mg/g). The ether extract content (EE) was 10.52%, and the major free fatty acid (FFA) was linolenic acid (C18:3n3; 55.97%) and the fat-soluble vitamins included γ-tocopherol (367.25 µg/100g). In addition, GPC contained minerals such as phosphorus (1.02%), potassium (0.83%), calcium (0.46%), and iron. However, crude fiber (CF) had a notably high content (24.43%). Increasing GPC levels in pig diets reduced the IVID of dry matter (DM), EE, and CF ($P<0.05$), especially the 20% GPC supplement. The IVID of the CP did not differ among the groups. Furthermore, the results for the ATTD of the CP and EE in the 5% GPC supplement group were significantly better than that of the other groups ($P<0.05$). We found the potential of GPC as an alternative protein source. Moreover, it contained high energy and polyunsaturated fatty acid.

Keywords: : Apparent total tract digestibility, Digestibility, Ground perilla cake, Growing pig, *In vitro* ileal digestibility, Nutritional value

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INTRODUCTION

Feedstuffs from plants and their by-products are widely used in pig diets (Skoufos et al., 2016), for example, canola, palm kernel, sesame, and perilla (Son et al., 2019). They are less expensive than animal protein sources, and they can supply nutritionally balanced diets at a minimal cost for pork, while at the same time, decreasing the import cost of other protein sources as fishmeal and soybean meal. Perilla (*Perilla frutescens* L.) of the Lamiaceae family is native to Asia and distributed across India, China, Japan, Korea, Laos, and Thailand, used as food and known as a good source of seed oil, the fat contains around 51% (Asif, 2011; Longvah and Deosthale, 1991). In Thailand, perilla seed production accounts for roughly 272 tons/year for perilla oil extraction, and approximately 60% remaining are perilla meal which is a by-product after oil extraction (Montha et al., 2021). Generally, the crude protein (CP) in whole perilla seed content is 17.2%, while it is 20.1% in kernels and 5% in the hull, while the defatted perilla seeds had a CP content 36.3%. (Longvah and Deosthale, 1998). Also, Son et al. (2017) found that perilla seed meal contains 43.2% CP, 1.08% EE, and 9.0% ash.

A few researchers presented that the perilla seed and meal could be used for diet formulation for improving the production and health of animals (Ruamrungsri et al., 2016; Arjin et al., 2020). Oh et al. (2020) reported that a diet contained 2% perilla seed meal can achieve improvement in growth performance, better meat quality, and fatty acid composition of thigh meat of broilers. Hadi and Sudiyono (2019) found that with 5% perilla seed in a duck diet, the ADG and fat content of meat were increased. Peiretti et al. (2010) showed that 10% perilla seed in the rabbit diet had better digestibility than other diets. In general, perilla meal comes from a solvent extraction method, resulting in less fat contents around 1–2 %. The extraction method of screw pressing provided a by-product after oil extraction with a higher oil residue (8–14%) than that from solvent extraction (Ionescu et al., 2015; Shrikanta Rao, 1980). Ground perilla cake (GPC) is obtained from perilla seeds by screw pressing from the oil refinery industry. To the best of our knowledge, few research studies have investigated the general use of GPC in pig diets. The same is true for evaluating the properties of GPC raw material in terms of nutritional value and digestibility for use as the protein source of pig diets. Therefore, this study assessed the nutritional values of GPC raw material and evaluated the potential of dietary GPC supplementation by using the nutrient digestibility as *in vitro* ileal digestibility (IVID) and apparent total tract digestibility (ATTD) in growing pigs.

MATERIALS and METHODS

Chemicals

The γ-tocopherol standard was obtained from Restek, USA, butylated hydroxytoluene (BHT), pepsin, pancreatin, chloramphenicol were obtained from Sigma-Aldrich Co. (St. Louis, MO, USA). All other chemicals used were of analytical grade. Dichloromethane, methanol, phenol, hexane, and isopropanol (HPLC grade) were purchased from Merck (Darmstadt, Germany). Sodium hydroxide and ethanol were obtained from RCI Labscan Limited (Bangkok,

Thailand). 5-Sulfosalicylic acid was purchased from Loba Chemie Pvt. Ltd. (Rd. Colaba, Mumbai, India). Fatty acid methyl esters (FAME) standard mixtures was purchased from Restek Corporation (PA, USA).

Raw materials and chemical composition analysis

The GPC used in this study was the by-product from the mechanical screw press of perilla oil extraction from the Putawan Nakhon Pathom limited partnership oil refinery industry located in Nakhon Pathom, Thailand. The chemical compositions of these feedstuffs were analyzed according to methods of the AOAC (2006): DM was determined via oven-drying at 95-100°C for 5 h (method 934.01). Ash was determined following the complete combustion of samples in a muffle furnace at 600°C for 2 h (method 942.05). The CP content was determined by the macro-Kjeldahl technique (method 2001.11) and calculated as nitrogen × 6.25. EE was extracted via Soxhlet extraction for 16 h using dichloromethane as a solvent (method 920.39). CF was determined following method 962.09, and gross energy (GE) was scaled using an adiabatic bomb calorimeter (CAL2k oxygen bomb calorimeter, Digital Data Systems, South Africa).

The amino acid profile was analyzed using the protein hydrolysis procedure according to Takenaka et al. (2010). A sample (100 mg) was weighed in a polytetrafluoroethylene container, followed by the addition of 10 mL of 6M HCl and 1–2 drops of phenol. Nitrogen gas was blown over the sample for approximately 15 min, and the lid was immediately closed. The sample was hydrolyzed using a microwave (TANK PRO, Sineo, China) for 30 min. Subsequently, the sample was filtered through Whatman paper, and the volume was adjusted to 50 mL with distilled water. The solution was evaporated with a rotary evaporator, followed by the addition of 2 mL of sample dilution buffer (SDB). The sample solution was filtered using a 0.45-μm syringe filter. Samples were analyzed with an Amino Acid Analyzer (membraPure, ARACUS, Germany).

The free fatty acid (FFA) profile was analyzed, and lipids were extracted from GPC by Soxhlet extraction (method 920.39). Fatty acid methyl esters were prepared as described by Morrison and Smith (1964). Gas chromatographic analysis was accomplished using the Shimadzu model GC-2030 (Kyoto, Japan) equipped with a 0.25 mm × 100 m × 0.25 μm wall-coated fused wax capillary column. Helium was used as the carrier gas. Oven temperature programming was increased from 100 °C and held for 4 min, and then increased from 100 to 240 °C at a rate of 3 °C /min, and then held at 240 °C for 20 min. The injector volume was 1 mL, and the flame ionization detector temperature was 250 °C. Chromatograms were processed using the Lab Solution (Shimadzu, Kyoto, Japan). Identification was accomplished by comparing the retention time of peaks from samples with those of FAME standard mixtures.

The γ -tocopherol content was analyzed according to Huang and Ng (2011). Briefly, 0.5 g of GPC has extracted with 3 mL of hexane and 0.1 mL of BHT (10 mg/mL) and stirred for 30 s. The test tubes were placed in a 60 °C for 20 min in a shaker water bath. After that, separation hexane layer by use centrifugation at 2,000 × *g* for 15 min. The upper layer was collected, and then the residue was twice extracted. Three separate extractions were combined and concentrated. Twenty microliters of the filtrate were injected into HPLC. The

HPLC system consisted of a Shimadzu (Kyoto, Japan) and fluorescence detector set at an excitation wavelength of 290 nm and an emission wavelength of 330 nm. Chromatographic separation was performed by Inertsil SIL100A (5 µm, 4.6 × 250 mm) column coupled with an Inertsil SIL 100A (5 µM, 4 × 10 mm) guard column (GL Sciences Inc., Tokyo, Japan), and a mobile phase composed of hexane/isopropanol/ethyl acetate/acetic acid (97.6:0.8:0.8:0.8, v/v/v/v). The flow rate was 1 mL/min. The γ-tocopherol was identified by retention time and quantified by the calibration curve of external standards (membraPure GmbH, Germany). Also, the β-carotene was analyzed following the method of Bohm et al. (2002).

In vitro ileal digestibility (IVID)

The growing pig diets were mainly maize, broken rice, rice bran, fishmeal, and soybean meal. The IVID performed to four levels of GPC supplementation in diets; 0% (GPC0), 5% (GPC5), 10% (GPC10), and 20% (GPC20), respectively. It was ensured that each diet had an amount of metabolizable energy (ME) and CP levels to meet the requirements of growing pigs, according to the National Research Council (NRC, 2012). The diet samples were sieved to particle size < 1 mm and subsequently stored in airtight containers until digestibility analysis.

IVID was determined according to methods described by Boisen and Fernández (1995) and Kong et al. (2015). At first, 1 g of sample was weighed into a 250-mL conical flask, added 25 mL of phosphate buffer (0.1 N, pH 6) and 10 mL 0.2 N HCl, adjusted pH to 2 using 1 N HCl and 1 N NaOH. Thereafter, 1 mL of freshly prepared pepsin (10 mg/mL; ≤ 250 U/mg) and 0.5 mL of chloramphenicol solution were added to the mixture. The mixture was incubated in a shaking incubator at 39°C for 6 h. Subsequently, 10 mL of phosphate buffer (0.2 M, pH 6.8) and 5 mL of 0.6 M NaOH were added and adjusted pH to 6.8 by 1 N HCl and 1 N NaOH. Then, 1 mL of a freshly prepared pancreatin solution (50 mg/mL) was added. The mixture was then incubated in a shaking incubator at 39°C for 18 h. After incubation, the enzymatic activity was terminated by adding 5 mL of 99% 5-sulfosalicylic acid solution. The mixture was stand at room temperature for 30 min, filtered through weighed glass filter crucibles (Kovitvadhi et al., 2019), and rinsed with 10 mL of 95% ethanol, followed by 99.5% acetone. The undigested residues were dried overnight at 100°C, and the residue was cooled in a desiccator and weighed. The chemical compositions of the residues were analyzed according to AOAC (2006). All digestion parameters were calculated from the difference between concentration in the sample and undigested residue after correcting.

Apparent total tract digestibility (ATTD)

This study was performed in strict accordance with the recommendations in the "Guide for the Care and Use of Agricultural Animals in Research and Teaching". All experimental protocols were reviewed and approved by the Animal Care and Use Committee, Chiang Mai University (2559/AG-0001). The experiment was conducted at Mea Hia Agriculture Resource Demonstrative and Training Center, Faculty of Agriculture, Chiang Mai University, Chiang Mai, Thailand. Twenty-four grower crossbred barrow pigs [('Large White' × 'Landrace') × 'Duroc'] with an initial average body weight of 26.32 ± 3.05 kg were used. Pigs were randomly designed to three dietary treatments (8 replicate

pens of 1 pig per pen) in completely randomized design (CRD). The pigs were individually raised in a metabolic cage (0.4 × 0.8 × 0.9 m) which contained drinking water and a feeding trough. The daily feed allowance was equivalent to 4% of body weight at the beginning of each period (Adeola, 2001). Water was provided *ad libitum*, and pigs were fed at 8:00 am and at 5:00 pm; before the experiment.

The diets were formulated to three levels of GPC supplement: 0% (GPC0), 5% (GPC5), and 10% (GPC10). Digestibility was determined by the indicator method using Cr_2O_3 (Kasprowicz and Frankiewicz, 2004). The experimental period included five days of adaptation followed by five days during which samples for ATTD were taken. The ATTD was determined according to Recharla et al. (2019). During the collection period, the feces of individual pigs were collected daily at approximately 08.00 h. After that, the next daily ration was provided. Each daily feces sample was placed in a two-layer plastic bag to prevent the loss of moisture; urine was collected by using plastic containers that contained 200 mL of 2 N HCl. All feces and urine samples were stored at –80°C immediately to constant weight after collection and until chemical analyses.

Chemical analysis of feed and feces

All samples were dried in an oven at 60°C for 48 h. After drying, samples were ground and passed through a 1-mm sieve. The samples were analyzed according to Kim et al. (2009) and Son et al. (2017): Approximately 3 mL of the urine sample was added to a cotton ball (0.3–0.4 g) placed in a crucible. The weight of the crucible, cotton ball, and urine was recorded. Samples were then dried for 24 h before GE analysis. The chemical compositions of all samples including DM, ash, CP, EE, fiber, and GE were conducted according to the AOAC (2006).

Calculation and statistical analysis

For all IVID samples, *in vitro* dry matter digestibility (IVDDM) was calculated according to Tiwari and Jha (2016). This method also allows for determining the *in vitro* ileal digestibility gross energy (IVDGE). IVDDM was calculated according to equation 1:

$$IVDDM = [(DWH – DWR)/DWH] \times 100 \quad (1)$$

where DWH is the dry weight of the sample before hydrolysis and DWR is the dry weight of the residue. The IVDGE was calculated according to equation 2:

$$IVDGE = [(g\ sample \times DMs \times GEs) – (g\ residue \times GEr)]/(g\ sample \times DMs \times GEs) \quad (2)$$

where DMs is the percentage of dry matter of the sample, GEs is the gross energy of the sample, and GEr is the gross energy of the residue.

$$DE = 4{,}151 – [(12.2 \times Ash) + (2.3 \times CP) + (3.8 \times EE) – (6.4 \times CF)] \quad (3)$$

$$ME = [(0.997 \times DE) – (0.68 \times CP) + (0.23 \times EE)] \quad (4)$$

$$NE = (0.843 \times DE) – 463 \quad (5)$$

Digestible energy (DE) and ME in feed formulation were calculated according to Noblet and Perez (1993), and net energy (NE) was determined as described by Noblet et al. (1994), according to equations 3-5:

The ATTD of nutrients was determined by the index method as described by Adeola (2001), and expressed as % digestibility according to equation 6:

$$\text{Digestibility (\%)} = 100 - [100 \times (Cr_2O_3 \text{ in feed} \times \text{N in feed})/\text{N in feces} \times Cr_2O_3 \text{ in feces})] \quad (6)$$

where N intake is the nutrient or energy (g or MJ kg^{-1}).

The IVID and ATTD digestibility were subjected to statistical analysis as a randomized complete design using SPSS for Windows version 23.0 (SPSS Inc., Chicago, IL, USA). Analysis of variance (ANOVA) was used to evaluate the effects of different GPC levels in the diet on IVID and ATTD. In addition, differences between groups were tested using Duncan's test with a $P < 0.05$ indicating statistically significant.

RESULTS

Chemical and amino acid composition and fatty acid profile of GPC

The chemical composition of GPC had a high content of CP (31.54%), CF (24.43%), and EE (10.52%), and it also had a high GE content (4,715 cal/g) and DM (92.10%) but a low ash content (6.62%). The GPC had many macrominerals such as phosphorus (1.02%), potassium (0.83%), calcium (0.46%), and magnesium (0.40%). Moreover, there were 468.43 mg/kg iron, 66.87 mg/kg manganese, 60.80 mg/kg zinc, and 7.64 mg/kg sodium as shown in Table 1.

Table 1 Chemical composition and minerals of ground perilla cake.

Items	Contents
Chemical composition	
Dry matter, %	92.10
Crude protein, %	31.54
Ether Extract, %	10.52
Ash, %	6.62
Crude fiber, %	24.43
Gross energy, cal/g	4,715
Mineral	
Calcium, %	0.46
Magnesium, %	0.40
Phosphorus, %	1.02
Potassium, %	0.83
Iron, mg/kg	468.43
Manganese, mg/kg	66.87
Sodium, mg/kg	7.64
Zinc, mg/kg	60.80
Vitamin	
β-carotene, µg/100g	nd.
γ- tocopherol, µg/100g	367.25

nd. = not detected; The β-carotene limit of detection is 0.02 µg /kg

The amino acid composition and fatty acid profile in GPC were analyzed and presented in Table 2. The total amino acid contents were detected as 439.34 mg/g. The essential amino acid (EEA) content was found to be around 138.34 mg/g. The EAA contents found that leucine was highest (28.87 mg/g). Also, lysine was the first limiting amino acid in pigs, it contained 19.52 mg/g, and methionine is the second limiting amino acid was 10.94 mg/g.

Table 2 Amino acids profile of ground perilla cake

Amino acid	Content (mg/g)
Essential amino acids (EAA)	
Histidine	13.73 ± 0.15
Isoleucine	10.92 ± 0.07
Leucine	28.87 ± 0.02
Lysine	19.52 ± 0.27
Methionine	10.94 ± 0.14
Phenylalanine	21.85 ± 0.06
Threonine	17.11 ± 0.01
Valine	15.41 ± 0.01
Σ EAA	**138.34**
Non-essential amino acids (NEAA)	
Alanine	22.81 ± 0.14
Arginine	47.61 ± 24.85
Aspartic acid	40.04 ± 0.24
Cystine	3.41 ± 0.26
Glutamic acid	87.73 ± 0.41
Glycine	23.13 ± 0.12
Hydroxy-L-proline	2.35 ± 0.21
Hydroxylysine	0.54 ± 0.03
Proline	16.29 ± 0.10
Serine	26.16 ± 0.09
Tyrosine	16.91 ± 0.20
Σ NEAA	**301.00**
Σ amino acids	**439.34**

Data for the amino acid compositions represent the mean of triplicates

The fatty acid profile found that GPC contained saturated fatty acid (SFA) levels lower than monounsaturated fatty acids (MUFA) (10.31% and 12.78%, respectively) (Table 3). The main SFA was palmitic acid (C16:0) 6.86%. The major fatty acid content was polyunsaturated fatty acid (PUFA), which was 76.91%. In this study, GPC contained mainly PUFA as linolenic acid (C18:3n3) at 55.97%, linoleic acid (C18:2n6) at 20.62%, and the major MUFA was oleic acid (C18:1n9c) at 12.37%. Moreover, we found the fat-soluble vitamin was γ- tocopherol (367.26 µg/100g). However, β-carotene content was not detected (LOD = 0.02 µg/kg).

Table 3 Fatty acid profile of ground perilla cake

Fatty acids profile	Content (%)
Saturated fatty acid (SFA)	
C16:0	6.86
C17:0	0.03
C18:0	3.13
C20:0	0.19
C22:0	0.03
C23:0	0.02
C24:0	0.02
Monounsaturated fatty acid (MUFA)	
C16:1	**0.19**
C18:1 n9t	**0.03**
C18:1 n9c	12.37
C20:1 n9	0.14
C22:1n9	0.03
Polyunsaturated fatty acid (PUFA)	
C18:2n6	20.62
C20:2	0.05
C18:3n6	0.21
C18:3n3	55.97
C20:3n3	0.04
C20:5n3	0.01
C22:6n3	0.01
Σ SFA	**10.31**
Σ MUFA	**12.78**
Σ PUFA	**76.91**

Compositions represent the mean of triplicates.

In vitro ileal digestibility of diet (IVID)

The IVID performed four levels of GPC supplementation in diets, as shown in Table 4. The results indicated that the IVID of GPC raw material had the potential for nutrient digestibility with CP (45.37%), and GE 46.47%, but there was low potential in the EE and CF with only 33.70% and 29.45%, respectively. In addition, there was 45.72% DM, shown in Table 5.

In the IVID of the diet formula, the GPC20 showed a lower potential of IVID than other groups in all parameters except CP. The IVID of CP in all levels of GPC supplement ranged from 72.94 to 74.65%. However, the IVID of CP was not significantly different between groups ($P>0.05$). The IVID of DM in the GPC0 group (80.89%) was the highest ($P<0.01$), and GPC5 (74.40%) was more elevated than the GPC10 group (69.35%). Besides, the IVID of GE was in the same direction, but GPC5 (77.77%) was not different from the GPC10 group (77.05%). Nevertheless, the IVID of CF and EE was not different in GPC0, GPC5, and GPC10 but higher than GPC20.

Table 4 The ingredients and chemical composition of diet formulation on a as is basis.

Items	GPC (% of diet)			
	GPC0	GPC5	GPC10	GPC20
Ingredient (%)				
Maize	31.00	29.67	29.34	28.00
Broken rice	31.00	29.22	29.00	27.68
Rice bran	9.92	12.00	12.00	13.00
GPC	0.00	5.00	10.00	20.00
Soybean meal (44%)	20.68	15.51	10.34	0.00
Fish meal (58%)	4.80	6.00	6.72	8.00
Dicalcium phosphate	2.00	2.00	2.00	2.00
Salt	0.25	0.25	0.25	0.25
Premix[a]	0.35	0.35	0.35	0.35
Total	100	100	100	100
Chemical composition (analyzed value)				
Dry matter, %	89.99	89.59	89.76	91.25
Crude protein, %	20.40	20.33	20.10	20.02
Ether Extract, %	3.97	4.31	4.58	7.50
Ash, %	4.37	6.51	6.78	6.32
Crude fiber, %	22.94	25.49	28.43	35.01
Chemical composition (calculated value)				
Gross energy, cal/g	4,056	4,400	4,668	4,890
Digestible energy, cal/g	4,013	3,972	3,950	3,924
Metabolizable energy, cal/g	3,999	3,958	3,936	3,911
Net energy, cal/g	3,037	3,001	2,983	2,960

Data for the chemical composition represent the mean of triplicates.
GPC = Ground perilla cake
[a] Vitamin premix (U or mg provided per kilogram of premix): vitamin A, 12,000U; vitamin D3, 4500U; vitamin E, 70U; vitamin K, 3.5mg; vitamin B1, 3mg; vitamin B2, 7.5mg; vitamin B3, 30mg; vitamin B5, 65mg; vita- min B6, 4.3 mg; vitamin B9, 2 mg; vitamin B12, 0.025 mg; biotin, 0.3 mg; choline chloride, 800 mg.

Table 5 *In vitro* ileal digestibility (IVID) of ground perilla cake and diet formulation in growing pig diets.

Items	GPC*	GPC0	GPC5	GPC10	GPC20	SEM	P-value
Dry matter, %	45.72	80.89[a]	77.77[b]	77.05b[c]	75.07[c]	0.21	< 0.001
Crude protein, %	45.37	72.94	74.02	74.65	73.78	0.31	0.297
Ether Extract, %	33.70	61.33[a]	59.19[a]	64.33[a]	50.06[b]	1.95	0.023
Crude fiber, %	29.45	79.06[a]	73.93[a]	71.07[ab]	64.37[b]	1.93	0.020
Energy, %	46.47	80.65[a]	74.40[b]	69.35[c]	67.02[d]	1.58	< 0.001

* GPC raw material not statistical analysis with diet group.
SEM is the standard error of the mean.
Different superscript letters in the same row indicate significant differences (P<0.05). Statistical analysis in GPC0, GPC5, GPC10, GPC20
GPC = Ground perilla cake

Apparent total tract digestibility (ATTD)

According to the IVID result, we performed ATTD analysis in growing pigs at three GPC supplementation levels (Table 6). The results showed that the DM digestibility was not significantly different between groups (P>0.05). The ATTD of CP and EE in GPC5 were significantly higher than in GPC0 (P<0.05) but not different from GPC10. Moreover, the CF digestibilities of GPC0 and GPC5 were not different (43.96 and 43.07%, respectively); however, GPC10 (25.89%) was significantly lower than among the other groups (P<0.05). In addition, the GE digestibility of GPC10 was the lowest (73.33%) in the other groups.

Table 6 Effect of ground perilla cake on apparent total tract digestibility of growing pigs.

Items	GPC0	GPC5	GPC10	SEM	P-value
Dry matter, %	72.56	72.49	68.20	1.07	0.165
Crude protein, %	68.45^{b}	74.19^{a}	72.53^{ab}	1.07	0.049
Ether Extract, %	54.24^{b}	62.93^{a}	59.21^{ab}	1.51	0.028
Crude fiber, %	43.96^{a}	43.07^{a}	25.89^{b}	3.27	0.007
Energy, %	80.49^{a}	75.75^{b}	73.33^{c}	1.05	<0.001

SEM is the standard error of the mean.
Different superscript letters in the same row indicate significant differences (P<0.05).
GPC = Ground perilla cake

DISCUSSION

GPC is a by-product of perilla seed after removing oil and is known as a good source of protein. It is very suitable for potential pig diets because of its high CP content, making it a potential alternative source of protein. In this study, the CP in GPC was 31.54%, which was excellent to be the protein source for the pig diet. Meanwhile, Oh et al. (2020) reported that perilla seed meal contained CP at 39.01%, which differed considerably from the results of Son et al. (2017), who found CP at 43.2%. In addition, amino acids play an important role in the growth rate of pigs, particularly EAA, especially lysine and methionine, the first and second most limiting AA in a cereal-grain-based diet for pigs. In this study, the major EAA in GPC was leucine that contained 28.87 mg/g (2.887%). In addition, the limiting AA lysine was 19.52 (1.952%), and methionine was 10.94 mg/g (1.094%). Similar to the report of Son et al. (2019) found that perilla meal contained leucine (2.25%), lysine (0.44%), and methionine (0.35%). However, the variation of AA among oilseeds may vary depending on several factors such as botanical varieties, geographical locations, climate, cultivation and processing treatment (Messad et al., 2016). Longvah and Deosthale (1998) reported that lysine of perilla seed cultivated in India was 37.13 mg/g (3.71%) and methionine 26.02 mg/g (2.60%), while perilla seed was cultivated in Fukushima, Japan was 5.2% lysine (Oita et al., 2008).

In the present study, the EE content in GPC was 10.52%, which is higher than the report of Oh et al. (2020), who found the EE content in perilla meal was 7.31%. The large variation of EE content of perilla meal from other reports as Son et al. (2017) showed EE content at 1.08%. The reason for that difference is from the oil extraction methods. Generally, when the oil extraction

method is solvent extraction, it resulted in a low oil content left in the by-product. Son et al. (2017) reported that the EE of perilla meal, canola meal, and soybean meal were 1.08%, 1.85%, and 0.74–2.46%, respectively. However, the GPC sample in this study was obtained as a by-product from the mechanical screw press oil extraction. Shrikanta Rao (1980) described that mechanical screw presses are relatively inefficient for oil recovery, leaving about 8–14% of the available oil in the cake. This process does not entirely extract the oil from the seeds, resulting in a higher EE content. Furthermore, genetic and environmental factors affect the properties of the final product and can result in varying EE contents. Based on this observation, it is hypothesized that differences in the EE content of the raw material used as sources of GPC also affected the CP content. Moreover, GPC also contain minerals such as phosphorus (1.02%), potassium (0.84%), calcium (0.46%), and magnesium (0.40%). there was reported by Hadi and Sudiyono (2019) reported that perilla meal is a rich source of phosphorus (2.03%) and calcium (2.45%).

The essential fatty acid content in GPC was notably high (76.59%) and mainly consisted of linolenic acid (C18:3n3; omega-3 fatty acid) and linoleic acid (C18:2n6; omega-6 fatty acid). The primary essential fatty acid was linolenic acid, of which it contained 55.97%. In the present work, the linolenic acid content of GPC was within the range previously reported for perilla seed oil contents (54–82.47%) (Kurowska et al., 2003; Maitree et al., 2015; Peiretti et al., 2011; Sirilun et al., 2016). The linoleic acid content in GPC in this study was within the range of the previous report, which contained 9.26–21.17% (Kurowska et al., 2003; Maitree et al., 2015; Peiretti et al., 2011; Sirilun et al., 2016). The fatty acid content variations of perilla seed oil may be depending on the growing conditions, location, and genotypes or cultivars (Siriamornpun et al., 2006). For fat-soluble vitamins, we were found γ- tocopherol (367.26 μg/100g), vitamin E is an antioxidant in biological membranes where it protects polyunsaturated fatty acids and other components of cellular membranes from oxidation by free radicals (Pinelli-Saavedra, 2003).

The IVID model was used to evaluate GPC in growing pig diets. In this study, the IVID of CP was the only parameter that did not statistically differ among the GPC supplement levels, ranging from 72.94 to 74.65%. The increase of GPC in growing pig diets resulted in decreased IVID levels of EE and CF; this was especially the case in the diet with an inclusion level of 20%. The digestibility of GE also decreased when the GPC level increased. This is in agreement with the results of Zhang et al. (2013), reported that the increasing of total dietary fiber effected to significantly decreased digestibility of DM, CP, and energy. Similarly, Regmi et al. (2009) reported a low digestibility of CF that influenced the physical and chemical characteristics of feedstuffs. However, the high CF level in the diet is a limitation as pigs are monogastric animals and have difficulties digesting diets with high CF levels (Cho and Kim, 2011). A high dietary fiber content in the diet is associated with a decreased nutrient use (Noblet and Le Goff, 2001). Bach Knudsen et al. (2001) explained that dietary fiber might reduce the digestibility of dry matter and energy at this site of the digestive tract because of its resistance to digestion with endogenous enzymes secreted into the small intestine.

The ATTD was determined in addition to the IVID to confirm that GPC has the potential in the growing pig diet at three supplement levels (0, 5, and 10% GPC, respectively). The 20% GPC supplement group was removed because of its high CF content and low IVID values of EE, CF, and GE. The ATTD of CP was highest in the 5% GPC supplement group (74.19%). Because the GPC had a low CP content than soybean meal, we adjusted to add the fish meal to balance the CP in diet formulation. The high content of fish meals in diet effect higher CP digestibility. Also, the ATTD of EE at 5% GPC supplement was higher than that of the basal diet (62.93 vs. 54.24%). Peiretti et al. (2010) reported that the EE digestibility increased with an increasing level of perilla seed, with the high value recorded for a rabbit diet containing 10% perilla seed (83.9%). Also, Pascual et al. (2002) reported that the EE apparent digestibility coefficient is generally higher when the level of dietary fat is increased, and its value usually depends on the type of added fat. The perilla seed fat (mono-, di- and tri-glycerides) is more digestible compared to fats (terpens, waxes, etc.) contained in the other raw materials commonly in animal diet (Peiretti et al., 2010). The ATTD of CF at 10% GPC supplement was lowest (25.89%). This was mainly because the CF of GPC was higher than that of soybean meal. The addition of GPC in the diet leads to increased fiber levels, resulting in a decreased ATTD. Arjin et al. (2021) reported that the ATTD of pigs fed a high-fiber diet (banana stem + concentrate and fermented banana stem + concentrate) was low, resulting in low overall nutrient digestibility. The increase in total fiber in the diet significantly decreased the ATTD levels of DM, organic matter (OM), CP, and GE (Freire et al., 2003; Wilfart et al., 2007). Le Goff and Noblet (2001) state that the digestibility of energy decreases linearly when the NDF content of the diet increases. The inclusion of fiber in the diet offered to pigs results in reductions in foregut and whole-tract digestibility of DM, leading to lower absorption of nutrients and energy in the small intestine of these monogastric animals (Bach Knudsen et al., 1991; Ngoc et al., 2012). According to the IVID and ATTD levels, the GPC shows the potential as a protein source that could replace SM in growing pig diets. However, the use of GPC in pig diets should be adjusted considering the CF content in the diet formulation.

CONCLUSION

The GPC raw material contained a high crude protein (CP) content (31.54%). The total essential amino acid (EAA) content was 138.34 mg/g and was mainly leucine (28.87 mg/g), and notably contained limiting amino acids for pigs, including lysine (19.52 mg/g) and methionine (10.94 mg/g). Ether extract content (EE) was 10.52%, and the major free fatty acid (FFA) was linolenic acid (C18:3n3; 55.97%) and fat-soluble vitamin contained γ-tocopherol (367.25 μg/100g). Also, enriched minerals as phosphorus (1.02%), potassium (0.83%), calcium (0.46%), and iron (468.43 mg/kg). However, crude fiber (CF) was notably high content (24.43%). Increasing GPC level in pig diet was reduced IVID of dry matter (DM), EE, and CF ($P < 0.05$), especially 20% GPC supplement. However, the IVID of the CP did not differ among the group. Furthermore, the results for the ATTD of the CP and EE in the 5% GPC supplement group were significantly better than that of the other groups ($P<0.05$). This is a relevant saving potential in the production of monogastric

animals in many Asian countries, where GPC is a cheaper source of protein. This study is an initial report about the potential of GPC in the diet of growing pigs, regarding its nutritional value. Further studies are needed to verify the efficiency of GPC as a protein source on the productive performance, health, and meat quality of pigs.

CONFLICT OF INTEREST

The authors declare that they hold no conflicts of interest

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