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Comparative Studies on Nutrient Value and Ileal Amino Acid Digestibility of Palm Kernel Cake for Broiler in Direct and Indirect Methods

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ABSTRACT

Two digestibility trials were performed to determine apparent ileal digestibility (AID) of crude protein (CP) and amino acids (AAs) of untreated palm kernel cake (uPKC) and Palm kernel cake treated with Lactobacillus plantarum RI11 (LAB-PKC) from 21 to 32 and 18 to 28 days in the first and second trial, respectively. PKC was treated by L. plantarum RI11 for 14 days with moisture to PKC ratio 1:1 (v/w) before to be fed to the broilers. Digestibility trials were performed in direct method and indirect method. A total of 36 broilers were fed diet containing 90.57% (w/w) uPKC and LAB-PKC as sole source of CP and AAs in direct method, whereas 108 broilers were fed with corn-soybean diet substituting with 25% (w/w) uPKC and LAB-PKC in indirect method. All diets in both method contained titanium dioxide as indigestible biomarker to determine the AID of CP and AAs of PKC. The results showed that both uPKC and LAB-PKC determined by the indirect method showed higher AID than that of the direct method ($P<0.05$). The CP and AA content of LAB-PKC were higher ($P<0.05$) than uPKC. However, LAB-PKC showed lower ($P<0.05$) fat content than uPKC. The reduction of NDF content in LAB-PKC could be considered as a significant factor for improving the nutritive value of the PKC and the digestibility of CP and AA in the current study. The findings showed that AID of CP and AA for LAB-PKC were improved in the direct method but not significant in the indirect method.

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INTRODUCTION

Palm kernel cake (PKC) is the by-product of the palm kernel fruit after the palm oil extraction and hence PKC is also known as palm kernel expeller (PKE). PKC was the byproduct of palm oil milling industries with total production recorded at 2.7 million tons in 2017 (MPOB, 2018). Because of its high energy content and low cost, PKC can be used as animal feed ingredient to reduce the cost of feed because most of the commercial feedstuffs including corn and soybean meal are imported from United State, China and Brazil. Besides, the global price of corn and soybean meal has been raised by 33.67% and 54.20%, respectively since the year of 2002 (MoA, 2014). PKC as substitute feed ingredient has been employed to poultry feed but the growth performance of broiler was declined as the inclusion level of PKC was increased (Zamani *et al*., 2017). The low content of crude protein (≥15-16%), apparent metabolizable energy (AME) and high fiber content make PKC less desirable as poultry feed ingredient (Abdollahi *et al*., 2015; Alshelmani *et al*., 2016). A digestibility trial conducted by Alshelmani *et al*., (2016) showed that 10% PKC led to decrease the digestibility on dry matter and CP in broiler diet. The digestibility of CP and AA in test ingredients was performed in direct and indirect method in this current study. According to Abdollahi *et al*. (2015), direct method is a conventional method of determining nutrient digestibility solely on the test ingredients. Indirect method is another method of substituting certain level of conventional diet to test ingredient in order to determine the nutrient digestibility. The result of AID for broiler fed diet containing solely PKC and 25% inclusion level of PKC using titanium dioxide (TiO_2) as a bio-indicator was used.

Increasing cost of commercial feed ingredient globally have moved toward to non-conventional feed ingredients as the alternative animal feed source; PKC is an examples as Malaysia is second palm oil producer in the world with the highest quality and inexpensive feedstuffs. Despite of interest, limited studies on ileal AA digestibility of PKC in broiler chicken were conducted (Abdollahi *et al*., 2015; Alshelmani *et al*., 2017a). It was reported by Alshelmani *et al*., (2017b, 2021a, 2021b) that inclusion of 10% PKC in broiler diets led to decrease the body weight gain and feed conversion ratio. Although, PKC have not proper potential for satisfying nutrient requirements in poultry but biological or physical treatment or both can be used to increase the AA digestibility of broilers (Sundu *et al*., 2006; Alshelmani *et al*., 2016). Ileal AA digestibility of fermented PKC by *Paenibacillus polymyxa* ATCC 842 and *P. curdlanolyticus* DSMZ 10248 was increased as compared to untreated PKC in broiler as reported by Alshelmani *et al*. (2017a). The objectives of the present study were to determine the apparent ileal digestibility (AID) of AA of PKC and fermented PKC in direct and indirect methods.

MATERIALS and METHODS

Preparation of untreated PKC (uPKC) and lactic acid bacteria-treated PKC (LAB-PKC)

PKC was purchased from local company in Malaysia. The used PKC as broiler feed was fermented by *Lactobacillus plantarum* RI11 for 14 days. Briefly, *L. plantarum* RI11 was revived actively and the optical density (OD) was adjusted to 1.0 at the wavelength of 600.0 nm reaching 9.0 log CFU/mL. 10% (v/v) inoculum size was inoculated into sterilize medium containing 25.08 g/L molasses and 107.26 g/L PKC and was incubated for 4 days at 30°C. 10% (v/v) inoculum medium was later mixed with PKC with the total moisture level of 100% (v/w) and incubated for 14 days at room temperature in sealed plastic bags. Untreated PKC (uPKC) was not undergone any fermentation process.

Broilers and experimental diets

The study conducted according to the guidelines of the Research Policy on Animal Ethics of University Putra Malaysia (IACUC-AUP-R101/2018).

Table 1. Composition of feed ingredients

	Direct method		Indirect method		
Composition (%)	**uPKC**	**LAB-PKC**	**Corn soybean**	**uPKC**	**LAB-PKC**
uPKC	90.57	-	-	25.00	-
LAB-PKC	-	90.57	-	-	25.00
Corn	-	-	58.26	43.70	43.70
Soybean meal	-	-	34.80	26.10	26.10
DCP	-	-	1.81	1.36	1.36
Palm oil	6.02	6.02	3.10	2.33	2.33
Calcium carbonate	1.71	1.71	0.95	0.71	0.71
Salt	0.40	0.40	0.40	0.30	0.30
Vitamin premix[1]	0.50	0.50	0.05	0.04	0.04
Mineral premix[2]	0.50	0.50	0.10	0.08	0.08
Choline chloride	0.30	0.30	0.07	0.05	0.05
L-LYS	-	-	0.21	0.15	0.15
DL-MET	-	-	0.19	0.14	0.14
L-THR	-	-	0.06	0.04	0.04
TOTAL	100	100	100	100	100
Calculated analysis					
Metabolizable energy (Kcal/Kg)	1723.05	2261.22	2995	2593..03	2741.74
Crude protein (% as fed)	13.30	17.04	20.38	18.96	20.00

[1]**Provided per kg diet**: vitamin A 6670 IU; vitamin D_3 1000 IU, vitamin E 23 IU; vitamin K_3 1.33 mg; cobalamin 0.03 mg; Thiamine 0.83 mg; riboflavin 2.0 mg; folic acid 0.33 mg; biotin 0.03 mg; pantothenic acid 3.75 mg; niacin 23.30 mg; pyriodoxine 1.33 mg.

[2]**Provided by kg diet**: Fe 100.0 mg; Mn 110.0 mg; Cu 20.0 mg; Zn 100.0 mg; I 2.0mg; Se 0.20 mg; Co 0.60 mg

Day-old male broiler chicks (Cobb 500) were raised in stainless steel tier cage with the stocking density of 30 kg/m^2 based on the Cobb broiler management guideline (2012). Following the recommendation from the industry, the dimension of the cage cell is 800 mm x 600 mm x 420 mm, with the average size of 600 sq. cm. Day-old male broiler chick were fed *ad-libitum* with commercial broiler diet in starter phase (0 day to 17 day) following by experimental diets. Two digestibility trials were conducted in this study.

In the first experiment, a total of 36 chicks 22-day-old were selected for the experimental diet in the direct method. The birds with uniform body weight were randomly distributed into two treatments with six replicates and three chicks per replicate. Day-old chicks were fed with commercial starter diets from 0 to 21 days. The chicks were later fed with solely uPKC feed (90.57%) and LAB-PKC (90.57%) from 22 to 28 days as shown in Table 1. All the chicks were freely access to the experimental diets and drinking water. Experimental diet with biomarker was later fed from 29 to 32 days which contained 0.3% (w/w) titanium dioxide (TiO_2).

In the second experiment, a total of 108 chicks were selected for the experimental diets in indirect method. The birds with uniform body weight were randomly distributed into two treatments with six replicates and six chicks per replicate. Another group of chicks were fed the reference diet (corn-soybean). Day old chicks were fed with commercial starter diet from 0 to 17 days. The chicks were fed with 100% corn-soybean feed, 25% uPKC feed and 25% LAB-PKC feed from 18 to 24 day as shown in Table 1. All chicks were freely access to the experimental diets and drinking water. Experimental diets with biomarker were later fed from 25 to 28 days which consisted of 0.5% (w/w) TiO_2 (Kareem et al., 2018).

Table 2. Details of digestibility trials used in direct and indirect method

	Direct method	**Indirect method**
Broiler strain	Cobb 500	Cobb 500
Gender	Males	Males
Replicate	6	6
Birds per replication	3	6
Dietary marker	Titanium dioxide	Titanium dioxide
Feeding period	1 week (experimental diets)	1 week (experimental diets)
Feeding period (biomarker)	4 days	4 days
Age at euthanasia, d	32	28
Method of euthanasia	*Halal* slaughtering	*Halal* slaughtering
Site of digesta collection	Merkel's diverticulum to 1 cm before ileo-cecal junction	Merkel's diverticulum to 1 cm before ileo-cecal junction
Collection of digesta	Gentle flushing with distilled water	Gentle flushing with distilled water
Processing of digesta	Oven-drying	Oven-drying

Ileal digesta of broilers performed in direct and indirect method were individually collected upon slaughtering (Alshelmani *et al*., 2016). The ileal digesta was collected

from the Meckel's diverticulum to 1 cm before ileo-cecal junction (Alshelmani *et al*., 2016) with gentle flushing of distilled water to collect the digesta (Alshelmani et al., 2017a). Ileal digesta of birds were individually collected and pooled the sample within each replicate (cage) in the pill bottles. The digesta were immediately kept at -20°C and subsequently dried at 50°C. The dried ileal digesta samples were ground to fine powder and stored at -20°C for the determination of crude protein and AAs.

Proximate analyses

Feed samples were taken from each replicate and ileal digesta from each bird was pooled into each replicate to determine the dry matter, (DM, %), ash (%), ether extract (%), crude protein (CP, %), NDF (%), ADF (%) and ADL as the protocol described by AOAC (1995) and Goering and Van Soast (1970).

Cellulose and hemicellulose of feed were determined as described by Goering and Van Soast (1970) and the formulae were shown below:

$$\% \, Cellulose = \% \, ADF - \%ADL$$

$$\% \, Hemicellulose = \%NDF - \%ADF$$

Amino acids content of PKC

A total of six feed samples and ileal digesta were digested by HCl acid hydrolysis. Briefly, A volume of 5 mL 6M HCl was added into the sample and were heated under air-forced condition at 110°C for 24 hours. As for methionine and cysteine were hydrolyzed as methionine and cysteic acid by oxidation with performic acid at 4°C for 16 hours as the protocol described by Moore (1963). The samples were later filtered through Whatman No.1 filter paper and rinsed with deionized water. The sample were later kept at -20°C.

AA content of feed and ileal digesta were determined by high performance liquid chromatography (HPLC) with post column derivatization (O-phthaldehyde) and fluorescence detection (Model 1100, Agilent Technologies, Inc., Santa Clara, CA). The protocol of AA determination was based on the protocol described by manufacturer (Alshelmani *et al*., 2017a).

Apparent ileal amino acids digestibility analyses

AID of PKC was determined using two methods, namely direct and indirect method. TiO_2 as biomarker was used in both digestibility assays and determined based on Short *et al*. (1996). In the direct method, PKC considered as sole source of AA, whereas only 25% PKC was included in the experimental diet in the indirect method. The AID (%) of PKC was calculated using the following formulae in the direct method (Baker *et al*., 2010):

$$AID = 100 - \left[100 \times \left(\frac{\% \, TiO_2 \, in \, feeds}{\% \, TiO_2 \, in \, digesta}\right) \times \left(\frac{\% \, nutrient \, digesta}{\% \, nutrient \, feed}\right)\right]$$

The other formula was used to determine the AID in the indirect method (Abdollahi *et al.*, 2015):

$$\text{AID of PKC} = \left[\frac{(\text{AID of PKC diet x AA of PKC diet}) - (\text{AID of reference diet AA of reference diet x } 0.75)}{(0.25 \text{ x AA of PKC})}\right]$$

Statistical analysis

The mean difference of obtained in each experiment was analyzed with student t test at significant level ($P<0.05$) by using SAS 9.4 software.

RESULTS and DISCUSSION

Table 3 showed the nutrient composition of test ingredients. The nutrient composition of sole source of untreated PKC (uPKC) and PKC fermented by *L. plantarum* RI11 (LAB-PKC) was determined in direct method whereas corn soybean diet, diet substituted with uPKC and LAB-PKC in indirect method. No significant difference of ash content was shown among different test ingredients ($P>0.05$).

Table 3. Nutrient composition of test ingredients (% dry matter basis)

Nutrient	Direct method		Indirect method		
	uPKC	LAB-PKC	Corn soybean	uPKC	LAB-PKC
Dry matter	89.46 ± 0.50	74.10 ± 1.85*	91.69 ± 0.89	92.18 ± 0.53	80.96 ± 0.16*
Ash	5.04 ± 0.46	7.28 ± 0.10NS	7.66 ± 0.50	6.91 ± 0.55	7.10 ± 0.51NS
Crude protein	14.68 ± 0.42	18.81 ± 0.18*	24.81 ± 0.54	23.67 ± 0.06	24.10 ± 0.37NS
EE	9.15 ± 0.47	4.55 ± 0.20*	3.18 ± 0.20	3.83 ± 0.24	2.18 ± 0.07*
Crude fiber	14.55 ± 1.02	12.94 ± 0.37NS	6.94 ± 0.46	7.26 ± 0.42	5.88 ± 0.22NS
NDF[1]	75.66 ± 0.25	66.87 ± 0.98*	43.86 ± 1.68	60.47 ± 0.73	55.67 ± 0.46NS
ADF[2]	34.53 ± 0.25	34.83 ± 0.32NS	14.08 ± 0.26	17.53 ± 0.35	17.14 ± 0.35NS
ADL[3]	12.18 ± 0.69	13.33 ± 1.04NS	7.91 ± 0.52	12.38 ± 0.47	11.81 ± 0.37NS
Hemicellulose	41.13 ± 1.36	32.05 ± 1.08	29.78 ± 1.86	42.93 ± 0.63	38.54 ± 0.79
Cellulose	22.36 ± 1.75	21.50 ± 0.81	6.16 ± 0.44	5.15 ± 0.20	5.33 ± 0.19
Indispensable AA (%)					
Lysine	0.45 ± 0.01	0.50 ± 0.02 NS	0.88 ± 0.02	0.74 ± 0.03	0.93 ± 0.03*
Leucine	0.97 ± 0.02	0.96 ± 0.02 NS	1.33 ± 0.06	1.13 ± 0.04	1.24 ± 0.05 NS
Isoleucine	0.37 ± 0.01	0.48 ± 0.01*	0.37 ± 0.03	0.39 ± 0.02	0.44 ± 0.01 NS
Valine	0.37 ± 0.01	0.39 ± 0.02 NS	0.30 ± 0.01	0.34 ± 0.02	0.31 ± 0.01 NS
Phenylalanine	0.50 ± 0.01	0.46 ± 0.02 NS	0.65 ± 0.03	0.57 ± 0.02	0.73 ± 0.03*
Threonine	0.44 ± 0.01	0.52 ± 0.02*	0.71 ± 0.01	0.69 ± 0.02	0.78 ± 0.01*
Histidine	0.68 ± 0.03	0.61 ± 0.02 NS	0.68 ± 0.03	0.67 ± 0.03	0.66 ± 0.02 NS
Methionine	0.30 ± 0.02	0.31 ± 0.01 NS	0.26 ± 0.02	0.34 ± 0.01	0.40 ± 0.02 NS
Arginine	2.56 ± 0.03	1.48 ± 0.01*	1.04 ± 0.04	1.28 ±0.03	1.09 ± 0.03*
Glycine	1.06 ± 0.00	0.94 ± 0.01*	0.70 ± 0.02	0.72 ± 0.02	0.70 ± 0.01 NS
Dispensable amino acids (%)					
Aspartic acid	0.16 ± 0.01	0.34 ± 0.02 NS	1.28 ± 0.01	0.79 ± 0.01	1.40 ± 0.12*
Glutamic acid	1.83 ± 0.05	1.83 ± 0.08 NS	1.99 ± 0.04	2.59 ± 0.10	2.71 ± 0.04 NS
Proline	0.53 ± 0.02	0.48 ± 0.02 NS	0.73 ± 0.00	0.71 ± 0.02	0.99 ± 0.02*
Serine	0.59 ± 0.02	0.57 ± 0.01 NS	0.77 ± 0.03	0.70 ± 0.04	0.80 ± 0.02 NS
Tyrosine	0.12 ± 0.01	0.16 ± 0.01 NS	0.39 ± 0.02	0.40 ± 0.02	0.46 ± 0.02 NS
Alanine	0.57 ± 0.01	0.71 ± 0.01*	0.79 ± 0.01	0.68 ± 0.01	0.84 ± 0.02*
Average[b]	**0.72**	**0.67**	**0.80**	**0.80**	**0.91**

[1]NDF: Neutral detergent fiber; [2]ADF: Acid detergent fiber; [3]ADL: acid detergent lignin; [b] Average of 16 amino acids and cysteine not detected. N = 6 (6 replicates per treatment); Means ± SE (standard error). * (P<0.05). [NS] Not significant.

Table 4. Apparent ileal digestibility of amino acid and crude protein in broiler chickens fed with untreated palm kernel cake and fermented palm kernel cake by *L. plantarum* RI11 in direct and indirect methods (dry matter basis)

AID (%)	Direct method		Differences	Indirect method			Differences
	uPKC	LAB-PKC		Corn soybean	uPKC	LAB-PKC	
Crude protein	50.23 ± 0.86	58.18 ± 0.62	7.95*	83.71 ± 0.95	77.83 ± 0.44	75.64 ± 0.30	2.19NS
Indispensable AAs							
Lysine	17.55 ± 1.01	30.27 ± 2.55	12.72*	85.00 ± 0.86	73.64 ± 1.08	72.78 ± 0.95	0.86NS
Leucine	60.98 ± 1.94	65.82 ± 0.10	4.84NS	86.55 ± 0.61	82.53 ± 1.18	78.61 ± 1.06	3.92NS
Isoleucine	70.87 ± 2.95	86.09 ± 0.76	15.22*	78.49 ± 3.37	75.10 ± 2.64	77.54 ± 1.64	2.44NS
Valine	79.94 ± 0.91	85.60 ± 1.0	5.66NS	77.45 ± 1.77	82.01 ± 2.27	71.63 ± 3.72	10.38*
Phenylalanine	69.90 ± 1.43	68.86 ± 2.02	1.04NS	91.19 ± 0.51	82.14 ± 1.62	95.58± 0.99	13.44NS
Threonine	58.85 ± 2.80	67.46 ± 2.34	8.61*	85.50 ± 0.38	70.97 ± 1.44	77.07 ± 0.76	6.10NS
Histidine	22.18 ± 1.47	17.70 ± 1.22	4.48NS	78.55 ± 1.40	66.04 ± 1.52	60.90 ± 0.77	5.14NS
Methionine	45.64 ± 2.24	51.19 ± 0.64	5.55NS	89.02 ± 0.70	85.38 ± 0.51	84.82 ± 1.55	0.56NS
Arginine	81.27 ± 0.49	53.72 ± 2.70	27.55*	89.88 ± 0.49	82.38 ± 1.22	74.16 ± 0.61	8.22*
Glycine	51.17 ± 0.12	50.01 ± 1.67	1.16NS	81.05 ± 0.67	72.41 ± 0.61	70.18 ± 0.88	2.23NS
Dispensable AAs							
Glutamic acid	83.27 ± 1.02	82.55 ± 0.69	0.72NS	87.65 ± 0.56	85.90 ± 0.36	79.09 ± 0.11	6.81*
Proline	63.87 ± 2.57	61.97 ± 2.48	1.90NS	87.14 ± 0.55	87.46 ± 0.71	86.62 ± 0.79	0.84NS
Serine	66.09 ± 0.85	73.44 ± 1.09	7.35*	89.46 ± 0.57	85.90 ± 0.36	80.88 ± 1.39	5.02NS
Tyrosine	57.08 ± 2.84	81.31 ± 1.68	24.23*	96.72 ± 0.96	67.31 ± 0.55	69.31 ± 0.81	2.00NS
Alanine	29.63 ± 2.91	48.91 ± 1.60	19.28*	76.99 ± 0.62	72.24 ± 1.36	68.65 ± 0.44	3.59NS
Average [a]	**57.22**	**61.66**	**4.44NS**	**85.38**	**78.58**	**76.56**	**2.02NS**

[a] Average of 15 amino acids. N = 6 (6 replicates per treatment); Means ± SE (standard error). * (P<0.05). [NS] Not significant.

Among the indispensable AA of PKC, arginine (ARG), leucine (LEU) and glysine (GLY) were the major AA whereas methionine (MET), threonine (THR), valine (VAL)

and isoleucine (ILE) were the AA with lowest content. However, AAs of LAB-PKC were significantly (P<0.05) increased as compared to uPKC such as ILE and THR in direct method. The AA content of PKC showed consistent result with Abdollahi *et al*. (2015) and Alshelmani *et al*. (2017) except aspartic acid which was higher content than that of the current study.

The process of solid state fermentation of PKC by *L. plantarum* RI11 showed the increased level of crude protein (CP) by 21.96%; from 14.68% to 18.81% in the sole LAB-PKC diet. PKC consisted of high NDF (%) and ADF (%). Moreover, most of the non-starch polysaccharides (NSPs) of PKC are not soluble; Abdollahi *et al*., 2015).

Apparent ileal digestibility (AID) of uPKC and LAB-PKC in both direct and indirect method was shown in Table 4. AID of CP in uPKC increased (P<0.05) as compared to LAB-PKC in direct method. LAB-PKC significant increase (P<0.05) in the digestibility of LYS, ILE, THR, SER, TYR and ALA in direct method, whereas only ARG significant decreased (P<0.05) of AID as compared to uPKC. AID of LYS, LEU, PHE, THR, HIS, MET, GLY, PRO, SER, TYR and ALA in uPKC was higher in indirect method than direct method. AID of LYS, LEU, PHE, THR, HIS, MET, ARG, GLY, PRO, SER and ALA in LAB-PKC was higher in indirect method than direct method.

The averages of AID of AA in uPKC determined by direct and indirect method were 57.22% and 78.58%, whereas LAB-PKC was 61.66% and 76.56%, respectively.

The nutrient compositions of PKC were within the range as reported by Sundu *et al*. (2006), Abdollahi *et al*. (2015) and Alshelmani *et al*. (2017). In the agreement of Alshelmani *et al*. (2017), the fermentation process of PKC increased the level of CP and AA as same results were shown in this study. CP of LAB-PKC was significantly higher than that of uPKC as indicated that the introduction of *L. plantarum* RI11 into PKC could improve protein availability of PKC. The findings are also in agreement with the results obtained by Muziana (2017).

Slightly reduction of cellulose was found in LAB-PKC as compared to uPKC. However, hemicellulose content of LAB-PKC was reduced by 22.08% as compared to uPKC (Table 3). This indicates that the fermentation of PKC by *L. plantarum* RI11 could biodegrade the hemicellulose into simpler sugars as energy source. Similar agreement was found by Muziana (2017) who reported LAB was capable to increase the protein availability in PKC as LAB was proteolytic enzyme producer (Muziana, 2017).

PKC consisted high fiber content and hence high ADF and NDF of uPKC was determined as shown in Table 3. With the process of solid state fermentation (SSF) in PKC, NDF of LAB-PKC was lower than uPKC by the difference of -8.79% indicating that *L. plantarum* RI11 exhibited the capability of furthering degrading hemicellulosic compounds in PKC. Significant reduction of hemicellulose content was determined in LAB-PKC as compared to uPKC (Table 3). Alshelmani *et al*. (2014) reported that the PKC fermented by cellulolytic and hemicellulolytic bacteria showed the reduced level of hemicellulose content as compared to untreated PKC. In the agreement with Wan

(2015) and Muziana (2017), LAB had the capability of producing β-mannanase and xylanase to biodegrade PKC via SSF process.

The content of AAs in uPKC showed comparable to the previous research studies (Perez *et al*., 2000; Sundu *et al*., 2006; Abdollahi *et al*., 2015; Alshelmani *et al*., 2017a) except histidine obtained higher and aspartic acid was lower than the current PKC. The current data clearly showed that the current PKC only consisted 113.56 mg/g of AAs (a total of 16 AAs) and these indicated that PKC is a poor source of AA as compared to the recommended minimum requirement of Cobb 500 broilers. Therefore, same agreement was reported by Abdollahi *et al*. (2015) with the concentration of 124 mg/g (total of 17 AAs) in PKC. Supplementation of AA to poultry such as lysine, methionine and threonine were needed to achieve balanced diet with healthy growth. Therefore, the introduction of 75% corn and soybean meal with different treatments of PKC in indirect method showed more balanced diets in term of nutritive composition and AAs content.

The AAs content of LAB-PKC was 107.4 mg/g (total of 16 AAs) and was slightly lower than that of uPKC and this showed that *L. plantarum* RI11 was utilized certain AAs as a source of nutrient for bacterial growth. LAB was able to utilize arginine by arginine deiminase pathway as reported by Savino *et al*. (2012). Therefore, *L. plantarum* RI11 could increase the availability of certain AAs in PKC via SSF process.

The AID of CP in uPKC showed significantly lower than LAB-PKC in direct method and AID of LAB-PKC had been increased by 7.95%. Therefore, the available crude protein to broiler in LAB-PKC was 10.94% as compared to only 7.37%. One of the possible reasons is that the *L. plantarum* RI11 in LAB-PKC had partially degrade the hemicellulosic by hemicellulolytic enzymes in order to increase the digestibility of CP. In the agreement with Alshelmani *et al*. (2017), PKC fermented by cellulolytic bacteria could increase the availability of metabolizable protein to broiler by 2.96% to 3.91%. The main reason attributing to low AID was the high content of NDF, ADF and ADL as anti-nutritional factor. According to Alshelmani *et al*. (2017a), high NDF brought negative effect on digestibility of CP and AA of soluble dietary fiber in PKC. PKC consisted high concentration of soluble dietary fiber such as β-mannan and glucomannan due to high crystallity and low degree of branching (Alshelmani et al., 2013). In addition, high concentration of NSPs in poultrys' intestine increased the gut viscosity attributing to increased residence time of digesta and low feed intake (Alshelmani *et al*., 2017b). However, no significant difference of AID in CP between uPKC and LAB-PKC was found ($P>0.05$) in the indirect method. This finding suggested that the value determined in the indirect method did not deliver the effect AID of CP in test ingredients. It was reported by Ravindran *et al*., (2017) that in case the oven-drying is used for digesta, it should be less than 65ºC to avoid destruction or binding of some AAs.

Digestibility of AAs in PKC and LAB-PKC in both direct and indirect method was shown in Table 4. Although ileal digesta is not recommended to be collected by slaughtering method (Ravindran *et al*., 2017), the findings in the current study is in agreement with Hakim *et al*., (2020), who used anesthesia to kill the chicks. This could be attributed to the indigenous loses from large intestine. Overall, poor AA digestibility of uPKC was shown in direct method with the average AID of 57.22% (total of 15 amino acids), along with the reported study by Abdollahi *et al*. (2015) and Alshelmani *et al*. (2017), formulating PKC diet solely based on crude protein content did not able to fulfill the bird growth nutrient requirements for a balanced diets. AID of LYS, ILE, VAL, THR, SER, TYR and ALA in LAB-PKC showed significant ($P<0.05$) improvement of nutrient digestibility in broiler. This could be due to the presence of proteolytic enzyme produced by LAB in which increased the availability of AAs. AAs digestibility of PKC in the indirect method showed relatively high than direct method of digestibility trial. The average of AAs AID of uPKC was 57.22% and 78.58% in the direct and in indirect method, respectively. These indicate that the AID of AAs was broadly increased by 21.36% leading to the misconception of improved AA and CP AID in PKC. Comparison of AID of CP and AAs for both uPKC and LAB-PKC determined by direct and indirect method, the results show that CP digestibility can be used as an indicator for AA digestibility.

CONCLUSIONS

Based on the current study, PKC is a poor source of energy and protein for broiler. However, PKC provided adequate amount of digestible AA to broiler. PKC fermented by *L. plantarum* RI11 reduced the content of fiber to improve the digestibility of AAs and CP. Comparison between direct and indirect method of CP and AA digestibility determination showed that the metabolizable nutrient in the indirect method overestimate the actual value of CP and AA digestibility.

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