Passage time, apparent metabolisable energy and ileal amino acids digestibility of treated palm kernel cake in broilers under the hot and humid tropical climate

Ali Hanafiah Hakima, Idrus Zulkiflia,b, Abdoreza Farjam Soleimania, Elmutaz Atta Awada, Norhani Abdullah, Wei Li Chen and Rosfarizan Mohamadd

Institute of Tropical Agriculture and Food Security, Serdang, Selangor, Malaysia; Department of Animal Science, Universiti Putra Malaysia, Serdang, Selangor, Malaysia; Department of Poultry Production, University of Khartoum, Khartoum North, Sudan; Department of Bioprocess Technology, Universiti Putra Malaysia, Serdang, Selangor, Malaysia

ABSTRACT
Four different palm kernel cake (PKC) including untreated PKC, enzyme fermented PKC (EPKC), extruded PKC (XPKC) and lactic acid bacteria fermented PKC (LPKC) were compared for their passage (ROP), apparent metabolisable energy (AME) and apparent ileal amino acid (AA) digestibility in broilers under the hot and humid tropical climate. The various PKC diets were formulated by replacing 25% of the basal diet with PKC, EPKC, XPKC or LPKC. The AME values of various PKC were enumerated by the difference between the AME of basal and PKC diets. The AME of EPKC, XPKC and LPKC were significantly increased by 39.2, 44.9 and 43.1%, respectively, compared to untreated PKC. In comparison with the untreated PKC, the ileal crude protein (CP) digestibility of LPKC and EPKC were significantly increased by 30.5% and 20.3%, respectively, while no difference was observed for XPKC. The average ileal AA digestibility of LPKC was significantly higher by 22.8% compared to the untreated PKC. In conclusion, bacterial fermentation, enzymatic fermentation and thermal extrusion improved the AME of PKC the hot and humid tropical climate, while only bacterial fermentation resulted in significant improvements in the CP and AA digestibility.

HIGHLIGHTS
- Nutrients availability of PKC can be improved by fermentation.
- Passage time of digesta may not change by 25% inclusion of treated PKC.

Introduction
Over the past few decades, there has been a dramatic increase in the prices of the conventional feedstuffs accompanied by a shortage in their supply. Hence, there is a necessity for the utilisation of other non-conventional ingredients such as palm kernel cake (PKC) in poultry feed formulation. PKC is being produced abundantly as a by-product of the palm oil industry and has the potential to serve as a feed ingredient for livestock, both ruminants and non-ruminants (Zulkifli et al. 2009). However, due to its high crude fibre content and several non-starch polysaccharides (NSP), PKC has limited nutritional value for chickens (Sundu et al. 2006). Consequently, it has been reported that PKC has a relatively low apparent metabolisable energy (AME) and digestibility of crude protein (CP) and amino acids (AA) (Alimon 2004; Abdollahi et al. 2015).

The presence of high NSP and fibre in poultry diet has been reported to increase the viscosity along the intestinal tract and subsequently to alter the feed passage time, which may contribute to the low digestibility of nutrients in NSP containing feed (Choct 1997; Alshelmani et al. 2016). Sieo et al. (2005) reported that chickens fed with a barley-based diet supplemented with transformed Lactobacillus strains had faster
passage rate compared to those provided control diet and this could be attributed to depolymerisation of the complex structure of NSP by the enzymes produced by the transformed bacteria. On the contrary, Rochell et al. (2012) found that feeding a diet containing higher insoluble fibre led to a faster passage time and lower apparent ileal amino acid digestibility (AIAAD). This inconsistency leads to an unclear explanation of the effects of PKC on the passage time in chicken’s gastrointestinal tract.

Various treatments have been proposed to improve the nutritional value of PKC, including solid-state fermentation (SSF), physical treatment and exogenous enzyme supplementation (Marini et al. 2005; Saenphoom et al. 2011; Alshelmani et al. 2014; Roslan et al. 2015). SSF of PKC by cellulolytic and hemicellulolytic bacteria has shown reduction of cellulose, hemicellulose, xylans and mannans molecules (Alshelmani et al. 2014). Besides, enzymatic hydrolysis of PKC via SSF has been reported to increase the monosaccharides reduction and enhanced the release of prebiotic oligosaccharides (Chen et al. 2015). Extrusion has also been proven to enhance the nutritive value of PKC as the reduction of total NSP and crude fibre were accompanied by the increase of mannose, glucose, fructose, 1,4-β-D-mannobiose, 1,4-β-D-mannotriose, 1,4-β-D-mannotetraose and 1,4-β-D-mannopentaose concentration (Roslan et al. 2017).

To date, only a limited number of studies on the AIAAD of treated PKC and its AME are available (Muangkeow and Chinajariyawong 2013; Saenphoom et al. 2013; Hanafiah et al. 2017). Moreover, Hanafiah et al. (2017) found that both AME and apparent ileal crude protein digestibility (AICPD) of PKC are highly affected by environmental temperature, as the PKC consumed by heat-stressed chickens had significantly lower AICPD and AME compared to their thermoneutral counterparts. Hence, the contribution of information on the AME, AICPD and AIAAD of treated PKC of broilers under tropical climate, an environment characterised by the hot and humid environmental conditions, would be an informative comparison with the prior findings.

Therefore, this study aims to compare the rate of passage (ROP), AME and AIAAD of the enzyme-fermented PKC, extruded PKC and lactic acid bacteria-fermented PKC in broilers under tropical climate.

**Materials and methods**

**Ethical note**

The experimental protocol was approved by the institutional animal care and use committee (IACUC) of the Universiti Putra Malaysia (UPM/IACUC/AUP-R021/2018).

**PKCs treatment**

PKC was purchased from a commercial kernel oil extraction factory in Klang, Selangor, Malaysia and ground to uniform size of about 3.0 mm before treatment. The PKC was subjected to the following treatments: (1) enzyme treatment (EPKC), (2) extrusion (XPKC); and (3) lactic acid bacteria treatment (LPKC).

The EPKC preparation started with the production of crude enzyme mixture containing endoglucanase, mannanase and xylanase, which were produced from solid-state fermentation of PKC using *Aspergillus terreus* K1 as an inoculum (Chen et al. 2013). Briefly, PKC was mixed with distilled water at the pH value of 5.8 to obtain 62.7% moisture content prior to inoculation with $6.0 \times 10^5$ *A. terreus* spore per gram of PKC, following an incubation period of 7 days at 30.5°C. Upon fermentation, the crude enzyme produced was extracted by shaking the inoculated PKC in distilled water (4°C) for 24 h. The mixture was centrifuged at 10,000 $\times$ g for 10 min and filtered using a filter paper (Whatman No. 1). The extracted crude enzyme was lyophilised and kept at 4°C for subsequent use.

Following the enzyme production, PKC was mixed with distilled water to achieve a moisture content of about 60% before adding 10 U of previously produced enzymes per gram of PKC for solid-state fermentation. The mixture was incubated at 55°C for 24 h. The moisture content was increased to 35% by adding 10 U of previously produced enzymes per gram of PKC and incubated at 55°C. After each fermentation cycle, the PKC was mixed with 6.0 $\times$ 105 spores per gram of PKC, followed by incubation at 55°C for 72 h or until achieving constant weight and kept at 4°C until later use (Chen et al. 2015).

The extrusion method was carried out following Roslan et al. (2017), with a few modifications, using a co-rotating twin-screw extruder with a screw diameter of 50 mm and length to diameter ratio of 24. The extruder was equipped with six thermocouples to measure the temperature of the barrel and the temperature inside of the barrel and three heaters to control the heating of the barrel. Two nozzles with a 13 mm diameter hole were fitted at the end of the barrel, for the extrudate to come out. The extrusion device was set up following several conditions; cooking temperature of 80°C, 90°C and 100°C orderly, a screw speed of 150 rpm and hopper speed of 4.5 Hz. The PKC was mixed with water to achieve 50% moisture content prior to extrusion treatment. Once the extrusion is completed, all extrudates were dried at 60°C for 3 days or until achieving a constant weight.

For LPKC preparation, lactic acid bacteria of strain *Weisella confusa* SR-17b was used as an inoculum for the PKC fermentation using solid-state fermentation technique following the procedure established by Tan (2016), with few modifications. Briefly, *W. confusa* SR-17b isolates was activated on the De Man, Rogosa and
Sharpe medium (MRS) agar containing (g/L): peptone from casein (10.0), meat extract (8.0), yeast extract (4.0), α-(+)-glucose (20.0), dipotassium hydrogen phosphate (2.0), Tween 80 (1.0), di-ammonium hydrogen citrate (2.0), sodium acetate (5.0), magnesium sulphate (0.2) and manganese sulphate (0.04). The seed culture broth was prepared by picking five colonies of 16–18 h W. confusa culture into a 100 mL MRS broth. Then, 2 mL of the cell suspension was transferred into another 100 mL MRS broth, and the culture was inoculated statically at 37°C for 14 h. A minimal salt solution (MSM) with a glucose concentration of 1.0% was prepared as a moisturising agent, and the pH of the solution was adjusted to pH 6.5. Upon completion, inoculum broth was used for the subsequent PKC fermentation where 500 g autoclaved PKC was inoculated with 250 mL of prepared inoculum broth, and MSM solution was supplemented at the ratio of 1:4 (w/v) to the weight of PKC. The mixture was incubated at 37.5°C for 96 h. As fermentation completed, LPKC was dried at 60°C for 3 days or until achieving a constant weight and kept at 4°C for subsequent use.

**Birds, housing and management**

A total of 300 day-old Cobb 500 male broiler chicks were obtained from a local hatchery. Upon arrival (day 1), chicks were weighed and randomly allocated to 25 battery cages (120 × 120 × 45 cm, length × width × height) with wire floors in a conventional open-sided house with 12 chicks per cage. The experimental temperature ranged from 34 ± 1.0°C to 21 ± 1.5°C, and the relative humidity was between 62 ± 1.0% and 88 ± 1.3%. Birds were vaccinated (intraocular route) against Newcastle disease (ND) on days 7 and 21. Birds had ad libitum access to drinking water and feed. The lighting programme consisted of continuous 24 h of light throughout the study.

**Experimental diets**

The AME of PKC was determined by the difference method (Abdollahi et al. 2015; Hanafiah et al. 2017) while ileal CP and AA digestibility of PKC were determined by the direct method (Jia et al. 2012; Ahmed et al. 2014). For the ROP assay, the same experimental diet from the AME assay was used. In the difference method, a corn soybean meal-based diet was used as the basal diet. Basal starter and finisher diet were formulated to meet or exceed the NRC (1994) requirements as appropriate. From day 1 to 14, birds were fed a standard broiler diet with 21.5% CP and 3035 kcal/kg ME (Table 1). Five experimental diets were then developed by including untreated PKC, EPKC, XPKC or LPKC at 25% of the basal diet (Table 2). While for the direct method, PKC, EPKC, XPKC or LPKC served as the sole source of CP and AA in the assay diets (Table 3). All diets contained titanium dioxide (TiO2) at 0.50%, as an indigestible index. All diets were randomised in a complete randomised design with five replicates per treatment.

**Experimental procedure**

The first 4 days (day 15 to 18) of feeding the experimental diets were considered as an adaptation period, followed by an assay to determine the ROP on day 19 (Rochell et al. 2012). Each PKC diet treatment group consisted of 5 replicates of 12 chicks. The assay was initiated with a 2 h fasting, followed by 2 h feeding of experimental diets with TiO2 before replaced with experimental diets without TiO2 to allow ad libitum feed intake for the remainder of the ROP assay. Consumption of diets containing TiO2 was used to

| Item | Starter |
|------|---------|
| Ingredient, % (fed basis) | |
| Corn | 56.74 |
| Soybean | 35.70 |
| Palm oil | 3.81 |
| MCP | 1.54 |
| Limestone | 1.32 |
| NaCl | 0.34 |
| α-Methionine | 0.19 |
| l-Lysine.HCL | 0.15 |
| Mineral premix | 0.10 |
| l-Threonine | 0.06 |
| Vitamin premix | 0.05 |
| Nutrient composition, % (unless stated otherwise) | |
| ME (kcal/kg) | 3035 |
| CP | 21.50 |
| CF | 3.75 |
| EE | 2.44 |
| Lysine | 1.20 |
| Calcium | 0.95 |
| Methionine + Cysteine | 0.90 |
| Threonine | 0.84 |
| Methionine | 0.51 |
| Available phosphorus | 0.43 |

**Table 1. Ingredient and nutrient compositions of starter (day 1–14).**

**MCP:** monocalcium phosphate; **ME:** metabolisable energy; **CP:** crude protein; **EE:** ether extract; **CF:** crude fibre.

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determine the total TiO\textsubscript{2} intake. Upon the 2 h of feeding index containing diet, total excreta collections were conducted at an interval of 1 h for 12 consecutive hours, where all excreta from each cage were collected, weighed and frozen until later analysis.

On day 20, each treatment group for the ROP assay was divided into two subgroups with six birds per cage arrangement. One group from all treatments has proceeded with AME assay, where the same experimental diets (from ROP assay) with TiO\textsubscript{2} were fed from day 20 until day 23. For 4 consecutive days, on a daily basis, feed intake was monitored, and the excreta were collected, weighed and pooled within a cage. The pooled excreta were mixed thoroughly, and representative samples were obtained for freeze-drying. Then, the dried excreta samples were stored in airtight plastic containers at \(-20\)\degree C for further laboratory analyses. Both dry matter and gross energy of the excreta samples were analysed for AME determination.

On the other hand, on day 20, the remaining group (except basal diet) was offered the assay diets of the direct method according to their previous PKC group during ROP assay. The birds were allowed to consume the feed for 4 days (day 20 until day 23) for an adaptation period. After the adaptation period ended, the birds were fasted for 24 h to remove the gastrointestinal content and induce appetite on the assay diet feeding. On day 25, the birds were allowed for ad libitum feeding of the assay diets for 1.5 h. After 4 h of feeding, all birds were euthanised by injecting their wing veins with sodium pentobarbitone. The ileal contents were collected by gentle flushing with distilled water and pooled within each cage. The samples were freeze-dried and stored in airtight containers at \(-20\)\degree C. The dry matter (DM), CP, AA and TiO\textsubscript{2} were analysed for ileal AA and CP digestibility determination.

All PKC and diets samples were analysed for their DM, CP, crude fibre (CF), ether extract (EE), organic matter (OM), ash, gross energy (GE), neutral detergent fibre (NDF), acid detergent fibre (ADF), acid detergent lignin (ADL), hemicellulose, cellulose, AA concentration and titanium concentration.

Table 2. Ingredient and nutrient compositions of experimental diets used in the rate of passage (ROP) and apparent metabolisable energy (AME) assays.

| Item | Basal diet | PKC diet\textsuperscript{a} | EPKC diet\textsuperscript{a} | XPKC diet\textsuperscript{a} | LPKC diet\textsuperscript{a} |
|------|------------|-----------------------------|-----------------------------|-----------------------------|-----------------------------|
| Ingredient, % (fed basis) | | | | | |
| Corn | 61.50 | 46.14 | 46.14 | 46.14 | 46.14 |
| PKC | – | 25.00 | 25.00 | 25.00 | 25.00 |
| Soybean | 29.90 | 22.40 | 22.40 | 22.40 | 22.40 |
| Palm oil | 5.30 | 3.98 | 3.98 | 3.98 | 3.98 |
| MCP | 1.35 | 1.01 | 1.01 | 1.01 | 1.01 |
| Limestone | 1.00 | 0.75 | 0.75 | 0.75 | 0.75 |
| NaCl | 0.35 | 0.26 | 0.26 | 0.26 | 0.26 |
| L-Lysine.HCL | 0.21 | 0.15 | 0.15 | 0.15 | 0.15 |
| α-Methionine | 0.19 | 0.14 | 0.14 | 0.14 | 0.14 |
| Mineral premix\textsuperscript{b} | 0.10 | 0.07 | 0.07 | 0.07 | 0.07 |
| Vitamin premix\textsuperscript{c} | 0.05 | 0.03 | 0.03 | 0.03 | 0.03 |
| Choline | 0.05 | 0.03 | 0.03 | 0.03 | 0.03 |
| Nutrient composition, % (unless stated otherwise) | | | | | |
| ME, kcal/kg | 3131 | 2749 | 2861 | 2874 | 2874 |
| CP | 19.40 | 18.25 | 18.50 | 18.25 | 18.52 |
| CF | 3.45 | 6.23 | 5.68 | 5.63 | 4.98 |
| EE | 2.58 | 7.14 | 7.31 | 7.26 | 7.26 |
| Lysine | 1.10 | 0.82 | 0.83 | 0.81 | 0.86 |
| Methionine + Cysteine | 0.79 | 0.63 | 0.64 | 0.62 | 0.65 |
| Calcium | 0.77 | 0.69 | 0.69 | 0.69 | 0.69 |
| Threonine | 0.69 | 0.51 | 0.54 | 0.50 | 0.54 |
| Methionine | 0.48 | 0.42 | 0.42 | 0.42 | 0.44 |
| Available phosphorus | 0.38 | 0.29 | 0.29 | 0.29 | 0.29 |

Titanium oxide was added (0.50%) to all experimental diets as an indigestible index.
PKC: palm kernel cake; LPKC: lactic acid bacteria-fermented PKC; EPKC: enzyme-fermented PKC; XPKC: extruded PKC; MCP: monocalcium phosphate; ME: metabolisable energy; CP: crude protein; EE: ether extract; CF: crude fibre.

\textsuperscript{a}PKC, EPKC, XPKC and LPKC diets were developed by replacing 25% of the basal diet of the particular PKC.

\textsuperscript{b}Supplied per kilogram of the diet: Mn, 70.0 mg; Fe, 35.0 mg; Zn, 30.0 mg; Cu, 8.0 mg; I, 1.00 mg; Se, 0.250 mg; Co, 0.200 mg.

\textsuperscript{c}Supplied per kilogram of the diet: vitamin A (retinyl acetate), 8000 U; vitamin D3 (cholecalciferol), 1000 U; vitamin E (\alpha-\textit{tocopherol}, 30.0 U; vitamin K3 (menadione dimethylpyrimidinol, 2.50 mg; vitamin B1, 2.00 mg; vitamin B2, 5.00 mg; vitamin B6, 2.00 mg; vitamin B12, 0.010 mg; niacin, 30.0 mg; D-biotin, 0.045 mg; vitamin C, 50.0 mg; folic acid, 0.500 mg.
**Table 3. Ingredient and nutrient compositions of experimental diets used in ileal crude protein and amino acids digestibility assays.**

| Item                  | PKC | EPKC | XPKC | LPKC |
|-----------------------|-----|------|------|------|
| Ingredient, % (fed basis) |     |      |      |      |
| PKC                   | 95.15 | 95.15 | 95.15 | 95.15 |
| Palm oil              | 2.00  | 2.00  | 2.00  | 2.00  |
| MCP                   | 1.35  | 1.35  | 1.35  | 1.35  |
| Limestone             | 1.00  | 1.00  | 1.00  | 1.00  |
| NaCl                  | 0.35  | 0.35  | 0.35  | 0.35  |
| Mineral premixa       | 0.10  | 0.10  | 0.10  | 0.10  |
| Vitamin premixb       | 0.05  | 0.05  | 0.05  | 0.05  |
| Nutrient composition, % (unless stated otherwise) |     |      |      |      |
| CP                    | 16.09 | 17.04 | 16.09 | 17.33 |
| CF                    | 16.18 | 14.09 | 13.9  | 11.42 |
| EE                    | 6.27  | 6.94  | 6.75  | 6.75  |
| Calcium               | 1.08  | 1.03  | 1.03  | 1.08  |
| Phosphorus            | 0.35  | 0.34  | 0.34  | 0.35  |
| Methionine + Cysteine | 0.20  | 0.27  | 0.15  | 0.39  |
| Methionine            | 0.19  | 0.16  | 0.16  | 0.25  |
| Threonine             | 0.17  | 0.28  | 0.13  | 0.22  |
| Lysine                | 0.15  | 0.12  | 0.05  | 0.29  |

Titanium oxide was added (0.50%) to all experimental diets as an indigestible index.

PKC: palm kernel cake; LPKC: lactic acid bacteria-fermented PKC; EPKC: enzyme-fermented PKC; XPKC: extruded PKC; MCP: monocalcium phosphate; ME: metabolisable energy; CP: crude protein; EE: ether extract; CF: crude fibre.

aSupplied per kilogram of the diet: Mn, 70.0 mg; Fe, 35.0 mg; Zn, 70.0 mg; Cu, 8.00 mg; I, 1.00 mg, Se, 0.250 mg; Co, 0.200 mg.

bSupplied per kilogram of the diet: vitamin A (retinyl acetate), 8000 U; vitamin D3 (cholecalciferol), 1000 U; vitamin E (α-tocopherol), 30.0 mg, vitamin K3 (menadione dimethylpyrimidinol, 2.50 mg; vitamin B1, 2.00 mg; vitamin B2, 5.00 mg; vitamin B6, 2.00 mg; vitamin B12, 0.010 mg; niacin, 30.0 mg; o-biotin, 0.045 mg; vitamin C, 50.0 mg; o-pantothenate, 8.00 mg, folic acid, 0.500 mg.

**Laboratory analyses**

Proximate analysis for DM, nitrogen content (N), CF, EE, OM and ash were determined following the procedure of (AOAC 1990). The CP was calculated as N × 6.25. The GE was analysed using a bomb calorimeter (IKA2000©, GmbH & Co.KG, Satufen, Germany). The NDF, ADF and ADL were determined according to the methods described by Goering and Van Soest (1970), while hemicellulose and cellulose were calculated as NDF – ADF and ADF – ADL, respectively. Amino acids were determined using high performance liquid chromatography (HPLC) (Law et al. 2018). Briefly, samples were hydrolysed by 6 N HCl at 110 °C for 24 h and followed by the addition of an internal standard (l-α-amino-N-butyric acid; AABA) to the hydrolysate. Then, samples were paper and syringe filtered, mixed with borate buffer and ACCQ reagent (Waters Corporation, Milford, MA, USA) prior to the peak’s separation by an AA column (AccQ Tag 3.9 × 150 mm; Waters). The latter was detected by a fluorescent detector (2475; Waters) using HPLC. Cysteine and methionine were analysed as cystic acid and methionine sulphone, respectively by oxidation with performic acid for 16 h at 4 °C and neutralisation with hydrobromic acid before hydrolysis. For titanium determination, the samples were ashed at 580 °C for 13 h to burn all organic material and the remaining minerals were digested (using 7.4 M sulphuric acid) to release titanium dioxide content, which was determined using a spectrophotometer (SPECORD PLUS©, Analytic Jena AG, Jena, Germany) at a wavelength of 410 nm (Short et al. 1996).

**Calculations**

The total titanium excreted by the birds was considered as a unity. The titanium excreted at each hourly collection was expressed as the cumulative fraction of the total titanium excreted. The cumulative data was plotted, and the time to excrete 50% of titanium (T50) was determined from the cumulative curve using the following model (Almirall and Esteve-Garcia 1994):

\[
y = \frac{t^m}{t^m + k}
\]

where \( t \) = time (hour), \( y \) = cumulative excreted titanium, \( m \) = slope of the line and \( k \) = correlation coefficient.

The goodness of fit of the data to the Hill-type equation was tested. Then, the curves were transformed into a linear equation to determine the value of \( m \) and \( k \), as follows:

\[
\ln \left( \frac{y}{1-y} \right) = m \ln (t) - \ln (k) \rightarrow Y = aX + b
\]

where \( Y = \ln \left( \frac{y}{1-y} \right) \), \( a = m \) (gradient of the line), \( X = \ln (t) \) and \( b = -\ln (k) \).

The mean retention time (MRT) was calculated according to the method established (Coombe and Kay 1965):

\[
MRT = \frac{\sum (x_i \times t_i)}{\sum x_i}
\]

where \( x \) is the amount of excreted titanium of the \( i \)th collection at the \( i \)th hour.

The AME, AICPD and AIAAD of PKC and treated PKC were determined following the calculations described by Abdollahi et al. (2015) and Awad et al. (2016):

\[
AME_{diet} = -\frac{(\text{Gross Energy}_{\text{diet}} \times \text{Feed Intake})}{\text{Gross Energy}_{\text{diet}} \times \text{Feed Intake}}
\]
AME\textsubscript{PKC} = \frac{AME_{\text{PKC diet}} - (0.75 \times AME_{\text{basal diet}})}{0.25} \\
AICPD/AIAAD\textsubscript{PKC} = 100 - \frac{\text{CP/AA digesta} \times T_{\text{digest}}}{\frac{\text{CP/AA}_{\text{PKC diet}} \times T_{\text{PKC diet}}}{100}}

Statistical analysis
All data were subjected to 1-way ANOVA using the GLM procedure of SAS software (SAS Institute Inc., Cary, NC, USA). The mean values of the four types of PKC were separated by Duncan’s multiple range test. Results were considered statistically significant at \( p < .05 \).

Results
The chemical compositions of untreated and treated PKC are shown in Table 4. The DM of EPKC, XPKC and LPKC were higher (\( p < .001 \)) than the untreated PKC. The GE of treated PKCs was improved (\( p < .001 \)) as compared to the untreated one, where the EPKC showed the highest value (4294 kcal/kg) followed by XPKC and LPKC which had similar values (4201 kcal/kg). The CP of EPKC and LPKC but not those of XPKC were higher (\( p < .001 \)) than the untreated PKC. The EE of EPKC was similar (\( p > .05 \)) to LPKC and XPKC, but significantly higher than untreated PKC. The CF value was lower (\( p < .001 \)) in LPKC, XPKC and EPKC compared to the untreated PKC. Except for XPKC, LPKC and EPKC had lower (\( p < .001 \)) NDF content than the untreated PKC. The ADF of LPKC was lower (\( p < .001 \)) than the other groups. The LPKC, EPKC and XPKC showed reductions (\( p < .001 \)) in their hemicellulose, cellulose and lignin contents as compared to untreated PKC.

The amino acid concentrations of various types of PKC are presented in Table 5. As compared to the untreated PKC, LPKC showed improved (\( p < .001 \)) lysine, methionine, valine, leucine, tyrosine, alanine, glutamic acid, aspartic acid and proline concentrations. The concentrations of glycine, alanine and aspartic acid of EPKC were higher (\( p < .001 \)) than the untreated PKC. Only alanine and glutamic acid concentrations were enhanced (\( p < .001 \)) in XPKC when compared to the untreated PKC. Irrespective of treatment, the concentrations of threonine, arginine, isoleucine, histidine, phenylalanine, cysteine and serine were not affected.

The type of PKC had negligible (\( p > .05 \)) effect on T50 or MRT (Table 6). The results of AME, AICPD and AIAAD of various PKC are shown in Tables 7 and 8. The AME values of EPKC, XPKC and LPKC were higher

| Table 5. Amino acid concentrations of various treated PKC. |
|-----------------------------------------------|
| Amino acid | PKC | EPKC | XPKC | LPKC | SEM | p Value |
|----------------|-----|------|------|------|-----|---------|
| Indispensable |     |      |      |      |     |         |
| Lysine | 0.50<sup>b</sup> | 0.45<sup>d</sup> | 0.46<sup>d</sup> | 0.54<sup>a</sup> | 0.008 | <.001 |
| Methionine | 0.30<sup>b</sup> | 0.27<sup>c</sup> | 0.28<sup>d</sup> | 0.33<sup>a</sup> | 0.006 | <.001 |
| Threonine | 0.54<sup>a</sup> | 0.53<sup>d</sup> | 0.50<sup>d</sup> | 0.55<sup>a</sup> | 0.005 | <.001 |
| Arginine | 1.94<sup>a</sup> | 1.78<sup>c</sup> | 1.95<sup>d</sup> | 1.90<sup>a</sup> | 0.015 | <.001 |
| Valine | 0.90<sup>b</sup> | 0.92<sup>ac</sup> | 0.87<sup>d</sup> | 0.93<sup>a</sup> | 0.006 | <.001 |
| Isoleucine | 0.60<sup>c</sup> | 0.57<sup>d</sup> | 0.55<sup>d</sup> | 0.60<sup>a</sup> | 0.006 | <.001 |
| Histidine | 0.31<sup>c</sup> | 0.25<sup>b</sup> | 0.31<sup>c</sup> | 0.29<sup>b</sup> | 0.006 | <.001 |
| Leucine | 1.08<sup>c</sup> | 1.07<sup>c</sup> | 1.05<sup>c</sup> | 1.14<sup>a</sup> | 0.008 | <.001 |
| Phenylalanine | 0.66<sup>c</sup> | 0.50<sup>c</sup> | 0.57<sup>d</sup> | 0.67<sup>a</sup> | 0.01 | <.001 |
| Dispensable |     |      |      |      |     |         |
| Cysteine | 0.36<sup>a</sup> | 0.20<sup>c</sup> | 0.17<sup>ac</sup> | 0.21<sup>b</sup> | 0.01 | <.001 |
| Tyrosine | 0.30<sup>b</sup> | 0.30<sup>c</sup> | 0.31<sup>b</sup> | 0.37<sup>a</sup> | 0.007 | <.001 |
| Serine | 0.75<sup>a</sup> | 0.71<sup>d</sup> | 0.74<sup>a</sup> | 0.76<sup>b</sup> | 0.006 | .012 |
| Glycine | 0.80<sup>c</sup> | 0.85<sup>d</sup> | 0.81<sup>d</sup> | 0.82<sup>b</sup> | 0.005 | <.001 |
| Alanine | 0.87<sup>a</sup> | 0.94<sup>c</sup> | 1.16<sup>b</sup> | 1.02<sup>b</sup> | 0.01 | <.001 |
| Glutamic acid | 3.06<sup>a</sup> | 3.07<sup>c</sup> | 3.17<sup>bc</sup> | 3.22<sup>b</sup> | 0.01 | <.001 |
| Aspartic acid | 1.14<sup>a</sup> | 1.18<sup>b</sup> | 1.15<sup>bc</sup> | 1.16<sup>b</sup> | 0.004 | <.001 |
| Proline | 0.57<sup>c</sup> | 0.57<sup>d</sup> | 0.53<sup>d</sup> | 0.63<sup>a</sup> | 0.008 | <.001 |

Values are means of five replicates (\( n = 5 \)).
PKC: palm kernel cake; LPKC: lactic acid bacteria-fermented PKC; EPKC: enzyme-fermented PKC; XPKC: extruded PKC; SEM: standard error of the mean; DM: dry matter; AID: apparent ileal digestibility; AICPD: apparent ileal crude protein digestibility; AIAAD: apparent ileal crude amino acid digestibility; AME: apparent metabolizable energy; MRT: mean retention time.

Table 6. Effect of various treated PKC on the time of 50% excretion (T50) and mean retention time (MRT) of feed in broiler chickens kept under tropical environment.

| Table 6. Effect of various treated PKC on the time of 50% excretion (T50) and mean retention time (MRT) of feed in broiler chickens kept under tropical environment. |
|-----------------------------------------------|
| Item | PKC | EPKC | XPKC | LPKC | SEM | p Value |
|----------------|-----|------|------|------|-----|---------|
| T50, hours | 4.24 | 3.94 | 4.01 | 4.02 | 0.05 | .29 |
| MRT, hours | 5.27 | 5.25 | 5.23 | 5.14 | 0.05 | .82 |

Values are means of five replicates (\( n = 5 \)).
PKC: palm kernel cake; LPKC: lactic acid bacteria-fermented PKC; EPKC: enzyme-fermented PKC; XPKC: extruded PKC; DM: dry matter; SEM: standard error of the mean; T50: time of 50% T0 excretion; MRT: mean retention time.

\* \* Means within a row with no common superscripts differ significantly at \( p < .05 \).
Table 7. Apparent metabolisable energy (AME) and apparent ileal crude protein digestibility (AICPD) of various treated PKC in broiler chickens kept under tropical environment.

| Item (DM basis) | PKC | EPKC | XPKC | LPKC | SEM | p Value |
|-----------------|-----|------|------|------|-----|--------|
| AME, kcal/kg    | 1397.60<sup>a</sup> | 1946.90<sup>a</sup> | 2025.30<sup>a</sup> | 1999.90<sup>a</sup> | 66.89 | <.001  |
| AICPD, %        | 45.90<sup>b</sup> | 55.20<sup>a</sup> | 45.40<sup>a</sup> | 59.90<sup>a</sup> | 1.74  | <.001  |

Values are means of five replicates (n = 5).

Table 8. Apparent ileal amino acids digestibility of various treated PKC in broiler chickens kept under tropical environment.

| Amino acids | PKC | EPKC | XPKC | LPKC | SEM | p Value |
|-------------|-----|------|------|------|-----|--------|
| Lysine      | 19.80<sup>b</sup> | 45.20<sup>a</sup> | 21.90<sup>a</sup> | 45.40<sup>a</sup> | 2.50  | <.001  |
| Methionine  | 39.60<sup>b</sup> | 50.90<sup>a</sup> | 37.90<sup>a</sup> | 47.10<sup>a</sup> | 2.01  | <.122  |
| Threonine   | 49.90<sup>b</sup> | 50.90<sup>a</sup> | 37.90<sup>a</sup> | 47.10<sup>a</sup> | 2.01  | <.001  |
| Arginine    | 54.80<sup>a</sup> | 57.50<sup>a</sup> | 50.90<sup>a</sup> | 63.60<sup>a</sup> | 1.51  | .061   |
| Valine      | 54.00<sup>b</sup> | 57.50<sup>a</sup> | 50.90<sup>a</sup> | 63.60<sup>a</sup> | 1.51  | .061   |
| Isoleucine  | 41.80<sup>b</sup> | 45.70<sup>a</sup> | 38.90<sup>a</sup> | 46.40<sup>a</sup> | 2.01  | <.122  |
| Histidine   | 50.00<sup>b</sup> | 55.20<sup>a</sup> | 47.10<sup>a</sup> | 61.30<sup>a</sup> | 1.58  | .001   |
| Leucine     | 45.20<sup>b</sup> | 46.70<sup>a</sup> | 42.90<sup>a</sup> | 56.00<sup>a</sup> | 1.93  | <.001  |

Values are means of five replicates (n = 5).
PKC: palm kernel cake; LPKC: lactic acid bacteria-fermented PKC; EPKC: enzyme-fermented PKC; XPKC: extruded PKC; SEM: standard error of the mean.

Discussion

In the current experiment, the experimental diets were designed to evaluate the effect of a single dietary component on the passage time of feed, besides determining AME and AIAAD of various treated PKC under the tropics. The present findings showed that the analysed values for NDF were 75.0, 72.6, 75.4 and 70.3%, for RPKC, EPKC, XPKC and LPKC, respectively. Whereas, the values for ADF were 37.3, 38.7, 39.3 and 33.8% for RPKC, EPKC, XPKC and LPKC, respectively. Although there were significant differences in the insoluble fibre content (NDF and ADF) in different types of PKC, no differences in T50 and MRT were observed among PKC diets. These findings suggest that the various types of PKC had no effect on ROP. Ravindran et al. (1984) observed faster ROP when swine fed increased insoluble fibre content (13.5 to 20.2%), which could be associated with increased digesta bulk. The lack of diet effect on ROP in the present study could be associated with the lower level of PKC inclusion in the diets. According to Mateos et al. (1982) there a negative relationship between the ROP and dietary fat content as higher levels of dietary fat was shown to increase the time taken for the first appearance of the index in excreta (slower ROP) in laying hens. In the current study, however, all the diets were supplemented with similar levels of oil (3.75%), and thus the ROP was not affected.

The determined AME of untreated PKC reported in this study was 1397.6 kcal/kg, which is comparable to the values reported by Saenphoom et al. (2013), Abdollahi et al. (2015) and Hanafi et al. (2017). However, it is lower than the AME determined by other researchers (Sundu et al. 2005). The variations in the AME values of PKC could be attributed to several factors such as the method of oil extraction, the crude fat content of PKC (Abdollahi et al. 2015), and the age of the experimental birds (Sundu et al. 2006).

The determined AME of EPKC, XPKC and LPKC were 1946.9, 2025.3 and 1999.9 kcal/kg, respectively. This improvement in the AME of LPKC, XPKC and EPKC could be explained by the reduction in CF content in treated PKC. In the present results, LPKC had a significantly lower CF compared to XPKC and EPKC, which suggests that the fermentation process with lactic acid bacteria led to a higher reduction in polysaccharides. The NSP of PKC is mostly appeared as linear, insoluble polysaccharides, which mostly found as mannose, methyl-glucuronoxylans, and 3% arabinoglycans (Daud and Jarvis 1992). Upon fermentation, a major part of fibres in PKC is reduced to various monosaccharides, which mostly found as mannose (Saenphoom et al. 2011; Alshelmani et al. 2014; Chen et al. 2015). However, it has been reported that the

(p < .001) than the untreated PKC. Both LPKC and EPKC showed higher (p < .001) AIDCP percentages than the untreated PKC. The total AA digestibility of LPKC but not those EPKC and XPKC was higher (p < .001) compared to the untreated PKC. On the other hand, all treatments had a negligible effect on the digestibility of methionine, arginine, histidine, cysteine and glycine.

Discussion

In the current experiment, the experimental diets were designed to evaluate the effect of a single dietary component on the passage time of feed, besides...
assimilation of mannose is much lower than glucose, particularly when glucose is existing in the system (Chen et al. 2015). Although LPKC had lower CF than XPKC and EPKC in the present study, no significant differences in AME values among the treated PKC groups were observed. These findings suggest that even though depolymerisation of PKC releases a great number of sugars, they are primarily mannose, which is weakly absorbed and thus of little benefit to the chicken’s energy metabolism (Saenphoom et al. 2013).

The AICPD of untreated PKC found in this study was 45.9%, a value that considered close to the 41.6% reported by Hanafiah et al. (2017) and Abdollahi et al. (2015), but was lower than the value reported by Bryden et al. (2009) who reported CP digestibility of 54%. In the present results, the average AIAAD of untreated PKC (45.1%) reported in this study was close to the value of 48.2% reported by Abdollahi et al. (2015). In terms of treated PKC, LAB fermentation resulted in a significant improvement in the digestibility of all AA except for methionine, glycine and histidine. On the other hand, enzymatic fermentation of PKC only improved the digestibility of lysine, threonine, leucine, alanine and aspartic acid. As for extrusion, a significant improvement was only observed for alanine.

The low AICPD and AIAAD of untreated PKC and XPKC could be related to the high ADF and NDF value, instead of crude fibre in general. Alshalmani et al. (2016) postulated that the abundance of NDF, ADF, insoluble fibres and NSPs might result in lower apparent ileal digestibility of nutrients in PKC for broilers. This argument was also supported by Szczeruk (2009), who suggested that the lower digestibility of lysine, threonine, arginine, cystine and some other AA in oilseed rape could be attributed to the high fibre content, and probably the high proportion of NSP and phytates. Consequently, the digestion and absorption of nutrients may be disrupted, due to altered viscosity of intestinal lumen and digesta passage time along the intestinal tract (Sioe et al. 2005; Szczeruk 2009; Rochell et al. 2012). On the other hand, the reduction in ADF, NDF and insoluble fibre as reported for EPKC and LPKC can lead to better CP and AA digestibility (Hanafiah et al. 2017).

We previously observed that the AME and digestibility of nutrients of PKC were highly reduced in chickens exposed to heat stress conditions. However, the AME value of PKC (1397.6 kcal/kg) observed in this study was substantially higher than that reported earlier (Hanafiah et al. 2017). The inconsistency could be attributed to differences in the severity of heat stress between the two environmental conditions.

**Conclusions**

From this experiment, we found that bacterial fermentation, enzymatic fermentation and thermal extrusion have the potential to improve the AME of PKC. Both bacterial and enzymatic fermentation enhanced the CP digestibility. Compared to untreated PKC, only bacterial fermentation improved the AA digestibility significantly. Our findings suggested that 25% inclusion of untreated or treated PKC in the diet had a negligible effect on the passage time of digesta, thus the measurement of passage time may not be a good indicator of nutritive quality of feed.

**Disclosure statement**

The authors certify that there is no conflict of interest with any financial organisation regarding the material discussed in the manuscript.

**Funding**

This work was supported by the Malaysian Ministry of Higher Education under the Long-term Research Grant Scheme.

**ORCID**

Idrus Zulkifli http://orcid.org/0000-0002-2082-7430
Elmutaz Atta Awad http://orcid.org/0000-0002-4312-501X

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