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Effect of solid state fermentation on nutrient content and ileal amino acids digestibility of palm kernel cake in broiler chickens

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ABSTRACT

Digestibility trial was conducted to determine the apparent ileal digestibility (AID) of crude protein (CP) and amino acids (AA) in untreated palm kernel cake (PKC) and fermented palm kernel cake (FPKC) on finisher broiler. *Paenibacillus polymyxa* ATCC 842 and *P. curdlanolyticus* DSMZ 10248 were used to produce FPKCa and FPKCb, respectively through solid state fermentation (SSF). Broiler male chickens were fed with diets containing 15% PKC from day one until 41 days of age. Birds (36) were selected with uniform body weight, and randomly distributed into 3 groups with 6 replicates in each treatment and 2 birds per replicate. The chickens were deprived from food overnight with free access to drinking water. The birds were fed PKC, FPKCa and FPKCb with indigestible marker. All the chickens were allowed free access to the test ingredients and drinking water for 4 days. The birds were slaughtered; ileal digesta were individually collected, pooled within each replicate in plastic cups; and immediately kept at –20°C for chemical analysis. The findings showed that the process of SSF by cellulolytic bacteria increased the levels of CP from 16.43% in the PKC to 16.68% and 16.80% in FPKCb and FPKCa, respectively. The AID of CP was increased in FPKC compared to the PKC. Additionally, there was an increase in the digestibilities of AA in FPKC compared to untreated PKC. The process of SSF decreased the fibres in FPKC, and there was improvement in the nutrient value of FPKC by cellulolytic bacterial cultures in terms of nutrient content and digestibility.

Key words: Amino acids, Broiler, Cellulolytic bacteria, Digestibility, Fermented palm kernel cake

The most common feedstuffs used in poultry diets are soybean meal and yellow corn as important sources for protein and energy, respectively. The soybean meal provides protein and most essential amino acids (AA), whereas the yellow corn is considered as a main source of energy for the chickens. It is also known that availability of AA in these feedstuffs is high, and therefore enable the chickens to exhibit high growth performance. There are attempts to substitute yellow corn and soybean meal with local feedstuffs in order to decrease the cost of feed, and to achieve the food security in the developing countries. However, the local feedstuffs are poor in nutrient digestibility, crude protein (CP) and energy content. Moreover, the local ingredients are high in crude fibres and non-starch polysaccharides (NSPs) as well as anti-nutritional factors.

Malaysia produces abundant amount of palm kernel cake (PKC) as an agro-industrial by-product from palm fruits during oil extraction. The nutritive value of PKC is 16% CP and contains high content of crude fibres as well as NSPs, especially mannans (Sundu and Dingle 2002, Alimon 2004, Fadil *et al.* 2014). The challenge point of using the PKC in broiler diets is the content of NSPs and coarse texture (McDonald *et al.* 1995, O'Mara *et al.* 1999, Sundu and Dingle 2002). Therefore, there were attempts to improve the nutritive quality of the PKC by using exogenous enzymes (Ng and Chong 2002, Iyayi and Davies 2005, Lawal *et al.* 2010, ´Alvarez-Cervantes *et al.* 2013, Chen *et al.* 2013, Kocher *et al.* 2002, Adebiyi and Olukosi 2015), or by applying solid state fermentation (SSF) using cellulolytic microorganisms (Marini *et al.* 2005, Iluyemi *et al.* 2006, Graminha *et al.* 2008, Lateef *et al.* 2008, Muangkeow and Chinajariyawong 2009, Sukaryana *et al.* 2010, Mirnawati *et al.* 2011, Alshelmani *et al.* 2013, Gao *et al.* 2013, Alshelmani *et al.* 2014, Alshelmani *et al.* 2016a, Alshelmani *et al.* 2016b). Although many researches were conducted by using fungi in SSF, the secondary metabolites resulting by fungi can suppress the growth of animals (Khin 2004, Marini *et al.* 2005, Moftah *et al.* 2012). Therefore, fermented PKC cellulolytic microorganisms in SSF may alleviate this problem.

The presence of NSPs in poultry diets may lead to increased viscosity, raise the speed of passage time of the ingesta in gastro-intestinal tract (GIT), and therefore

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decrease the absorption and utilization of nutrients in the small intestines. Consequently, it appears to be important to examine this by-product in poultry nutrition, and study the AA digestibility to the PKC after fermentation process. Since *P. polymyxa* and *P. curdlanolyticus* exhibited high capabilities in degrading crude fibre and NSPs in our previous studies (Alshelmani *et al*. 2013, 2014), the objective of this experiment was to investigate the digestibility of CP and AA in the fermented PKC (FPKC) by cellulolytic bacteria.

MATERIALS AND METHODS

Preparation of fermented palm kernel cake: PKC was fermented by *Paenibacillus polymyxa* ATCC 842 and *P. curdlanolyticus* DSMZ 10248. The fermentation process was conducted as described by Alshelmani *et al.* (2013, 2014). The bacterial cultures were revived (sub-cultured), and the optical density (OD) was adjusted to 1.00 at 600 nm using spectrophotometer. Inoculum [10% (v/w)] was then transferred into each conical flask. The conical flasks inoculated with cellulolytic bacteria were incubated for 9 days under humidified conditions by placing sterile distilled water inside the incubator. The FPKC was autoclaved and dried after SSF.

Birds and experimental design: The study was conducted according to the guidelines of the Research Policy on Animal Ethics of the Universiti Putra Malaysia. Day-old male broiler chicks (Cobb 500) were fed with diet containing 15% PKC in starter and finisher phase. The birds received a commercial broiler starter diet (21.84% crude protein) from days 1 to 21 and finisher diet (20.02% crude protein) from 22 to 41 days (Table 1). Thirty six birds were selected with uniform body weight (1837.80 ±100 g) for this trial. The birds were randomly distributed into three treatments with six replicates in each treatment, and two birds per replicate. The chickens were deprived from food overnight with free access to drinking water in order to evacuate their GIT from any previous feed. After a fasting period, the birds were fed untreated PKC, FPKCa and FPKCb (Table 2). All the chickens were allowed free access to the test ingredients and drinking water for 4 days. The birds were slaughtered, and ileal digesta were gently squeezed and few drops of distilled water were added to the ileum to collect the digesta (Ravindran *et al.* 2005). The ileal digesta was collected from the Meckel's diverticulum to 1 cm before ileo-cecal junction (Loh *et al.* 2008).

Ileal digesta of birds were individually collected, and pooled within each replicate (pen) in plastic containers resulting in 6 composite samples per test ingredients. The digesta were immediately kept at –20°C and subsequently dried at 60°C. The dried ileal digesta samples were ground to pass through a 0.5 mm sieve and stored at –20°C for chemical analyses.

Chemical analyses: Amino acids content in test ingredients and ileal digesta were determined by high performance liquid chromatography (HPLC) with post-column derivatization (*O*-phthaldeyde) and fluorescence detection (Model 1100, Agilent Technologies, Inc., Santa Clara, CA). The samples were hydrolysed with 6 M hydrochloric acid (HCl) at 110°C for 24 h, whereas methionine and cysteine were hydrolysed as methionine sulfone and cysteic acid by oxidation with performic acid at 4°C for 16 h (Moore 1963). The concentration of N, crude fibre (CF), dry matter (DM) and ash were determined based

Table 1. Ingredient and nutrient compositions of starter and finisher diet

Ingredient (%)	Starter	Finisher
Yellow corn	39.15	43.84
Palm oil	8.24	7.32
PKC[1]	15.00	15.00
Soybean meal 44% CP	28.54	24.60
Fish meal 55% CP	5.00	5.00
DCP[2] 18%	1.70	1.80
Calcium carbonate	0.37	0.37
DL-Methionine	0.25	0.28
L-Lysine	0.22	0.23
L-Thrionine	0.00	0.03
Salt	0.30	0.30
Vitamin premix[a]	0.50	0.50
Mineral premix[b]	0.50	0.50
Toxin binder	0.14	0.14
Antioxidant[c]	0.01	0.01
Choline chloride	0.08	0.08
Total	100.00	100.00
Calculated analyses[d]		
ME* (kcal/kg)	3000.71	3000.00
Methionine + Cystine (%)	0.95	0.90
Lysine (%)	1.30	1.21
Arginine (%)	1.54	1.43
Lys:Arg ratio	1;1.18	1: 1.18
Calcium (%)	1.08	1.14
Available phosphorus (%)	0.57	0.58
Chemical analyses (%)		
DM	94.11	91.00
CP	21.84	20.02
NDF[3]	19.30	19.12
ADF[4]	11.42	11.20
EE	10.30	11.73
Ash	4.77	5.10
NFE	58.16	58.47

[a]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; Thiamin, 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; pyridoxine, 1.33 mg. [b]Provided per kg diet: Fe, 100.0 mg; Mn, 110.0 mg; Cu, 20.0 mg; Zn, 100.0 mg; I, 2.0 mg; Se, 0.20 mg; Co, 0.60 mg. [c]Butyrated hydroxytoluene (BHT). DM, dry matter; CP, crude protein; EE, ether extract; NFE, nitrogen free extract. [1]PKC, Palm kernel cake; [2]DCP, Dicalcium phosphate; [3]NDF, Neutral detergent fibre; [4]ADF, Acid detergent fibre; *ME, Metabolizable energy. [d]Diets were formulated using FeedLIVE software (FeedLIVE 1.52, Thailand).

Table 2. Composition of test ingredients in the digestibility trial

Item	Test ingredients (%)		
	PKC	FPKCa	FPKCb
PKC[1]	90.30	0.00	0.00
FPKCa[2]	0.00	90.30	0.00
FPKCb[3]	0.00	0.00	90.30
Palm oil	6.00	6.00	6.00
$CaCO_3$	1.70	1.70	1.70
Salt	0.40	0.40	0.40
Vitamin premix[4]	0.50	0.50	0.50
Mineral premix[5]	0.50	0.50	0.50
Choline-Cl	0.30	0.30	0.30
TiO_2	0.30	0.30	0.30
Total	100.0	100.0	100.0

[1]PKC, Untreated palm kernel cake.[2]FPKCa, fermented by *P. polymyxa* ATCC 842. [3]FPKCb, fermented by *P. curdlanolyticus* DSMZ 10248.[4]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; pyridoxine, 1.33 mg.[5]Provided per kg diet: Fe, 100.0 mg; Mn, 110.0 mg; Cu, 20.0 mg; Zn, 100.0 mg; I, 2.0 mg; Se, 0.20 mg; Co, 0.60 mg.

on the method described by AOAC (1995). The CP was calculated as N × 6.25 AA, CP and other nutrients were presented in percentages as dry matter basis. Neutral detergent fibres (NDF) and acid detergent fibres (ADF) were determined based on the method described by Goering and Van Soest (1970). Titanium dioxide was determined in the feed and digesta based on the method described by Short *et al.* (1996).

Calculation: Apparent ileal digestibility (AID) of CP and AA was calculated based on CP, AA and TiO_2 concentrations of test ingredients and ileal digesta using the following formula (Son *et al.* 2014):

AID = 100 – [100 × (% TiO_2 in feed/ % TiO_2 in digesta) × (% nutrient digesta/ % nutrient feed)

Statistical analysis: Data were analysed using One-way ANOVA to compare the differences among the nutrients in the test ingredients. The experimental design was based on complete randomized design (CRD) in the digestibility trial. Data were analyzed by using General Linear Model procedure of the statistical analysis system (SAS 2003). Tukey's test was used to compare the means of treatment at 5% probability (P<0.05). The statistical model used for the trial was $Y_{ijk} = \mu + T_{ij} + E_{ijk}$, where, $Y_{ijk,}$ observation; μ, population mean; T_{ij}, effect of test ingredients; E_{ijk}, experimental error

RESULTS AND DISCUSSION

The content of nutrients in the PKC and FPKC by cellulolytic bacteria are shown in Table 3. The process of SSF by cellulolytic bacteria led to increased levels of CP and AA in the FPKC compared to the untreated PKC. The CP increased (P=0.0722) from 16.43% in the untreated PKC to 16.68% and 16.80% in FPKC by *P. curdlanolyticus* DSMZ 10248 and *P. polymyxa* ATCC 842, respectively. The AA were significantly increased (P<0.05) in the FPKC as compared to the untreated PKC. For instance, the concentration of isoleucine (P=0.0239), phenylalanine (P=0.0192), threonine (P=0.0118), histidine (P=0.0150), methionine (P=0.0003), arginine (P=0.0312), glycine (P=0.0489), aspartic acid (P=0.0155), glutamic acid (P= 0.0033) and serine (P=0.0150) were increased in FPKC compared to untreated PKC. On the other hand, the SSF decreased the NDF (P<0.0001), ADF (P=0.0003), CF (P<0.0001), hemicellulose (P=0.0010) and cellulose (P=0.0010) in FPKC compared with untreated PKC. The AA content in the untreated PKC were consistent with Sulabo *et al.* (2013) and Son *et al.* (2014). It was also consistent with Muangkeow and Chinajariyawong (2009) except histidine, arginine, glutamate and tyrosine in which they were higher than the current PKC. The increase of CP and AA content in the FPKC by cellulolytic bacteria could be due to the reduction of NDF (12.87% in FPKCa and

Table 3. Nutrient content of palm kernel cake and fermented palm kernel cake by cellulolytic bacteria (dry matter basis)*

Nutrient (%)	PKC	FPKCa[1]	FPKCb[2]	SEM[3]	P-values
Crude protein	16.43	16.80	16.68	0.04	0.0822
Dry matter	91.42	92.62	92.44	0.38	0.5228
Ash	4.74	4.67	4.80	0.13	0.2201
Neutral detergent fibre (NDF)	82.29^{b}	71.70^{a}	73.54^{a}	0.52	<0.0001
Acid detergent fibre (ADF)	51.48^{b}	47.27^{a}	47.45^{a}	0.58	0.0003
Hemicellulose	30.81^{b}	24.43^{a}	26.42^{a}	0.75	0.0010
Cellulose	35.55^{b}	31.85^{a}	31.41^{as}	0.62	0.0010
Indispensable amino acids					
Lysine	0.37	0.41	0.38	0.02	0.1325
Leucine	0.89	0.94	0.95	0.02	0.0551
Isoleucine	0.50^{b}	0.59^{a}	0.53^{a}	0.02	0.0239
Valine	0.69	0.78	0.72	0.03	0.1433
Phenylalanine	0.57^{b}	0.66^{a}	0.63^{ab}	0.02	0.0192
Threonine	0.41^{b}	0.51^{a}	0.46^{ab}	0.02	0.0118
Histidine	0.23^{b}	0.29^{a}	0.24^{ab}	0.02	0.0150
Methionine	0.22^{b}	0.27^{a}	0.26^{a}	0.01	0.0003
Arginine	1.60^{b}	1.76^{a}	1.69^{ab}	0.04	0.0312
Glycine	0.60^{b}	0.78^{a}	0.71^{ab}	0.04	0.0489
Dispensable amino acids					
Aspartic acid	1.12^{b}	1.27^{a}	1.23^{ab}	0.03	0.0155
Glutamic acid	2.48^{b}	2.80^{a}	2.76^{a}	0.08	0.0033
Proline	0.44^{b}	0.59^{a}	0.52^{ab}	0.02	0.0018
Serine	0.56^{b}	0.69^{a}	0.66^{ab}	0.04	0.0150
Tyrosine	0.25	0.24	0.24	0.01	0.4435
Cysteine	0.20	0.22	0.21	0.01	0.3632
Alanine	0.62	0.70	0.71	0.06	0.3892

[1]FPKCa, fermented palm kernel cake by *P. polymyxa* ATCC 842; [2]FPKCb, fermented palm kernel cake by *P. curdlanolyticus* DSMZ 10248; [3]Pooled standard error of means.*Means±SEM. [a,b]Means with different superscripts in the same row differ significantly (P<0.05). n= 6 (6 replicates per treatment with 2 birds per replicate).

Table 4. Amino acids and crude protein digestibility of palm kernel cake and fermented palm kernel cake by cellulolytic bacteria (dry matter basis)*

Nutrient (%)	PKC	FPKCa[1]	FPKCb[2]	SEM[3]	P-values
Crude protein	57.92[b]	61.83[a]	60.88[a]	0.63	0.0014
Indispensable amino acids					
Lysine	65.94	69.57	70.63	2.12	0.1479
Leucine	65.47	68.04	63.89	1.39	0.1375
Isoleucine	69.59	70.47	66.39	2.58	0.5152
Valine	62.89[b]	70.42[a]	65.08[b]	1.26	0.0022
Phenylalanine	68.77	70.76	68.51	1.96	0.6802
Threonine	61.38	64.98	61.69	1.73	0.2935
Histidine	56.99[b]	71.50[a]	64.83[ab]	2.77	0.0076
Methionine	61.67[b]	71.92[a]	69.20[a]	0.90	<0.0001
Arginine	75.75[b]	81.15[a]	76.30[b]	0.95	0.0019
Glycine	47.44	45.52	52.96	4.16	0.4424
Dispensable amino acids					
Aspartic acid	56.87[b]	64.30[a]	61.74[a]	1.20	0.0018
Glutamic acid	62.64[b]	72.37[a]	65.45[b]	0.91	<0.0001
Proline	53.76	58.73	51.20	3.06	0.2401
Serine	65.76	69.78	67.58	2.10	0.4186
Tyrosine	59.04[b]	67.58[a]	61.93[ab]	1.84	0.0155
Cysteine	33.34[b]	41.45[a]	37.46[ab]	2.01	0.0393
Alanine	52.07[b]	66.87[a]	59.84[ab]	2.49	0.0029

[1]FPKCa, Fermented palm kernel cake by *P. polymyxa* ATCC 842.[2]FPKCb, fermented palm kernel cake by *P. curdlanolyticus* DSMZ 10248.[3] Pooled standard error of means. * [a,b]Means±SEM. Means with different superscripts in the same row differ significantly (P<0.05). n= 6 (6 replicates per treatment with 2 birds per replicate).

10.63% in FPKCb), ADF (8.18% in FPKCa and 7.83% in FPKCb) and NSPs.

The findings were in agreement with Muangkeow and Chinajariyawong (2009), who found that the rest of AA were increased when the PKC was fermented by *Aspergillus wentii* TISTR 3075. These findings were also consistent with Gao *et al*. (2013), who reported that SSF by *Lacobacillus brevis* led to increased CP and AA in soybean meal, or by *L. salivarius* in canola meal (Ahmed *et al.* 2014). The increase in CP in FPKC by *P. polymyxa* ATCC 842 may be due to its capability of producing proteolytic enzymes (Alvarez *et al.* 2006). Therefore, proteolytic enzymes may produce more peptides and AA, and consequently the digestible and soluble protein could be increased (Pranoto *et al.* 2013).

Digestibility of CP and AA in the PKC and FPKC by cellulolytic bacteria as is presented in Table 4. The digestibility of CP was significantly (P<0.05) increased in FPKC by cellulolytic bacteria as compared to the untreated PKC.

The CP digestibilities were 57.92%, 60.88% and 61.83% in the untreated PKC, FPKC by *P. curdlanolyticus* DSMZ 10248 and FPKC by *P. polymyxa* ATCC 842, respectively. There was also significant increase in the digestibilities of valine (P=0.0022), histidine (P=0.0076), methionine (P<0.0001), arginine (P=0.0019), aspartic acid (P=0.0018), glutamic acid (P<0.0001), tyrosine (P=0.0150), cysteine (P=0.0393) and alanine (P=0.0029) in FPKC compared to the untreated PKC. Overall, there was improvement in the FPKC by cellulolytic bacterial cultures as mentioned above in terms of CP and AA digestibility.

The digestibility of CP and AA in the PKC is poorer compared to other common feedstuffs used in poultry feed such as soybean meal and yellow corn. The digestibility of CP in the PKC was consistent with Mustafa *et al.* (2004), who mentioned that digestibility of CP in the PKC was 53.37% in male Muscovy ducks. On the other hand, the digestibility of CP and AA were improved in the FPKC by cellulolytic bacteria, especially *P. polymyxa* ATCC 842.

The findings were in agreement with Sulabo *et al.* (2013), who reported that reduction in CP and AA digestibility in the growing pigs may be due to presence of high content of fibres. They also attributed the reduction of AID in the PKC to the high content of NDF, ADF and lignin. Sulabo *et al.* (2013) also reported that digestibility of CP was decreased by 0.3–0.8% as NDF increased by 1% in the diet. In addition, the negative effect of the soluble fibres in monogastrics is greater than the insoluble (Dégen *et al.* 2007, Sulabo *et al.* 2013). Sulabo *et al.* (2013) reported that PKC, with exception of guar meal, has greatest levels of α-mannans among all feedstuffs. It was also claimed by Annison and Choct (1991) and Aya *et al.* (2013) that presence of NSPs in poultry diet depressed the nutrient utilization.

The improvement in CP and AA digestibility in FPKC by cellulolytic bacteria in the current study may be attributed to the reduction of NDF, ADF, hemicellulose and cellulose as a result of their capabilities of producing cellulolytic enzymes such as cellulase, xylanase and mannanase. These findings were consistent with Liu *et al.* (2013), who reported that *Bacillus licheniformis* derived protease led to better CP and AA digestibility of broiler chickens fed with sorghum-based diets. Additionally, the improvement in AID could be attributed to the cellulolytic enzymes (Ravindran *et al.* 2007, Selle *et al.* 2009, Masey-O'neill *et al.* 2014).

The presence of NSPs in poultry diets may lead to increase in the speed of food passage in the gastrointestinal tract (GIT). Therefore, the utilization of AA and other nutrients will be decreased. The findings were also in agreement with Lawal *et al*. (2010), who reported that reduction in NSPs of FPKC by fungi led to better nutrient digestibility in broiler chickens.

Based on the current results, it can be concluded that nutritive value of FPKC by cellulolytic bacteria improved. The NDF and ADF were decreased by 16.92%, 12.87% and 8.18%, respectively in FPKCa. The bacterial culture of *P. polymyxa* ATCC 842 showed higher ability in degradation and improvement of the PKC during SSF.

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