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Effect of feeding different levels of palm kernel cake fermented by *Paenibacillus polymyxa* ATCC 842 on nutrient digestibility, intestinal morphology, and gut microflora in broiler chickens

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ABSTRACT

A feeding trial was conducted to investigate the effect of palm kernel cake fermented (FPKC) by *Paenibacillus polymyxa* ATCC 842 on nutrient digestibility, villi height, and gut microflora. A total of 245 one-day-old broiler chicks (Cobb500) were purchased from a local hatchery and raised in an open house system in the research unit at Universiti Putra Malaysia. The chicks were individually wing-banded, weighed and randomly distributed into seven groups with five replicates (pens) and seven birds per pen. The birds were fed diets containing 0, 5, 10 and 15% palm kernel cake (PKC) and 5, 10, 15% FPKC. All the birds were fed *ad libitum* during the feeding trial, which lasted 42 days. One bird and two birds per replicate in each dietary treatment were randomly selected at 3 and 6 weeks, respectively. Ileal digesta and small intestinal tissues were collected. The findings showed that feeding of 10% or 15% PKC led to a significant decrease ($P < 0.05$) in nutrient digestibility compared with the control group, whereas feeding of 10% or 15% FPKC led to a significant improvement ($P < 0.05$) in nutrient digestibility (except in nitrogen free extract at 3 weeks of age) compared with those groups of birds fed with PKC. No significant differences ($P > 0.05$) were observed between the dietary treatments in terms of intestinal morphology. However, gut microflora were improved ($P < 0.05$) in a group of birds fed with 15% FPKC compared to the other dietary treatments. In conclusion, the current experiment showed that FPKC by *P. polymyxa* ATCC 842 could be fed to broiler chickens up to 15% in their rations without any adverse effect on nutrient digestibility.

Abbreviations: CFU, colony forming unit; ENT, enterobacteriaceae; FPKC, fermented palm kernel cake; GIT, gastrointestinal tract; LAB, lactic acid bacteria; NSPs, non-starch polysaccharides; PKC, palm kernel cake; SSF, solid state fermentation.

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1. Introduction

Malaysia has an abundant amount of palm kernel cake (PKC), which is considered to be an agro-industrial waste derived from the extraction process of oil from palm fruits. However, PKC contains high levels of insoluble fibers, a coarse texture and gritty appearance (McDonald et al., 1995; O'Mara Mulligan et al., 1999; Sundu and Dingle, 2002), and non-starch polysaccharides (NSPs), such as mannan, xylan and cellulose (Sundu and Dingle, 2002; Alimon, 2004; Francesch and Brufau, 2004). The presence of high levels of fiber in poultry diets may lead to a decrease in the surface, width and height of intestinal villi (Moharrery and Mohammadpour, 2005; Kalmendal et al., 2011). Thus, the nutrient utilization may be negatively affected. The effect of dietary fiber on villi height is variable and depends on the physio-chemical characteristics of the fibers, levels, duration of the dietary fiber in the diet, animal species, age (Montagne et al., 2003) and health status of the bird (Mateos et al., 2012). It is suggested that PKC could also increase the non-pathogenic bacteria, such as lactic acid bacteria (LAB) (Zulkifli et al., 2009). At the same time, the *Enterobacteriaceae* group (ENT), which is considered to be pathogens, may decrease in birds fed diets containing PKC. The colonization of LAB in the intestinal tract may be influenced by the composition of the diet (Wenk, 2001; Zulkifli et al., 2009). It was reported by Zulkifli et al. (2009) that the presence of 78% NSPs in PKC might have a profound effect on gut microflora. Wenk (2001) and Chen et al. (2014) reported that long-term feeding of high ratios of dietary fiber could alter the physiological and anatomical characteristics of the gastrointestinal tract (GIT) of pigs. The soluble dietary fibers may increase ileal and post-ileal microbial fermentation (Wenk, 2001). The reduction of nutrient digestibility could be attributed not only to the presence of insoluble fibers, but also to the high content of soluble fibers such as NSPs. These molecules can increase the passage rate of ingesta and may decrease nutrient absorption. Consequently, high levels of dietary fiber lead to a reduction in the digestibility of energy, starch, protein and lipids in monogastrics (Montagne et al., 2003). In contrast, the digestibility of amino acids has been improved in broiler chickens when PKC was fermented with *Aspergillus wentii* TISTR 3075 (Muangkeow and Chinajariyawong, 2009). In addition, the nutritive value of PKC fermented by cellulolytic microorganisms was improved, and it could be beneficial in terms of feed cost per kilogram diet and poultry performance (Marini et al., 2005). Because there is increasing interest to improve the nutritive quality of PKC using cellulolytic microorganisms via solid state fermentation (SSF), it seems to be vital to investigate the effect of PKC fermented by cellulolytic microbes. Therefore, the objective of the current trial was to investigate the effect of PKC fermented by *Paenibacillus polymyxa* ATCC 842 (FPKC) on nutrient digestibility, intestinal morphology and gut microflora in broiler chickens.

2. Materials and methods

2.1. Birds and experimental diets

The study was conducted according to the guidelines of the Research Policy on Animal Ethics of the Universiti Putra Malaysia. A total of 245 male broiler one-day-old chicks (Cobb 500) were purchased from a local hatchery. The chicks were individually weighed, wing banded and randomly allocated into 35 pens of seven chicks per pen. The birds were raised in an open house system in the Poultry Unit, Department of Animal Science, Faculty of Agriculture, Universiti Putra Malaysia (UPM). Seven dietary treatments were formulated in the feed factory at the Poultry Unit, and the PKC levels were 0%, 5%, 10%, 15%, whereas the FPKC levels were 5%, 10% and 15%. The FPKC was produced using *Paenibacillus polymyxa* ATCC 842 under SSF for nine days (Alshelmani et al., 2014). Each treatment consisted of five replicates, and the feeding trial lasted for 42 days. The birds had access to the feed and drinking water *ad libitum*. The birds were vaccinated against infectious bronchitis disease and Newcastle disease, and Gumboro (infectious bursal) disease at 7 and 21 days, respectively. The starter (Table 1) and finisher (Table 2) diets were offered to the chickens from 0 to 21 days and 22 to 42 days, respectively. All the feed were formulated as isocaloric and iso-nitrogenous based on NRC (1994) recommendation, and the nutrient content of PKC and FPKC were considered for feed formulation. The birds were raised in the conventional open-sided house with cyclic temperature (maximum 35 °C and minimum 24 °C) and humidity (maximum 91% and minimum 65%). Diets were formulated using FeedLIVE software (FeedLIVE 1.52, Thailand). Dry matter (DM), CP, ash, and ether extract (EE) were determined using procedure 934.01, 976.05, 942.05, 920.39 of AOAC (1990), respectively, while acide tetergent fiber (ADF) and neutral detergent fiber (NDF) were determined based on the procedure of Goering and Van Soest (1970).

2.2. Samples and data collection

One bird and two birds per replicate in each dietary treatment were randomly selected at 3 and 6 weeks, respectively. The birds were slaughtered by *halal* neck cut for sampling of the ileal digesta (Ahmed et al., 2014) and small intestines for further analysis. The ileal digesta was collected from the Meckel's diverticulum to 1 cm before ileo-ceco junction. The ileal digesta samples were taken in order to determine the lactic acid bacteria (LAB) and *Enterobacteriaceae* (ENT) counts, as well as nutrient digestibility. The small intestine samples were collected for measuring villi height and crypt depth.

2.3. Nutrient digestibility

Titanium dioxide (TiO_2) was added to the feed as an indigestible marker at 0.3% before slaughtering the birds for four days during the end of the starter and finisher phases. Ileal digesta were collected at 3 and 6 weeks of age. The TiO_2 was

Table 1
Ingredient and chemical compositions of broiler starter diet.

Ingredient (%)	PKC[1] level (%)				FPKC[2] level (%)		
	0	5	10	15	5	10	15
Yellow corn	55.94	50.05	44.51	39.15	50.05	44.51	39.15
Palm oil	3.46	5.16	6.73	8.24	5.16	6.73	8.24
PKC	0.00	5.00	10.00	15.00	0.00	0.00	0.00
FPKC	0.00	0.00	0.00	0.00	5.00	10.00	15.00
Soybean meal 44% CP	31.95	30.71	29.62	28.54	30.71	29.62	28.54
Fish meal 55% CP	5.00	5.00	5.00	5.00	5.00	5.00	5.00
DCP[3] 18%	1.60	1.80	1.80	1.70	1.80	1.80	1.70
Calcium carbonate	0.30	0.37	0.37	0.37	0.37	0.37	0.37
DL-Methionine	0.20	0.28	0.28	0.25	0.28	0.28	0.25
L-Lysine	0.00	0.10	0.16	0.22	0.10	0.16	0.22
Salt	0.32	0.30	0.30	0.30	0.30	0.30	0.30
Vitamin Premix[a]	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Mineral Premix[b]	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Toxin binder	0.14	0.14	0.14	0.14	0.14	0.14	0.14
Antioxidant[c]	0.01	0.01	0.01	0.01	0.01	0.01	0.01
Choline chloride	0.08	0.08	0.08	0.08	0.08	0.08	0.08
Total	100.00	100.00	100.00	100.00	100.00	100.00	100.00
Calculated analyses[d]							
ME[6] (kcal/kg)	3000.10	3000.03	3000.14	3000.71	3000.03	3000.14	3000.71
Methionine + Cystine (%)	0.94	1.00	0.99	0.95	1.00	1.00	0.96
Lysine (%)	1.19	1.25	1.27	1.30	1.25	1.27	1.30
Arginine (%)	1.43	1.47	1.50	1.54	1.47	1.50	1.54
Lys: Arg ratio	1:1.2	1:1.18	1;1.18	1;1.18	1:1.18	1;1.18	1;1.18
Calcium (%)	1.07	1.08	1.09	1.08	1.09	1.10	1.12
Available phosphorus (%)	0.56	0.57	0.58	0.57	0.57	0.58	0.57
Chemical analyses							
DM	93.52	93.47	93.71	94.11	93.17	93.40	93.98
CP	22.50	22.53	22.25	21.84	22.20	22.40	22.60
NDF[4]	8.72	12.20	15.73	19.30	11.67	14.67	17.68
ADF[5]	4.45	6.67	9.09	11.42	6.55	8.66	10.79
EE	6.44	7.64	9.35	10.30	7.34	8.53	9.98
Ash	3.59	4.51	4.59	4.77	4.92	4.10	4.40
NFE	64.58	61.44	59.40	58.16	61.84	60.88	58.81

DM, dry matter; CP, crude protein; EE, ether extract; NFE, nitrogen free extract.
[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).
[d] Diets were formulated using FeedLIVE software (FeedLIVE 1.52, Thailand).
[1] PKC: Palm kernel cake.
[2] FPKC: Fermented palm kernel cake.
[3] DCP: Dicalcium phosphate.
[4] Neutral detergent fiber.
[5] Acid detergent fibe.
[6] ME: Metabolizable energy.

determined based on the method described by Short et al. (1996). Proximate analysis was applied to the feed and digesta to calculate the DM, CP, EE, ash NDF and ADF digestibilities based on the methods mentioned above.

2.4. Morphology of small intestines

The procedure for measuring the villi height and crypt depth was according to the method described by Choe et al. (2012). Five samples were taken at three weeks and ten samples at six weeks of age. Segments of approximately 5–6 cm long were removed from three different parts of the small intestine. The portion of duodenum was taken from the middle part of the duodenum loop, and for the jejunum, from midway between the end of the duodenal loop and Meckel's diverticulum, whereas the segment for ileum was taken from midway between Meckel's diverticulum and the ileo-cecal junction. All intestinal segments were flushed with 10% (v/v) neutral buffered formalin solution. The sections were filled out with the neutral buffered formalin, and banded before being kept in the formalin buffer solution for further morphometric analysis. The intestinal segments were excised to approximately 3 mm, and transferred into plastic cassettes in order to measure villus height and crypt depth. The whole excised segments were kept overnight in neutral buffer formalin solution. Then, the intestinal samples were dehydrated in tissue processing machine (Leica ASP 3000, Japan), and embedded in paraffin wax (Leica EG 1160, Japan). The intestinal samples were trimmed at 15 μm. Later, the samples were sectioned at 5 μm, and

Table 2
Ingredient and chemical compositions of broiler finisher diet.

Ingredient (%)	PKC[1] level (%)				FPKC[2] level (%)		
	0	5	10	15	5	10	15
Yellow corn	60.50	54.95	49.39	43.84	54.95	49.39	43.84
Palm oil	2.60	4.20	5.77	7.32	4.20	5.77	7.32
PKC	0.00	5.00	10.00	15.00	0.00	0.00	0.00
FPKC	0.00	0.00	0.00	0.00	5.00	10.00	15.00
Soybean meal 44% CP	28.16	26.80	25.70	24.60	26.80	25.70	24.60
Fish meal 55% CP	5.00	5.00	5.00	5.00	5.00	5.00	5.00
DCP[3] 18%	1.69	1.80	1.80	1.80	1.80	1.80	1.80
Calcium carbonate	0.30	0.37	0.37	0.37	0.37	0.37	0.37
DL-Methionine	0.20	0.25	0.28	0.28	0.25	0.28	0.28
L-Lysine	0.00	0.10	0.16	0.23	0.10	0.16	0.23
L-Threonine	0.00	0.00	0.00	0.03	0.00	0.00	0.03
Salt	0.32	0.30	0.30	0.30	0.30	0.30	0.30
Vitamin Premix[a]	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Mineral Premix[b]	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Toxin binder	0.14	0.14	0.14	0.14	0.14	0.14	0.14
Antioxidant[c]	0.01	0.01	0.01	0.01	0.01	0.01	0.01
Choline chloride	0.08	0.08	0.08	0.08	0.08	0.08	0.08
Total	100	100	100	100	100	100	100
Calculated analyses[d]							
ME[6] (kcal/kg)	3000.5	3000.2	3000.5	3000.0	3000.2	3000.5	3000.0
Methionine + Cystine (%)	0.90	0.91	0.91	0.90	0.90	0.91	0.90
Lysine (%)	1.10	1.15	1.18	1.21	1.15	1.18	1.21
Arginine (%)	1.33	1.36	1.40	1.43	1.36	1.40	1.43
Lys: Arg ratio	1:1.21	1:1.18	1:1.19	1:1.18	1:1.18	1:1.19	1:1.18
Calcium (%)	1.01	1.09	1.08	1.14	1.07	1.05	1.14
Available phosphorus (%)	0.57	0.58	0.57	0.58	0.58	0.57	0.58
Chemical analyses							
DM	90.00	90.50	91.60	91.00	91.10	91.61	90.10
CP	20.12	20.31	19.97	20.02	20.00	20.20	20.10
NDF[4]	8.58	12.07	15.60	19.12	11.54	14.54	17.53
ADF[5]	4.22	6.52	8.85	11.20	6.31	8.42	10.54
EE	5.70	7.27	9.67	11.73	7.86	9.66	11.53
Ash	6.29	5.19	5.71	5.10	5.21	6.02	5.27
NFE	64.76	63.27	60.23	58.47	63.86	60.12	59.05

DM, dry matter; CP, crude protein; EE, ether extract; NFE, nitrogen free extract.
[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).
[d] Diets were formulated using FeedLIVE software (FeedLIVE 1.52, Thailand).
[1] PKC: Palm kernel cake.
[2] FPKC: Fermented palm kernel cake.
[3] DCP: Dicalcium phosphate.
[4] Neutral detergent fiber.
[5] Acid detergent fiber.
[6] ME: Metabolizable energy.

fixed on slides in hot plate at 60 °C. The slides were stained with hematoxylin and eosin, mounted and examined under microscope.

The morphometric variables being examined were villi height and crypt depth. The villus height was measured from the tip of the villus to the base, whereas the crypt depth was measured as the depth of invagination between adjacent villi. Ten villi and crypts were measured from each slide (Five and ten slides at 3 and 6 weeks of age, respectively) and the average was taken.

2.5. Gut microflora count

2.5.1. Ileal lactic acid bacteria count

The gut microflora count was carried out based on the method described by Foo et al. (2003) using sterile peptone water; 10% (w/v) of ileal digesta were diluted and left at room temperature for one hour. Then, followed by ten-fold serial dilutions (v/v) using sterile peptone water. To determine the colony forming unit (CFU), LAB 100 μl was pipetted into MRS agar (Lactobacillus-Agar De Man, ROGOSA, and SHAPE) (Merck®, KGaA, Darmstadt). Hence, the plates were spread using a sterile L-shape and incubated in an anaerobic jar at 30 °C for 48 h.

Table 3
Effect of fermented palm kernel cake on ileal nutrient digestibility on broiler chickens.

Nutrient (%)	PKC[1] level				FPKC[2] level				Contrast, P-values	
	0%	5%	10%	15%	5%	10%	15%	SEM[5]	Linear[3]	Quadratic[4]
21 days										
DM	61.32^{a}	59.26^{b,c}	58.38^{c}	57.89^{c}	60.63^{a,b}	60.59^{a,b}	60.77^{a,b}	0.38	<0.0001	0.0006
CP	80.87^{a}	79.89^{a}	76.21^{b,c}	75.01^{c}	79.93^{a}	78.97^{a,b}	79.73^{a}	0.65	<0.0001	0.0024
NDF	35.81^{a}	36.10^{a}	35.03^{a,b}	33.85^{b}	36.06^{a}	35.38^{a,b}	35.10^{a,b}	0.35	0.0049	0.2194
ADF	20.50	20.00	19.88	19.75	20.38	20.50	20.75	0.48	0.7199	0.7489
EE	54.95^{a}	54.11^{a,b}	53.20^{b,c}	52.50^{c}	54.86^{a}	54.52^{a,b}	53.96^{a,b,c}	0.36	0.0006	0.0910
NFE	63.93^{a}	62.80^{a,b}	58.94^{c}	58.51^{c}	63.59^{a,b}	61.92^{b}	61.79^{b}	0.40	<0.0001	0.0128
Ash	34.74	34.42	33.88	33.60	34.94	34.68	34.37	0.40	0.2387	0.4863
42 days										
DM	67.11^{a}	64.59^{b}	62.08^{c}	61.38^{c}	65.95^{a,b}	66.22^{a,b}	68.30^{a}	0.51	<0.0001	<0.0001
CP	77.87^{a}	77.08^{a}	65.93^{b}	61.93^{c}	76.01^{a}	76.20^{a}	75.60^{a}	0.61	<0.0001	0.0001
NDF	48.30^{a}	47.26^{a,b}	45.20^{c,d}	44.43^{d}	46.68^{a,b,c}	45.76^{b,c,d}	46.52^{a,b,c}	0.44	<0.0001	0.0088
ADF	25.88^{a}	25.13^{a,b}	23.25^{c,d}	22.25^{d}	24.88^{a,b,c}	24.12^{b,c}	24.20^{a,b,c}	0.54	<0.0001	<0.0001
EE	69.22^{a}	68.00^{a,b}	65.90^{b}	63.63^{c}	68.77^{a}	67.81^{a,b}	68.42^{a}	0.48	<0.0001	0.0029
NFE	67.83^{a}	66.96^{a}	63.11^{b}	62.77^{b}	67.70^{a}	66.55^{a}	66.08^{a}	0.49	<0.0001	0.0220
Ash	55.48^{a}	54.18^{a,b}	53.04^{a,b}	51.83^{b}	54.22^{a,b}	55.34^{a}	53.96^{a,b}	0.71	0.0216	0.0677

DM; dry matter, CP; crude protein, NDF; neutral detergent fiber, ADF; acid detergent fiber, EE; ether extract, NFE; nitrogen-free extract.
n = 5 at 3 weeks and 10 at 6 weeks of age.
^{a,b,c,d} Means ± SEM. Means with different superscripts in the same row differ significantly (P< 0.05).
[1] PKC: Palm kernel cake.
[2] FPKC: Fermented palm kernel cake.
[3] Linear and quadratic responses, respectively, to PKC or FPKC inclusion levels.
[4] Linear and quadratic responses, respectively, to PKC or FPKC inclusion levels.
[5] Pooled standard error.

2.5.2. Ileal Enterobacteriaceae count

The petri dishes were spread plated in Eosin-methylene blue lactose sucrose agar (Merck®, KGaA, Darmstadt) in order to determine the ENT count, and incubated anaerobically at 37 °C for 24 h. The CFU was expressed as the base of 10 logarithms of CFU per gram digesta, and all samples were repeated five times.

2.6. Experimental design and data analysis

The experimental design was based on completely randomized design, and data were analyzed using the General Linear Model procedure of the statistical analysis system (SAS, 2003). Orthogonal polynomial contrasts were used to determine the linear and quadratic effects of dietary increasing proportion of inclusion PKC or FPKC against the control group on nutrient digestibility and gut microflora. Tukey's test was used to compare the means of treatment with probability 5% (P < 0.05). The statistical model used was $Y_{ijk} = \mu + T_{ij} + E_{ijk}$ where, Y_{ijk}, response variables; μ, the overall mean; T_{ij}, the effect of dietary treatment; E_{ijk}, the experimental error.

3. Results

3.1. Nutrient digestibility

The effect of feeding FPKC on the nutrient digestibility of broiler chickens is shown in Table 3. The findings showed that feeding 10% or 15% PKC in the starter and finisher phases led to a significant linear decrease (P < 0.05) in the digestibility of dry matter, crude protein, ether extract and nitrogen free extract compared to those birds fed with the negative control. On the other hand, no significant differences (P > 0.05) were observed in the dry matter, crude protein, and ether extract digestibility for those fed with 10% or 15% FPKC in comparison with the negative control in two phases, whereas a significant linear and quadratic improvement (P < 0.05) in the nutrient digestibility was found for those birds fed with 10% or 15% FPKC compared to those groups of birds fed with PKC.

A significant linear decrease (P < 0.0049) at starter phase in NDF digestibility, and both linear (P < 0.0001) and quadratic decrease (P < 0.0088) at finisher phase in NDF digestibility was observed for broilers fed with 15% PKC compared to those birds fed with the negative control diet. A significant linear and quadratic decrease (P < 0.0001) was also observed in ADF digestibility at finisher phase.

The digestibility of ash was linearly lower (P = 0.0216) than the negative control for chickens fed with 15% PKC. However, the digestibility of ash did not significantly (P > 0.05) differ for those groups of birds fed with 10% or 15% FPKC as compared to the negative control at the sixth week of age.

Table 4
Effect of fermented palm kernel cake on villus height and crypt depth in broiler chickens.

Dietary treatment	Villi height (μm)			Crypt depth (μm)		
	Duodenum	Jejunum	Ileum	Duodenum	Jejunum	Ileum
		21 days				
0% PKC[1]	1025.10	548.12	333.70	187.23	130.33	103.25
5% PKC	1031.21	568.93	383.63	196.06	144.83	101.95
10% PKC	1105.13	549.99	386.51	184.60	137.26	106.71
15% PKC	1109.33	550.32	358.70	188.53	131.90	109.22
5% FPKC[2]	1123.28	529.04	365.29	194.93	133.00	112.35
10% FPKC	1107.53	517.05	349.67	204.21	141.37	110.94
15% FPKC	1127.36	545.62	391.42	191.99	137.20	106.11
SEM[a]	27.31	21.40	15.10	6.60	8.06	2.82
Contrast, P-values						
Linear[3]	0.0757	0.7228	0.0788	0.052	0.8599	0.0998
Quadratic[4]	0.0687	0.4934	0.5506	0.0622	0.9754	0.518
		42 days				
0% PKC	1473.14	735.56	614.34	227.67	213.26	197.92
5% PKC	1458.61	736.38	609.41	229.80	219.06	201.04
10% PKC	1440.86	730.43	601.30	226.15	212.13	178.67
15% PKC	1439.65	734.91	614.37	224.68	202.88	194.18
5% FPKC	1471.21	732.08	613.14	233.26	217.13	208.23
10% FPKC	1480.77	731.34	607.71	231.04	217.71	200.81
15% FPKC	1482.87	733.50	669.30	234.35	216.01	211.75
SEM	30.50	15.50	17.30	5.52	5.43	9.54
Contrast, P-values						
Linear[3]	0.8782	1.000	0.1233	0.8586	0.4177	0.2840
Quadratic[4]	0.4200	0.7939	0.0656	0.4706	0.6052	0.1759

n = 5 at 3 weeks and 10 at 6 weeks of age.
[1] PKC: Palm kernel cake.
[2] FPKC: Fermented palm kernel cake.
[3] Linear and quadratic responses, respectively, to PKC or FPKC inclusion levels.
[4] Linear and quadratic responses, respectively, to PKC or FPKC inclusion levels.
[a] Pooled standard error.

Table 5
Effect of fermented palm kernel cake on ileal lactic acid bacteria (LAB) and *Enterobacteriaceae* (ENT) count in broiler chickens.

Bacterial count, $\log_{10}$ CFU/g		PKC level				FPKC level				Contrast, P-values	
		0%	5%	10%	15%	5%	10%	15%	SEM[3]	Linear[1]	Quadratic[2]
21 days											
	LAB count	5.20	5.25	5.27	5.17	5.28	5.24	5.30	0.03	0.0599	0.1899
	ENT count	$4.19^{a,b}$	$4.24^{a,b}$	4.27^{a}	$4.22^{a,b}$	$4.20^{a,b}$	$4.13^{b,c}$	4.03^{c}	0.02	<0.0001	<0.0001
42 days											
	LAB count	5.17^{b}	$5.26^{a,b}$	$5.27^{a,b}$	$5.44^{a,b}$	$5.43^{a,b}$	$5.44^{a,b}$	5.56^{a}	0.07	0.0266	0.0007
	ENT count	4.38	4.67	4.60	4.42	4.26	4.31	4.45	0.22	0.8077	0.7240

n = 5 at 3 weeks and 10 at 6 weeks of age.
[a,b,c] Means ± SEM. Means with different superscripts in the same row are differ significantly ($P < 0.05$).
[1] Linear and quadratic responses, respectively, to PKC or FPKC inclusion levels.
[2] Linear and quadratic responses, respectively, to PKC or FPKC inclusion levels.
[3] Pooled standard error.

3.2. Morphology of small intestines

The effect of feeding FPKC on villus height and crypt depth in broiler chickens is shown in Table 4. The data obtained did not show any significant differences ($P > 0.05$) among the dietary treatments in villus height and crypt depth for three segments of the small intestine – duodenum, jejunum and ileum – in the starter or finisher phase.

3.3. Gut microflora

The effect of feeding FPKC on ileal LAB and ENT counts in broiler chickens at three and six weeks of age is presented in Table 5. The results showed that the ENT counts were significantly lower (linear and quadratic, $P < 0.0001$) for the birds fed with 15% FPKC compared to the negative control group or those birds fed with different levels of PKC. No significant difference was observed (linear, $P = 0.0599$; quadratic, $P = 0.1899$) between the dietary treatments in the LAB count at three weeks of age. In contrast, LAB count was significantly higher (linear, $P = 0.0266$; quadratic, $P = 0.0007$) for birds fed with 15%

FPKC than the negative control group at six weeks of age, whereas, no significant difference was observed (linear, P = 0.8077; quadratic, P = 0.7240) between the dietary treatments in ENT counts at the sixth week of age.

4. Discussion

4.1. Nutrient digestibility

The nutrient digestibility declined in broiler chickens fed with 10% or 15% PKC for both phases. The reduction of apparent ileal digestibility of nutrients could be attributed to the presence of the high levels of NDF, ADF, insoluble fibers and NSPs in the PKC (Sulabo et al., 2013; Son et al., 2014). Indeed, the viscosity would increase in the small intestines and speed up the passage rate of ingesta (Dégen et al., 2007), and the apparent ileal digestibility of the nutrients would decrease. These findings are consistent with Aya et al. (2013), who reported that the inclusion of 10% PKC in the broiler starter diet led to a significant decrease ($P < 0.05$) in nutrient digestibility. The findings are also in agreement with Lawal et al. (2010), who referred to the reduction of nutrient digestibility for broiler chickens fed with 10% PKC compared to those chickens fed with 10% PKC treated with cellulolytic crude enzymes that hydrolyze fibers. Similar findings were obtained by Mok et al. (2013), who showed that dry matter, organic matter and energy digestibility in growing pigs fed with 10% PKC were significantly ($P < 0.05$) lower than those growing pigs fed with 10% PKC supplemented with β-mannanase.

The results are also in agreement with Brenes et al. (2002), who mentioned that feeding young chicks with a diet containing high levels of fibers (Lupin) had a significantly ($P < 0.05$) adverse effect on the apparent digestibility of protein, whereas a diet supplemented with enzymes (0.1% Novozyme®) led to improved crude protein digestibility. The supplementation of enzyme to a lupin-based diet enhances the apparent ileal digestibility of NSPs in broiler chickens (Brenes et al., 2002).

The findings are in agreement with Fasuyi et al. (2014), who reported that apparent protein digestibility was significantly ($P < 0.05$) lower in broiler chickens fed with 10% PKC compared to the chickens fed with the negative control diet. On the other hand, the apparent protein digestibility was improved for those chickens fed with 10% PKC supplemented with enzymes (cellulase, glucanase, and xylanase). It has been reported that *P. polymyxa* ATCC 842 was able to produce high cellulase, xylanase, and mannanase activities on different substrates (Alshelmani et al., 2013).

Similar findings were obtained by Selle et al. (2009), who claimed that a diet supplemented with xylanase significantly ($P < 0.05$) enhanced the ileal digestibility of protein for broiler chickens fed with a wheat-based diet compared to those chickens fed with the control diet. It has been reported that apparent ileal digestibility of crude protein can be reduced by 0.3%–0.8% per every percentage point in NDF increase (Sulabo et al., 2013). In addition, the adverse effect of soluble fibers (NSPs) is greater than the insoluble fibers in terms of nutrient digestibility (Dégen et al., 2007; Sulabo et al., 2013). Based on our previous work, the reducing sugars have been significantly increased ($P < 0.05$) as a result of NSPs degradation by *P. polymyxa* ATCC 842 (Alshelmani et al., 2014). This can interpret the improvement in nutrient digestibility of FPKC.

The reduction of nutrient digestibility could be attributed not only to the presence of insoluble fibers, but also to the high content of soluble fibers, such as NSPs. These molecules can increase the speed passage rate of ingesta, which may result in decreased nutrient absorption. Therefore, high levels of dietary fiber lead to a reduction in the digestibility of energy, starch, protein and lipids in monogastric animals (Montagne et al., 2003; Walugembe et al., 2014). In the current experiment, it seems that the full genetic potential of the commercial meat-type chickens may not be able to be expressed because of environmental constraints (Awad et al., 2014).

4.2. Morphology of small intestines

It has been reported that feed containing a high content of dietary fiber can decrease the surface area, width and height of intestinal villi (Moharrery and Mohammadpour, 2005; Kalmendal et al., 2011; Carabaño et al., 2013). The effect of dietary fiber on villi height is variable and depends on the physio-chemical characteristics of the fibers, levels, duration of the dietary fiber in the diet, animal species, age (Montagne et al., 2003) and health status of the birds (Mateos et al., 2012).

In the current study, no significant differences were found for the villi height and crypt depth in the small intestine. However, these findings are consistent with Zulkifli et al. (2009), who claimed that no significant difference ($P > 0.05$) was observed in the intestinal villi height for broiler chickens fed 25% PKC in the diets.

4.3. Gut microflora

The important role of gastrointestinal microflora in the health of the animals has been investigated. LAB are considered to be the normal microflora of the GIT. LAB are known for their ability to produce a wide variety of anti-bacterial substances, such as acetate, lactate, propionate, ethanol and bacteriocins (Loh et al., 2009). These compounds are able to control and inhibit the growth of intestinal pathogenic bacteria (Foo et al., 2003; Nguyen et al., 2010; Choe et al., 2012; Loh et al., 2014). No significant difference in the results was found among the dietary treatments in the LAB count at three weeks of age, whereas the LAB count significantly ($P < 0.05$) increased in the birds fed with 15% FPKC compared to the negative control in the finisher phase (six weeks of age). These findings are inconsistent with Zulkifli et al. (2009), who reported that the LAB count increased significantly ($P < 0.05$) in the ileum and caecum for finisher broilers fed with 25% PKC. On the other hand,

these findings are consistent with the results of Gao et al. (2008), who claimed that there were no significant differences in the LAB count for broilers fed with a wheat-based diet in the starter phase.

The colonization of LAB in the intestinal tract may be influenced by the composition of the diet (Wenk, 2001). It was reported by Zulkifli et al. (2009) that the presence of 78% NSPs in PKC might have a profound effect on gut microflora. Wenk (2001), and Chen et al. (2014) reported that long-term feeding of high ratios of dietary fibers could alter the physiological and anatomical characteristics of the GIT of pigs. The soluble dietary fibers may increase ileal and post-ileal microbial fermentation (Wenk, 2001).

The ileum ENT counts were significantly ($P < 0.05$) decreased at 3 weeks of age, while the LAB count increased at 6 weeks of age in the current study. These findings are in agreement with Zulkifli et al. (2009), who mentioned that an increase in the LAB count can depress the growth of enteropathogens in the gut. The findings are also consistent with Loh et al. (2009), and Choe et al. (2012) who reported that there is a negative correlation between LAB and ENT, in that an increased LAB population may reduce the ENT count.

5. Conclusion

PKC fermented (FPKC) by *P. polymyxa* ATCC 842 can be offered up to 15% in broiler rations without causing any adverse effects on nutrient digestibility (except in nitrogen free extract at 3 weeks of age). The intestinal villi height and crypt depth were not affected by the inclusion of FPKC in the diet, whereas the LAB count was increased in broilers fed with 15% FPKC. In this way, the inclusion of 15% FPKC can replace up to 30% of yellow corn in the birds' diets, which can be reflected in the cost saving of feed in the poultry industry.

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