Feeding fermented palm kernel cake with higher levels of dietary fat improved gut bacterial population and blood lipid concentration but not the growth performance in broiler chickens

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ABSTRACT
The study aimed to determine the effects of Weisella confusa SR-17b fermented PKC (LPKC) and different levels of dietary fats on the growth performance, caecal microbial population, and blood lipid profile concentration in broiler chickens raised in the tropical environment. During the starter period, all birds received the same basal diet. From d 22 to 35, broiler chickens were randomly fed with either (1) 20% LPKC based diet with 5% palm oil, (2) 20% LPKC-based diet with 9.5% palm oil, (3) 20% PKC-based diet with 5% palm oil or (4) 20% PKC-based diet with 9.5% palm oil. No significant difference was observed between LPKC and PKC diets on broilers' growth performance. LPKC-based diet reduced the caecal population of E. coli and serum triglyceride concentration. In terms of oil supplementation, both PKC- and LPKC-based diets required higher levels of dietary fat to maintain optimum growth performance. A higher level of dietary fat in the LPKC-/PKC-based diet reduced serum levels of triglycerides and lower-density lipoprotein cholesterol and lowered the caecal E. coli population.

HIGHLIGHTS
- Feeding finisher diet based on fermented palm kernel cake has no effect on the overall growth performance but reduces gut E. coli population, and serum triglycerides levels.
- Incorporating 9.5% palm oil in the finisher diet is beneficial in improving final body weight and feed efficiency, reducing gut E. coli population, and modifying serum lipid profile.
- Diet with fermented palm kernel cake and 9.5% palm oil shows the best feed cost/kg of chicken produced.

Introduction
Palm kernel cake (PKC) is an agro-industrial by-product of palm oil production, which holds a moderate amount of essential nutrients for animal consumption, such as energy, protein, and fat. The use of PKC in poultry diets has been actively investigated, especially in palm oil-producing countries, due to its abundance, non-seasonal availability, and low pricing. However, PKC has some drawbacks that limited its inclusion level in poultry diets, including low essential AA concentrations and high insoluble fibre content, mainly in the form of mannan non-starch polysaccharides (Sharmila, Alimon, et al. 2014a; Alshelmani et al. 2021). The high fibre content and gritty texture of PKC has been reported to result in low metabolisable energy (ME) and low crude protein (CP) and amino acids (AA) digestibility in broiler chickens (Abdollahi et al. 2015; Alshelmani et al. 2017). Feedstuffs with higher fibre content may also exacerbate heat stress problems in poultry (Aljubori et al. 2017). Thus, it may restrict the use of PKC in poultry raised in a hot and humid environment.

Earlier work on PKC in poultry diets involved higher levels of dietary fat in compensating the lack of metabolisable energy supplied by PKC, especially when a higher level of PKC was incorporated (Saenphoom et al. 2013; Abdollahi et al. 2016; Alshelmani et al. 2017; Chen et al. 2019). Saenphoom et al. (2013) included palm oil at 3% in a PKC-free diet, whereas the inclusion of 20 and 30% of PKC...
increased the level of palm oil used to 6.6 and 8.7%, respectively, which nearly 3-folds higher than the control diet for the latter. Zulkifli et al. (2003) suggested that the higher addition of palm oil in a PKM-based diet for heat-stressed broilers may alleviate the adverse effects caused by fibre in PKM. Earlier reports suggested depressive effects of fibre on the growth of chicken by affecting the nutrient utilisation via changes in the intestinal environment and reduction in feed retention time (Choct et al. 1996, 1999; Hetland and Svihus 2001). Higher dietary fat has been reported to increase the nutrient retention time in broilers, which may improve the nutrient digestion and absorption, subsequently the animal growth rate (Mateos et al. 1982; Brue and Latshaw 1985; Rochell et al. 2012). Thus, there is a possibility that the heat stress problem associated with the use of fibrous PKC could be alleviated by a higher inclusion level of dietary fat (Zulkifli et al. 2003).

However, high dietary fat consumption in chicken has seen not only improved feed efficiency but accompanied by high body fat deposition (Rivas and Firman 1994), which may lead to drawbacks in the post-production stage, including decreases of carcass’s yield and influencing the consumer’s preferences (Emmerson 1997). There is a lack of documented work on the relationship between dietary fat and dietary fibre in broiler chickens’ diet. Such information is essential to promote the use of a PKC-based diet in the poultry industry.

Solid-state fermentation has been widely used to produce bioactive secondary metabolites such as antibiotics, growth promoters, peptides, and enzymes (Robinson et al. 2001) for various industries, including pharmaceuticals (Demain 1999) and the food industry (Wang 2009; Dharmaraj 2010). In the animal feed industry, solid-state fermentation has been used for nutrient improvement in medium to low-quality agro-industrial waste for animal consumption (Robinson and Nigam 2003). Lactic acid bacteria (LAB) have been found to release functional hemicellulolytic and cellobolytic enzymes secondary metabolites such as mannanase, cellulase, and xylanase (Alshelmani et al. 2013). SSF of PKC with LAB has been shown to improve nutrient availability and subsequent growth performance in broilers, which indicated higher NSP degradation (Alshelmani et al. 2014; Tan 2016). Besides its ability to produce degrading enzymes, fermentation with LAB can lengthen the shelf-life of the fermented product, down to the anti-fungal properties of LAB. LAB fermentation has shown the ability to produce anti-fungal metabolites such as lactic and acetic acids (Londero et al., 2014). LAB also exhibited high antimicrobial activity as it has also been used to control pathogen contamination on animal feed such as E. coli, Campylobacter, Salmonella, and Clostridium (Heres et al., 2003 ; Murry Jr. et al. 2004). Besides, mannan-oligosaccharides of NSP-degraded PKC may improve the growth of beneficial gut bacteria and immune response (Chen et al. 2015; Rezaei et al. 2015). Jahromi et al. (2016) reported supplementation of oligosaccharides extracted from PKC enhanced the populations of beneficial bacteria by at least 1.3-folds in Lactobacillus and Bifidobacterium, and half-fold in Enterococcus, while reduced the pathogenic E. coli and Enterobacter’s population by 3- and 1-fold(s), respectively, in the caecum of chickens. A previous report from our laboratory found that the CP and AA digestibility values of Weisella confusa SR-17b fermented PKC (LPKC) were higher than the untreated PKC and enzyme-fermented PKC in broilers reared under hot and humid environmental conditions (Hakim et al. 2020). Accompanied with higher AME values (1800 kcal/kg) than the untreated PKC, LPKC has great potential to be included in broilers’ diet. Aljubori et al. (2017) reported that solid-state fermentation with Lactobacillus salivarius reduced the fibre content of canola meal by 16%, and it can be fed up to 30% to heat-stressed broilers without any detrimental effect on growth performance. Hence, we hypothesised that with the lower fibre content of LPKC, the inclusion level of dietary fat can be reduced, and LPKC could be a suitable feedstuff for broilers in the hot and humid tropical environment. Therefore, the objective of this study was to determine the effects of feeding an untreated/fermented PKC-based diet at different levels of dietary fats on the growth performance, caecal microbial population, and blood lipid profile concentration in broiler chickens raised under the hot and humid environmental conditions. We also determined the feed cost/kg of chicken produced for each diet.

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).

Birds and management

A total of 300 1-d-old Cobb 500 male broiler chicks were obtained from a local hatchery. Upon arrival (d 1), the chicks were weighed and randomly allocated
to 30 battery cages (240 × 120 × 45 cm, length × width × height) with wire floors in a conventional open-sided house with 10 chicks per cage. The temperature and relative humidity were from 24 to 36 °C and 62 to 88%, respectively (Table 1). Birds were vaccinated (intratraqueal route) against Newcastle disease (ND) and Infectious bronchitis (IB) on d 7 and 21. Birds were fed ad libitum, and water was always available.

**Experimental design and diets**

The experimental design was a 2 × 2 factorial arrangement of treatments, including two types of PKC (PKC and LPKC) and two levels of oil inclusion (5 and 9.5%). PKC was obtained from a commercial kernel oil extraction factory in Klang, Selangor, Malaysia, and ground to a uniform size of about 3 mm before treatment. LPKC was prepared following the procedure established by Tan (2016), inoculating lactic acid bacteria of strain *Weisella confusa* SR-17b with PKC via solid-state fermentation technique. Briefly, *W. confusa* SR-17b isolates were 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), D (+) 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 *W. confusa* SR-17b with PKC via solid-state fermentation technique. Briefly, *W. confusa* SR-17b isolates were 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), D (+) 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).

### Table 1. Profile of environmental temperature and humidity during experimental period.

| Experimental period | Temperature, °C | Relative humidity, % |
|---------------------|-----------------|----------------------|
|                     | Minimum | Maximum | Minimum | Maximum |
| Week 1              | 24      | 33      | 58      | 88      |
| Week 2              | 23      | 33      | 64      | 90      |
| Week 3              | 23      | 31      | 68      | 95      |
| Week 4              | 24      | 34      | 62      | 90      |
| Week 5              | 25      | 36      | 62      | 88      |

The birds were fed with a standard broiler starter ration from d 1 to 21 (Table 2). From d 22 to 35, equal numbers of birds were randomly assigned to one of four dietary treatments: 20% LPKC with 5% Palm Oil (LPKC-LO), 20% LPKC with 9.5% Palm Oil (LPKC-HI), 20% PKC with 5% Palm Oil (PKC-LO) and 20% PKC with 9.5% Palm Oil (PKC-HI) (Table 2). Each dietary group had six replicates in a complete randomised design. All diets were isocaloric and isonitrogenous, following or exceeding the nutrient requirements suggested for Cobb 500 broilers (Cobb-Vantress Inc., Siloam Springs, AR, USA). The formulation of PKC and LPKC diets was based on nutrient and digestible

### Table 2. Feed ingredients and chemical composition of the starter and finisher broiler diets.

| Ingredient | Starter (day 1–21) | Finisher (day 22–35) |
|------------|-------------------|----------------------|
|            | LPKC-LO | LPKC-HI | PKC-LO | PKC-HI |
| Corn       | 51.26   | 45.53   | 39.63  | 42.91  | 35.77 |
| Corn gluten meal | – | 6.67    | –      | 7.96   | –     |
| Soybean meal | 40.00  | 7.93    | 27.56  | 19.18  | 12.23 |
| Fullfat soybean meal | 11.22  | 20.43   | 12.23  | 20.43  | 12.23 |
| Fermented PKC | –     | 20.00   | 20.00  | –      | –     |
| PKC        | –      | –       | 20.00  | 20.00  | –     |
| Palm oil   | 5.00    | 5.00    | 9.50   | 9.50   | –     |
| L-Lysine   | 0.07    | 0.41    | 0.13   | 0.45   | 0.15  |
| DL-methionine | 0.28  | 0.21    | 0.25   | 0.21   | 0.27  |
| L-Cysteine | –      | 0.08    | 0.06   | 0.09   | 0.08  |
| DCP        | 1.82    | 1.53    | 1.48   | 1.53   | 1.47  |
| Limestone | 0.92    | 0.65    | 0.65   | 0.65   | 0.63  |
| Salt       | 0.50    | 0.35    | 0.35   | 0.35   | 0.35  |
| Vitamin premixa | 0.05 | 0.05    | 0.05   | 0.05   | 0.05  |
| Mineral premic | 0.10  | 0.10    | 0.10   | 0.10   | 0.10  |
| Choline chloride | –     | 0.08    | 0.05   | 0.07   | 0.03  |
| Antioxidant | 0.10   | 0.10    | 0.10   | 0.10   | 0.10  |
| Toxin binder | 0.10   | 0.10    | 0.10   | 0.10   | 0.10  |

PKC: palm kernel cake; DCP: dicalcium phosphate; ME: metabolisable energy; CP: crude protein; EE: ether extract; CF: crude fibre; NDF: neutral detergent fibre; Dig Lys: digestible lysine; Dig Met + Cys: digestible methionine + cysteine; Dig Thr: digestible threonine.

### Nutrients composition (% unless stated otherwise)

| Ingredient         | Starter | Finisher |
|--------------------|---------|----------|
|                    | LPKC-LO | LPKC-HI  |
| ME (kcal/kg)       | 3065.00 | 3177.00  |
| CP                 | 22.36   | 19.67    |
| EE                 | 7.52    | 9.98     |
| CF                 | 4.56    | 6.36     |
| DCP                | 11.62   | 22.86    |
| NDF                | 1.18    | 0.96     |
| Dig Lys            | 0.88    | 0.75     |
| Dig Met + Cys      | 0.77    | 0.65     |

**Premixed administered vitamins per kilogram of the diet:** vitamin A (retinyl acetate), 8000 IU; vitamin D3 (cholecalciferol), 1.000 IU; vitamin E (DL-α-tocopherol), 30.0 IU; vitamin K3 (menadione dimethylpyrimidinol, 2.50 mg; vitamin B1, 2.00 mg; vitamin B2, 5.00 mg; vitamin B6, 2.00 mg; vitamin B12, 0.01 mg; niacin, 30.0 mg; d-biotin, 0.045 mg; vitamin C, 50.0 mg; d-pantothenate, 8.00 mg, folic acid, 0.500 mg.

### Premixed administered minerals 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.

The birds were fed with a standard broiler starter ration from d 1 to 21 (Table 2). From d 22 to 35, equal numbers of birds were randomly assigned to one of four dietary treatments: 20% LPKC with 5% Palm Oil (LPKC-LO), 20% LPKC with 9.5% Palm Oil (LPKC-HI), 20% PKC with 5% Palm Oil (PKC-LO) and 20% PKC with 9.5% Palm Oil (PKC-HI) (Table 2). Each dietary group had six replicates in a complete randomised design. All diets were isocaloric and isonitrogenous, following or exceeding the nutrient requirements suggested for Cobb 500 broilers (Cobb-Vantress Inc., Siloam Springs, AR, USA). The formulation of PKC and LPKC diets was based on nutrient and digestible
nutrient composition, pre-determined in our laboratory (Hakim et al. 2020).

**Samples and data collection**

Bodyweight (BWG) and feed intake (FI) were recorded weekly. Feed conversion ratios (feed/gain) were calculated. Mortality was recorded as it happened. On d 35, two birds per cage were randomly selected and weighed individually. The birds were slaughtered humanely according to the halal procedure (Farouk et al. 2014). Blood samples were collected, centrifuged at 4,000 x g at 4°C for 20 minutes, and the harvested serum samples were stored at −80°C for determination of serum triglycerides (TG), total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), and low-density lipoprotein cholesterol (LDL-C). Caecal contents were collected and immediately rapid-frozen in liquid nitrogen to determine the intestinal bacterial population.

**Blood lipid profile analysis**

Serum concentrations of TG, TC, LDL-C, and HDL-C were analysed using an automated chemistry analyser (Hitachi 902 Automatic Analyser: Hitachi, Tokyo, Japan) and commercial test kits (Roche Diagnostics, Basel, Switzerland).

**Gut microflora population determination**

The numerations of Lactobacillus spp., E. coli, and Clostridium spp. populations in the caecum were done according to the real-time PCR (q-PCR) method described by Navidshad et al. (2016). DNA extraction from caecal samples was carried out using QIAamp Fast DNA Stool Mini Kit (QIAGEN, Germany), following the manufacturer’s protocol. Purity and concentration of the extracted DNA samples were measured using a Nanodrop ND-1000 spectrophotometer after the PCR products being purified using the MEGA quick-spinTM (Intron Biotechnology, Inc). Standard curves were formed using a serial dilution of PCR products from pure cultures of Lactobacillus, E. coli, and Clostridium. For the targeted bacterial quantifications, the sequences of the primer reported in Table 3 were used. The q-PCR was performed with the BioRad CFX96 Touch (BioRad, USA) using optical grade plates. The PCR reaction was carried out in 20 μl total volumes using the QuantiNova SYBR Green PCR kit (QIAGEN, Germany). Each reaction consisted of 10 μl SYBR Green Supermix, 1 μl of each Primer, 2 μl of DNA samples and 6 μl molecular H2O. The reaction conditions for amplification of DNA were 94°C for 5 min, followed by 40 cycles of 94°C for 20 s, 58°C (Lactobacillus) or 60°C (E. coli and Clostridium) for 30 sec, and 72°C for 20 sec. The amplification melting curve analysis was carried out after the last cycle of each amplification to confirm the specificity of amplification melting curve analysis.

**Economic efficiency analysis**

Economic efficiency was determined following the method described by Soares et al. (2020), which considered the cost of the ration and the cost of the chick, and included the performance variables of feed intake, body weight, feed conversion ratio, and viability. The average cost per kilogram of chicken produced was determined as the sum of feed cost/kg of chicken produced and chick cost/kg of chicken produced.

The cost was calculated according to the following equation:

\[
\text{Cost (USD)} = (\text{feed conversion ratio} \times \text{feed cost}) + \left(\frac{\text{chick cost}}{\text{total number of birds} \times \text{body weight} \times \text{viability}}\right)
\]

**Statistical analyses**

Data were analysed using the GLM procedure of SAS 9.4 (SAS 2005) (SAS Institute Inc., Cary, NC, USA). Two-way ANOVA was used in a 2 × 2 factorial arrangement with type of PKC, level of oil, and their interactions as the main effects. The Duncan’s multiple range test was used to separate the means. Differences were considered significant if p < .05.
Table 4. Growth performance of broilers fed LPKC-and PKC-based diets at different levels of oil.

| Types of PKC | Level of oil | BWG, g/bird | FCR | SEM | d22-35 | d1-35 | d22-35 | d1-35 | d22-35 | d1-35 |
|-------------|-------------|-------------|-----|-----|--------|--------|--------|--------|--------|--------|
| Low         | Low         | 1047.61b    | 134  | 1.49 | 0.02   | 0.01   | 0.02   | 0.01   | 0.02   | 0.01   |
| High        | High        | 1099.39     | 134  | 1.49 | 0.02   | 0.01   | 0.02   | 0.01   | 0.02   | 0.01   |

Values are means of six replicates (n = 6). PKC: palm kernel cake; LPKC: Weisella confusa SR-17b fermented PKC; Low, 5% oil; High, 9.5% oil; FCR: feed conversion ratio; SEM: standard error of means.

Results

Growth performance

Data on the effects of types of PKC and level of oil on growth performance are presented in Table 4. From days 22-35, FI was not significantly (p = .479, p = .250) affected either by types of PKC or by level of oils. The birds fed LPKC had higher (p = .024) BWG and lower (p = .027) FCR compared to the PKC fed groups. Similarly, the higher oil supplemented group showed an increase (p = .045) BWG and subsequently better (p < .001) FCR compared to their lower oil supplemented group counterpart. No interaction was observed between different types of PKC and levels of oils on FI, BWG, and FCR during days 21-35.

In terms of overall growth performance, types of PKC and level of oil had no effects (p = .676, p = .923) on the FI. Likewise, LPKC-fed birds showed similar (p = .163) BWG and (p = .079) FCR to the PKC-fed group. On the other hand, birds fed on higher oil supplemented diet exhibited better (p = .037) BWG and (p = .002) FCR than the birds fed on a lower oil supplemented diet. No interaction was noted throughout the rearing period between different types of PKC and levels of oils on FI, BWG, and FCR.

Economic efficiency analysis

Table 5 shows the economic efficiency of broiler production as affected by PKC types and oil inclusion level. Diets with a higher level of oil inclusion significantly improved the viability rate of broilers (p = .054). The viability rate of birds was not affected by the types of PKC (p = .959). The LPKC based diets had significantly (p = .002) lower feed cost/kg of chicken produced than those with PKC. Feeding diets with a higher level of oil supplementation was significantly (p < .001) cheaper than those with a lower level of oil inclusion. Comparison among the four diets showed that the PKC-LO diet had the worst feed cost/kg of chicken produced, followed by PKC-HI and LPKC-LO (both had similar costs), and LPKC-HI.

Caecal microbial population

Table 6 shows the caecal bacteria populations of broilers affected by types of PKC and level of oil inclusion. Lactobacillus spp. and Clostridium spp. populations were not affected by both types of PKC (p = .379, p = .822) and level of oils (p = .224, p = .962). As for E. coli populations, birds fed on LPKC based diets and higher oil supplemented diets exhibited lower (p = .020, p = .015) values than the PKC-based diets and lower oil supplemented diets fed birds, respectively. No interaction was observed between different types of PKC and levels of oil on the caecal population of Lactobacillus spp., E. coli, and Clostridium spp. in broilers.

Blood serum lipid profile

The effect of types of PKC and level of oil inclusion on the blood serum lipid profile of broilers are presented in Table 7. Both types of PKC and level of oil had

Table 5. Viability rate and economic efficiency of broilers fed LPKC-and PKC-based diets at different levels of oil.

| Types of PKC | Level of oil | Viability | Cost, USD |
|-------------|-------------|-----------|-----------|
| PKC         | Low         | 0.90      | 0.71      |
| LPKC        | Low         | 0.98      | 0.68      |
| PKC         | High        | 0.92      | 0.68      |
| LPKC        | High        | 0.97      | 0.65      |
| SEM         | Low         | 0.02      | 0.01      |
| LPKC        | High        | 0.02      | 0.01      |

Values are means of six replicates (n = 6). LPKC-LO: 20% Weisella confusa SR-17b fermented palm kernel cake with 5% palm oil; LPKC-HI: 20% Weisella confusa SR-17b fermented palm kernel cake with 9.5% palm oil; PKC-LO: 20% palm kernel cake with 5% palm oil; PKC-HI: 20% palm kernel cake with 9.5% palm oil; PKC: palm kernel cake; LPKC: Weisella confusa SR-17b fermented PKC; Low, 5% oil; High, 9.5% oil; MYR: Malaysian Ringgit; FI: feed intake; BWG: body weight gain; FCR: feed conversion ratio; SEM: standard error of means.


**Table 6.** Caecal bacteria populations of broilers fed LPKC-and PKC-based diets at different levels of oil.

| Diet         | Lactobacillus spp., 10^5 cfu/g | E. coli, 10^5 cfu/g | Clostridium spp., 10^5 cfu/g |
|--------------|-------------------------------|------------------|-------------------------------|
| Types of PKC |                               |                  |                               |
| PKC          | 7.28                          | 6.11*            | 8.03                          |
| LPKC         | 7.39                          | 5.81*            | 8.11                          |
| SEM          | 0.06                          | 0.07             | 0.21                          |
| Level of oil |                               |                  |                               |
| Low          | 7.26                          | 6.10*            | 8.07                          |
| High         | 7.41                          | 5.78*            | 8.08                          |
| SEM          | 0.06                          | 0.07             | 0.21                          |

*p* Value

| Types of PKC | .397 | .020 | .822 |
| Level of oil | .224 | .015 | .962 |

SEM .06 0.07 0.21

Values are means of six replicates (n = 6).

PKC: palm kernel cake; LPKC: Weisella confusa SR-17b fermented PKC; Low, 5% oil; High, 9.5% oil; SEM: standard error of means.

*a* Means within a column with no common superscripts differ at *p* < .05.

**Table 7.** Blood serum lipid profile of broilers fed LPKC-and PKC-based diets at different levels of oil.

| Diet         | TC (mmol/L) | TG (mmol/L) | LDL-C (mmol/L) | HDL-C (mmol/L) |
|--------------|-------------|-------------|----------------|----------------|
| Types of PKC |             |             |                |                |
| PKC          | 3.23        | 0.35*       | 0.46           | 2.60           |
| LPKC         | 2.95        | 0.25*       | 0.50           | 2.31           |
| SEM          | 0.12        | 0.02        | 0.02           | 0.08           |
| Level of oil |             |             |                |                |
| Low          | 3.29        | 0.36*       | 0.57*          | 2.55           |
| High         | 2.88        | 0.24*       | 0.39*          | 2.36           |
| SEM          | 0.12        | 0.02        | 0.02           | 0.08           |

*p* Value

| Types of PKC | .223 | <.001 | .153 | .074 |
| Level of oil | .083 | <.001 | <.001 | .247 |
| PKC < oil    | .912 | .789  | .910  | .705 |

Values are means of six replicates (n = 6).

PKC: palm kernel cake; LPKC: Weisella confusa SR-17b fermented PKC; Low, 5% oil; High, 9.5% oil; TC: total cholesterol; TG: triglycerides; LDL-C: low-density lipoprotein cholesterol; HDL-C: high-density lipoprotein cholesterol; SEM: standard error of means.

*a* Means within a column with no common superscripts differ at *p* < .05.

The current study evaluated the effects of feeding fermented PKC with different levels of palm oil inclusion on a range of variables in broilers in the hot and humid tropical environment. The study is relevant and important because Malaysia is one of the world’s largest producers of PKC and palm oil (Foreign Agricultural Service 2020; Malaysian Palm Oil Board 2020). The higher fibre content of PKC has limited its use in poultry feed, particularly in the tropics, because a fibrous diet may aggravate heat stress problems in chickens. Solid-state fermentation appears to be a possible technology to improve the nutritional value of PKC (Saenphoom et al. 2011; Chen et al. 2013; Alshelmani et al. 2014; Navidshad et al. 2016; Tan 2016). Thus, LPKC, in combination with palm oil as a source of dietary fat, may enhance its utilisation as a poultry feedstuff, particularly in the tropics.

The inclusion of 20% untreated PKC or higher levels in broiler diets throughout the rearing period has been reported to negatively affect the BWG and feed efficiency compared to 0% PKC diet (Zulkifli et al. 2003; Soltan 2009; Mardhati et al. 2011). Feeding broilers with diets containing 10 or 15% PKC for 42 days may also have adverse effects on BWG and FCR (Alshelmani et al. 2017). Therefore, the experimental diets were only fed during the finisher period in the current study rather than throughout 35 days. Feeding of LPKC during the finisher phase did not affect the overall (day 1–35) BWG and FCR as compared to the PKC group (Table 4), despite significant improvement observed during the finisher phase (day 22–35). This could be pointed out to the short treatment duration as PKC and LPKC diets were fed for 14 days out of 35 days rearing period. A similar outcome was observed when less shell PKC, untreated PKC, and commercial corn-soy-based diets were fed during the finisher phase for 10 days (Jahari et al. 2020). The author postulated a prolong feeding period may result in greater differences in the growth performance. Regardless of different PKC types, the chickens’ overall BWG and FCR were significantly improved upon feeding of a higher oil supplemented diet (Table 4). To date, there is a paucity of information on the influence of dietary fat concentration in a higher fibre diet on the growth performance in broiler chickens. Works in PKC diets involved different levels of oil supplementation were documented widely, but with different PKC level inclusion (Saenphoom et al. 2013; Abdollahi et al. 2016; Alshelmani et al. 2017; Chen et al. 2019), thus a fair comparison between different oil concentration were not valid. Different concentration of oil per se in conventional corn-soy based diet has no direct effects on the growth of chicken, instead, the level of energy concentration in a diet is more of concern (Fuller and Rendon 1979; Sanz et al. 2000;
However, in the current study, the inclusion level of dietary fat appears to be important in determining the feed efficiency of broilers fed PKC- or LPKC-based diets. This could be attributed to the physicochemical characteristics of the oil and PKC as both ingredients may affect the passage time of the diet and viscosity of the digesta, subsequently, alter the efficiency of nutrient digestion and absorption (Mateos et al. 1982, 2012; Choc and Annison 1992; Seio et al. 2005; de Vries et al. 2012). Because dietary fat had lower heat increment than protein and carbohydrate (Austic 1985), in a hot and humid environment, the inclusion of high dietary fat may reduce the detrimental effects of fibrous material in PKC diets on thermal load in broiler chickens (Zulkifli et al. 2003). The current study shows supplementing diets with a higher level of oil improved the viability of broilers in the hot and humid tropical environment. These findings confirmed Zulkifli et al. (2003, 2006) and Ghazalah et al. (2008) that higher dietary fat was beneficial to heat-stressed chickens. Higher dietary fat may help reduce the heat production from digestion while increasing energy density in the diets to cope better with heat stress (Dale and Fuller 1979; Daghir 2009).

The primary focus of intensive poultry production is reducing the feed cost to optimise economic benefits (Hinrichs and Steinfeld 2007). Feed is the main factor determining the total production cost, and energy is one of the fundamental cost constituents of poultry feed. The present findings showed that the LPKC-HI and PKC-LO diets had the best and worst feed cost/kg of chicken produced, respectively. The noted improved weight gain and FCR in the birds fed diets with LPKC and 9.5% oil compared to those provided PKC and 5% oil, could justify the above findings. The significantly higher production cost of the birds fed PKC-LO diet could also be attributed to the higher inclusion of alternative energy sources (full-fat soybean meal) to replace the energy from palm oil and to compensate for the lower energy available in untreated PKC.

*Escherichia coli* is an adaptive bacterial species that may compromise both commensal and versatile pathogens that commonly cause enteric infections. In the present study, feeding of LPKC reduced the *E. coli* population in the caeca of chickens regardless of dietary fat level compared to the PKC (Table 6). Previously, exogenous enzyme-treated PKC with fewer shells content reduced caecal *E. coli* population in chicken, which the author postulated the effects could be attributed by the presence of mannan-oligosaccharides in reduced-NSP PKC (Navidshad et al. 2016). Degradation of PKC has been reported to increase the mannan oligosaccharides and monosaccharides concentrations via depolymerisation of mannan NSP of PKC (Chen et al. 2015; Roslan et al. 2017). Besides that, a fermented by-product in LPKC may provide a hostile environment for pathogenic microorganisms’ growth, thus reducing the caecal *E. coli* populations (Liang et al. 2012). The phenomenon could be associated with the high concentration of lactic acid and low pH in fermented feeds (Murry Jr. et al. 2004). This finding suggests that LPKC is more effective than PKC in lowering the growth of gut *E. coli*. On the other hand, the current study exhibited a higher level of dietary fat is required to reduce the caecal *E. coli* counts when PKC/LPKC were fed to the chickens (Table 5). The inclusion of fat can alter the intestinal viscosity and digesta passage rate, and thus it may affect the life cycle of bacteria (Mateos et al. 1982; Sharmila, Azhar, et al. 2014b) and reduce their population in the intestine, concomitantly.

In the current study, higher oil level supplemented diets showed lower LDL-C values than their lower oil level supplemented diets counterparts, while no differences were noted between PKC and LPKC diets (Table 7). On the contrary, feeding the birds with LPKC and higher oil level supplemented diets resulted in lower TG levels than the PKC and lower oil level supplemented diet fed birds, respectively (Table 6). These results suggest that the LPKC is more beneficial in lowering the overall blood lipid profile in chickens than the PKC. Similarly, broilers fed on fermented rapeseed meal had reduced serum concentration of triglycerides compared to those provided control and raw rapeseed meal diets (Ashayerizadeh et al. 2018). In another study, Sumarsih et al. (2010) observed reduced serum concentrations of triglycerides and when broilers were given LAB-fermented fish but no effects on total cholesterol and HDL-C levels. There are few possible mechanisms in manipulating lipid metabolisms, such as hindering the Acetyl CoA synthesis enzyme that is necessary for the biosynthesis of fatty acids (Puvača et al. 2015) and inhibition of hepatic cholesterol synthesis by LAB via short-chain fatty acid production such as propionate (Homayouni et al. 2012). Particularly in poultry, suppression of cholesterol biosynthesis via a reduction in hepatic 3-hydroxy-3-methylglutaryl-CoA reductase, cholesterol 7-α-hydroxylase, and fatty acid synthetase has been associated with lower cholesterol levels in White Leghorn pullet fed with garlic oil (Qureshi et al. 1983). The lower serum TG and LDL-C in higher oil level supplemented diets compared to their lower oil level
supplemented diets counterpart (Table 6) may suggest the benefits of higher dietary fat percentage in PKC/LPKC based diet. Such response could be associated with the physicochemical characteristics of fats, which may affect the intestinal viscosity and digesta passage time, reducing the lipid digestion process (Mateos et al. 1982, 2012). Elevated plasma TG concentration has also been linked to the consumption of high carbohydrates (particularly simple sugars and starches with high glycaemic index) and a low-fat diet, followed by an increase of LDL concentration (Siri and Krauss 2005). This may also suggest an interaction between PKC/FPKC and oil fibre in lowering the TG and LDL-C levels. This agrees with Jørgensen et al. (1996), where lower total tract retention of crude fat was observed in a high level of pea-fibre feeding to broiler chickens, which contains a higher NSP level than wheat and oat bran.

Conclusions

In conclusion, the results of the study showed that feeding LAB fermented PKC to broiler chickens reduced the caecal population of E. coli and serum levels of TG but had no effect on the final growth performance. Additionally, irrespective of type of PKC, diets with 9.5% palm oil had a beneficial impact on the final growth performance, caecal E. coli population and serum lipid profile. Thus, based on these findings, finisher diets with 20% LPKC and 9.5% palm oil are potentially suitable for broiler chickens in hot and humid tropical environments.

Acknowledgments

This work was financially supported by fund granted by the Ministry of Higher Education, Malaysia, under the Long Term Research Grant Scheme [Project number: UP M/700-1/3/LRGS] and Universiti Putra Malaysia.

Ethical Approval

The experimental protocol was approved by the Institutional Animal Care and Use Committee of Universiti Putra Malaysia.

Disclosure statement

We certify that there is no conflict of interest with any financial organisation regarding the material discussed in the manuscript. No potential conflict of interest was reported by the author(s).

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