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RESEARCH ARTICLE
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Valorization and Optimization of Protein in Fermented Palm Kernel Cake: Influence on Broiler Chicks Growth

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ABSTRACT

Palm kernel cake (PKC) is recognized for its potential as an animal feed ingredient, but its moderate protein content (14-19%) compared to soybean meal (48%) curbs its benefits. However, the PKC protein content can be enhanced through microbial fermentation. Thus, this study investigated the factors influencing the protein content in PKC fermented with *Rhizopus oryzae* ME01 (fPKC), using response surface methodology (RSM). This study also evaluated the effect of feeding fPKC on live performance of young broilers. Temperature, moisture content, and inoculum concentration were the optimized factors. This study revealed that the optimal fermentation conditions for maximum protein content in fPKC were 35ºC, 60% (v/w) moisture content, and 7.17mg/mL inoculum concentration. Under these conditions, protein content was 19.81%, which was very close to the predicted value (20.28%). Unfortunately, growth performance analysis showed low body weight gain (BWG) ($P \leq 0.05$) of broiler chicks fed with 30% fPKC and 30% PKC, respectively, compared to broiler chicks fed with the commercial diet. However, an analysis of the amino acids of the optimized fPKC showed that fermentation successfully increased branched-chain amino acids (BCAA) and lysine. These findings concluded that the optimized fPKC could be given to broiler chicks without detrimental health effects.

Keywords: Animal feed, Essential amino acid, Broiler chickens growth performance, Oil palm byproduct, Palm kernel cake, *Rhizopus oryzae*

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INTRODUCTION

The high price of livestock feed worldwide is a dire problem affecting the human food chain. Prices of imported raw materials, such as corn, soybean meal, and fish meal, have increased tremendously due to the increase in production costs. Moreover, a shortage of animal protein in the food chain is observed in developing countries, especially after the Covid-19 pandemic, as producers have to cull the farm animals due to a shortage of supply (Aday & Aday, 2020). Therefore, research interest has been stimulated in seeking an alternative feed source, particularly palm kernel cake (PKC), as substitute to the conventional animal feed. PKC is a by-product of palm kernel oil extraction process from the palm kernel crushing plant, which can be abundant from local palm oil mills. Malaysia produced 2.14 and 2.3 million tons of PKC in 2022 and 2023, respectively (Parveez et al., 2023). PKC offers about 14–18% crude protein, 12–20% crude fiber, 3–9% ether extract, and also various minerals that can partially substitute soybean meal and corn in poultry nutrition (Azizi et al., 2021). The fibrous material in PKC also consists of insoluble mannose-based polysaccharides, making it unfavorable for monogastric animals such as broilers due to nutrient digestibility issues (Saenphoom et al., 2013; Rohaya et al., 2021). In addition, Sharmila et al. (2014) and Olukomaiya et al. (2019) reported that PKC has an anti-nutritional factor (ANF), referring to high fiber content, which can affect animal growth.

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To solve these issues, solid-state fermentation (SSF) is deemed as an optimistic method to enhance the nutritive value of PKC with the use of microorganisms. SSF refers to biotechnological process, where the growth of microorganisms occurs in solid substrates without the presence of free water (Azizi et al., 2021). This method is capable of hydrolyzing and reducing cellulose, hemicellulose and lignin content in PKC as well as increasing its application in livestock feed (Mohd Firdaus et al., 2021; Devi et al., 2023). To further enhance the nutritive value of PKC, SSF stimulates the production of a product with low cellulose and hemicellulose content but high in protein content (Lee et al., 2019). In animal feed application, mainly broiler chickens, SSF is beneficial in improving growth performance and gut health of broiler chickens through enhanced nutrient bioavailability and improved gastrointestinal tract microecology, including lactic acid bacteria (LAB) and gut pathogenic bacteria inhibition (Alshelmani et al., 2016; Olukomaiya et al., 2019).

Fungi are good source of nutrients, especially digestible nitrogen, used by animals through appropriate metabolic and physiological adaptations, but amino acid content varies according to species (Wallis et al., 2012). Common fungi that have been exploited in the fermentation of PKC includes *Aspergillus niger, Trichoderma spp, Sclerotium rolfsii, Rhizopus stolonifera, Rhizopus oryzae* ME01 and *Rhizopus* sp. Strain PKC12B2 (Iluyemi et al., 2006; Suriani et al., 2008; Marini et al., 2008, Lateef et al., 2008; Abdeshahian et al., 2011; Lee et al., 2011; Mohd Firdaus et al., 2021; Riyadi et al., 2024). According to Alshelmani et al. (2016), *Paenibacillus polymyxa* ATCC 842 showed higher ability in degradation and improvement of the PKC through SSF. This is also supported by Mohd Firdaus et al. (2021) that *Rhizopus oryzae* ME01 assisted SSF of PKC in which the crude protein and ash content increased by 3.45 and 18.26%, respectively. The effects of fermented PKC (fPKC) on broiler chickens' growth have been delineated by several previous research. Alshelmani et al. (2016) incorporated 15% PKC fermented by *Paenibacillus polymyxa ATCC 842* in broiler rations and demonstrated no adverse effect on nutrient digestibility. In addition, Sundu et al. (2021) employed three different types of fungi, namely *Aspergillus niger, Trichoderma viride* and *Pleorotus ostreatus,* in their broiler diets. The study revealed that the weight gain of broiler chickens fed with 20% *Aspergillus niger*-fermented palm kernel meal (fPKM) was higher than that fed with 20% unfermented PKM. The gizzards of broiler chickens fed with 20% *Aspergillus niger*- and *Trichoderma viride*-fPKM were larger than that fed with control diet (0% PKM).

Nevertheless, factors influencing protein content enhancement in PKC for broiler chicks growth performance have not been widely explored. To serve the purpose, response surface methodology (RSM) could be applied. RSM is regarded as a useful mathematical and statistical technique for development, improvement and optimization of process variables which involves plans, conducts, analyses and interprets factors that affect parameters studied (Veza et al., 2023; Selim et al., 2024). It provides more factors to be examined with fewer experimental runs compared to conventional optimization which requires more time and laborious (Manojkumar et al., 2022; Abdullah et al., 2024). It determines the effect of independent variables and solves the multi-variable data obtained from a well-designed experiment specifically to find the answer to multi-variable equations (Chen et al., 2013; Morales-Borrell et al., 2021). RSM also helps in optimization through economic and improved process efficiency (Pereira et al., 2021). Thus, this study aimed to investigate the factors influencing the protein content in PKC fermented with *Rhizopus oryzae* ME01 using RSM. This study also ascertained the effect of feeding fPKC on live performance of young broiler chicks.

MATERIALS & METHODS

Microorganism and Fermentation of PKC

A locally isolated *R. oryzae*, strain ME01 was used in this study. SSF parameters and culture media were prepared according to Iluyemi et al. (2006) and Mohd Firdaus et al. (2021). SSF was carried out in 5000mL sterile containers by inoculating the seed culture with minimal medium containing 2000g sterile PKC supplemented with 1% ammonium sulphate (NH_4SO_4) as simple nitrogen source and 0.3% potassium dihydrogen phosphate (KH_2PO_4) on 1:1 (w/v) basis to get initial 50% (v/w) moisture level. KH_2PO_4 is needed for fungal buffering activity on the medium (Senthilkumar et al., 2005). Fermentation was performed for 10 days at ambient temperature with filtered aeration supply without agitation. SSF of PKC was repeated after the optimization until the desired quantity to be used as broiler chicks feed was obtained.

Optimization of Protein Content in fPKC

Optimization of factors associated with fungal growth was conducted using central composite design (CCD) via Design Expert software. Three factorial levels were selected to identify the best combination for fungal growth. Temperature, moisture content and inoculum concentration were three independent variables to be optimized. Response levels of the range and midpoint values of the three factors are summarized in Table 1. All experiments were randomized to minimize the effect of unanticipated variability. The state of the system is shown in the following Equation 1.

$$y = b_0 + b_1x_1 + b_2x_2 + b_3x_3 + b_{11}x_1^2 + b_{22}x_2^2 + b_{33}x_3^2 + b_{12}x_1x_2 + b_{13}x_1x_3 + b_{23}x_2x_3 \quad (1)$$

where, the growth factors were selected in response to Y (dependent constants*),* while *b0, bi, bii* and *bij* are the coefficients estimated by the model, and X_1, X_2 and X_3 are the response factors.

Amino Acid Analysis of the Optimized fPKC

Amino acid assay of the optimized fPKC was performed using Dedicated Amino Acid Analyzer L-8800 (Hitachi, Japan). One mg of sample was weighed and added to 1mL of 6N hydrochloric acid (HCl) in hydrolysis tube. The mixture was hydrolyzed at 110˚C for 22 hours before drying the mixture using rotary evaporator. One mL of 0.02N HCl was added to the sample and centrifuged for 30min to separate the supernatant and its residue. The supernatant was collected and then filtered with 0.45µm membrane filter before injection into the analyzer.

Table 1: Experimental range of three level factors in CCD

Code	Factor	Unit	Response level		
X_1	Temperature	℃	25	30	35
X_2	Moisture content	% (v/w)	40	50	60
X_3	Inoculum concentration	mg/mL	6.9	7.4	7.9

Effects of fPKC on Growth Performance of Broiler Chicks

Birds and Housing

In total, 90 broiler chicks of the Cobb breed were mixed from day 1 to day 7 in Climatic Controlled House (Speecht, Jerman). Chicks were given commercial feed in their diet, which did not contain PKC from day 1 to day 7, but starting from day 8, chicks were randomly assigned into different dietary groups with each group was triplicated. Ten broiler chicks were placed in a cage for each replication. Broiler chicks were fed according to the dietary group starting from day 8 until the completion of the trial.

Experimental Diets

Experimental feed diets were formulated based on the modified method of Carrión et al. (2011) and Purba et al. (2018), where 30% commercial feed was substituted with 30% PKC and 30% fPKC, respectively. This resulted in three dietary groups for broiler chicks, namely commercial feed diet as a control, commercial feed diet substituted with fPKC at ratio of 700g : 300g (w/w) as the first experimental diet, and commercial feed diet substituted with PKC at ratio of 700g : 300g (w/w) as the second experimental diet. Broiler chicks obtained feed and water in ad libitum throughout the experimental period, and antibiotics were not directly given in water or feed.

Determination of Growth Performance

Broiler's growth performance was determined according to Severin et al. (2024). Feed intake was recorded weekly and determined by the difference between the amount of feed served and leftovers at the end of the week. Broilers were weighed at the beginning of the experiment and a weekly basis while body weight gain was recorded every two days to avoid animal stress. Feed conversion ratio was obtained by dividing the amount of feed intake by the weight gain of the same week.

Chemical Composition Analysis

Proximate analysis was conducted to determine the chemical composition of the diet and broiler's digesta according to the Association of Official Analytical Chemists (AOAC, 2005) as described previously by Mohd Firdaus et al. (2021).

Data Analysis

Data for feed dietary, body growth performance and digestibility were analyzed using the General Linear Model (GLM) procedure in the Statistical Analysis Software (SAS) software (SAS Institute Inc., USA, NC). Significant differences were accepted if $P \leq 0.05$ and represented by different superscripts.

RESULTS & DISCUSSION

Optimization of Protein Content in fPKC

The experimental design of the three variables (temperature, moisture content and inoculum concentration) via Central Composite Design (CCD) is shown in Table 1. Twenty trials were conducted to ascertain the optimum conditions for maximum protein production via SSF by *R. oryzae* ME01 as shown in Table 2. Table 3 shows the analysis of variance (ANOVA) of the quadratic model of CCD. The model is significant or fit for all responses if the adjusted R^2 is high (Chen et al., 2013; Hossain et al., 2022). The calculated R^2 value of 0.8963 suggested the mathematical equation can explain 89.63% of the variations in the experiment and ensured the model was satisfactory the experimental trial as explained previously by Hossain et al. (2022). Merely 10.37% of the total variations could not be explained by the model. The value of the adjusted R^2 (Adj R^2) was high (0.8493), reflecting a fit model between the actual and predicted responses.

Table 2: Results of coded level factor for three variables via CCD and protein yield

Runs	X_1	X_2	X_3	Protein yield
1	-1	-1	-1	18.63
2	1	-1	-1	19.19
3	-1	1	-1	18.56
4	1	1	-1	18.60
5	-1	-1	1	18.62
6	1	-1	1	12.47
7	-1	1	1	13.32
8	1	1	1	18.13
9	0	-1.682	0	17.99
10	0	1.682	0	22.83
11	1.682	0	0	19.27
12	-1.682	0	0	18.54
13	0	0	1.682	17.82
14	0	0	-1.682	15.39
15	0	0	0	18.62
16	0	0	0	19.57
17	0	0	0	19.18
18	0	0	0	19.10
19	0	0	0	18.79
20	0	0	0	15.70

Abbreviation: X_1= Temperature, X_2= Moisture content; X_3= Inoculum concentration

Table 3: ANOVA of quadratic model of CCD

Source	Sum of square (SS)	Degree of freedom (DF)	Mean square	F-value	P value > F
Model	65.878	3	7.320	1.604	0.175
Residual	100.365	6	4.562		
Lack of fit	90.483	5	5.323	2.693	0.1387
Pure error	9.883	5	1.977		
Total	166.243	19			

R^2= 0.8963; Adj R^2= 0.8493, Adeq. Precision = 5.195

Lack of fit was evaluated to determine if the model is adequate. The insignificant lack of fit of this model ($P>0.05$) confirmed that the model was fit and could be used as a model. Lack of fit is insignificant when the pure error from the experiment is high compared to the residual error indicating fewer issues in the model (Veza et al., 2023). The adequate precision value is a ratio of signal to noise and it can be used for the design if all the responses show ratio greater than 4 (Hossain et al., 2022). The value of Adeq. precision of this model was 5.195 and this model could be used as a guide in surface area design.

The second-order polynomial equation for protein optimization was obtained through multiple regression data analysis. The quadratic model is shown by the following equation:

$$Protein = 18.835 + 0.0357X_1 + 0.574X_2 + 0.1206X_3 - 0.0138X_1^2 + 0.518X_2^2 - 0.8269X_3^2 + 0.055X_1X_2 - 0.2425X_1X_3 + 0.1275X_2X_3 \quad (2)$$

where, X_1, X_2 and X_3 represent the coded levels of temperature, moisture content and inoculum concentration, respectively.

Effect of Variables on Protein Yield

Response surface plot is important in understanding the effect of significant factors and interactions between variables, medium components and optimum levels of variables (Xu et al., 2008; Zhu et al., 2012; Abdullah et al., 2024). Fig. 1 shows the response surface plots of protein content in the fermented PKC with *R.oryzae* ME01 to examine the interaction of temperature, moisture content and inoculum concentration. Moisture content and inoculum concentration were proportional to the protein increment with increase in temperature. However, the increase in temperature after 7.4mg/mL inoculum concentration had no effect on the protein yield as inoculum concentration was the limiting factor. The optimum inoculum concentration for protein production was 7.4mg/mL, and subsequent increase in the inoculum concentration caused the protein content to decrease.

Validation of Model

It is crucial to compare the fitted model with real system to validate that the fitted model provides adequate approximation (Xu et al., 2008). Triplicate experiments were performed and the optimum protein yield was 19.81% at 35°C temperature, 60% (v/w) moisture content and 7.17mg/mL inoculum concentration, as shown in Table 4. Meanwhile, prediction of the model demonstrated maximum protein yield of 20.28%, which corroborated the validity and effectiveness of the model.

Table 4: Experimental value under optimized condition

Parameter	Optimum condition	Predicted value (%) (w/w)	Experimental value (%) (w/w)
Temperature (°C)	35 ± 2		
Moisture content (%)	60 ± 10	20.28	19.81
Inoculum concentration (mg/mL)	7.17 ± 0.5		

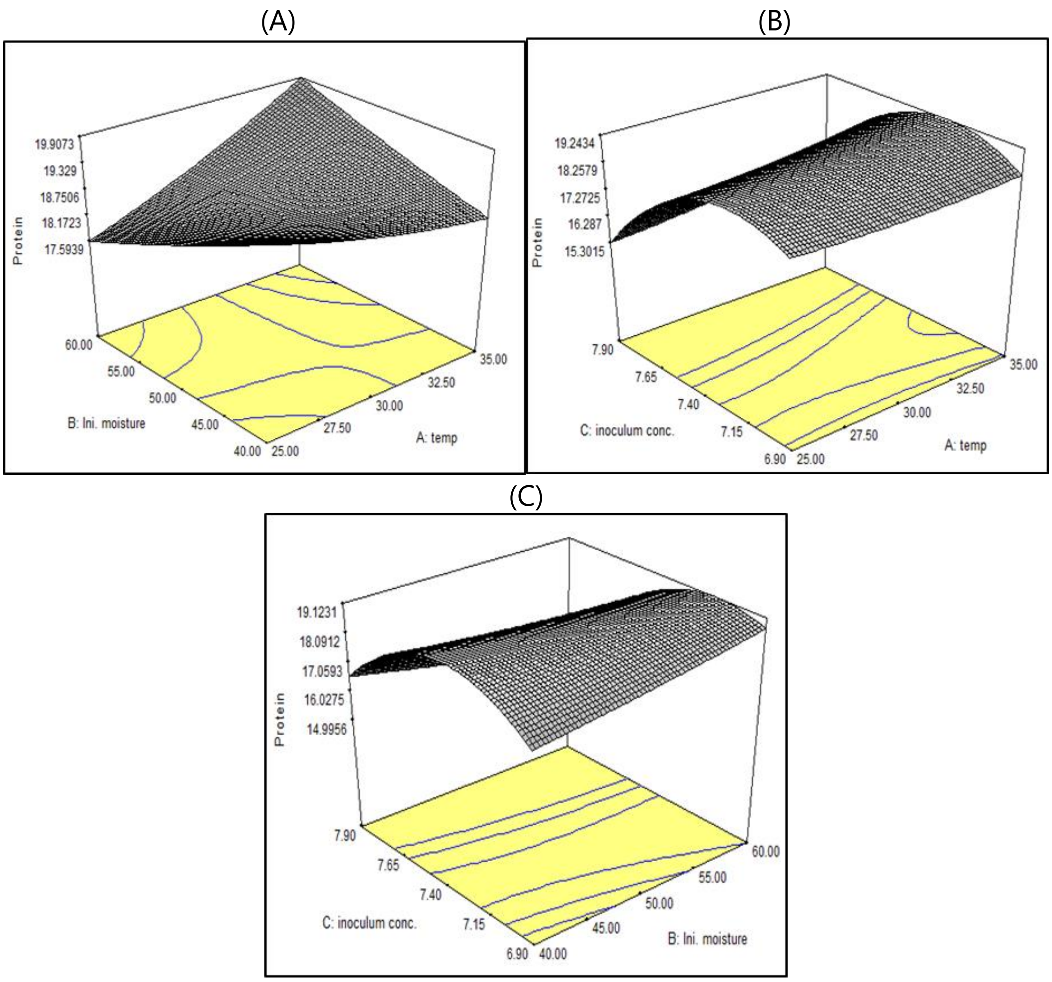

Fig. 1: Response surface plot (a, b and c) of protein content in the fermented PKC showing interaction effects of fermentation temperature, inoculum concentration and moisture content.

Aeration or oxygen supply to microbes is a significant parameter influencing the success of fermentation, according to Morales-Borrell et al. (2021), and it was supplied throughout the study. Moisture content is an important factor to determine product quality, texture and appearance (Nurilmala et al., 2023). The optimum temperature and moisture content obtained through this validation was in agreement with previous finding that *R.oryzae* gave high lactic acid yield at 35ºC, and moisture content between 50% and 65% relative humidity (RH) did not show any significant difference as the fungus prefers low humidity levels for its proper growth (Groff et al., 2024). The least increase in crude protein content was recorded at 30% (v/w) moisture content for SSF of probiotics-treated composite flour (Koyum et al., 2023). The protein yield production was directly proportional to the increase in inoculum concentration from 6.9 to 7.17mg/mL. A similar pattern of result was demonstrated by Hussin et al. (2023) who reported that any increase in the inoculum percentage used would increase the production of culture metabolites.

Amino Acid Analysis of the Optimized fPKC

Amino acid composition of food material is an essential indicator of the quality of raw materials. Amino acids are the important building blocks of life found either as free monomers or combined into peptides to form complex molecules, and there are 20 common amino acids encoded by human genetics, which can be obtained from food whether as essential or non-essentials amino acid (Yates et al., 2023). The quality of protein is shown by the essential amino acid content of total amino acid (Galla et al., 2012). According to Djenontin et al. (2012) and Cabanos et al. (2021), soy bran is the main source of protein as it has the highest protein content (35-40%) among other legumes.

There were 17 amino acids found in the optimized fPKC and PKC as shown in Table 5. Glutamic acid, arginine and aspartic acid from the non-essentials amino acid group were rich in both products but deficient in cysteine. This finding is in agreement with Kok et al. (2011) and Manaf and Dian (2021) except that lysine was the least amino acid in their studies. Lysine is the most limiting amino acid in sesame meal and flaxseed meal (Almeida Sá et al., 2021). Moreover, the digestibility of lysine is poor due to the Mailard reaction through overheating in the drying process of palm kernel (Stein et al., 2015). Interestingly, fermentation of PKC in this study successfully increased the lysine content by twofold. Valine, isoluecine and leucine, which are branched chain amino acids (BCAAs), were improved through this fermentation. BCAAs are essentials components for various biogenesis signaling pathways or human nutrient modulators (Dullius et al., 2020). According to Cabanos et al. (2021), fermentation is an efficient and cheaper method to produce bioactive peptides, but it may not completely hydrolyze complex protein molecules.

Total amino acid content of fPKC was 18.13% and higher than total amino acid of PKC (16.23%). This finding showed that the total amino acid of fPKC increased by 6.3% compared to PKC as a result of fermentation by *Rhizopus oryzae* ME01. However, the decrease in cysteine, tyrosine and phenylalanine was observed as they were utilized by *R.oryzae* for its own metabolism. Gao et al. (2013) reported that changes in amino acid composition of soybean bran fermented with *Aspergillus niger* were due to synthesis and hydrolysis by the respective microorganisms. The observed increase in amino acid composition is consonance with previous works that SSF by *A.niger and R.oryzae* have improved protein contents of PKC and plantain (*Musa paradisiacea*), respectively, due to microbial biomass, secretion of various proteins and extracellular enzymes released from the fermentation (Marini et al., 2008; Anigboro et al., 2022; Riyadi et al., 2024).

Table 5: Amino acid composition of PKC and fermented PKC (fPKC)

Amino Acids	Amino acid (%)	
	PKC	fPKC
Essential Amino Acids (EAA)		
Threonine	0.62±0.01	0.61±0.04
Cysteine	0.19±0.01	0.14±0.07
Valine	0.88±0.01	0.93±0.02
Methionine	0.34±0.02	0.28±0.07
Isoleucine	0.55±0.00	0.66±0.01
Leucine	1.19±0.02	1.23±0.01
Tyrosine	0.52±0.00	0.38±0.02
Phenylalanine	0.81±0.01	0.74±0.01
Lysine	0.45±0.02	0.90±0.06
Histidine	0.33±0.00	0.32±0.01
Non-Essential Amino Acids (EAA)		
Aspartic Acid	1.49±0.03	1.75±0.06
Serine	0.77±0.01	1.10±0.07
Glutamic acid	3.86±0.09	4.08±0.49
Glycine	0.84±0.01	0.89±0.01
Alanine	0.77±0.01	0.87±0.04
Arginine	2.16±0.01	2.59±0.43
Proline	0.51±0.06	0.65±0.05
Total AA	16.23±0.11	18.13±0.18

Note: Results were the average of three replicates (mean±SD).

Effect of fPKC Diet on Growth Performance of Broiler Chicks

Chemical Analysis of Feed

Table 6 shows the proximate compositions of the control, fPKC and PKC diets. The amount of crude protein, ash and ether extract for all treatments were in compliance with the calculated values and was balanced. The addition of PKC and fPKC in the starter diet increased the crude protein and ether extract in the fPKC and PKC diet, respectively. The increase in crude protein and ether extract is in coherence with the work of Chukwukaelo et al. (2018) who reported the same trend after replacing maize with 28% PKC. The addition of PKC in the diet led to higher hemicellulose and cellulose content in the PKC-based diet compared to the control diet in this study. This was due to the fact that more than half of the PKC composition is carbohydrate, and it can be broken down into non-starch polysaccharides, such as cellulose (24%), hemicellulose (24.6%), ADF (35.6%), NDF (60.2%) and lignin (7.7%) apart from anti-nutritional factor, such as tannin (3500ppm), oxalate (13.4ppm) and phytate (109.5ppm) (Mohd Firdaus et al., 2021; Koranteng et al., 2022). Crude fiber is a fraction of carbohydrate which contains cellulose, lignin and hemicellulose (McDonald et al., 2011).

Table 6: Chemical compositions of broiler chicks' diet

Chemical analysis	Dietary Treatments		
	Control	fPKC	PKC
Crude protein	19.454^{a}	20.397^{a}	19.857^{a}
Crude fiber	13.58^{a}	16.37^{b}	14.52^{ab}
Ether extract	3.635^{b}	4.153^{ab}	4.496^{a}
Ash	5.466^{a}	5.7026^{a}	5.011^{b}
NDF	7.421^{c}	27.532^{b}	32.581^{a}
ADF	4.066^{b}	14.624^{a}	14.403^{a}
ADL	4.046^{b}	10.539^{a}	10.154^{a}
Hemicellulose	3.219^{b}	10.321^{a}	16.144^{a}
Cellulose	0.342^{b}	2.808^{ab}	4.506^{a}

^{a,b,c}Mean for each superscript was significantly different (P≤0.05) in row

Crude fiber content in fPKC diet was higher than the control (P≤0.05) as the commercial diet itself had high crude fiber content (13.58%). From the literature, the crude fiber for the control diet was 14.77% as it composed of various fibers from maize, soybean meal, wheat offal, fish meal and bone meal (Chukwukaelo et al., 2018). Ingredients used also have certain quantity of anti-nutritional factors which are not easily degraded by monogastric animals (Zamani et al., 2017). Walugembe et al. (2014) contended that the inclusion of high fiber ingredients of 60-120g/kg or up to 12% in the broiler diets did not affect the growth performance. The inclusion of 30% PKC and 30% fPKC in the basal diet resulted in 14.52% and 16.37% of crude fiber in the feed, respectively. This finding is in line with that reported by Yusrizal et al. (2013) that the inclusion of up to 30% PKC in the corn-soybean meal (basal) diet resulted in an increase in dietary fiber from 7.95 (0% PKC) to 15.43% (30% PKC).

Effect of fPKC Diet on Growth Performance of Broiler Chicks

Fig. 2 shows the growth of broiler chicks fed with distinctive dietary treatments. Broiler chicks fed with 30% PKC and 30% fPKC showed no significant difference in terms of growth performance during starter phase. This study is in agreement with study conducted by Noraini et al. (2009). In another study, feeding 5 to 15% PKC to Ross 308 type of broilers exhibited no effect on broiler performance in the starter phase (Yaophakdee et al., 2018).

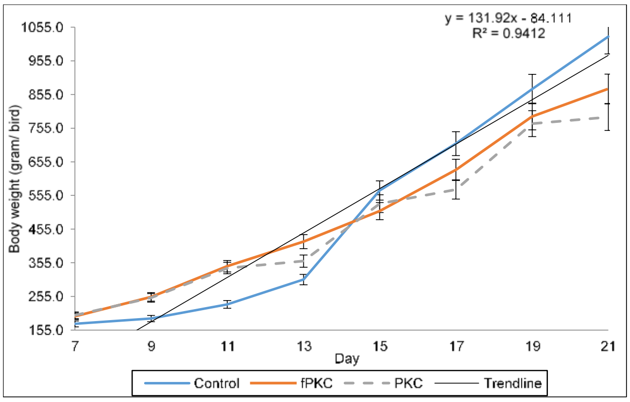

Fig. 2: Growth of broiler chicks subjected to different dietary treatments over time.

Broiler chicks consuming fPKC and PKC indicated higher body weight (P≤0.05) than the control from day 7 to day 13. However, broiler chicks given with control diet began to show vivid growth from day 14 and reached highest final body weight (FBW) of 1027g compared to chicks fed with fPKC (871g) and PKC (831g). Body weight gain (BWG) of the control scored the highest (854g) and significantly different (P≤0.05) from diet with fPKC (672g) and PKC (632g) on day 21 as shown in Table 7.

Table 7: Growth performance of broiler chicks during age of 1-21 days

Parameters	Diets		
Body weight gain, BWG (g/bird)	853.47^{ab}	671.6^{ab}	631.8^{ba}
Daily body weight gain, DBWG (g/bird/d)	60.96^{ab}	47.97^{ab}	45.13^{ba}
Feed intake, FI (g/bird)	659.47^{b}	860^{a}	812^{a}
Average daily feed intake, ADFI (g/bird/d)	47.1^{a}	61.42^{a}	58^{a}
Feed conversion ratio, FCR	1.29^{a}	0.78^{b}	0.78^{b}
Mortality (%)	0	0	0

^{a,b,c}Mean for each superscript was significantly different (P≤0.05) in row

Table 7 also shows feed intake (FI) of broiler chicks on the fPKC diet was the highest among all diets and had no difference with PKC diet (P>0.05), while FI of the control diet was the lowest (P≤0.05). The FI of broiler chicks given the fPKC diet increased by 200.53g/bird compared to FI of the control (659.47g/bird). This finding is in agreement with previous studies that incorporation 20% to 30% PKC, fPKC or enzymatic treated PKC did not increase broiler weight except for increased FI compared to commercial diet (Chukwukaelo et al., 2018; Purba et al., 2018; Ramiah et al., 2019; Hakim et al., 2021). However, the inclusion of endo-β enzyme at 0.06% in PKC diet has obtained better broiler growth than the diet with PKC alone (Purba et al., 2018). Broiler chickens fed with PKC and fPKC needed to consume more feed to convert to live weight ratio compared to the control due to palatability problem (Ekalle et al., 2015; Obirikorang et al., 2015). Feed conversion ratio (FCR) of each treatment was low and differed significantly (P≤0.05) from the control diet. No mortality was recorded in all treatments throughout this study. From cellular and enzymological viewpoint, it was postulated that the small intestinal epithelium is not fully matured during the first 14 DOA of broiler chicks (Yaophakdee et al., 2018).

The utilization of up to 25% PKC not only improved Ca, Mg and potassium in the diet, and more important, it has no negative effect in gene expression for growth and metabolism as the expression of genes involved in mTOR (GRB2, GRB10), FoxO (FOXO3) and insulin (PRKCZ) signalling pathways, glycolysis/gluconeogenesis (ENO1), fructose and mannose metabolism and apoptosis (RHOBTB2, LOC101750363) were significantly up-regulated in broilers fed with untreated PKC (12% shell), low shell PKC (4% shell) or enzymatic treated PKC (Okon & Ayuk 2014; Ramiah et al., 2019). Therefore, the utilization of 30% fPKC or PKC in broiler chicks in this study only affected the growth performance than the diet without PKC. It is relevant for economic reason to reduce the cost of imported materials such as soy bean meal. Recent works have demonstrated that the application of PKC or treated PKC could improve or maintain broilers gut microflora, such as *Lactobacillus spp.* and *Clostrodium spp.*, from 10 to 15% addition in the diet (Alshelmani et al., 2021; Hakim et al., 2021; Koranteng et al., 2023). It was also suggested to feed 5 to 15% PKC to broilers in the starter phase for 21 days (Yaophakdee et al., 2018). Thus, PKC or treated PKC is recommended as prebiotic to promote gut-beneficial growth in chicken.

Conclusion

The PKC fermented with locally isolated *R.oryzae* ME01 was optimized via RSM before being tested on broiler chicks in the starter phase. Protein content of fPKC was 19.81% and close to the predicted value of 20.28% under optimum conditions of 35°C temperature, 60% (v/w) moisture content and 7.17mg/mL inoculum concentration. The fPKC diet was given to young broiler chicks and showed lower BWG and higher FI (P≤0.05) compared to the diet without PKC. However, this study found that BCAAs and lysine were improved through the fermentation of PKC with *R.oryzae* ME01.

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