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Research Article

Utilization of Fermented Palm Kernel Meal with a Combination of *Bacillus subtilis* and *Lactobacillus fermentum* In Broiler Rations

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Abstract | This study aimed to evaluate the effect of a *Bacillus subtilis* and *Lactobacillus fermentum* mixture (6:4 ratio) in fermented palm kernel meal (FPKM) on the performance of broiler chickens. One hundred day-old broiler chick of the *Lohmann* strain MB-202 Platinum, were used in this study. The cages used were 20 units of box cages, each measuring 80 x 80 x 60 cm filled with 5 chickens, equipped with places to eat and drink. This study used a complete randomized design (CRD) experimental method with 4 treatments and 5 replications consisting of R1 (control), R2 (25% FPKM with *B. subtilis* and *L. fermentum* (6:4)), R3 (30% FPKM with *B. subtilis* and *L. fermentum* (6:4)) and R4 (35% FPKM with *B. subtilis* and *L. fermentum* (6:4)). Parameters measured were feed consumption, body weight gain (BWG), feed conversion, body weight, carcass percentage and abdominal fat percentage. The results of the analysis showed that the treatment ration R1, R2, R3 and R4 had a highly significant (P<0. 01) effect on feed intake, BWG, body weight and had no significant effect (P>0.05) on feed conversion, carcass percentage and abdominal fat percentage. In conclusion fermented palm kernel cake with combination of *B. subtilis* and *L. fermentum* can be used up to 30% in broiler ration. It can be seen from the feed intake of 815.27 g/chick/week; BWG 470.45 g/chick/week; feed conversion 1.75; body weight 1895.20 g; carcass percentage 76.48% and abdominal fat percentage 1.03%, cholesterol of chicken thigh meat 263.54%, nitrogen retention 59.39%, crude fiber digestibility 56.61% and energy metabolism 2283.95% kcal/kg.

Keywords | *B. Subtilis*, Broiler, Fermentation, Growth performance, *L. fermentum*, PALM kernel meal

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INTRODUCTION

Palm oil is one of the major plantation commodities that significantly contributes to Indonesia's economic activities, particularly due to its role in producing vegetable oil demanded by various sectors. Indonesia is currently the world's largest producer of palm oil. According to the Directorate General of Plantation (2021), Indonesia's crude palm oil (CPO) production rose from 42.9 million tons in 2018 to 49.7 million tons in 2021, with oil palm plantations spanning approximately 15 million hectares. Along with the increase in CPO production, the volume of by-products such as palm kernel meal (PKM) has also grown. PKM, in particular, shows promise as an alternative feed ingredient in poultry production due to its nutritional content and availability.

Palm kernel meal contains notable levels of nutrients, such as crude protein (17.31%), crude fiber (27.62%), ether extract (7.14%), calcium (0.27%), phosphorus (0.94%), and copper (48.4 ppm) (Mirnawati *et al*., 2018). However, its use in poultry diets is limited to approximately 10% due to the high content of crude fiber, particularly β-mannan (Azman *et al*., 2016), which cannot be efficiently digested by poultry because they lack endogenous fiber-degrading enzymes. One approach to enhance the nutritional value of PKM is through microbial fermentation using cellulolytic and mannanolytic bacteria, which can reduce fiber content and improve digestibility (Sari *et al*., 2021).

Fermentation is a biological process that breaks down complex macromolecules such as carbohydrates, proteins, and fats into simpler forms through enzymatic activity of microbes. This transformation not only improves digestibility but also enhances the nutritional quality and palatability of feed ingredients (Azizi *et al*., 2021). Previous studies have demonstrated that fermentation of PKM using Bacillus subtilis improves nutrient profiles, enzyme activity, and broiler performance. For instance, Mirnawati *et al*. (2019a, 2019b) reported increased crude protein (24.65%), nitrogen retention (68.47%), and enzymatic activities of mannanase (24.27 U/mL) and cellulase (17.13 U/mL). In vivo studies showed that up to 25% inclusion of PKM fermented with B. subtilis in broiler rations yielded positive effects (Mirnawati *et al*., 2020).

Recent advances have also identified Lactobacillus fermentum as a promising cellulolytic and mannanolytic lactic acid bacterium. Isolated from acidified PKM, this bacterium demonstrated enzyme activities comparable to B. subtilis, with added benefits of probiotic functionality that supports gut health and improves nutrient absorption in poultry (Mirnawati *et al*., 2022; Karlyshev *et al*., 2015). The fermentation of PKM with L. fermentum yielded improvements in nutrient content, particularly crude protein (25.81%) and a reduction in crude fiber to 16.90% (Mirnawati *et al*., 2023).

Several studies indicate that combining B. subtilis and L. fermentum in a fermentation consortium can result in synergistic effects. Safitri *et al*. (2021) reported improved amino acid concentrations and nutrient profiles when both bacteria were used together. Devi *et al*. (2023) further demonstrated that fermentation of PKM with a 6:4 ratio of B. subtilis to L. fermentum produced optimal enzymatic activities and nutritional outcomes, including higher protein content and lower crude fiber.

Given the complementary properties of *B. subtilis* and *L. fermentum*, it is hypothesized that their combined use in a 6:4 ratio could enhance the nutritional value of PKM more effectively than individual strains. However, the biological response of broilers to diets containing different inclusion levels of this fermented PKM (FPKM) has not been thoroughly evaluated. Therefore, this study aims to determine the optimal inclusion level of FPKM fermented with a combination of *B. subtilis* and *L. fermentum* (6:4) in broiler diets and to evaluate its impact on growth performance, feed efficiency, nutrient digestibility, and economic returns. The findings are expected to support the development of cost-effective, high-nutrient poultry feeds based on agro-industrial by-products and probiotic biotechnology.

MATERIALS AND METHODS

Ethical Approval

The present study was approved by the Animal Ethics Committee of Andalas University, West Sumatera, Indonesia. The study was conducted from August 2022-January 2023 in field laboratory at Animal Science Faculty, University of Andalas.

Making Inoculum

Inoculum preparation was carried out using 100 g of bran substrate. Then, 70 ml of distilled water was added to each treatment and sterilized in an autoclave at 121ºC and 1 atm pressure for 30 minutes, followed by cooling to room temperature (31ºC). The bacterial uspension was then diluted in 7 ml of a modified mineral-nutrient solution, as described by Brook *et al*., which is intended to support bacterial activation prior to inoculation. The composition of this solution per liter is as follows: $MgSO_4$ (0.25 g), $FeSO_4$ (0.10 mg), $ZnSO_4$ (0.10 mg), thiamin hydrochloride (12.50 mg), $MnSO_4$ (0.10 mg), H_2PO_4 (1.00 g), urea (1.5 g), and distilled water up to 1 liter. The bacterial inoculum was standardized to 5.4×10^8 CFU/ml before inoculation. After inoculation, the samples were incubated for 4 days at 31ºC.

Making Fermented Palm Kernel Meal

Palm kernel meal (PKM) and bran are used as substrates in a ratio of 80%: 20%. The bran and PKM were mixed then sterilized using an autoclave at 121ºC, 1 atm pressure for 30 minutes. Then colded down to room temperature. It was then inoculated with 10% *B. subtilis* inoculum (Mirnawati *et al*., 2019b) and *L. fermentum* with a ratio (6:4) and incubated for 3 days. This ratio was chosen based on preliminary studies indicating optimal enzyme activity and fermentation efficiency compared to other ratios such as (4:6) (Devi *et al*., 2023). The *Lactobacillus fermentum* strain was isolated from decomposed palm kernel cake and identified using the 16S rRNA molecular method. Meanwhile, the *Bacillus subtilis* strain was obtained commercially from the Indonesian Institute of Sciences (LIPI). Then the bacteria were killed by placing them in an oven (80ºC for 2 hours) until the sample weight remained constant so that the activity of the bacteria could stop during the fermentation process.

Research Materials

A total of 100, day-old chicks (1 day old) of the *Lohmann* MB-202 Platinum strain were obtained from PT Japfa Comfeed Indonesia, without separating males and females (unsex). As many as 20 box cage units are used in the experiment, assigned to 4 dietary treatments with 5 replicates each. Each replicate consisted of one cage containing 5 chickens The experimental units were cages measuring 80 cm × 80 cm × 60 cm. Each cage was equipped with a feeder, a drinker, and a 60-watt incandescent lamp, which served as both a heat source and lighting. Additional equipment used during the study included digital scales (for weighing chickens and feed), buckets, plastic sheets (for covering or handling materials), brooms (for cleaning), excreta containers, and ration waste containers.

Research Design

This study employed an experimental design consisting of four dietary treatments with five replicates each. The treatments were as follows:

R1 (Control): Standard ration without fermented palm kernel meal (FPKM)
R2: Ration containing 25% FPKM fermented with B. subtilis and L. fermentum (6:4 ratio)
R3: Ration containing 30% FPKM fermented with B. subtilis and L. fermentum (6:4 ratio)
R4: Ration containing 35% FPKM fermented with B. subtilis and L. fermentum (6:4 ratio)

The rations were formulated to contain 22% crude protein and 3000 kcal/kg metabolizable energy. The percentage composition of feed ingredients for each treatment is presented in Table 1, while Table 2 shows the detailed nutrient composition and metabolizable energy values.

Table 1: Composition of feed ingredients (%) of treatment ration.

Feed Ingredients	Treatment Rations			
	R1	R2	R3	R4
Yellow Corn	51	40	37.8	35.15
Soybean meal	25.75	11.65	8.85	6.35
FPKM	0	25	30	35
Fish flour	20	20	20	20
Coconut oil	2.75	2.85	2.85	3
Vitmin-mineral Mix	0.5	0.5	0.5	0.5
TOTAL	100	100	100	100

FPKM: fermented palm kernel meal.

Table 2: Food substance content (%) and metabolizable energy (kcal/kg) treatment rations.

Food Substance Content				
	R1	R2	R3	R4
Crude protein	22.07	22.01	22.00	22.08
Crude Fat	6.60	5.93	5.77	5.79
Crude Fiber	1.55	5,31	6.06	6.80
Calcium	0.98	1.05	1.06	1.08
Phosphor	0.01	0.55	0.55	0.55
Metabolizable Energy	3004	3001.92	3000.17	3003.19

Parameters Measured

Parameters measured were feed intake (FI) g/chick/week, body weight gain (BWG), ration conversion (FCR), body weight (BW), percentage of carcass (PC), crude fiber digestibility (CFD), abdominal fat percentage (AFP), nitrogen retention (NR), metabolizable energy (ME) and chicken thigh cholesterol.

Feed intake (FI): Feed consumption was calculated over a period of one week. The weekly feed consumption was obtained by subtracting the leftover feed from the total ration given during the week (Marcu *et al*., 2013). The average consumption was then calculated each week by summing it with the previous weeks. Feed consumption was calculated using the formula:

$$Feed\ consumption\ (g/bird) = \frac{(feed\ given - left\ over\ (g))}{total\ number\ of\ chickens}$$

Body weight gain (BWG): Weight gain was measured by weighing the chickens weekly before they are fed throughout the study (Marcu *et al*., 2013). At the time of harvest, the average weight gain was calculated using the formula:

$$WG = Wtt - Wt{-}1$$

Explanation;
WG = Weight Gain
Wt = Body weight at time tt
Wt−1 = Body weight at previous time
t = In poultry farming, usually one week

Feed conversion ratio (FCR):
The feed conversion ratio (FCR) was calculated by dividing the average feed intake by the average body weight gain:

$$FCR = \frac{XFC}{XWG}$$

Explanation;
FCR = Feed conversion ratio

FC = Average feed consumption (g/bird)
WG = Average weight gain

Body weight (BW): Live weight was measured by weighing each broiler chicken based on treatment and replication, following a 12-hour fasting period prior to weighing (Zhang *et al.*, 2023).

Percentage of Carcass (PC): Carcass percentage was measured by comparing the weight of the chicken without feathers, blood, head, neck, feet, and internal organs (g), except for the heart, liver, and gizzard, to the live weight (g), and then multiplying by 100%.

$$Carcass\ Percentage\ (\%) = \frac{Carcass\ Percentage\ (\%)}{Body\ Weight}$$

Abdominal fat percentage (AFP): The percentage of abdominal fat was determined by weighing the fat obtained from around the gizzard and the layer adhering between the abdominal muscles and intestines, then weighing it (Salam *et al.*, 2013). Sembiring (2001) states that the quality of broiler carcasses was determined by the amount of abdominal fat present in the broilers. Moreover, the amount of fat content will affect meat shrinkage. A good carcass should contain a lot of meat, be of good edible quality, and have a low fat content. The percentage of abdominal fat (g) was obtained by comparing the weight of abdominal fat with the carcass weight (g) multiplied by 100%.

$$Abdominal\ Fat\ Percentage\ (\%) = \frac{Abdominal\ Fat\ Weight\ (g) \times 100\%}{Body\ Weight}$$

Statistical Analysis

All data obtained in this study were analyzed using analysis of variance (ANOVA) to determine the significant effect of the treatments (Steel and Torie, 2002). If there was a significant difference from the treatment, it was followed by Duncan's Multiple Range Test (DMRT).

RESULT AND DISCUSSION

Average FI, BWG and FCR, BW, PC, AFP, CFD, NR and cholesterol in broiler thigh meat during the study can be seen in Table 3.

Feed Intake (FI)

Based on Table 3, the addition of up to 35% FPKM fermented with *B. subtilis* and *L. fermentum* (6:4) in the diet had a highly significant effect (P<0.01) on broiler feed intake. However, feed intake in treatments R2 and R3 (25% and 30% FPKM) was not significantly different from the control (R1). This can be attributed to the improved digestibility and palatability of fermented products, which undergo physical changes in flavor, texture, and color. According to Mirnawati *et al.* (2022) and Ciptaan *et al.* (2022), fermentation enhances nutritional quality and improves taste, aroma, and texture compared to the unfermented form. Thus, the inclusion of FPKM can improve feed digestibility in broilers. In treatments R1, R2, and R3, feed intake remained relatively similar, allowing for consistent nutrient utilization. However, in R4, feed intake was significantly reduced due to the higher crude fiber content (6.8%), which exceeds the optimal tolerance level for broilers and negatively impacts feed palatability and digestibility.

Table 3: Performance of broilers fed varying levels of fermented palm kernel meal (FPKM) supplemented with a *Bacillus subtilis* and *Lactobacillus fermentum* mixture at a 6:4 ratio in the diet.

Parameter	R1 (0% FPKM)	R2 (25% FPKM)	R3 (30% FPKM)	R4 (35% FPKM)	S.E
Feed Consumption (g/head)	842.60[a]	819.46[a]	816.27[a]	705.53[b]	27.59
Body Weight Gain (g/head)	479.92[a]	465.79[a]	470.45[a]	400.88[b]	15.22
Feed Conversion	1.76	1.77	1.75	1.77	0.073
Body Weight (g/head)	1977.00[a]	1899.00[a]	1895.20[a]	1704.40[b]	55.44
Carcass Percentage (%)	79.7	78.55	76.48	73.26	1.25
Abdominal Fat Percentage (%)ns	1.01	1.00	1.03	1.02	0.05
Chicken Thigh Cholesterol (%)	384.74[a]	263.54[b]	151.36[c]	131.14[c]	8.11
Nitrogen Retention (%)	61.68[a]	59.92[a]	59.39[a]	52.20[b]	1.11
Crude Fiber Digestibility (%)	58.85[a]	57.68[a]	56.61[a]	48.96[b]	2.03
Metabolizable Energy (kcal/kg)	2365.17[a]	2304.41[a]	2283.95[a]	2052.16[b]	27.48

Note: Different superscripts in a row show highly significantly different effects (P<0.01). SE = Standard Error.

The low of FI in treatment R4 was due to the treatment using rations containing up to 35% FPKM. This is due to the high CF content in the ration, namely 6.8%, where CF is voluminous, so that chickens get full quickly so the feed is eaten in small quantities. This is in accordance to Indonesian national standards NSI (2006), that the maximum limit for giving crude fiber to broilers is 6%. The difference in results between treatments R3 and R4, despite their similar feed compositions, can be attributed to the crude fiber content. In R3, the crude fiber level did not exceed the maximum tolerance limit of 6% for broilers. This level of crude fiber is still within the broilers' capacity to tolerate and digest effectively, thus maintaining good growth performance. In contrast, R4 had a crude fiber content exceeding this threshold, which may have negatively affected nutrient digestibility and feed utilization. High crude fiber levels can reduce feed efficiency by limiting nutrient absorption and increasing gut fill, leading to lower weight gain compared to R3. Therefore, even with similar overall feed compositions, differences in crude fiber concentration explain the variation in performance between these two treatments. Broilers cannot digest high crude fiber which will cause a decrease of feed efficiency (Tejeda and Kim, 2021). High CF causes the rate of digestion to be faster, so that the digestibility value of other nutrients will alleviate, which comes out with the excreta and results in an lessen in ration consumption.

In this study, the average weekly consumption of broiler rations was 815.27 g/chick. This outcome is greater than that of earlier studies that employed FPKM in conjunction with *B. subtilis*, yielding an average feed consumption of 527 g/chick/week (Mirnawati *et al*., 2020).

Body Weight Gain (BWG)

Table 3 demonstrates that adding of *B. subtilis* and *L. fermentum* (6:4) to 35% FPKM has a highly significant (P<0.01) impact on broiler BWG. Because the treatment included fermented items, where the fermented material had better quality and nutritional content and could improve digestibility thereby increasing BWG. According to Mirnawati *et al*. (2019a) and Mirnawati *et al*. (2020), fermented products have easier-to-digest components, which enhances the nutritional value of a food. Added by Abeddargahi *et al*. (2022) and Ibrahim *et al*. (2021) that fermentation can increase digestibility, so that leading in improvement of body weight.

The average broiler BWG obtained in this study was 470.45 g/head/week. These results were higher than in previous studies using rations containing fermented palm kernel meal with *B.subtilis* without adding humic acid in drinking water which resulted in an average BWG of 280 g/chick/week (Mirnawati *et al*., 2020).

The significant difference in broiler BWG in treatments R1, R2 and R3 was also due to the administration of FPKM with a combination of *B. subtilis* and *L. fermentum* in this treatment which resulted in high nitrogen retention. The higher the nitrogen retention, the higher the resulting BWG. Study from Yu *et al*. (2024) and Mirnawati *et al*. (2023) stated that the amount of nitrogen retained in the animal's body and BWG are correlated so NR can be a useful tool for estimating growth.

The low raise in body weight in the R4 treatment was due to consumption factors which greatly influenced BWG because the less ration consumed, the lower the resulting body weight. Marchesi *et al*. (2021) stated that an increase in body weight is related to feed intake, the higher the feed intake, the higher BW, conversely, the lower FI lead to the lower BW.

Feed Conversion Ratio (FCR)

Based on Table 3, it shows that the use of FPKM with *B. subtilis* and *L. fermentum* (6: 4) up to a level of 35% in the ration had no significant effect (P>0.05) on the conversion of broiler rations. The insignificant effect on FCR in treatments R1, R2, R3 and R4 is also due to the comparison of the amount of ration consumed and the resulting BWG being balanced between treatments, where high consumption is also followed by high BWG and vice versa low consumption is also followed low BWG too. Meanwhile conversion is a comparison between consumption and BWG, therefore ration conversion is the same between treatments. Liu *et al*. (2021) stated that feed conversion is the ratio between ration consumption and BWG gained over a certain period of time.

In contrast, a lower ration conversion value indicates a smaller ration required to increase body weight per unit weight. A low FCR indicates the efficient use of rations. This is backed up by the viewpoint of Ciptaan *et al*. (2021) who stated that a low FCR value exhibit good efficiency in using rations.

The outcomes of statistical analyses indicated that there were no significant variations; yet, variations in feed conversion within each treatment were believed to be attributed to other factors, such as adequate energy content, appropriate nutrients in the feed, ambient temperature, and health conditions. Environmental factors that can influence feed conversion are uncomfortable temperatures and humidity (Oke *et al*., 2024; Quintana-Ospina *et al*., 2023), limited access to quality of feed and water (Reski *et al*., 2021), maintenance management (Reski *et al*., 2021), cage density (Kyriazakis *et al*., 2024) and disease (Oke *et al*., 2024).

The average FCR obtained in this study ranged from 1.77-1.82. These results were lower than previous studies using FPKM rations with *B.subtilis* resulting in an average ration conversion of 1.91 (Mirnawati *et al*., 2020).

Body Weight (BW)

Based on Table 3, the inclusion of FPKM fermented with *B. subtilis* and *L. fermentum* (6:4) up to 35% in the ration had a very significant effect (P<0.01) on broiler body weight (BW). However, there were no significant differences in BW among treatments R1 (0% FPKM), R2 (25% FPKM), and R3 (30% FPKM). A reduction in live weight was observed in treatment R4 (35% FPKM), which is likely due to the higher crude fiber content (6.8%) in the ration.

According to Sekh and Karki (2022), the recommended crude fiber level in broiler rations is between 3–6%, while for laying hens it is up to 8%. Bedford *et al.* (2023) and Sekh and Karki (2022) reported that increased crude fiber in feed reduces carbohydrate digestibility in poultry, as crude fiber is difficult to digest. Therefore, the decrease in live weight in the R4 treatment can be attributed to the higher crude fiber content negatively affecting nutrient digestibility.

Previous studies by Mirnawati *et al.* (2020) reported that broiler performance on a diet containing 25% FPKM fermented with *B. subtilis* resulted in a feed intake of 527.10 g/bird/week and body weight gain of 279.80 g/bird/week. In contrast, the present study using FPKM fermented with *L. fermentum* at the same inclusion level (25%) showed improved feed intake (733.69 g/bird/week) and weight gain (393.84 g/bird/week). Moreover, Devi *et al.* (2023) demonstrated that the combination of *B. subtilis* and *L. fermentum* (6:4) further improved the nutritional quality of FPKM. These findings suggest a potential synergistic effect of the bacterial consortium, which may be superior to monoculture fermentation in enhancing broiler performance. However, direct comparative trials are recommended to validate the synergistic effect.

Carcass Percentage (CP)

As shown in Table 3, the use of FPKM fermented with *B. subtilis* and *L. fermentum* (6:4) up to 35% in the ration had no significant effect (P>0.05) on carcass percentage (CP). The CP values in treatments R1 (control), R2 (25% FPKM), R3 (30% FPKM), and R4 (35% FPKM) were not significantly different. This result may be due to the improved nutritional quality of the fermented product in R2–R4, which allowed these treatments to match the performance of the control group.

According to Calvillo *et al.* (2022) and Aini *et al.* (2023), fermentation improves nutrient digestibility by breaking down complex compounds into simpler, more absorbable forms, and also contributes to the synthesis of vitamins and growth factors such as riboflavin, vitamin B12, and provitamin A. These enhancements may help maintain carcass performance similar to that of standard rations. Furthermore, the lack of significant difference in carcass percentage among R1, R2, and R3 corresponds with the similar live weights observed in these treatments. As reported by Bulkaini *et al.* (2022), carcass percentage is closely related to live weight—higher live weights generally lead to higher carcass yields, and vice versa.

Over the course of the six weeks of this trial, the average percentage of carcasse was 76.48%. These results were higher than previous studies which used a 25% FPKM ration with *B. subtilis* resulting in an average carcass percentage of 70.24% (Mirnawati *et al.*, 2020). It is reasonable to say that the research's average carcass % is within the typical range. Aini *et al.* (2023) stated that 60-67% is the average percentage of broiler carcasses.

Abdominal Fat (AF)

The inclusion of fermented palm kernel meal (FPKM) with *B. subtilis* and *L. fermentum* in a 6:4 ratio, up to 35% in the diet, had no significant effect (P > 0.05) on the abdominal fat percentage in broiler chickens (Table 3). The energy content of the treatment rations was essentially constant, which produced the same proportion of AF, which also had an inconsequential affect on the percentage of AF in each treatment. According to Mirnawati *et al.* (2020), energy consumption has a direct impact on the amount of AF that broiler chicks accumulate on their bodies. Apart from that, age also affects the percentage of AF in broilers. Broilers at 5-6 weeks of age are still growing so that the chicken's body uses the resources it has taken in for growth, and not much fat gets stored.

The average percentage of AF obtained in the study gave results that tended to be better, namely around 1.00-1.03% lower than previous research which used rations of 25% FPKM with *B. subtilis* resulting in a carcass percentage of around 1.61% (Mirnawati *et al.*, 2020). Furthermore, Ciptaan *et al.* (2021) also added that the percentage of AF in broiler carcasses ranges from 1.75%.

Chicken Thigh Cholesterol (CTC)

The results presented in Table 3 demonstrated that, increasing the inclusion level of fermented palm kernel meal (FPKM) in the diet, up to 35%, resulted in a decrease in the carcass total cholesterol (CTC) of broiler chickens. The low cholesterol content is due to PKM fermentation using LAB (*L. fermentum*) in which microorganisms from the LAB group can produce the enzyme bite salt hydrolase (BSH) to inhibit fat digestion. The addition of probiotics in broiler rations can expand the activity of enzymes such as BSH (Yulianto *et al.*, 2020; Liang *et al.*, 2020; Risna *et al.*, 2020). Bite salt hydrolase can deconjugate bile salts which results in fat not being emulsified and absorbed by the body so it comes out through the excreta. According to Sudha *et al.* (2009), the HMG-Coa reductase enzyme, which is involved in the synthesis of mevalonate during the cholesterol synthesis process, can be inhibited by the BSH enzyme. Javed *et al.* (2023) also stated that giving probiotics that produce the BSH enzyme has an effect on reducing cholesterol levels.

Nitrogen Retention (NR)

Based on the results of the variance analysis (F-test), the inclusion of fermented palm kernel meal (FPKM) with *B. subtilis* and *L. fermentum* in a 6:4 ratio in the broiler diet had a significantly different effect (P > 0.05) on nitrogen retention (NR). The results showed that NR in treatments R1, R2, and R3 was not significantly different (P>0.05), but was significantly (P<0.05) higher than R4. Based on the NR in Table 3, the use of FPKM up to 30% can equal the nitrogen retention of the control diet (R1) while in the R4 treatment with 35% FPKM there was an alleviate in NR.

There was no significant difference in nitrogen retention in treatments R1, R2, and R3 due to the fermentation process which produces a lot of amino acids, where amino acids are compounds that are more easily digested in the poultry digestive tract. This is in accordance with Mirnawati *et al.* (2023) fermented products will have better quality and complete amino acids so that nitrogen retention will also increase.

The significant reduction in nitrogen retention in R4 was likely due to the increased inclusion level of FPKM (35%), which elevated the crude fiber content (6.80%) and consequently reduced the overall quality of the ration. In line with Rizal (2006) opinion who stated that the tolerance for crude fiber content in broiler chicken rations is 3-6%. The high CF content in R4 disrupts the protein digestion process and results in low nitrogen retention. According to Ginindza *et al.* (2022), the CF content of the ration influences the absorption of other food substances, such as protein, which will later influence nitrogen retention.

Crude Fiber Digestibility (CFD)

According to the results of the variance analysis, the use of FPKM with *B. subtilis* and *L. fermentum* in a 6:4 ratio resulted in no significant difference (P > 0.05) in CFD. The CFD in treatments R1, R2, and R3 was not substantially different (P>0.05), but it was significantly (P<0.05) greater than R4, according to the findings of the Duncan Multiple Range Test (DMRT). The CFD in Table 3 indicates that the use of FPKM up to treatment R3 (30%) can match the CFD in the control diet (R1), but the CFD is alleviated in treatment R4 with 35% FPKM.

There was no significant difference in CFD in R1, R2, and R3. This is caused by fermented products that improve the quality of the ration and are easily digestible from the original ingredients. Mirnawati *et al.* (2023) stated that fermented products have high digestibility, complete amino acids so that the digestibility of crude fiber will be good and livestock will find it easier to digest the material.

However, at R4 (35% FPKM in ration), crude fiber digestibility experienced a significant decrease due to the high CF content in the ration (6.80%) which also affected digestibility. According to Mirnawati *et al.* (2019b), the crude fiber concentration of the feed ingredients determines the CFD; the higher the CF content, the lower the CFD because of the poultry's limited ability to digest CF. The assertion made by Sekh and Karki (2022) that broiler chicken feeds should have a 3-6% CF content tolerance lends credence to this.

The average crude fiber digestibility obtained in this study was 56.61%. This result is higher than previous research which used a ration of 25% FPKM with *B. subtilis* resulting in an average CFD of 49.51% (Mirnawati *et al.*, 2020).

Metabolizable Energy (ME)

Results in Table 3 showed that FPKM with *B. subtilis* and *L. fermentum* (6:4) had significant effects (P>0.05) on ME, according to the diversity analysis results using F Calculation. The energy metabolism in treatments R1, R2, and R3 was not substantially different (P>0.05), but it was considerably different (P<0.05) and higher than that of R4, according to the results of the *Duncan Multiple Range Test* (DMRT). According to Table 3 metabolizable energy, using up to 30% FPKM can match the amount of the control ration (R1) consumed, however using 35% FPKM in the R4 treatment resulted in a notable decrease in metabolizable energy.

The difference in metabolizable energy in treatments R1, R2, and R3 is related to the content of food substances in the treatment rations which have high digestibility, because many components are easily digested as a result of PKM fermentation by *B. subtilis* and *L.fermentum* (6:4) which experienced a proportional increase in quality, namely by increasing protein and decreasing CF. Mirnawati et al. (2022) stated that increasing the digestibility of fiber so that other nutrients such as protein and fat can be digested and utilized by broiler livestock as an energy source, low digestibility causes a lot of energy to be lost through excreta.

The low metabolizable energy value in the R4 treatment (35% FPKM) is thought to be caused by the relatively high CF content in the ration. Thus, the absorption of food substances is not optimal, especially the absorption of energy in the ration consumed becomes less, which is indicated by a tendency to decrease the value of metabolizable energy in this treatment. Feed containing high crude fiber produce low energy value (Wisbech *et al*., 2023). Study from Chouhan *et al*. (2020) stated that a high CF content will reduce the ME of feed ingredients, because there is an alleviate in the digestibility of the ingredients so that the absorption of substances does not run optimally. The average metabolizable energy obtained in this study was 2283.95Kcal/kg.

CONCLUSIONS AND RECOMMENDATIONS

Fermented palm kernel meal (PKM), using a combination of Bacillus subtilis and Lactobacillus fermentum, can be included up to 30% in broiler rations. This level of inclusion was shown to support optimal broiler performance, as evidenced by an average feed intake of 815.27 g/head/week; body weight gain of 470.45 g/head/week; feed conversion ratio of 1.75; live weight gain of 1895.20 g/head/week; carcass percentage of 76.48%; abdominal fat percentage of 1.03%; thigh meat cholesterol level of 131.14mg/dL; nitrogen retention of 59.39%; crude fiber digestibility of 56.61%; and metabolizable energy of 2283.95 kcal/kg.

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NOVELTY STATEMENTS

This study is the first to evaluate the synergistic effect of Bacillus subtilis and Lactobacillus fermentum (6:4 ratio) in fermenting palm kernel meal (PKM) and its impact on broiler performance. The research provides novel insights into the optimal inclusion rate of this fermented product in broiler diets and presents evidence of improved nutritional quality and metabolic efficiency, which is crucial for cost-effective poultry production using agro-industrial by-products.

AUTHOR'S CONTRIBUTIONS

Mirnawati, Gita Ciptaan, Yetti Marlida, Yan Heryandi, and Montesqrit were participating in conceiving the idea and study design and also reviewed the manuscript. Pratiwi Chintya Devi established the experiment, analyzed data and wrote the manuscript. Anifah Srifani and Hayumi Azzahra wrote and finalized the manuscript.

Conflict of Interest

The authors have declared that no competing interests exist.

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