

Research Article



Solid-State Fermentation to Improve the Nutrient Value, Supplementation Level, and Digestibility of Palm Kernel Meals as Feed Ingredients for Red Nile Tilapia (*Oreochromis niloticus*)

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ARTICLE INFO

Article history:

Received October 27, 2025

Received in revised form January 14, 2026

Accepted February 9, 2026

Available Online May 18, 2026

KEYWORDS:

palm kernel meals,
solid-state fermentation,
tilapia,
feed ingredient,
Paenibacillus polymyxa

ABSTRACT

This study investigated the potential of palm kernel meal (PKM) as a sustainable feed ingredient for red tilapia. Palm kernel meal is a byproduct of palm oil production, abundant in Indonesia. Like other plant-based feed ingredients, PKM contains a lot of non-starch polysaccharides (NSP), making it difficult to digest, especially for monogastric animals. This study aimed to improve the nutritional value, digestibility, and dietary supplementation levels of PKM for red tilapia through solid-state fermentation (SSF). PKM was fermented using *Paenibacillus polymyxa* BR25, a fibrolytic bacterium isolated from the buffalo rumen. SSF enhanced the nutritional value of PKM, as indicated by an increase in dry matter, crude protein, calcium, phosphorus, and ash. Essential amino acids, including threonine and methionine+cysteine, were also increased, accompanied by decreases in hemicellulose and cellulose, indicating effective NSP degradation. In addition, oleic acid and linoleic acid increased, providing essential fatty acids for fish health and development. Unfermented PKM negatively affected growth at inclusion levels up to 10%, whereas fermented PKM maintained growth performance at a 15% dietary inclusion. In addition, SSF significantly increased the digestibility of dry matter, protein, and lipid in tilapia, demonstrating its potential to enhance PKM utilization in aquafeeds.



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1. Introduction

Tilapia is a leading aquaculture commodity in Indonesia. Tilapia production in 2023 ranked second among aquaculture production (1.36 million tons), followed by catfish (1.13 million tons) (KKP RI 2025). Tilapia has the potential to be cultivated, especially in developing countries such as Indonesia, because it grows quickly; has a wide range of adaptations to various environmental conditions (temperature, salinity, and low oxygen levels); is resistant to stress and disease; is able to reproduce at a high capacity and short generation time;

has a low trophic level; and readily accepts artificial feed immediately after hatching (El-Sayed 2020).

A significant barrier to aquaculture development in developing countries is the restricted access of sustainable feed formulations, despite the abundance of resources available from agriculture and animal waste in these regions (Admasu *et al.* 2017). The availability of quality feed at affordable prices significantly reduces production costs and increases profits. The use of local feed ingredients that are inexpensive, abundant, and sustainable will support the availability of cheaper and sustainable feeds. One of the feed ingredients with potential for development in Indonesia is palm kernel meal (PKM), a byproduct of palm kernel oil extraction. Indonesia is the largest palm oil-producing country in the

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world, with a production volume of 47.5 million metric tons in 2024 (Siahaan 2025).

This PKM has the potential to be used as a feed ingredient because of its year-round availability, relatively low cost, high crude protein content (14-18%), a fairly complete amino acid profile, and varying levels of various minerals (Azizi *et al.* 2021). However, like other plant feed ingredients, the use of PKM in aquaculture remains rare. Most plant feed ingredients contain a high level of non-starch, which makes them difficult to utilize in aquafeeds, especially for carnivorous fish (Vieira *et al.* 2023). NSP is a well-known antinutritional factor that inhibits the digestion and absorption of nutrients in animal intestines (Choi *et al.* 2021).

Numerous studies have been conducted to enhance the nutritional value and digestibility of PKM. Findings show that solid-state fermentation of palm kernel meal (PKM) using bacteria or fungi enhances its nutritional value by reducing crude fiber (CF) levels while increasing crude protein (CP), amino acids, and energy content (Azizi *et al.* 2021). Solid-state fermentation of PKM with effective microorganisms has been proven to increase the nutritional value and level of substitution for soybean meal in tilapia feed (Wattanukul *et al.* 2021). Fermented feed has beneficial effects on the ecosystem and morphology of the gastrointestinal tract (GIT), minimizing GIT pathogen colonization, improving immune responses, oxidative status, resistance to environmental stressors, growth performance, and feed utilization of aquatic animals (Dawood & Koshio 2020). In general, microbes used for the solid-state fermentation of plant feed ingredients must be able to degrade NSP. Previous research by Sari *et al.* (2021) successfully isolated fibrolytic bacteria from buffalo rumen. Screening results based on the formation of clear zones on screening media with single carbon sources, namely microcrystalline cellulose, locust bean gum, and PKM, showed that these bacteria had the ability to hydrolyze cellulose, mannan, and fiber in PKM. Based on the 16S rRNA gene sequence, Isolate BR25 with the highest hydrolytic activity was identified as closely related to *Paenibacillus polymyxa* (Accession: KR780413.1) with a similarity of 98.57%. In addition to their cellulolytic and mannolytic activities, these bacteria have proteolytic and lipolytic activities (Sari *et al.* 2022). Based on its ability to degrade fiber, proteins, and lipids, *P. polymyxa* BR25 has the potential to be applied to solid-state PKM

fermentation. *Paenibacillus polymyxa* has been shown to be safe for animals and has potential as a probiotic, producing antibacterials to fight pathogens (Gong *et al.* 2021; Zhou *et al.* 2024). *Paenibacillus polymyxa* (LM31), as a new feed additive, had antioxidant and antimicrobial activity, increased growth performance, and improved health status of growing Japanese quail (Alagawany *et al.* 2021). This study aimed to improve the nutritional value, supplementation level, and digestibility of PKM in red tilapia by the application of a solid-state fermentation process utilizing *P. polymyxa* BR25.

2. Materials and Methods

2.1. Preparation of Inoculum

Paenibacillus polymyxa BR25 was isolated from buffalo rumen by Sari *et al.* (2021). A total of 2.5 mL of bacterial culture in NB medium was inoculated into 22.5 mL of NB medium and incubated at 34°C with agitation at 120 rpm for 18 hours or until an optical density of 1 at a wavelength of 600 nm (Alshelmani *et al.* 2014; Sari *et al.* 2021).

2.2. Solid-State Fermentation of Palm Kernel Meals

Solid-state fermentation was carried out under conditions according to the optimization results in the preliminary study, namely by adding 0.2% urea, a substrate: water ratio of 1:2.5 (w/v), and a temperature of 30°C, and was incubated for 8 days. The PKM was dried at a temperature of 50°C, ground, and then sieved with a 40-mesh sieve. A total of 100 g of PKM was added to a 1 L Erlenmeyer flask, 0.2 g of urea was added, and distilled water was added at a ratio of 1:2.5 (w/v). The fermentation medium was sterilized by autoclaving at a temperature of 121°C and a pressure of 1 atm for 15 minutes. A total of 10 mL of inoculum was added to 100 g of fermentation medium (10% v/w), then mixed with a sterile spatula until evenly distributed. The mixture was incubated for 8 days at 30°C.

2.3. Experimental Design

This research used a completely randomized design with 6 feed composition treatments (each with 3 replications), namely:

1. T0: Standard feed control without the addition of palm kernel meal (PKM) or fermented palm kernel meal (FPKM)
2. TA1: Standard feed with 5% PKM supplementation
3. TA2: Standard feed with 10% PKM supplementation
4. TB1: Standard feed with 5% FPKM supplementation
5. TB2: Standard feed with 10% FPKM supplementation
6. TB3: Standard feed with 15% FPKM supplementation

2.4. Experimental Animals

This research used red tilapia var. Nilasa has a weight of approximately 13–14 g and a length of between 8.5 and 9 cm. Red tilapia were obtained from the Balai Pengembangan Teknologi Perikanan Budidaya (BPTPB), Sleman, Daerah Istimewa Yogyakarta, Indonesia.

2.5. Fish Culture

The fish were kept in 18 plastic ponds with a volume of 125 L, each equipped with an aeration and water-circulation system. Each pond was filled with 20 fish. Acclimatization was carried out for 7 days on a standard feeding regimen. The feed was given twice a day, at 08:00 and 17:00, at a rate of up to 4% of the fish's weight. The feeding treatment lasted 45 days.

2.6. Feed Composition and Preparation

The feed composition was formulated according to the nutrient requirements of tilapia (NRC 1993). The composition of the feed ingredients is shown in Table 1. A total of 6 feed formulas (Table 2) were formulated with a crude protein (CP) content of approximately 32% (isoprotein) and digestible energy (DE) of 3,000 kcal DE/kg (isoenergy).

Table 1. Nutrient composition of feed ingredients

Ingredient	DW (%)	CP (%)	DE (Kcal/kg)	Ca (%)	P (%)	Met+Cys (%)	Lys (%)	Trp (%)
Fish Meal	92	50.52	4200	3.73	2.43	2.56	5.04	0.75
SBM	90	40.55	3010	0.3	0.65	1.27	2.85	0.64
PKM	91.48*	13.33*	2890	0.4*	1.17*	0.74*	0.48*	0.37*
FPKM	90.31*	16.43*	3000	0.46*	1.23*	1.25*	0.99*	0.4*
Starch	88	0	2700	0	0	0	0	0
Rice bran	91	11.27	2110	0.11	1.37	0.41	0.54	0.21
Fish oil	99	0	8800	0	0	0	0	0
Vitamine C	100	0	0	0	0	0	0	0
Mineral mix	100	0	0	32.5	22	0	0	0

Table 2. Feed formulation and nutrient composition of the experimental diets

Ingredient	Proportion (%)	Proportion (%)	Proportion (%)	Proportion (%)	Proportion (%)	Proportion (%)
Ingredient	T0	TA1	TA2	TB1	TB2	TB3
Fish Meal	40	40	40	40	40	40
SBM	32	32	32	32	30	30
PKM	0	5	10	0	0	0
FPKM	0	0	0	5	10	15
Starch	9	9	9	9	9	7
Rice bran	16	11	6	11	8	3
Fish oil	2	2	2	2	2	2
Vitamine C	0.5	0.5	0.5	0.5	0.5	0.5
Mineral mix	0.5	0.5	0.5	0.5	0.5	0.5
Total	100	100	100	100	100	100
Nutrient composition						
Digestible energy (kcal/kg)	3107.78	3143.96	3180.14	3147.24	3170.92	3162.86
Drymatter (%)	90.56	90.58	90.61	90.53	90.51	88.72
Crude protein (%)	31.91	32.01	32.10	32.14	31.84	32.07
Ca (%)	1.48	1.49	1.50	1.49	1.50	1.52
P (%)	1.28	1.27	1.26	1.27	1.28	1.27
Methionine+Cysteine (%)	1.37	1.38	1.40	1.41	1.43	1.47
Lysine (%)	2.75	2.75	2.75	2.77	2.75	2.77
Tryptophan (%)	0.49	0.50	0.51	0.50	0.50	0.51

2.7. Chemical Analysis

Dry matter (DM), crude fiber (CF), crude protein (CP), crude lipid (CL), phosphorus, calcium, and ash contents were determined using the AOAC 2005 method (Horwitz 2006). Nutrient contents are presented as percentages on a dry-matter basis. The gross energy was analyzed with a bomb calorimeter (Azarm and Lee 2014). Fiber composition, including acid detergent fiber (ADF), neutral detergent fiber (NDF), lignin detergent fiber (LDF), cellulose, and hemicellulose, was determined according to the methods of Van Soest *et al.* (1991). The amino acid profile was analyzed by using a precolumn HPLC gradient system. The free fatty acid composition was analyzed via GC-MS (Wathne *et al.* 2018).

2.8. Analysis of Growth Performance and Feed Utilization Parameters

Weight was measured every 15 days. The growth performance and feed utilization parameters were determined via the following formulas (Adjanke *et al.* 2016):

Daily weight gain (DWG) (g/day)

$$DWG = (W_f - W_i) / \text{maintenance duration}$$

Note: W_f is the final weight, whereas W_i is the initial weight.

Specific growth rate (SGR) (%/day)

$$SGR = 100 * (\ln W_f - \ln W_i) / \text{maintenance duration}$$

2.9. Measuring Feed Digestibility

The feed digestibility was determined by the indirect method. Acclimatization was carried out for 7 days by providing standard feed, followed by feeding according to the treatment in the growth test for 14 days. On the 15th day, the tilapia were fed a diet supplemented with 0.5% Cr_2O_3 (Sigma). The feed was given at 08:00 and 17:00, as much as 4% of the weight of the fish. Before feeding, the ponds were cleaned by siphoning. The remaining feed was removed by siphoning after 2 hours of feeding. Fish feces were collected by siphoning at 07:00 and 19:00. The feces were centrifuged at $3,000 \times g$ for 15 minutes and then stored at -20°C . Fecal collection was carried out for 10 days. After the feces were collected, the dry weight, crude protein content, total fat content, and Cr_2O_3 content in the feces and treatment feed were analyzed. Cr_2O_3 levels were analyzed by using an atomic absorption spectrophotometer (AAS). Feed digestibility was calculated using the formula described by Vidal *et al.* (2015).

2.10. Statistical Analysis

The data obtained were analyzed by one-way analysis of variance (ANOVA) at $p > 0.05$. If there was a significant difference, the analysis continued with the Duncan multiple-range test (DMRT).

3. Results

3.1. Chemical Composition of Palm Kernel Meal (PKM) and Palm Kernel Meal Fermented by *Paenibacillus polymyxa* BR25 (FPKM)

The nutrient composition of PKM before and after fermentation with *Paenibacillus polymyxa* BR25 is shown in Table 3. The dry matter (DM), crude protein (CP), calcium (Ca), phosphorus (P), and ash contents relatively increased after solid-state fermentation. Changes in fiber composition following SSF indicate the NSP-degradation process. The crude fiber, acid detergent fiber (ADF), and lignin contents increased by 3.3%, 6.38%, and 22.54%, respectively, whereas the neutral detergent fiber (NDF), hemicellulose, and cellulose contents decreased by 0.06%, 34.48%, and 2.21%, respectively. The essential amino acid content increased, except for arginine, phenylalanine, and valine. Threonine and methionine+cysteine increased by 76.09% and 68.92%, respectively, whereas valine decreased by 11.25%. Long-chain saturated fatty acids, including lauric (C12), myristic (C14), palmitic (C16), and stearic (C18) acids, decreased, whereas short saturated fatty acids, namely, capric acid (C10), increased.

3.2. Growth Performance

The growth curve of the tilapia after 45 days of rearing is shown in Figure 1. The growth of the control feed and all the treatments during the first 15 days of maintenance seemed to coincide, and the growth curve for the 10% PKM supplementation group was flatter than that for the other group.

Table 4 presents the effects of PKM and FPKM supplementation on survival and growth performance. Supplementation with PKM and FPKM did not affect survival rate but significantly improved growth performance ($P < 0.05$). The growth performance in the 5% PKM supplementation group did not differ significantly from that in the control group. However, when PKM supplementation was increased to 10%, growth was significantly lower than that in the control

Table 3. Nutrient content of palm kernel meal and after solid-state fermentation (FPKM) using *Paenibacillus polymyxa* BR25

Ingredients	PKM	FPKM	Increased (+) Decreased (-)
Dry matter (%)	94.09	94.37	+0.3%
Crude protein (%)	13.33	16.02	+20.18%
Crude lipid (%)	19.25	15.41	-19.95%
Calcium (%)	0.4	0.46	+15%
Phosphorus (%)	1.17	1.23	+5.13%
Gross energy(Kal/g)	4856	4925	+1.42%
Ash (%)	4.22	4.77	+13.03
Crude fiber (%)	11.25	11.61	+3.2%
Neutral detergent fiber (NDF),	69.78	69.74	-0.06%
Acid detergent fiber (ADF)	33.08	35.34	+6.83%
Hemicellulose	36.70	22.96	-34.48
Cellulose	23.48	22.96	-2.21%
Lignin	8.96	10.98	+22.54%
Essential amino acids			
Arg		0.7	-62.57
His	1.87	0.65	+140.7
Ile	0.27	0.72	+26.32
Leu	0.57	1.18	+0.8
Lys	1.17	0.99	+106.25
Met	0.48	0.68	+17.24
Phe	0.58	0.67	-12.99
Thr	0.77	0.81	+76.09
Trp	0.46	-	-
Val	-	0.71	-11.25
Nonessential amino acids	0.8		
Ala		0.72	+14.29
Asp	0.63	1.28	-11.11
Cys	1.44	0.57	+256.25
Glu	0.16	2.17	-9.21
Gly	2.39	0.81	+88.37
Pro	0.43	0.92	+338.1
Ser	0.21	0.77	+108.11
Tyr	0.37	0.64	+156
Arg:lys	0.25	0.71	-81.79
Met+Cys	3.90	1.25	+68.92
Fatty acid	0.74		
Caprylic acid (8:0)		Ttd	
Capric acid (10:0)	0.86	2.12	+46.21
Lauric acid (12:0)	1.45	45.58	-0.83
Myristic acid (14:0)	45.96	17.92	-26.62
Palmitic acid (16:0)	24.42	10.33	-69.92
Stearic acid (18:0)	34.34	0.05	-84.85
Arachidonic acid (20:0)	0.33	Ttd	
Oleic acid (18:1(9))	Ttd	24	+57.58
Linoleic acid (18:2(9,12))	15.23	5.21	+9.92
Linolenic acid (18:3(9,12,15))	4.74	Ttd	
Total fatty acids (%)	Ttd	105.21	17.37
	127.3		

feeding and all the treatments. Solid-state fermentation of PKM with *P. polymyxa* BR25 increased the level of PKM supplementation. This was demonstrated by the maintenance of growth performance at the FPKM supplementation level of 15%.

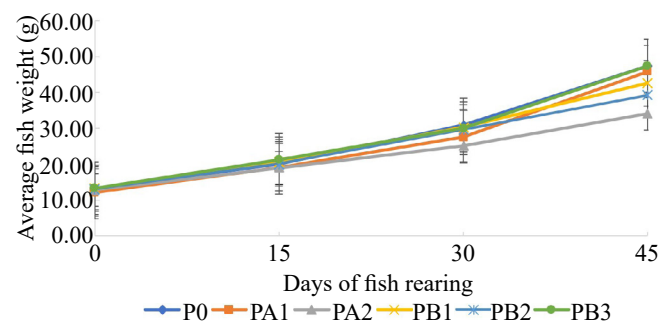


Figure 1. Growth curves of tilapia that were given control feed (T0), supplemented with 5% (TA1) and 10% (TA2) of palm kernel meal, supplemented with 5% (TB1), 10% (TB2), and 15% (TB3) of fermented palm kernel meal

3.3. Feed Digestibility

The effects of PKM and FPKM supplementation on feed digestibility in tilapia are presented in Table 5. The lowest feed digestibility (dry matter, lipids, proteins) among all the treatments occurred with 10% PKM supplementation, whereas the highest digestibility occurred with 15% FPKM supplementation. Digestibility with 10% FPKM supplementation was lower than with 5% and 15% FPKM supplementation, but still higher than with 10% PKM supplementation. These findings indicate that SSF with *P. polymyxa* BR25 can increase the digestibility of PKM in Nile tilapia.

4. Discussion

The solid-state fermentation of palm kernel meal with *Paenibacillus polymyxa* BR25 enhanced the supplementation level of palm kernel meal. The maintenance of growth at a 15% supplementation level of FPKM suggests the impact of SSF in enhancing PKM's nutritional value, presumably through fiber reduction, mitigation of antinutritional factors, and improved nutrient bioavailability. The findings of this study align with those of Alshelmani *et al.* (2016, 2021), who reported that the administration of 10% or 15% palm kernel meal (PKM) resulted in a significant decrease in nutrient digestibility relative to the control group, while the inclusion of 10% or 15% FPKM utilizing *P. polymyxa* ATCC 842 significantly enhanced nutrient digestibility in broiler chickens. Adjanke *et al.* (2016) indicate that PKM can constitute up to 30% of the diet for *O. niloticus* when subjected to technological processing to reduce fiber and antinutritional components. It has been shown that PKM can comprise up to 10% of tilapia diets without

Table 4. The effects of supplementing with palm kernel meal (PKM) and fermented palm kernel meal (FPKM) on the survival and growth performance of Nile tilapia

Treatment	Survival rate (%)	growth performance		growth performance
Treatment	Survival rate (%)	Total weight gain (W) (g)	Daily weight gain (DWG) (g/day)	Specific growth rate (SGR) (%/day)
T0	94.44±1.93 ^{ab}	34.37±2.17 ^a	0.767±0.050 ^a	2.87±0.12 ^{ab}
TA1	93.33±0.00 ^{ab}	33.71±1.70 ^a	0.750±0.036 ^{ab}	2.97±0.30 ^a
TA2	85.56±9.62 ^a	21.24±3.70 ^c	0.470±0.082 ^d	2.16±0.26 ^c
TB1	94.44±3.85 ^{ab}	29.31±3.31 ^b	0.653±0.075 ^{bc}	2.60±0.2 ^{bd}
TB2	91.67±3.33 ^{ab}	26.13±1.50 ^b	0.580±0.030 ^c	2.44±0.08 ^{cd}
TB3	96.67±3.34 ^b	34.11±1.42 ^a	0.757±0.031 ^a	2.82±0.11 ^{ab}

Different letters in one column indicate significant differences at an α of 5% ($p < 0.05$). T0: control feed, TA1: 5% PKM supplementation, TA2: 10% PKM supplementation, TB1: 5% FPKM supplementation, TB2: 10% FPKM supplementation, TB3: 15% FPKM supplementation

Table 5. Effects of palm kernel meal (PKM) and fermented palm kernel meal (FPKM) supplementation on the digestibility of dry matter, protein, and lipid in tilapia

Treatment	digestibility of dry matter (%)	Protein digestibility (%)	Lipid digestibility (%)
P0	87.27±0.21 ^a	85.83±0.55 ^a	92.77±0.09 ^a
PA1	81.88±0.31 ^b	80.72±0.81 ^b	87.24±0.04 ^b
PA2	71.59±1.08 ^c	63.93±2.16 ^c	75.89±2.23 ^c
PB1	85.08±0.40 ^d	81.02±0.03 ^b	86.56±1.14 ^b
PB2	74.64±0.19 ^c	65.30±0.30 ^c	76.15±4.31 ^c
PB3	85.64±0.19 ^d	82.97±0.22 ^d	91.74±0.40 ^a

different letters in one column indicate significant differences at α 5% ($p < 0.05$), P0: control feed, PA1: 5% PKM supplementation, PA2: 10% PKM supplementation, PB1: 5% FPKM supplementation, PB2: 10% FPKM supplementation, PB3: 15% FPKM supplementation

impacting apparent nutrient digestibility, growth, or overall nutritional composition (León *et al.* 2022). The current study did not assess levels above 15% FPKM to avoid potential confounding effects of residual fiber, amino acid imbalance, and diminished palatability. The main goal was to determine whether SSF could increase the supplementation level. Subsequent research using elevated inclusion levels, together with digestibility and gut health evaluations, is necessary to establish the maximum threshold of FPKM application in tilapia diets.

An increase in the level of PKM supplementation could occur due to an increase in the quality and availability of nutrients after SSF using *P. polymyxa* BR25. Solid-state fermentation of PKM using *P. polymyxa* BR25 has been shown to increase DM, CP, GE, P, and Ca, but decrease CL. Increases in CP and DM also occurred after SSF of PKM with *P. polymyxa* ATCC 842 and *P. curdlanolyticus* DSMZ 10248 (Alshelmani *et al.* 2017). Fermentation of PKM using effective microorganisms (such as photosynthetic bacteria, lactic acid bacteria, nitrogen-fixing bacteria, yeast, and *Bacillus* sp.) increased crude protein and total energy content, but crude fat content decreased (Wattanukul *et al.* 2021). The rise in CP content after SSF of PKM with *P. polymyxa* BR25 can be related to two complementary mechanisms: the contribution of microbial biomass and the breakdown of non-

starch polysaccharides (NSP) (Zhu *et al.* 2021; Feng *et al.* 2023). During SSF, *P. polymyxa* BR25 actively proliferates on PKM, leading to the accumulation of microbial cell material rich in proteins, nucleic acids, and intracellular enzymes. This microbial biomass directly contributes to the observed increase in crude protein, as microbial cells naturally contain significant amounts of nitrogenous compounds. At the same time, *P. polymyxa* produced fibrolytic enzymes capable of hydrolyzing complex carbohydrate fractions in PKM, thus decreasing the proportion of structural polysaccharides and elevating the relative concentration of proteins and other nutrients.

The enhancement of essential amino acid (EAA) profiles during SSF further substantiates the role of microbial biosynthesis. The significant increases in threonine and methionine+cysteine (76.09% and 68.92%, respectively) indicate active de novo synthesis of amino acids by *P. polymyxa* during SSF, alongside enhanced release of bound amino acids from complex protein-fiber matrices. The partial reduction in valine and certain other amino acids may indicate their preferred use by the bacterium for growth and metabolic processes.

The reduced levels of valine and certain other amino acids may suggest their preferential use by the bacteria for growth and metabolic functions. The improved EAA composition indicates a qualitative enhancement

in protein, which is nutritionally important for tilapia, as substantial amounts of lysine and threonine are required. Deficiencies in leucine, threonine, methionine, and phenylalanine have been associated with diminished growth performance in tilapia (do Nascimento *et al.* 2020; Rodrigues *et al.* 2020). The reduction of crude fat during fermentation is associated with the ability of microorganisms to metabolize lipids. *Paenibacillus polymyxa* BR25 has lipolytic activity, facilitating the breakdown of complex lipids for use as energy sources in microbial growth (Sari *et al.* 2022). After SSF of PKM, the level of unsaturated fatty acids, notably oleic (18:1 cis-9) and linoleic acid (18:2n-6) acids, rose. This increase is most convincingly attributed to a combination of microbial lipid modification and concentration effects rather than de novo fatty acid production. The partial degradation of carbohydrates in solid-state fermentation reduces dry matter, leading to a relative enrichment of the residual lipid fractions, while the selective microbial use of saturated fatty acids modifies the fatty acid profile towards unsaturated forms. This transition is beneficial nutritionally, as linoleic and linolenic acids are necessary fatty acids for tilapia and other vertebrates (Ng and Romano 2013). The linoleic acid requirement for tilapia is 0.5% (Takeuchi *et al.* 1983). Long-chain polyunsaturated fatty acids (LC-PUFAs), particularly omega-3 (n-3) and omega-6 (n-6), are essential for the nutrition of both fish and humans. Certain biomolecules are classified as essential fatty acids (EFAs) because they cannot be produced de novo and must be obtained from dietary sources (Carr *et al.* 2023). In general, freshwater and euryhaline fish species can synthesize LC-PUFA from C18 substrates, namely 18:3n-3 and 18:2n-6, while most marine predatory fish cannot do so (Xu *et al.* 2020). In fish, LC-PUFAs play an important role in cell membranes and cellular synthesis, ionic regulation, pigmentation, proper development and function of the nervous system, reproduction, intestinal barrier protection, control of metabolic functions, endocrine pathways, and immune function (Carr *et al.* 2023).

The increasing supplementation levels also reflect the greater digestibility of feed ingredients. SSF of PKM using *P. polymyxa* BR25 has been proven to increase digestibility (dry weight, lipids, and protein) in tilapia. Digestibility with 10% PKM supplementation was the lowest among all treatments, whereas 15% FPKM supplementation yielded the highest digestibility. The antinutritional effects of plant-based feed ingredients can be caused by the content of nonstarch structural polysaccharides

(NSP), which can increase the viscosity of feed in the digestive tract and inhibit digestion. Aquatic animals generally do not contain structural polysaccharide-degrading enzymes in their digestive tract (Kumar and Barman 2012). *Paenibacillus polymyxa* BR25 is a bacterium that exhibits cellulolytic and hemicellulolytic activities (Sari *et al.* 2021). During SSF, this bacterium decomposed cellulose and hemicellulose, resulting in a reduction of 34.48% in hemicellulose content and 2.21% in cellulose content. The results of this study were similar to those of the fermentation of a soybean meal and corn mixture using *Bacillus subtilis* and *Enterococcus faecium*, which reduced NDF and hemicellulose by 38.93% and 53.2%, respectively, while ADF increased by 2.58% (Shi *et al.* 2017). In addition, *P. polymyxa* BR25 has lipolytic and proteolytic activities (Sari *et al.* 2022). During SSF of PKM using *P. polymyxa* BR25, proteins and lipids were broken down into simpler components, promoting increased digestibility for the fish. Arte *et al.* (2015) confirmed protein breakdown during fermentation using protein profile analysis by SDS-PAGE, supported by HPLC results from Zhao *et al.* (2017). *Paenibacillus polymyxa* BR25 produces enzymes such as cellulases, hemicellulases, proteases, and lipases that may facilitate the degradation of certain substrates, such as cellulose, hemicellulose, protein, and lipid. This may reduce the viscosity of the digesta, facilitating nutrient absorption. Although direct evaluations of gut physiological alterations were not performed, these pathways may help clarify the improved digestibility and continuous growth observed at higher FPKM inclusion levels.

Our findings indicate that SSF could serve as a scalable, cost-efficient bioprocess that local feed manufacturers can readily adopt to enhance the nutritional quality of PKM. Solid-state fermentation (SSF) of palm kernel meal (PKM) may enhance nutrient digestibility and mitigate the adverse effects of elevated fiber levels, thereby improving feed efficiency and perhaps decreasing feed conversion ratios (FCR) in real agricultural settings. The current study demonstrated sustained growth performance under controlled experimental conditions; however, further validation through extended feeding trials is necessary. Assessments conducted in ponds or recirculating aquaculture systems are crucial for evaluating performance consistency, feed efficiency, fish health responses, and the economic feasibility of SSF on a commercial scale.

References

- Adjanke, A., Tona, K., Melecony, C., Toko, I.I., Gbeassor, M., 2016. Effect of dietary inclusion of palm kernel meal on feed intake, growth and body composition of Nile Tilapia, *Oreochromis niloticus* reared in concrete tanks in Togo. *International Journal of Fisheries and Aquatic Studies*. 4, 642–646.
- Admasu, F., Getahun, A., Wakjira, M., 2017. Supplemental feed formulation for the best growth performance of Nile tilapia, *Oreochromis niloticus* (Linnaeus, 1758) (Pisces: Cichlidae) in pond culture system. *Journal of Chemical Biological and Physical Sciences*. 7, 599–611.
- Alagawany, M., Madkour, M., El-Saadony, M.T., Reda, F.M., 2021. *Paenibacillus polymyxa* (LM31) as a new feed additive: antioxidant and antimicrobial activity and its effects on growth, blood biochemistry, and intestinal bacterial populations of growing Japanese quail. *Animal Feed Science and Technology*. 276, 114920. <https://doi.org/10.1016/j.anifeedsci.2021.114920>
- Alshelmani, M.I., Kaka, U., Abdalla, E.A., Humam, A.M., Zamani, H.U., 2021. Effect of feeding fermented and non-fermented palm kernel cake on the performance of broiler chickens: a review. *World's Poultry Science Journal*. 77, 377–388. <https://doi.org/10.1080/00439339.2021.1910472>
- Alshelmani, M.I., Loh, T.C., Foo, H.L., Lau, W.H., Sazili, A.Q., 2014. Biodegradation of palm kernel cake by cellulolytic and hemicellulolytic bacterial cultures through solid state fermentation. *The Scientific World Journal*. 2014, 1–8. <https://doi.org/10.1155/2014/729852>
- Alshelmani, M.I., Loh, T.C., Foo, H.L., Sazili, A.Q., Lau, W.H., 2016. Effect of feeding different levels of palm kernel cake fermented by *Paenibacillus polymyxa* ATCC 842 on nutrient digestibility, intestinal morphology, and gut microflora in broiler chickens. *Animal Feed Science and Technology*. 216, 216–224. <https://doi.org/10.1016/j.anifeedsci.2016.03.019>
- Alshelmani, M.I., Loh, T.C., Foo, H.L., Sazili, A.Q., Lau, W.H., 2017. Effect of solid state fermentation on nutrient content and ileal amino acids digestibility of palm kernel cake in broiler chickens. *Indian Journal of Animal Sciences*. 87, 1135–1140. <https://doi.org/10.56093/ijans.v87i9.74331>
- Arte, E., Rizzello, C.G., Verni, M., Nordlund, E., Katina, K., Coda, R., 2015. Impact of enzymatic and microbial bioprocessing on protein modification and nutritional properties of wheat bran. *Journal of Agricultural and Food Chemistry*. 63, 8685–8693. <https://doi.org/10.1021/acs.jafc.5b03495>
- Azarm, H.M., Lee, S., 2014. Effects of partial substitution of dietary fish meal by fermented soybean meal on growth performance, amino acid and biochemical parameters of juvenile black sea bream *Acanthopagrus schlegeli*. *Aquaculture Research*. 45, 994–1003. <https://doi.org/10.1111/are.12040>
- Azizi, M.N., Loh, T.C., Foo, H.L., Chung, E.L.T., 2021. Is palm kernel cake a suitable alternative feed ingredient for poultry? *Animals*. 11, 1–15. <https://doi.org/10.3390/ani11020338>
- Carr, I., Glencross, B., Santigosa, E., 2023. The importance of essential fatty acids and their ratios in aquafeeds to enhance salmonid production, welfare, and human health. *Frontiers in Animal Science*. 4, 1–7. <https://doi.org/10.3389/fanim.2023.1147081>
- Choi, W.J., Kim, J.H., Kim, H.W., Kim, K.E., Kil, D.Y., 2021. Effects of dietary palm kernel meal and β -xylanase on productive performance, fatty liver incidence, and excreta characteristics in laying hens. *Journal of Animal Science and Technology*. 63, 1275–1285. <https://doi.org/10.5187/jast.2021.e111>
- Dawood, M.A.O., Koshio, S., 2020. Application of fermentation strategy in aquafeed for sustainable aquaculture. *Reviews in Aquaculture*. 12, 987–1002. <https://doi.org/10.1111/raq.12368>
- do Nascimento, T.M.T., Mansano, C.F.M., Peres, H., Rodrigues, F.H.F., Khan, K.U., Romaneli, R.S., Sakomura, N.K., Fernandes, J.B.K., 2020. Determination of the optimum dietary essential amino acid profile for growing phase of Nile tilapia by deletion method. *Aquaculture*. 523, 735204. <https://doi.org/10.1016/j.aquaculture.2020.735204>
- El-Sayed, A., 2020. *Tilapia Culture: Current State and Future Potential in Tilapia Culture*, second ed. Academic Press, London, UK; San Diego, CA, USA. <https://doi.org/10.1016/B978-0-12-816541-6.00001-5>
- Feng, X., Ng, K., Ajlouni, S., Zhang, P., Fang, Z., 2023. Effect of solid-state fermentation on plant-sourced proteins: a review. *Food Reviews International*. 40, 2580–2617. <https://doi.org/10.1080/87559129.2023.2274490>
- Gong, L., Wang, B., Zhou, Y., Tang, L., Zeng, Z., Zhang, H., Li, W., 2021. Protective effects of *Lactobacillus plantarum* 16 and *Paenibacillus polymyxa* 10 against *Clostridium perfringens* infection in broilers. *Frontiers in Immunology*. 11, 1–12. <https://doi.org/10.3389/fimmu.2020.628374>
- Horwitz, W., 2006. *Official Methods of Analysis of AOAC International*, eighteenth ed. AOAC International, Gaithersburg, MD, USA.
- KKP RI. 2025. Produksi Perikanan. Available at: <https://portaldata.kkp.go.id/portals/data-statistik/prod-ikan/summary>. [Date accessed: 15 April 2025]
- Kumar, V., Barman, D., 2012. Anti-nutritional factors in plant feedstuffs used in aquafeeds. *World Aquaculture*. 43, 64–68.
- León, A.B., Aguilar, Y.M., Viana, M.T., Ojeda, M.O., Montañón, C.M., Corría, K.P., Méndez-Martínez, Y., Martí, B.V., 2022. Effect of palm kernel cake in the nutrition for tilapia fry (*Oreochromis niloticus*). *Revista MVZ Cordoba*. 27, e2527. <https://doi.org/10.21897/rmvz.2527>
- [NRC] National Research Council, 1993. *Nutrient Requirements of Fish*. National Academy Press, Washington, DC, USA.
- Ng, W.K., Romano, N., 2013. A review of the nutrition and feeding management of farmed tilapia throughout the culture cycle. *Reviews in Aquaculture*. 5, 220–254. <https://doi.org/10.1111/raq.12014>
- Rodrigues, A.T., Mansano, C.F.M., Khan, K.U., Nascimento, T.M.T., Boaratti, A.Z., Sakomura, N.K., Fernandes, J.B.K., 2020. Ideal profile of essential amino acids for Nile tilapia (*Oreochromis niloticus*) in the finishing growth phase. *Aquaculture Research*. 51, 4724–4735. <https://doi.org/10.1111/are.14819>
- Sari, S.L.A., Triyanto, T., Zuprizal, Z., Prijambada, I.D., 2021. Cellulolytic and mannanolytic aerobic bacteria isolated from Buffalo rumen (*Bubalus bubalis*) and its potency to degrade fiber in palm kernel meal. *Biodiversitas Journal of Biological Diversity*. 22, 2829–2837. <https://doi.org/10.13057/biodiv/d220733>
- Sari, S.L.A., Triyanto, T., Zuprizal, Z., Prijambada, I.D., 2022. Lipolytic and proteolytic activities of fibrolytic bacteria from buffalo (*Bubalus bubalis*) rumen. *Advances in Biological Sciences Research*. 22, 437–442. <https://doi.org/10.2991/absr.k.220406.061>
- Shi, C., Zhang, Y., Lu, Z., Wang, Y., 2017. Solid-state fermentation of corn-soybean meal mixed feed with *Bacillus subtilis* and *Enterococcus faecium* for degrading antinutritional factors and enhancing nutritional value. *Journal of Animal Science and Biotechnology*. 8, 50. <https://doi.org/10.1186/s40104-017-0184-2>
- Siahaan, M., 2025. Indonesia: oil palm production volume 2024 | Statista. Available at: <https://www.statista.com/statistics/706786/production-of-palm-oil-in-indonesia>. [Date accessed: 27 October 2025]
- Takeuchi, T., Satoh, S., Watanabe, T., 1983. Requirement of Tilapia nilotica for essential fatty acids. *Bulletin of the Japanese Society of Scientific Fisheries*. 49, 1127–1134.
- Van Soest, P.J., Robertson, J.B., Lewis, B.A., 1991. Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. *Journal of Dairy Science*. 74, 3583–3597. [https://doi.org/10.3168/jds.S0022-0302\(91\)78551-2](https://doi.org/10.3168/jds.S0022-0302(91)78551-2)
- Vidal, L.V.O., Xavier, T.O., De Moura, L.B., Graciano, T.S., Martins, E.N., Furuya, W.M., 2015. Apparent digestibility of soybean coproduct in extruded diets Nile Tilapia, *Oreochromis niloticus*. *Aquaculture Nutrition*. 23, 228–235. <https://doi.org/10.1111/anu.12383>

- Vieira, L., Filipe, D., Amaral, D., Magalhães, R., Martins, N., Ferreira, M., Ozorio, R., Salgado, J., Belo, I., Oliva-Teles, A., Peres, H., 2023. Solid-state fermentation as green technology to improve the use of plant feedstuffs as ingredients in diets for European Sea Bass (*Dicentrarchus labrax*) Juveniles. *Animals*. 13, 2692. <https://doi.org/10.3390/ani13172692>
- Wathne, A.M., Devle, H., Naess-andresen, C.F., Ekeberg, D., 2018. Identification and quantification of fatty acids in *T. viridissima*, *C. biguttulus*, and *C. brunneus* by GC-MS. *Journal of Lipids*. 2018, 3679247. <https://doi.org/https://doi.org/10.1155/2018/3679247> Research
- Wattanakul, W., Thongprajukaew, K., Hahor, W., Suanyuk, N., 2021. Optimal replacement of soybean meal with fermented palm kernel meal as protein source in a fish meal-soybean meal-based diet of sex reversed red tilapia (*Oreochromis niloticus* × *O. mossambicus*). *Animals*. 11, 2287. <https://doi.org/10.3390/ani11082287>
- Xu, H., Turchini, G.M., Francis, D.S., Liang, M., Mock, T.S., Rombenso, A., Ai, Q., 2020. Are fish what they eat? a fatty acid's perspective. *Progress in Lipid Research*. 80, 101064. <https://doi.org/10.1016/j.plipres.2020.101064>
- Zhao, H.M., Guo, X.N., Zhu, K.X., 2017. Impact of solid state fermentation on nutritional, physical and flavor properties of wheat bran. *Food Chemistry*. 217, 28–36. <https://doi.org/10.1016/j.foodchem.2016.08.062>
- Zhou, L., Abouelezz, K., Momenah, M.A., Bajaber, M.A., Baazaoui, N., Taha, T.F., Awad, A.E., Alamoudi, S.A., Beyari, E.A., Alanazi, Y.F., Allohibi, A., Saad, A.M., 2024. Dietary *Paenibacillus polymyxa* AM20 as a new probiotic: improving effects on IR broiler growth performance, hepatosomatic index, thyroid hormones, lipid profile, immune response, antioxidant parameters, and caecal microorganisms. *Poultry Science*. 103, 103239. <https://doi.org/10.1016/j.psj.2023.103239>
- Zhu, X., Wang, L., Zhang, Z., Ding, L., Hang, S., 2021. Combination of fiber-degrading enzymatic hydrolysis and lactobacilli fermentation enhances utilization of fiber and protein in rapeseed meal as revealed in simulated pig digestion and fermentation *in vitro*. *Animal Feed Science and Technology*. 278, 115001. <https://doi.org/10.1016/j.anifeedsci.2021.115001>