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RESEARCH ARTICLE
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Optimal Fermentation of Sago Pith with Cassava Leaves Using *Bacillus subtilis* Maintains Quail Performance and Egg Quality

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ABSTRACT

The potential of sago pith as an abundant and low-cost carbohydrate source in Indonesia remains underutilized, yet its nutritional value and applicability as poultry feed can be greatly enhanced through fermentation technology. This study investigated the optimization of fermented sago pith to enhance its nutritional content and the subsequent impact on quail performance. This study was conducted in two stages. The first stage employed a 3×3 factorial design with three replicates to evaluate various substrates, sago pith mixed with cassava leaves, indigofera leaves, or tofu dregs, and fermentation duration (2, 4, or 6 days) using *Bacillus subtilis* inoculum. The second stage involved 200 *Coturnix coturnix japonica* quails (8 weeks old, ±10% production), with five treatments and four replications assessing different inclusion levels of fermented sago pith-cassava leaf (FSP-CL) in their diets (0-30%). The result in the first stage showed a highly significant interaction ($P<0.001$) observed between substrate type and fermentation duration, leading to enhanced cellulase and protease activities, as well as improvements in crude protein, crude fiber, crude fiber digestibility, and nitrogen retention. In the feeding trial, inclusion of 25% FSP-CL maintains daily egg production, egg mass, feed conversion, egg weight, and also affects egg yolk color and cholesterol. Optimal results were obtained with a 25% FSP-CL inclusion, resulting in an average daily consumption of 19.96g/head, a 44.41% daily egg production, a 4.54g/head/day egg mass, and a feed conversion ratio of 4.54. The study concluded that fermenting sago pith with 20% cassava leaves for 4 days yields the optimal substrate. Its inclusion in quail feed at 25% effectively maintains quail productivity while reducing feed costs.

Keywords: Fermented sago pith, Cellulase, Protease, Enzyme activity, *Coturnix-coturnix japonica*, Performance.

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INTRODUCTION

The sago pith is the inner portion of the sago trunk after the removal of the outer skin. Sago pith is a relatively inexpensive and readily available source of carbohydrates. In 2021, sago plantations in Indonesia covered 206,150ha, producing 381,065 tonnes (Databoks, 2021). Despite its considerable potential, only 15–25% of sago is utilized, and its application as chicken feed remains underexploited by the community. Sago pith has a low nutritional content, comprising 4.45% crude protein, 1.83% crude fat, 18.22% crude fiber, 0.24% calcium, and 0.65% phosphorus (Fajrona et al., 2023). Processing using fermentation technology is necessary to enhance the value and benefits of the sago pith. The ability of microorganisms to convert starch into protein through fermentation improves the nutritional value and quality of feed materials (Mirnawati et al., 2022a; 2023a; 2024). Fermented feed is more digestible and has an extended shelf life without loss of nutritional value (Ciptaan et al., 2022; Ciptaan et al., 2024). Fermentation

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requires a nutrient-rich substrate as a medium for microbial growth. Djulardi et al. (2023) improved sago pith quality by fermenting a 50:50 mixture of sago pith and Indigofera leaves for three days. This treatment yielded the best results, with a crude protein content of 25.45%, crude fat of 0.02%, crude fiber of 6.40%, nitrogen retention of 59.72%, crude fiber digestibility of 57.34%, and metabolizable energy of 2658.44kcal/kg.

The addition of probiotics has been shown to maintain carcass weight and egg quality in poultry while increasing feed intake (Rafian et al., 2024). *Bacillus subtilis* exhibited the highest protease activity (2.16U/mL) after 46h of incubation (Efendi et al., 2017). *B. subtilis* plays a positive role in the quail diet by suppressing the growth of pathogenic bacteria in the digestive tract, improving serum triglyceride and cholesterol profiles, and enhancing yolk quality (Ermakova et al., 2021). Moreover, *B. subtilis* improves feed quality from agricultural byproducts (Mirnawati et al., 2019; Mirnawati et al., 2022b). Ciptaan et al. (2024) demonstrated that fermentation of 80% soy milk dregs with 20% Indigofera leaves using *B. subtilis* for six days yielded optimal results, including increased phytase enzyme activity (6.71U/mL), crude protein content (41.82%), nitrogen retention (61.41%), crude fiber (10.39%), crude fiber digestibility (56.51%), and metabolizable energy (2199.80kcal/kg).

Several factors must be considered to optimize fermentation results, including substrate composition and fermentation duration. The substrate serves as a growth medium for the microbes and contains the nutrients required for their development. As sago pith has a low protein content, it must be supplemented with high-protein ingredients, such as cassava leaves with 32.13% crude protein, Indigofera leaves with 28.89% crude protein, and tofu dregs with 25.36% crude protein (Djulardi et al., 2023). Another critical factor that influences fermentation is the duration of fermentation, which provides microbes with an opportunity to proliferate. Microbial growth and activity increase with longer fermentation periods (Mirnawati et al., 2019). This extended fermentation process enhances the production of enzymes that break down complex nutrients into simpler forms, thereby improving the protein content and quality of the fermented products.

The most effective substrate identified was subsequently utilized as feed for quails to evaluate its impact on quail performance. The hypothesis of this research is that fermented sago pith with *Bacillus subtilis* can be used up to 30% in quail rations. Therefore, this study aimed to determine the optimal combination of feed ingredients, fermentation period, and quail supplementation levels to maximize egg production and quality.

MATERIALS & METHODS

Inoculum Preparation

The inoculum was rejuvenated using nutrient agar. To prepare the medium, nutrient agar powder was mixed with distilled water, cooked until clear, dispensed into 5mL test tubes, and filled to one-quarter of their volume. The test tubes were sealed with cotton plugs and aluminum foil and sterilized in an autoclave at 121°C for 15 min. After sterilization, the tubes were cooled to a tilted position. Subsequently, pure cultures of *B. subtilis* were inoculated into the tubes using sterile inoculation needles and incubated at room temperature for 24h. The bacterial growth and colony counts were observed following the 24h incubation.

The *B. subtilis* inoculum was prepared using a substrate consisting of 100g of rice bran mixed with 70mL of distilled water. The substrate was sterilized by autoclaving at 121°C and 1atm pressure for 15 min, and then cooled to room temperature (~24°C). The bacteria were diluted in a test tube containing 7mL of physiological NaCl solution before being inoculated onto the substrate. The inoculated substrate was incubated for 4d, after which the inoculum was ready for use in its fresh form.

Substrate and Fermentation Preparation

The substrate consisted of a mixture of sago pith (SP), cassava leaves (CL), indigofera leaves (IL), and tofu dregs (TD) in the following treatment ratios. The SP was initially oven-dried at a temperature of 50–60°C for 24–48h until reaching a constant weight. Subsequently, the dried SP were ground to a fine consistency. The IL was used directly as a substrate mixture without further processing. For each treatment, 100g of substrate was weighed according to the specified composition and placed in 15×25cm polypropylene plastic bags. Subsequently, 70mL of distilled water was added to each bag. The mixtures were sterilized in an autoclave at 12°C and 1atm for 15min, followed by cooling to room temperature. The sterilized substrates were subjected to fermentation.

The sterilized substrate was cooled and inoculated with 10% *B. subtilis* inoculum (Ciptaan et al., 2024), followed by incubation according to the treatment. After harvesting the fermented sago pith, protease activity was assessed, and the product was dried at 50–60°C until a constant weight was achieved. The dried fermented product was then pulverized and prepared for subsequent analysis before being fed to quails to evaluate the digestibility of crude fiber and nitrogen retention values.

Enzyme Activity

Protease activity was measured as described previously (Cupp-Enyard, 2008). First, 2.5mL of 1% casein solution was pipetted into a test tube and mixed with 0.1 M phosphate buffer. The mixture was homogenized using a vortex mixer and incubated in a water bath at 37°C for 10min. Subsequently, 1mL of the enzyme extract was added, and the mixture was incubated again in a water bath at 50°C for 10 min. The solution was then centrifuged at 5000rpm for 15min at 4°C, and the supernatant was filtered. Next, 2mL of the supernatant was pipetted into a test tube, followed by the addition of 5mL of 0.5N NaOH and 0.5mL of Folin-Ciocalteu reagent. The mixture was allowed to stand for 10min, and the absorbance was measured using a spectrophotometer at a wavelength of 650nm.

The cellulase activity assay began with the extraction of crude enzymes. For each treatment, 10g of the sample was weighed and transferred into an Erlenmeyer flask, followed by the addition of 90mL of 0.05M phosphate buffer. The flask was sealed with aluminum foil and placed in a shaker for 2h. After shaking, the mixture was removed from the shaker, filtered, and centrifuged at 5000rpm for 15min at 4°C. The supernatant was collected to measure cellulase activity. Next, 1mL of the crude enzyme was mixed with 1mL of the substrate solution (0.5mL CMC + 10mL acetate buffer) and incubated at 40°C for 30min in a shaking water bath. After incubation, 1mL of the reaction mixture was mixed with 1mL of acetone and heated in boiling water for 20min. After cooling, 1mL phosphomolybdate and 7mL distilled water were added. Absorbance was measured at 575nm.

Chemical Analysis

Chemical analysis of the experimental feed materials was conducted according to the AOAC guidelines (AOAC, 2006). Nitrogen retention and crude fiber digestibility were measured using the methodology described by Sibbald (1975). The chemical compositions of the feed ingredients are presented in Table 1, and the experimental feed compositions are presented in Table 2.

Table 1: Composition of feed ingredients, content of nutrients, and metabolic energy (kcal/kg) treatment rations

Feed ingredients	Composition of experimental feed (%)				
	R_1	R_2	R_3	R_4	R_5
Corn	45.5	39	37	36	32.5
Soybean meal	16	10	8	6	4
Rice bran	18	18	18	18	18
FSP-CL*	0	15	20	25	30
Coconut oil	14.5	12	11	9	9.5
Fish meal	2	2	2	2	2
Mineral	2.5	2.5	2.5	2.5	2.5
Bone meal	1.5	1.5	1.5	1.5	1.5
Total	100	100	100	100	100

*Fermented sago pith and cassava leaves

Table 2: Chemical content of feed substances (% DM) and metabolic energy (kcal/kg) of experimental feed

Item	Experimental feed				
	R_1	R_2	R_3	R_4	R_5
Crude protein (%)	20.03	20.15	20.20	20.24	20.28
Crude fat (%)	5.12	5.16	5.17	5.19	5.20
Crude fiber (%)	4.01	4.12	4.14	4.04	4.24
Calcium (%)	2.59	2.52	2.49	2.43	2.44
Phosphorus (%)	0.94	0.95	0.95	0.95	0.96
Carotenoid (%)	20.92	31.52	35.28	39.04	42.80
Methionine (%)	0.57	0.52	0.51	0.49	0.48
Lysine (%)	1.57	1.46	1.42	1.39	1.35
Metabolic energy (kcal/kg)	2812.60	2818.20	2822.40	2840.60	2846.55

Formulation of the Experimental Diets

The first stage aimed to determine the substrate composition and optimal fermentation duration to enhance the content and quality of sago pith fermented with *B. subtilis*. The treatments included Factor A and substrate compositions: A_1 = 80% SP + 20% CL, A_2 = 80% SP + 20% IL, and A_3 = 80% SP + 20% TD. Factor B: fermentation time with three durations tested: B_1 = 2d, B_2 = 4d, and B_3 = 6d.

In the second stage, the effects of the optimal substrate on quail performance, egg production, and quality were assessed. The treatments consisted of different levels of fermented sago pith-cassava leaf (FSP-CL) inclusion in the quail diet: R_1 (0%), R_2 (15%), R_3 (20%), R_4 (25%), and R_5 (30%) FSP-CL. All diets were formulated to contain 20% iso-protein and 2800kcal/kg iso-energy. The treatment ratio was formulated to contain 20% crude protein and 2,800kcal/kg metabolic energy (Djulardi, 1995). The nutrient composition (%) and metabolic energy (kcal/kg) of the treatment groups are presented in Table 1.

Housing

This experiment involved 200 *Coturnix-coturnix japonica* quails aged 8 weeks, with an average production rate of approximately 10%. The experimental setup consisted of 20 box cages constructed of wood and wires. Each cage measured 65cm×45cm×25cm and was equipped with feeders and drinkers. Ten quail were housed in each cage. For nighttime illumination, a 60watt incandescent lamp was utilized. To maintain cleanliness, each cage was lined with a triplex base covered in plastic to collect the excreta.

Feed Intake

Feed intake was calculated by subtracting the amount of leftover feed at the end of the week from the total feed provided and then dividing the result by the number of birds to determine weekly consumption. To derive the daily ration consumption, this value was further divided into seven categories.

$$\text{Feed intake} = \left(\frac{\text{Total Feed Given} - \text{Leftover Feed}}{10}\right)/7$$

Daily Egg Production

Daily egg production was calculated by dividing the number of eggs laid each day by the number of live quail and multiplying by 100 to express the results as a percentage.

Feed Conversion

The feed conversion was calculated by dividing the total feed consumed by the total egg mass.

Egg Mass Production

Egg mass was calculated by multiplying the daily egg production by the average egg weight.

Egg Weight

The egg weight was measured daily throughout the study period. Weekly averages were computed, and these values were further averaged to derive the mean egg weight over the four-week study period.

Egg Yolk Color

Egg yolk color was evaluated using a Roche Yolk Color Fan, which provides a standardized color score ranging from 1 to 15.

Egg Yolk Cholesterol

Cholesterol content was determined by extracting cholesterol using the Lieberman-Burchard method (Xiong et al., 2007).

Statistical Analysis

The feed fermentation experiment was conducted using a completely randomized design (CRD) with three replicates in a 3×3 factorial arrangement. The quail experimental data were analyzed using CRD with five treatments, each replicated four times. Differences between treatments were assessed using Duncan's Multiple Range Test. The correlation between enzyme activity and several fermentation parameters was evaluated by regression statistics using the JASP software (Goss-Sampson, 2025).

RESULTS

Protease Activity

As illustrated in Fig. 1, the results demonstrated a highly significant effect ($P<0.001$) of the fermentation period (Factor B), whereas no significant effect ($P>0.05$) was observed for feed composition (Factor A). More specifically, a significant difference ($P<0.05$) was identified between the fermentation periods B_2 and B_3. The data indicate that treatment A_1 (80% SP + 20% CL) yielded higher protease activity than treatments A_2 (80% SP + 20% IL) and A_3 (80% SP + 20% TD) across all fermentation periods. Optimal protease activity was observed in the A1B2 treatment, which comprised 80% SP + 20% CL with a fermentation period of 4 days.

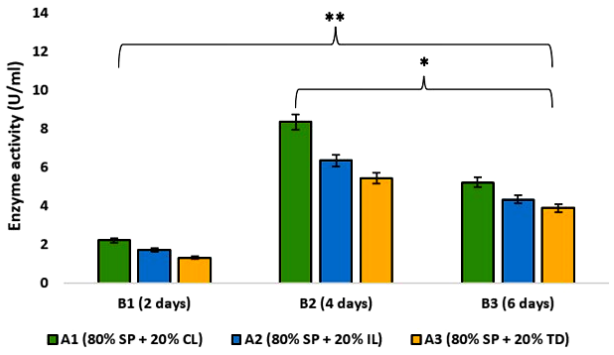

Fig. 1: Protease enzyme activity of substrate with different fermentation period. **: P<0.001; *: P<0.05. Abbreviations: SP: sago pith; IL: indigofera leaves: TD: tofu dregs.

Cellulase Activity

The analysis revealed a significant interaction ($P<0.01$) between substrate composition and fermentation duration, with both factors exhibiting a highly significant impact ($P<0.01$) on cellulase activity (Fig. 2). However, no significant difference ($P>0.05$) was observed between fermentation periods B_1 and B_3. The addition of 20% cassava leaves (A1) to the substrate resulted in the highest cellulase activity for all fermentation durations. Optimal cellulase activity was observed with the A_1B_2 treatment.

Chemical Content of Fermentation Substrate

The results in Table 3 indicate a significant interaction ($P<0.001$) between Factors A (substrate composition) and B (fermentation duration), with each Factor A exhibiting a significant effect ($P<0.01$) on the crude protein content. As shown in Table 3, treatment A_1 yielded a higher crude protein content than treatments A_2 and A_3 across all fermentation durations. The optimal crude protein content was observed in treatment A_1B_2, which comprised 80% SP + 20% CL with a fermentation duration of 4 days.

Table 3: Effect of substrate composition with fermentation time on crude protein, crude fiber, nitrogen retention and digestibility of crude fiber of fermented sago pith

Parameters (% DM)	Factor A (substrate composition)	Factor B (fermentation period)			SEM	SE
		B_1 (2 days)	B_2 (4 days)	B_3 (6 days)		
Crude protein	A_1 (80% SP + 20% CL)	20.58^{cA}	24.29^{aA}	22.18^{bA}	1.08	0.11
	A_2 (80% SP + 20% IL)	19.28^{cB}	21.27^{aB}	20.03^{bB}	0.58	
	A_3 (80% SP + 20% TW)	17.42^{cC}	20.58^{aC}	19.12^{bC}	0.91	
Crude fiber	A_1 (80% SP + 20% CL)	8.15^{aC}	6.31^{cC}	7.36^{bC}	0.53	0.07
	A_2 (80% SP + 20% IL)	10.27^{aB}	7.24^{cB}	9.20^{Bb}	0.89	
	A_3 (80% SP + 20% TW)	11.12^{aA}	9.37^{cA}	10.38^{bA}	0.51	
Nitrogen retention	A_1 (80% SP + 20% CL)	50.71^{cA}	57.25^{aA}	54.68^{bA}	1.90	0.25
	A_2 (80% SP + 20% IL)	48.43^{cB}	53.22^{aB}	50.27^{bB}	1.40	
	A_3 (80% SP + 20% TW)	45.37^{cC}	50.47^{aC}	46.97^{bC}	1.51	
Crude fiber digestibility	A_1 (80% SP + 20% CL)	52.23^{cA}	57.60^{aA}	55.42^{bA}	1.56	0.27
	A_2 (80% SP + 20% IL)	47.38^{cB}	56.10^{aB}	49.35^{bB}	2.64	
	A_3 (80% SP + 20% TW)	45.47^{cC}	53.31^{aC}	47.13^{bC}	2.38	

Different lowercase letters in the same row and different uppercase letters in the same column indicate significant differences ($P<0.001$).

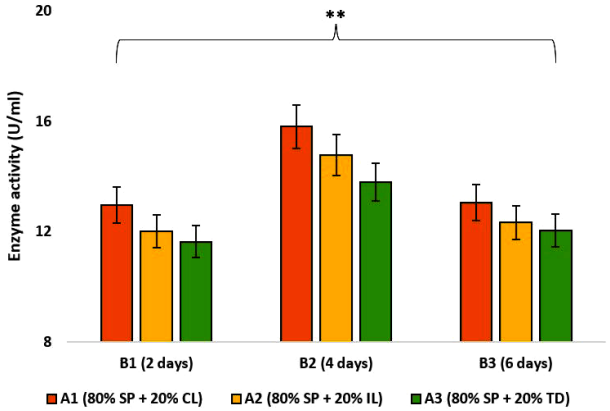

Fig. 2: Cellulase enzyme activity of substrate with different fermentation period. **: P<0.001. Abbreviations: IL: indigofera leaves: CL: cassava leaf; TD: tofu dregs.

The findings revealed a significant interaction ($P<0.01$) between Factors A and B, with each factor exhibiting a highly significant impact ($P<0.01$) on crude fiber content. As illustrated in Table 3, treatment A_1 yielded higher nitrogen retention than treatments A_2 and A_3 for all fermentation durations (B_1, B_2, and B_3). Optimal nitrogen retention was observed in treatment A_1B_2, which comprised 80% SP + 20% CL, with a fermentation duration of 4days. During fermentation, the addition of cassava leaves to the substrate resulted in the highest digestibility of crude fiber. The A_1B_2 treatment demonstrated the best fiber digestibility value, with the inclusion of 20% cassava leaves contributing to the highest crude protein levels, followed by 20% Indigofera leaves (A_2) and 20% tofu dregs (A_3). Regression analysis (Fig. 3) indicated a positive effect of substrate fermentation by *B. subtilis*. Beneficial enzyme activity in animals further enhances production-supporting parameters such as nutrient digestibility and nitrogen retention.

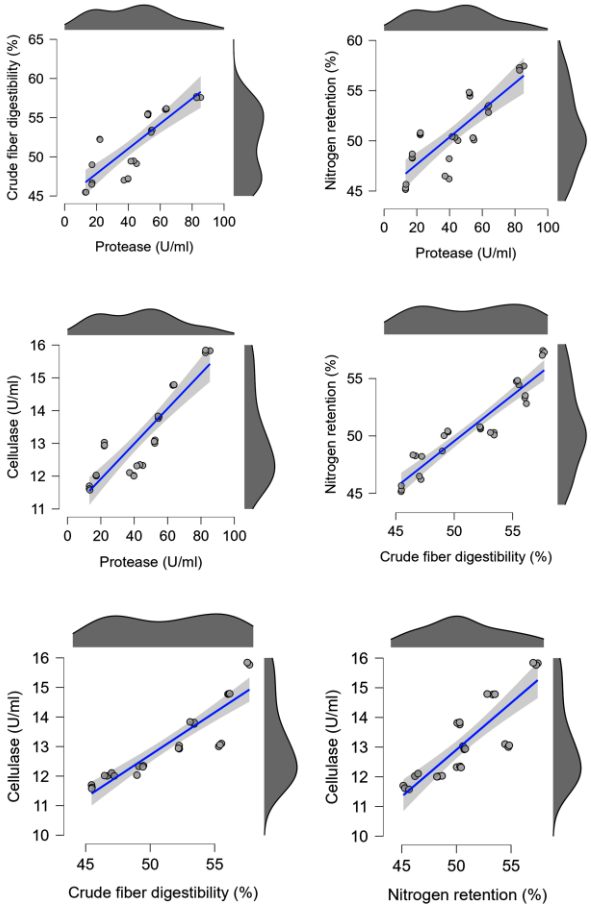

Fig. 3: Correlation between enzymes activity with crude protein, crude fiber, nitrogen retention and crude fiber digestibility.

Feed Intake

The inclusion of FSP-CL in quail rations had a significant effect on feed intake ($P<0.001$). The average feed consumption in treatment R_1 was not significantly different ($P>0.05$) from that in treatments R_2 and R_3, but it was significantly higher ($P<0.05$) than that in R_4 and was significantly higher ($P<0.001$) than that in R_5.

Daily Egg Production

Diversity analysis revealed that the inclusion of FSP-CL in quail diets had a significant effect ($P<0.001$) on egg production. As shown in Table 4, the lowest average daily egg production was observed in the R_5 (30% FSP-CL) treatment at 40.68%, whereas the highest average was recorded in the R_1 (0% FSP-CL) treatment at 45.44%.

Egg Mass

The inclusion of sago pith and cassava leaves fermented with *B. subtilis* (FSP-CL) in quail diets had a significant effect on egg mass production ($P<0.001$). As illustrated in Table 4, the lowest average egg mass was observed in the R_5 (30% FSP-CL) treatment at 4.11g/head/day, while the highest average was recorded in the R_1 (0% FSP-CL) treatment at 4.69g/head/day.

Feed Conversion

The inclusion of fermented sago pith and cassava leaves with *B. subtilis* (FSP-CL) in quail rations had a significant effect on feed conversion ($P<0.05$). As shown in Table 4, the lowest average feed conversion ratio was observed in R_1 (0% FSP-CL) at 4.39, whereas the highest average was recorded in R_5 (30% FSP-CL) at 4.78.

Table 4: Effect of providing FSP-CL in quail production and egg quality

Parameters	Experimental feed					SE
	R_1 (0%)	R_2 (15%)	R_3 (20%)	R_4 (25%)	R_5 (30%)	
Feed intake (g/head/day)	20.27^a	20.06^b	20.02^b	19.96^b	19.28^c	0.08
Daily egg production (%)	45.44^a	44.76^a	44.46^a	44.41^a	40.68^b	0.62
Egg mass (g/head/day)	4.69^a	4.60^a	4.55^a	4.54^a	4.11^b	0.06
Feed conversion	4.39^b	4.44^b	4.43^b	4.54^b	4.78^a	0.07
Egg weight (g)	10.31^a	10.28^a	10.26^a	10.24^a	10.12^b	0.02
Egg yolk color	7.17^c	7.67^{bc}	7.83^{bc}	8.33^b	9.42^a	0.22
Cholesterol (mg/100g)	403.33^a	372.78^{ab}	348^{bc}	314.98^c	299.58^c	17.05

Description: ^{a,b,c} = Means with different superscripts indicate a significant difference ($P<0.01$). SE = Standard Error.

Egg Weight

The inclusion of fermented sago pith and cassava leaves with *B. subtilis* had a significant effect ($P<0.001$) on the average weight of the quail eggs. As detailed in Table 4, the lowest average egg weight was observed in the R_5 treatment (30% FSP-CL) at 10.12g/egg, while the highest average was recorded in the R_1 treatment (30% FSP-CL) at 10.31g/egg.

Egg Yolk Color

The inclusion of sago pith and fermented cassava leaves in the ration had a significant effect ($P<0.01$) on the average color of quail egg yolk. As illustrated in Table 4, the lowest average egg yolk color score was observed in R_1 treatment (0% FSP-CL) at 7.17, while the highest average score was recorded in R_5 treatment (30% FSP-CL) at 9.42.

Cholesterol

Substituting quail rations with FSP-CL had a significant effect ($P<0.01$) on the average cholesterol content of quail egg yolks. As presented in Table 4, the lowest average cholesterol level was observed in the R_5 treatment (30% FSP-CL) at 299.58mg/100g, while the highest average was recorded in the R_1 treatment (0% FSP-CL) at 403.33mg/100g.

DISCUSSION

The high protease activity observed in FSP-CL could be attributed to its large microbial population. Substantial microbial growth was facilitated by the higher crude protein content of cassava leaves compared to that of Indigofera leaf flour and tofu dregs. The protein in the substrate serves as a nutrient source for microbial growth; thus, a higher substrate protein content promotes increased microbial proliferation. In turn, greater microbial growth enhances enzyme production, particularly protease production. This observation aligns with the findings of Fitriana and Asri (2022), who reported that enzyme secretion by bacteria increases proportionally with bacterial cell growth. Furthermore, elevated protease activity was consistent with the characteristics of *B. subtilis*,

a known protease-producing bacterium. This corroborates the assertion of Efendi et al. (2017) that *B. subtilis* exhibits high proteolytic activity, producing a peak protease yield of 2.16U/mL at the 46-h incubation.

The 4-day fermentation period yielded the highest protease activity compared with the 2-day and 6-day fermentation periods. During the 2-day period, the microorganisms were still in the adaptation phase, resulting in limited growth and consequently reduced protease activity. Conversely, the 6-day fermentation period was excessively prolonged, causing the microbial population to enter the dying phase. Fermentation provides microbes with an opportunity to grow by utilizing the available nutrients in the substrate; however, an extended fermentation period depletes these nutrients, thereby inhibiting microbial growth. The total number of bacterial colonies declined during the 6-day fermentation period, directly reducing enzyme activity, particularly protease activity.

The higher cellulase activity was attributed to substantial microbial growth, as evidenced by the total colony count. This proliferation was facilitated by the inclusion of 20% cassava leaves in the substrate, which provided the highest crude protein content compared with substrates containing 20% indigofera leaves or 20% tofu dregs. Crude proteins supply nitrogen, which is critical for microbial cell growth and enzyme synthesis. This aligns with the findings of Spohn et al. (2016) who demonstrated that substrate protein availability was directly correlated with enzyme production. Further supporting this, Fitriana and Asri (2022) noted that bacterial enzyme secretion increased with increasing cell growth. Notably, *B. subtilis*, as highlighted by Bilyartinus and Siswanto (2021), is a prolific cellulase producer that outperforms other bacterial strains in terms of enzyme yield.

This phenomenon aligns with the findings of Krishna and Devi (2005), who demonstrated that fermentation can elevate crude protein levels due to the contribution of microbial biomass, yielding single-cell protein products containing 40–65% protein. The fermentation duration further modulated the crude protein dynamics. While the 2-day and 4-day periods enhanced the protein content, the 6-day period led to nutrient depletion and reduced microbial viability. The 4-day fermentation period was optimal, striking a balance between microbial growth and substrate integrity. Beyond this period, prolonged fermentation exhausts nutrients, diminishes microbial populations, and subsequently, enzyme production.

The lowest crude fiber content was observed when cassava leaves were incorporated into the substrate during fermentation. This was particularly evident in FSP-CL, which yielded the optimal crude fiber concentration. The addition of 20% cassava leaves provided the highest crude protein content, surpassing that of the substrates with 20% indigofera leaves and 20% tofu dregs. Increased protein availability in the substrate enhances enzyme production, particularly that of cellulase, which hydrolyzes cellulose into glucose, thereby reducing crude fiber production by the end of the fermentation. This aligns with the results of Sudharmono et al. (2016), who noted that crude fiber reduction correlates with subsequent cellulase synthesis. High enzyme production will lead to high digestibility. This relationship aligns with the findings of Widodo et al. (2023), who identified a negative correlation between fiber fraction and digestibility; lower crude fiber concentrations correspond to higher digestibility. Similarly, Mirnawati et al. (2023) emphasized that crude fiber digestibility diminishes as its content in feed increases.

Yunus and Zubaidah (2015) noted that shorter fermentation periods limit enzyme production due to insufficient microbial growth, whereas excessively long periods deplete substrate nutrients, undermining microbial viability. However, this result contrasts with the findings of Ciptaan et al. (2024), who reported a higher crude fiber content (10.49%) after 6 days of fermentation using *B. subtilis* and a substrate mixture of indigofera leaves and soymilk dregs.

FSP-CL exhibited notable nitrogen retention, which was attributed to the enzymatic activity of *B. subtilis*. This microorganism synthesizes proteases that hydrolyze proteins into absorbable amino acids, thereby enhancing the nutritional quality of the fermented protein mixture composed of cassava leaves and sago pith. Elevated nitrogen retention is directly correlated with improved protein quality (Mahfudz, 2018). Their research highlighted microbial enzymatic activity in simplifying complex compounds (e.g., proteins into amino acids), facilitating nutrient absorption and nitrogen retention in livestock.

The low nitrogen retention observed during the 6-day fermentation can be attributed to the low crude protein content of the substrate. Nitrogen retention values are intrinsically linked to the crude protein content of the material because the nitrogen assimilated by livestock corresponds directly to the protein available in the feed. Furthermore, nitrogen excreted in feces inversely influences nitrogen retention, as reported by Maynard et al. (2005). This result contrasts with that of Ciptaan et al. (2024), who reported 61.41% nitrogen retention for a substrate composed of 80% soymilk dregs and 20% Indigofera leaf flour fermented with *B. subtilis* over a 6-day period.

This study demonstrated a decline in feed intake concomitant with the increased incorporation of FSP-CL in quail rations. However, this reduction was not statistically significant, as the fermentation process enhanced the aroma, taste, and texture of the rations, rendering them palatable. This aligns with the findings of Muslim and Mirzah (2012), who noted that fermented feed products often exhibit superior sensory attributes compared with their unprocessed counterparts. Moreover, the energy content of the rations remained consistent across treatments, which likely contributed to the uniformity of feed consumption. As highlighted by Djulardi (2022), poultry regulate their feed intake based on energy sufficiency, and cease consumption once their metabolic needs are met.

The 30% FSP-CL substitution resulted in a marked reduction in feed intake attributable to the diminished use of corn and soybean meal, which altered the color of the ration. As posited by Sari and Yoshi (2019), poultry exhibit

a preference for lighter-colored rations, which enhance palatability, and consequently, feed intake. The observed quail feed intake ranged from 19.28–20.27g/head/day, surpassing the values reported by Lokapirnasari et al. (2024) (14.38–19.22g/head/day) for rations supplemented with probiotics and acidifiers. Conversely, these results fell below the 24.13–24.45g/head/day documented by Sukri and Intan (2022) in studies involving papaya leaf flour as an alternative feed additive.

This study revealed a declining trend in the daily quail egg production as the proportion of FSP-CL in the feed rations increased. However, as shown in Table 4, the differences in egg production across the treatments were not statistically significant. This uniformity suggests that the nutrient intake from the rations was comparable, leading to similar levels of egg production. The fermentation process enhances protein quality and digestibility, facilitating better nutrient absorption by quails. This aligns with the findings of Mirnawati et al. (2023b) who demonstrated that fermentation improves protein digestibility, thereby increasing the availability of amino acids for egg production. The key factors influencing egg production include genetic strain, age at first laying, feed consumption, and nutrient composition of the ration (Djulardi, 2022).

The observed reduction in egg production at the 30% FSP-CL level could be attributed to lower ration consumption and reduced protein intake. In the present study, egg production rates remained relatively low, which may reflect the quail's pre-peak production phase. Daily egg production in this study ranged from 40.68–45.44%, which exceeded the 14–22% reported by Zahra et al. (2012) in their free-choice feeding experiment. The lower production rates in this study may stem from the quails' developmental stage, as they had not yet reached their peak production age. Additionally, despite its benefits, the nutrient profile of FSP-CL may not fully compensate for reduced protein consumption at higher inclusion levels.

This study indicated a declining trend in egg mass production as the inclusion of FSP-CL in quail diets increased. This reduction was primarily due to egg production rates averaging only 40% as quails were still in the early stages of laying. The non-significant differences in egg mass production across treatments could be attributed to similar egg production rates and uniform protein intake from the rations. The lowest egg mass production in the 30% FSP-CL treatment resulted from suboptimal egg weight and production rates. In this study, egg mass production ranged from 4.11–4.69g/head/day, which is higher than the 3.39–4.60g/head/day reported by Azizen et al. (2022) under feed-restriction conditions.

The non-significant difference in egg production across treatments indicated that FSP-CL were relatively efficient in supporting egg production. This aligns with the findings of Raoda et al. (2024), who reported that lower feed conversion was correlated with higher feed efficiency. The quality of the ration plays a pivotal role in feed conversion, as highlighted by Zampiga et al. (2021), with factors such as genetics and feed quality (amino acids and feed enzymes) influencing the outcomes. The elevated feed conversion observed in the 30% cassava leaf treatment can be attributed to a decline in egg mass production because feed conversion is directly influenced by ration consumption and egg mass. Notably, quail exhibit higher feed conversion values (3.3–4.9) compared to broilers (1.3–2.2), as documented by Khalil (2015). In this study, the feed conversion ranged from 4.39 to 4.78, which is higher than the 2.14–2.86 reported by Sukri and Intan (2022) in studies involving papaya leaf flour as an alternative feed.

The declining trend in egg weight increased with the inclusion of FSP-CL in quail rations. This phenomenon may be attributed to several factors, including age at first egg laying, as noted by Syaroni et al. (2020), who observed that egg weight was influenced by quail age at first laying, with older birds producing heavier eggs. The tendency for egg weight to decrease with increasing FSP-CL in quail rations is thought to be due to several factors, such as protein and amino acids in the feed. This is consistent with the opinion of Patterson & Burley (2017) that adequate protein and amino acids in the feed are the main factors influencing egg size. The increase in total and average egg weight is due to the addition of supplements containing the essential amino acid methionine, which cannot be produced in the body and therefore must be obtained from the feed. Methionine is an essential amino acid that influences egg weight. Table 4 shows non-significant differences in egg weight across treatments, likely due to uniform protein and ration consumption. Protein intake is a key determinant, with Azizen et al. (2022) highlighting that optimal protein levels enable quails to maximize egg weight.

In the present study, the average egg weight ranged from 10.12 to 10.31g/egg, which aligns with the findings of Djulardi (2022), who reported that quail egg weight typically falls within the range of 9–10g/egg. These results are higher than those of Satria et al. (2021), who reported an average egg weight of 8.08–8.96g/egg in quails fed cassava leaf flour silage. Furthermore, the egg weight observed in this study exceeds the 9.57g/egg reported by Fajrona et al. (2023) for quails fed rations containing palm kernel cake fermented with *B. subtilis*. The increase in egg yolk color observed in this study can be attributed to the elevated carotenoid content of the diets, which ranged from 20.92-42.80mg/kg. This aligns with the findings of Dansou et al. (2023), who noted that carotenoids, including β-carotene, zeaxanthin, and lutein, contribute to yellow, orange, and red hues in egg yolks, thereby enhancing color quality. In the present study, the yolk color values ranged from 7.17-9.42. However, these results remain lower than the 9.00–11.50 documented by Nuraini and Latif (2012) for quails supplemented with sago dregs and tofu dregs fermented with *Neurospora crassa*.

The observed increase in egg yolk pigmentation can be attributed to the elevated carotenoid content in the treatment ration, primarily derived from cassava leaves, which contain 154mg/kg of β-carotene as reported by Mustakim et al. (2023). β-carotene, an active compound, plays a significant role in reducing cholesterol levels in quail eggs; higher dietary β-carotene intake correlates with

lower egg cholesterol content. This mechanism is supported by Nuraini and Djuliardi (2019), who demonstrated that β-carotene inhibits the activity of the HMG-CoA reductase enzyme, a key regulator of cholesterol biosynthesis. Furthermore, Lachenmeier et al. (2012) showed that HMG-CoA reductase catalyzes the conversion of HMG-CoA to mevalonate, a precursor of hepatic cholesterol synthesis, and that its inhibition reduces cholesterol production. In this study, the incorporation of a 30% mixture of sago pith and cassava leaves fermented with *B. subtilis* in the ration reduced quail egg yolk cholesterol by 21.9% (314.98mg/100g). This result exceeds the 213.31mg/100g reported by Reski et al. (2023) for quails fed a ration containing fermented *Turbinaria murayana* seaweed.

Conclusion

In conclusion, fermentation of a substrate composed of 80% sago pith and 20% cassava leaves with *Bacillus subtilis* for four days markedly improved its nutritional value, as shown by increased enzyme activity, higher crude protein, and better digestibility. When included at 25% in quail diets, the fermented product matched the conventional feed in terms of daily egg production, egg mass, feed conversion, and egg weight, while enhancing yolk color and lowering egg cholesterol.

This study provides novel evidence that FSP-CL can serve as a sustainable, low-cost feed ingredient derived from locally available resources. Its application offers a practical alternative for smallholder quail farmers seeking to reduce feed costs and improve production performance. Future work should focus on evaluating the long-term effects of FSP-CL on quail health and reproductive traits, its influence on broader egg quality parameters, and the economic feasibility of its use under field conditions.

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Data Availability: The data presented are available within the article.

Ethics Statement: Animal maintenance standards in this study followed the Regulations of the Minister of Agriculture of the Republic of Indonesia Number 33/Permentan/OT.140/2/2014 concerning the Guidelines for Good Quail Farming.

Author's Contribution: M and GC supervised and coordinated this study. RKR and GY designed and conducted the experiments. M and AS drafted the manuscript. MM performed statistical analyses and interpreted the data.

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