2023, Scienceline Publication
World's Veterinary Journal
World Vet J, 13(4): 580-586, December 25, 2023
ISSN 2322-4568
DOI: https://dx.doi.org/10.54203/scil.2023.wvj62

Improving the Quality and Nutritional Value of a Mixture of Sago Pith and Indigofera Leaves Fermented with *Rhizopus oligosporus*

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ORIGINAL ARTICLE
pii: S232245682300062-13
Received: 28 September 2023
Accepted: 20 November 2023

ABSTRACT

The nutritional value of sago pith is limited due to its low protein content, making it less suitable for poultry feed. To increase the benefit values of the sago pith, it is necessary to process it through fermentation. The current study aimed to determine the effects of substrate composition and fermentation time of fermented sago (*Metroxylon sagu*) pith (SP) and *Indigofera* (*Indigofera zollingeriana*) leaves (IL) mixture using *Rhizopus oligosporus* as an inoculum on crude protein, crude fat and crude fiber content of fermented SP and IL, nitrogen retention, crude fiber digestibility, and energy metabolism in broiler chickens. The study was performed on 30 broiler chickens, average weighing ± 1.5 kg at 6 weeks of age, along with SP, IL, and *R. oligosporus*. This experiment was conducted using a randomized design in a 3×3 factorial with three replications. Substrate composition, or factor A, was made up of A1 (80% SP + 20% IL), A2 (60% SP + 40% IL), and A3 (50% SP + 50% IL). Fermentation time as Factor B entailed B1 (2 days), B2 (3 days), and B3 (4 days). The findings demonstrated a significant interaction between the time of fermentation and the composition of the substrate in relation to crude protein content, nitrogen retention, crude fat, crude fiber digestibility, and energy metabolism. It can be concluded that the composition of substrate 50% SP and 50% IL with 3 days of fermentation yielded the best result, with crude protein at 25.45%, nitrogen retention at 59.72%, crude fat at 0.020%, crude fiber at 6.40%, crude fiber digestibility at 57.34%, and metabolic energy at 2658.44 kcal/kg.

Keywords: Broiler chickens, Crude protein, *Indigofera*, Sago pith, *Rhizopus oligosporus*

INTRODUCTION

Sago pith extracted from the inner part of the sago stem after removing the outer skin is a readily accessible and economical source of carbohydrates (Syartiwidya, 2023). Indonesia, in particular, boasts a substantial supply, with sago pith covering 206,150 hectares in 2021 and producing 381,065 tons (Ministry of Agriculture, 2021). Despite its vast potential, only 15-20% of sago is consumed in the human diet. The pith is an abundant by-product in sago processing with great potential as a substitute poultry feed. The nutritional content of sago pith includes 4.45% crude protein, 1.83% crude fat, 8.22% crude fiber, 0.24% calcium, 0.65% phosphorus, and metabolic energy of 2,803 kcal/kg (Fajrona et al., 2023). Therefore, it can be inferred that the nutritional content of sago pith is of low quality because the protein content is too low, limiting its use for poultry feed. To increase the benefit values of the sago pith, it is necessary to process it by fermentation.

Fermentation is a process that can increase the protein content of feed through microorganisms that can convert starch into protein (Mirnawati et al., 2022). Fermented feeds are easier to digest and last longer without losing the nutritional value of the feed (Ciptaan et al., 2022). In the study conducted by Welvidani (2012), sago pith was subjected to fermentation using *Bacillus amyloliquefaciens* supplemented with Zinc (Zn), urea, and sulfur. The combination treatment of Zn (0.0025%), urea (3.0%), and sulfur (0.2%) yielded the best results. This treatment reduced crude fiber content from 18.61% to 12.425%, increased crude fiber digestibility from 29.74% to 53.336%, and increased metabolic energy from 1,777 kcal/kg to 2,525 kcal/kg.

In the current study, *Rhizopus oligosporus* (*R. oligosporus*) was used as the microorganism inoculum, a mold that is widely used in the production of fermented food, such as tempeh. Being saprophytic, it is widely found in nature. Protease, lipase, alpha-amylase, glutaminase, and alpha-galactosidase are all produced by *R. oligosporus* (Han et al.,

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2003). These enzymes play a pivotal role in facilitating the substrate hydrolysis, enhancing its digestibility in the poultry digestive tract. Wattiheluw (2012) used *R. oligosporus* to ferment a mixture of 90% chicken manure and 10% bran. The results showed an increase in crude protein content of 34.43%, a decrease in crude fiber content of 10.99%, and a decrease in crude fat content of 2.71%.

There are several factors to consider in the fermentation process, namely the composition of the substrate and the fermentation time. The nutrient-rich substrate is a growing medium for microorganisms. Availability of nutrients directly influences microbial growth; when ample nutrients are present, microorganisms proliferate accordingly. Moreover, the duration of fermentation plays a pivotal role. The amount of produced enzymes depends on the duration of fermentation. More microbes can proliferate and grow during longer fermentation times (Mirnawati et al., 2019a). More enzymes are produced when microbes grow, allowing more complex nutrients to be broken down into simpler forms and improving the quality and digestibility of the fermented product (Ciptaan et al., 2022).

Since the protein content of sago pith is low, it needs to be mixed with high-protein-content ingredients, such as Indigofera leaves. Indigofera are forages that are quite productive and have a high protein content. According to Sirait et al. (2012), *Indigofera zollingeriana* accounts for an average production of 63.57% of the total fresh yield, indicating its great potential as an alternative feed. Moreover, Indigofera also has relatively high nutritional content, including 21.75% crude protein, 0.19% crude fat, 26.99% crude fiber, 1.32% calcium, and 0.24% phosphorus. In addition, Indigofera leaves also contain carotenoids and xanthophylls that act as antioxidants in broiler rations (Akbarillah et al., 2008). The fermentation of a mixture of sago pith and Indigofera leaves is expected to compensate for the lack of nutritional content in sago pith, leading to the optimal use of this substrate mixture in poultry rations.

The current study aimed to obtain a combination of a mixture of sago pith and Indigofera leaves with the optimal period of fermentation with *R. oligosporus* on the nutritive value of fermented sago pith and Indigofera leaves, nitrogen retention, crude fiber digestibility and metabolic energy in broilers.

MATERIALS AND METHODS

Ethical approval

The broiler-rearing methods used in this study follow the regulation of the Minister of Agriculture of the Republic of Indonesia No. 31/Permentan/OT.140/2/2014 on guidelines for standard broiler and layer poultry farming.

Study materials

The current study was performed using sago pith, Indigofera leaves, *R. oligosporus* inoculant obtained through a mixture of rice bran and cassava leaf meal in a ratio of 9:1 (Annisa et al., 2020). Additionally, distilled water, NaOH, H_2SO_4, indicator methyl orange, Na_2CO_3, acetone, selenium, alcohol, and chemical substances for proximate analysis were employed. The study involved 30 female broiler Cobb 500 strain aged 6 weeks, with an average weight of 1.5 kg, consisting of 27 animals for treatment and 3 animals for correction factor. The sample used was a sago pith from a sago trunk sales company in Padang City, Indonesia. The sago pith was chopped and then sun-dried for 24 - 48 hours. The dried sago pith was then ground into fine granules. Indigofera leaves were obtained from the teaching farm, Faculty of Animal Science, Andalas University, Padang City, Indonesia.

Feed and substrate preparation

The substrate used was sago pith (SP) and Indigofera leaves (IL) in the proportions of 80% SP + 20% IL, 60% SP + 40% IL, and 50% SP + 50% IL. The experimental procedure involved weighing the substrate to 100 grams based on its composition and placing it in polypropylene plastic containers. Subsequently, 70 ml of distilled water was added. The mixture was then sterilized in an autoclave for 15 minutes at 1 atm pressure and 121°C and left to cool down. After the sterile substrate cooled, 0.02 grams of *R. oligosporus* inoculum was added, and it was incubated for 2 days for treatment B1, 3 days for treatment B2, and 4 days for treatment B3. At the end of the incubation period, the substrate was harvested and dried in an oven at 50-60°C until the weight remained constant. Then, the fermented product was analyzed for nutritional value following AOAC (1990) guidelines. Nitrogen retention, crude fiber digestibility, and metabolic energy according to Sibbald (1975) method.

Experimental design

The study was conducted using a completely randomized factorial design with three A factors as substrate composition (A1, A2, A3) × three B factors as fermentation period (B1, B2, B3), and three replications of each treatment. The A1 was 80% sago pith (SP) + 20% Indigofera leaves (IL), A2 was 60% SP + 40% IL, and A3 was 50% SP + 50% IL. Fermentation of the substrate contained B1 (2 days), B2 (3 days), and B3 (4 days).

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Statistical analysis

To determine the effect of treatment, data were statistically processed using analysis of variance (ANOVA) according to Steel and Torrie (1991). Differences between treatments were followed by the Duncan multiple range test. The p-value less than 0.05 was considered significant.

RESULTS AND DISCUSSION

Table 1 shows that factor A (substrate composition) and factor B (fermentation time) interacted significantly, and each factor could significantly affect the crude protein content ($p < 0.05$). It can be seen that adding Indigofera leaves (IL) at different concentrations (20%, 40%, and 50%) on days 2 and 3 resulted in higher crude protein content. However, on day 4, the crude protein content decreased compared to days 2 and 3. The high crude protein content in A3 and B2 was due to increased mold growth, evident from the total number of colonies (1.4×10^{11} cfu/gr). More mold growth results in a higher protein contribution, supporting the findings of Ciptaan et al. (2022) and Mirnawati et al. (2022), indicating that mold bodies contain protein and an increase in crude protein content is correlated with an increase in mold growth. Enhanced expansion yields single-cell protein products or cell biomass with between 40 and 65% protein (Krishna et al., 2005). Moreover, the crude protein content can increase due to microbes producing enzymes, where the enzyme is a protein. Enzymes are produced in greater quantities as microbes proliferate. During the fermentation process, microbes, as a source of single-cell protein, produce enzymes that are proteins. The amount of crude protein can rise following fermentation. To explain, *R. oligosporus* is a multienzyme organism that includes lipase, protease, a-amylase, glutaminase, and a-galactosidase (Han et al., 2003).

Table 1. The results of the nutritional content of a mixture of sago pith and Indigofera leaves fermented with *Rhizopus oligosporus* included in broiler chicken diet

Parameters	Factor A (Composition substrate)	Factor B (Fermentation time)			Average
		B1 (2 days)	B2 (3 days)	B3 (4 days)	
Crude protein (%)	A1 (80% SP + 20% IL)	14.66 ^{cC}	18.33 ^{aC}	16.10 ^{bC}	16.36
	A2 (60% SP + 40% IL)	18.30 ^{cB}	22.84 ^{aB}	19.46 ^{bB}	20.20
	A3 (50% SP + 50% IL)	22.39 ^{cA}	25.45 ^{aA}	23.98 ^{bA}	23.94
Crude fat (%)	A1 (80% SP + 20% IL)	0.034 ^{aA}	0.031 ^{aA}	0.033 ^{abA}	0.0331
	A2 (60% SP + 40% IL)	0.033 ^{aA}	0.024 ^{bB}	0.030 ^{cB}	0.0291
	A3 (50% SP + 50% IL)	0.030 ^{aB}	0.020 ^{bC}	0.022 ^{bC}	0.0244
Nitrogen retention (%)	A1 (80% SP + 20% IL)	35.77 ^{cC}	41.88 ^{aC}	39.63 ^{bC}	39.63
	A2 (60% SP + 40% IL)	46.31 ^{cB}	54.04 ^{aB}	52.25 ^{bB}	50.87
	A3 (50% SP + 50% IL)	56.42 ^{bA}	59.72 ^{aA}	57.86 ^{cA}	58.00
Crude fiber (%)	A1 (80% SP + 20% IL)	11.56 ^{aA}	9.41 ^{cA}	10.42 ^{bA}	10.46
	A2 (60% SP + 40% IL)	10.47 ^{aB}	7.64 ^{cB}	9.92 ^{bB}	9.35
	A3 (50% SP + 50% IL)	8.74 ^{aC}	6.40 ^{cC}	7.51 ^{bC}	7.55
Crude fiber digestibility (%)	A1 (80% SP + 20% IL)	45.32 ^{cC}	51.84 ^{aC}	47.32 ^{bC}	48.16
	A2 (60% SP + 40% IL)	47.43 ^{cB}	54.84 ^{aB}	48.64 ^{bB}	50.19
	A3 (50% SP + 50% IL)	51.60 ^{cA}	57.34 ^{aA}	55.26 ^{bA}	54.73
Energy metabolism (kcal/kg)	A1 (80% SP + 20% IL)	1455.22 ^{bB}	1829.41 ^{aB}	1537.77 ^{bC}	1607.46
	A2 (60% SP + 40% IL)	1624.89 ^{bB}	1956.51 ^{aB}	1778.97 ^{aB}	1786.79
	A3 (50% SP + 50% IL)	1831.32 ^{cA}	2658.44 ^{aA}	22 54.56 ^{bA}	2248.11

Different lowercases in rows and uppercases in the same column are significantly different ($p < 0.05$). A1: 80% sago pith + 20% Indigofera leaves, A2: 60% sago pith + 40% Indigofera leaves, A3: 50% sago pith + 50% Indigofera leaves. B1: 2 days of fermentation, B2: 3 days of fermentation, B3: 4 days of fermentation.

The longer fermentation period was the reason for the low crude protein content on day 4. Enzymes break down more material during extended fermentation, leading to reduced crude protein content. However, as fermentation duration increases, the nutrients in the media become less available, the microbes eventually die, and the amount of crude protein declines (Agustina et al., 2015). The A3 and B2 group treatments in this study yielded the best crude protein, that is, 25.45% (93% increase). This outcome is greater than that of Annisa et al. (2020), reporting a 28.47% increase in cassava leaves and tofu dregs fermented with *R. oligosporus* for 3 days.

Crude fat content throughout treatments indicated a highly significant interaction between factors A (length of fermentation) and B (substrate composition, $p < 0.01$). Factors A and B both demonstrate a highly significant difference ($p < 0.05$). The results indicated a decrease in crude fat content with increasing additions of Indigofera leaves (20%, 40%, and 50%), attributed to significant mold growth during fermentation, as evidenced by the proliferation of mold colonies (1.4×10^{11} cfu/gr). With increased mold growth, more lipase enzymes were produced, breaking down fat into glycerin and fatty acids. Microbes utilized these fatty acids as an energy source, leading to a reduction in crude fat content post-fermentation. On day 4, the high crude lipid resulted from the prolonged fermentation. Lengthening the fermentation time decreased nutrient availability in the substrate, hindering microbial growth and ultimately resulting in

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increased crude lipid content. In accordance with the present findings, Agustiana et al. (2021) reported that longer fermentation time could decrease nutrient availability in the substrate, leading to a decrease in microbial growth and an increase in fat content.

Fermentation time (factor B) and substrate composition (factor A) had a significantly different interaction ($p < 0.05$) regarding their influence on nitrogen retention ($p < 0.05$) as presented in Table 1. It can be seen that increasing the addition of Indigofera leaves (20%, 40%, and 50%) resulted in higher nitrogen retention. This high nitrogen retention can be attributed to the increased protein availability in the substrate for microbial growth during the 2-3 day period. This phenomenon is facilitated by several enzymes produced by mold that can improve the quality of sago pith protein, and Indigofera leaves after fermentation. This aligns with the findings of Mahfudz et al. (2004) and Mirnawati et al. (2019b), revealing that the ability of microbes to break down proteins into amino acids for easier digestion can increase nitrogen retention. The increased growth of microbes leads to an increase in enzyme activity. The enzyme converts proteins into amino acids, improving the quality of the final product. In addition, the high nitrogen retention is caused by the protein content in the diet. This supports the findings of McDonald et al. (2002) and Ciptaan et al. (2022), indicating that the amount of protein in the ration impacts the quality of nitrogen retention. The nitrogen retention value increases with the ration's higher protein content. The protein quality in the ration was the reason for the low nitrogen retention on day 4. According to Corzo et al. (2005), ration consumption, particularly protein consumption and digestibility, balances nitrogen consumption and ration metabolic energy. These factors also affect the percentage of nitrogen retention.

Factor A (substrate composition) and Factor B (fermentation time) indicated a highly significant difference in their interaction ($p < 0.05$), considering their influence on crude fiber content ($p < 0.05$). It is evident from the above data that the crude fiber content decreased when more Indigofera leaves were added to the substrate. The decrease occurred in A3 and B2, namely with a substrate composition of 50% sago pith and 50% Indigofera leaves. The decrease in crude fiber content was due to the addition of Indigofera leaves, which have a high protein content, and thus satisfy the nutrients in the substrate composition. When the nutrient content of the substrate was available, the microbes grew more (1.4×10^{11} cfu/gr). More cellulase can be generated by accelerating microbial growth. Cellulase can lower the amount of crude fiber in a substrate by breaking it down into glucose to produce energy (Mirnawati et al., 2019b). Similarly, Sudarmono et al. (2016) claimed that when microbes proliferated, more cellulase was generated to convert cellulose into simple sugars, lowering the amount of crude fiber during fermentation. The longer the fermentation time, the lower the crude fiber content observed until day 3. However, on day 4, the crude fiber content was high for all substrate compositions (80% SP + 20% IL, 60% SP + 40% IL, and 50% SP + 50% IL). The extended fermentation period (4 days) in the B3 treatment decreased the substrate's nutrient content and microbial growth, which produced fewer enzymes. This extended fermentation period was the cause of the treatment's high crude fiber content. On day 3, the microbes were still growing well and producing the cellulase enzyme to break down cellulose into glucose, so the crude fiber increased at the end of fermentation. However, by day 4, the microbes decreased, and the cellulase enzyme was no longer produced, so the crude fiber content increased after fermentation. In accordance with the opinion of Fardiaz (1992), fermentation time that exceeds the optimum limit causes a lower availability of nutrients for microbial growth.

For substrate compositions of 80% SP + 20% IL, 60% SP + 40% IL, and 50% SP + 50% IL, the crude fiber content decreased with increasing fermentation time up to day 3 but increased on day 4. The extended 4-day fermentation period reduced substrate nutrient content and microbial growth, resulting in minimal enzyme production and causing high crude fiber content in the B3 treatment. On day 3, the microbes were still growing well and producing the cellulase enzyme to break down cellulose into glucose, so the crude fiber increased at the end of fermentation. However, by day 4, the microbes decreased and the cellulase enzyme was no longer produced, so the crude fiber content increased after fermentation. This aligns with Fardiaz's (1992) observation that fermentation duration beyond the optimum limit could restrict nutrient availability for microbial growth, decreasing microbial growth. The present results indicated that the lowest crude fiber content was found in the A3 and B2 treatment with a substrate composition of 50% SP + 50% IL and a fermentation of 3 days that was 6.40%. This result indicated a lower crude fiber content value, compared to the results of a study by Nensih (2006), where fermentation was performed with a substrate composition of 80% SP + 20% tofu dregs with the mold *Neurospora* sp. The crude fiber content obtained was 7.31%. This difference is due to the different composition and fermentation times.

The interaction between factors B (fermentation time) and A (substrate composition) was significantly different with regard to the crude fiber digestibility parameter ($p < 0.05$; Table 1). Since enough microbes were growing, there was an increase in the digestibility of crude fiber in A3 and B2, specifically with a substrate composition of 50% SP and 50% IL and a fermentation time of 3 days. In other words, adding 50% Indigofera leaves provided sufficient nutrient availability in the substrate for the mold to grow in large numbers (1.4×10^{11} cfu/gr). After fermentation, less crude fiber was left because as microbes multiplied, more cellulase enzymes were created to convert cellulose to glucose. This aligns with the findings of Ciptaan et al. (2022), who observed a negative correlation between crude fiber and digestibility, indicating that higher crude fiber content leads to decreased digestibility. The lower crude fiber digestibility on day 4 was due to the prolonged fermentation time, resulting in high crude fiber content. With reduced microbial

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growth due to extended fermentation, enzyme production was limited, leading to minimal cellulose degradation. Present results indicated the highest crude fiber digestibility in the A3 and B2 treatment with a substrate composition of 50% SP + 50% IL and a fermentation time of 3 days (57.34%).

Regarding energy metabolism, there was a significant between factor A (substrate composition) and factor B (fermentation time, $p < 0.05$). Each factor displayed a highly significant difference. An increase in metabolic energy content could be associated with a decrease in crude fiber content and an increase in the digestibility of crude fiber, resulting in an increased energy level used by the animal's body. The crude fiber in fermented sago pith and Indogefera leaves was converted to glucose by cellulase, and this glucose was counted as an energy source, so it produced metabolic energy in fermentation products. According to Sembiring (2006), it facilitates the conversion of challenging-to-digest components like cellulose and hemicellulose into simpler sugars, thereby increasing metabolic energy. This energy boost results from the substrate's rich nutrient content, supporting robust microbial growth. However, an excessively long fermentation period can hinder microbial growth due to decreased substrate nutrients, leading to a decline in metabolic energy content. Yunus and Zubaidah (2015) state that a shorter fermentation period results in less microbial growth and the production of fewer enzymes, whereas a longer fermentation period causes the microbial population to decline due to the substrate's nutrient depletion. The results of this study showed that after 3 days of fermentation and a substrate composition of 50% SP and 50% IL, the A3 and B2 treatment had the maximum metabolic energy of fermentation of a mixture of SP and IL with *R. oligosporus*, 2658.44 kcal/kg.

CONCLUSION

Based on the current findings, it can be determined that the best results in terms of crude protein (25.45%), crude fat (0.02%), nitrogen retention (59.72%), crude fiber (6.4%), crude fiber digestibility (57.34%), and metabolic energy (2658.44 kcal/kg) could be obtained from fermented sago pith and Indigofera leaves with *R. oligosporus* at substrate composition level of 50 + 50 with fermentation time of 3 days. Additional study is required to determine the effects of fermenting 50% sago pith and 50% Indigofera leaves with *R. oligosporus* on the productivity and product quality of broilers and other poultry as a sustainable alternative feed source.

DECLARATIONS

Availability of data and materials

The study data and materials are accessible by request.

Ethical consideration

Before being published in this journal, all of the authors have reviewed ethical concerns such as data fabrication, double publication and submission, redundancy, plagiarism, consent to publish, and misconduct.

Funding

PTNBH of Andalas University provided financial support for this study (T/5/UN.16.17/PT01.03/Pangan-RPT/2022).

Acknowledgments

For all of the help and resources needed to complete this study, the authors are appreciative of the Faculty of Animal Science, Andalas University. The authors also want to express their appreciation to Septia Dila, Suci Sri Rahmawati, and all the faculty staff who worked so hard on this project but whose names could not be mentioned individually.

Authors' contribution

The final draft of the manuscript was written and revised with assistance from Ade Djulardi, Lovita Adriani, Mirnawati, and Malik Makmur. Anifah Srifani, Ridho Kurnia, and Gita Ciptaan were involved in designing the manuscript, experimental procedure, and data analysis. Every author confirmed the final revised manuscript.

Competing interests

No conflicts of interest have been disclosed by the authors.

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To cite this paper: Djulardi A, Mirnawati, Ciptaan G, Kurnia R, Srifani A, Adriani L, and Makmur M (2023). Improving the Quality and Nutritional Value of a Mixture of Sago Pith and Indigofera leaves Fermented with *Rhizopus oligosporus*. *World Vet. J.*, 13 (4): 580-586. DOI: https://dx.doi.org/10.54203/scil.2023.wvj62

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To cite this paper: Djulardi A, Mirnawati, Ciptaan G, Kurnia R, Srifani A, Adriani L, and Makmur M (2023). Improving the Quality and Nutritional Value of a Mixture of Sago Pith and Indigofera leaves Fermented with *Rhizopus oligosporus*. *World Vet. J.*, 13 (4): 580-586. DOI: https://dx.doi.org/10.54203/scil.2023.wvj62