



Nutritional Evaluation and Phytochemical Profile of Rice Straw and *Indigofera zollingeriana* Mixture under Different Fermentation Periods

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ABSTRACT

This study aimed to evaluate the nutritional content and phytochemical profile of a mixture of rice straw and *Indigofera zollingeriana* at different fermentation periods. The research was conducted in Mamuju Regency using an experimental method with a Completely Randomized Design (CRD) consisting of four treatments and four replications. The treatments included P0 = without fermentation (0 days), P1 = 7 days fermentation, P2 = 14 days fermentation, and P3 = 21 days fermentation. The feed mixture consisted of 70% rice straw and 30% *Indigofera zollingeriana*. Observed parameters included dry matter (DM), crude protein (CP), crude fiber (CF), crude fat, ash, nitrogen-free extract (NFE), and phytochemical compounds including tannins, saponins, flavonoids, alkaloids, and total phenols. Data were analyzed using analysis of variance (ANOVA) followed by Duncan's multiple range test. The results showed that fermentation duration significantly affected ($P < 0.05$) the nutritional content and phytochemical profile of the feed mixture. Fermentation for 14 days (P2) resulted in the highest crude protein, flavonoid, saponin, alkaloid, and total phenol contents, as well as the lowest crude fiber and tannin contents compared to other treatments. Crude protein increased from 10.45% in P0 to 15.76% in P2, while crude fiber decreased from 32.56% to 26.15%. Tannin content also decreased from 2.45% to 1.88% after 14 days of fermentation. It can be concluded that 14 days of fermentation is the optimal period to improve the nutritional quality and phytochemical profile of rice straw and *Indigofera zollingeriana* mixture as ruminant feed.

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Introduction

Feed is one of the primary factors determining the productivity of ruminant livestock, particularly in tropical farming systems that still heavily rely on locally available feed resources. The inconsistent availability of high-quality forage throughout the year has encouraged farmers to utilize agricultural by-products as alternative feed resources, one of which is rice straw. That is what is then exploited through the use of rice straw, which has great potential because of its extraordinary abundance and availability so that it can be obtained more easily from agricultural production centers like Mamuju. Nevertheless, there are still limitations of rice straw being used as ruminant feed such as low crude protein

content, high levels of crude fiber and high levels Lignin and silica contributing to poor nutritional value and digestibility (Mayulu et al., 2018; Wina et al., 2020). Therefore, efforts to improve the quality of rice straw are essential to optimize its utilization as a feed resource for ruminant animals.

Fermentation technology is a widely used method of increasing the nutritional value of agricultural residues. Fermentation can improve the chemical properties of feedstuffs by exerting enzymes produced by microbes that can degrade complex fibers such as cellulose and hemicellulose into more simple compounds (Nahrowi et al., 2019). Fermentation can improve the nutritional quality, aroma, texture, palatability of feed ingredients, which can increase the feed intake by livestock (Wahyuni & Bijanti, 2021). Fermentation time is an important parameter in determining fermentation success due to its direct correlation with microbes degrading organic matter and fermenting specific metabolites. While too short of fermentation periods may not provide quality degradation of nutrients, excessive prolonged NF could reduce nutritional gases due to the loss of organic matter and breakdown of functional elements (Sari et al., 2020)

Fermentation technology and inclusion of high-quality leguminous plants may also contribute to an effective way for improving the quality of feeding. *Indigofera zollingeriana* is an important tropical legume species with high crude protein, high biomass yield i.e. 138.6 g/m² and environmental adaptation potential (Abdullah, 2019). Several studies reported that this plant contained a high level of approximately 24–31% crude protein, so it is considered as one possibility alternative protein sources for ruminant livestock (Suharlina et al., 2016). In addition to its high nutrient profile, *Indigofera zollingeriana* is rich in different phytochemical such as flavonoids, saponins, tannins, alkaloid and total phenolic (Ampapon et al 2019) that could beneficial animal health and metabolisms efficiency. However, some phytochemicals are anti-nutritional when present in high concentrations and they have negative effects on nutrient utilization if not controlled (Rahman et al., 2022).

Both the nutritional composition and phytochemical profiles of fermented feed materials strongly affect their quality and safety, necessitating a thorough confirmation. Nutrition Studies, such as Dry Matter (DM), Crude Protein (CP), Crude Fiber (CF), Crude Fat, Ash and Nitrogen Free Extracts(NFE) are required for a complete animal nutrition evaluation of feedstuffs(AOAC 2016). Conversely, phytochemical analysis is performed to pinpoint bioactive compounds that can affect rumen microbial activity, nutrient digestibility and animal health (Makkar, 2016). Most previous studies investigating rice straw fermentation and incorporation of *Indigofera zollingeriana* have primarily focused on animal performance and nutrient digestibility, but little data are available regarding shifts in nutritional composition and phytochemical profiles as a function of fermentation duration, particularly for feed formulations where these two feed resources are combined.

Inspired by the above background, this study was performed with the aim of identifying nutritional content as well as phytochemical profiles of fermented rice straw and *Indigofera zollingeriana* mixtures at various fermentation periods. This research is expected to provide scientific knowledge regarding the effects of fermentation time on nutritional quality changes and bioactive compound compositions in feedstuff, supplying a scientific foundation for developing more efficient, high-quality, and sustainable feed technologies based on local resources for ruminant livestock.

Data Analysis

These activities took about 8 weeks to be done in Mamuju covering material preparation, fermentation process, laboratory analysis and data processing. Analyses of nutritional composition and phytochemical profile were performed at the Animal Nutrition and Feed Chemistry Laboratory. This study used an experimental method with a Completely Randomized Design (CRD) with 4 treatment and 4 replications. Use of CRD was justified as all experimental units were assumed to be homogeneous ensuring equal chances for applying each treatment (Steel & Torrie, 2015).

The raw materials used in this study were rice straw and *Indigofera zollingeriana* leaves. The composition of feed was 70% rice straw and 30% *Indigofera zollingeriana* leaves. Before fermentation, the rice straw was chopped to a length of about 3–5 cm to facilitate mixing and fermentation. Anaerobic fermentation was performed using local microbial inoculum with molasses as an alternative source of energy for fermentative microorganisms. All materials were mixed well until homogenous and then transferred into an airtight containers for anaerobic conditions.

For this study, fermentation duration was an independent (X) variable and the nutritional composition and phytochemical profile of the rice straw and *Indigofera zollingeriana* mixture, were dependent (Y) variables. The treatments used were,

P0 = no fermentation (0 days)

P1 = fermentation for 7 days

P2 = fermentation for 14 days

P3 = fermentation for 21 days

The fermentation was performed at room temperature anaerobically. After the fermentation time corresponding to each treatment was completed, samples of the feeds were collected for nutritional and phytochemical analyses.

Study variables recorded were nutritional content and phytochemical profile. Nutritional assays included dry matter (DM), crude protein (CP), crude fiber (CF), crude fat, ash and nitrogen-free extract (NFE). They were performed based on the methods established by AOAC (2016), using proximate analysis techniques. The oven-drying method was used for dry matter content, Kjeldahl method for crude protein, Soxhlet extraction method for crude fat and gravimetric method for crude fiber.

The nitrogen-free extract (NFE) value was calculated using the following equation:

$$\text{NFE}(\%) = 100 - (\text{Ash} + \text{CP} + \text{EE} + \text{CF})$$

Where:

NFE = Nitrogen-Free Extract

CP = Crude Protein

EE = Ether Extract (Crude Fat)

CF = Crude Fiber

We examined the phytochemical content of the feed materials, like tannins, saponins, flavonoids, alkaloids and total phenolics. Folin Denis method for tannin analysis, aluminum chloride method for flavonoids and Folin Ciocalteu method (Makkar, 2016) for total phenolics were carried out.

Alkaloid and saponin analyses were quantitatively performed using spectrophotometric methods based on the procedures described by Harborne (1998).

The data obtained from the study were analyzed using Analysis of Variance (ANOVA) according to the Completely Randomized Design (CRD) model to determine the effects of

different fermentation durations on the nutritional composition and phytochemical profile of the feed materials.

If the analysis indicated a significant effect ($P < 0.05$), Duncan's Multiple Range Test (DMRT) was subsequently performed to identify differences among treatments. All statistical analyses were conducted using IBM SPSS Statistics and Microsoft Excel.

Results and discussion

Data analysis and discussion

a. Results of Nutritional Composition Analysis

An analysis was conducted on the nutritional composition and phytochemical profile of a mixture of rice straw and *Indigofera zollingeriana* under different fermentation periods. The results demonstrated that fermentation duration influenced changes in both the nutritional composition and phytochemical compounds of the feed material. The mean values of the nutritional composition and phytochemical profile analyses are presented in Table 1.

Table 1 Nutritional Composition of Rice Straw and *Indigofera zollingeriana* Mixture under Different Fermentation Periods

Parameters	P0 (0 Days)	P1 (7 Days)	P2 (14 Days)	P3 (21 Days)
Dry Matter (DM, %)	89.12 ± 0.54 ^a	87.45 ± 0.48 ^b	85.76 ± 0.50 ^c	84.95 ± 0.45 ^c
Crude Protein (CP, %)	10.45 ± 0.31 ^a	12.88 ± 0.36 ^b	15.76 ± 0.42 ^c	14.90 ± 0.39 ^{bc}
Crude Fiber (CF, %)	32.56 ± 0.65 ^c	29.40 ± 0.58 ^b	26.15 ± 0.60 ^a	27.02 ± 0.55 ^{ab}
Crude Fat (%)	2.85 ± 0.12 ^a	3.10 ± 0.15 ^b	3.42 ± 0.14 ^c	3.25 ± 0.13 ^{bc}
Ash (%)	14.56 ± 0.30 ^a	13.88 ± 0.28 ^b	13.10 ± 0.25 ^c	13.22 ± 0.27 ^c
Nitrogen-Free Extract (NFE, %)	39.58 ± 0.72 ^a	41.14 ± 0.68 ^b	41.57 ± 0.70 ^b	41.66 ± 0.65 ^b

Notes: DM = Dry Matter; CP = Crude Protein; CF = Crude Fiber; NFE = Nitrogen-Free Extract. Values followed by different superscripts within the same row indicate significant differences ($P < 0.05$).

Dry Matter (DM) Parameter

The results of this study demonstrated that fermentation duration had a significant effect ($P < 0.05$) on the dry matter (DM) content of the rice straw and *Indigofera zollingeriana* mixture. The DM content decreased progressively with increasing fermentation periods. Treatment P0 exhibited the highest DM value, whereas treatments P2 and P3 produced the lowest DM values. The reduction in DM content during fermentation was associated with microbial activity utilizing organic substrates as an energy source for microbial growth and cellular metabolism. Throughout the fermentation process, a portion of the organic matter was degraded into simpler compounds, carbon dioxide, and water, resulting in a reduction of the dry matter fraction (Zayed, 2018).

The reduction in DM content also indicates that the fermentation process occurred actively and efficiently. Fermentative microorganisms, such as lactic acid bacteria and cellulolytic fungi, are capable of degrading structural components of plant cell walls into simpler compounds. This activity alters the chemical composition of the feed material, resulting in a relative increase in moisture content and a subsequent decrease in DM concentration (Islam et al., 2022). A decline in dry matter during fermentation is a common characteristic of agricultural by-product-based feed materials due to nutrient utilization by microorganisms throughout the anaerobic incubation process.

In the present study, the 14-day fermentation treatment (P2) demonstrated relatively optimal conditions, as the reduction in DM content was accompanied by an increase in crude

protein content and a decrease in crude fiber content. These findings indicate that fermentation not only reduced DM content but also improved the overall nutritional quality of the feed material. Previous studies have reported that the fermentation of rice straw supplemented with leguminous plants could enhance both the physical and chemical quality of silage, particularly at optimal fermentation durations ranging from 14 to 21 days (Kirana et al., 2022).

Crude Protein (CP)

The values of crude protein (CP) increased significantly ($P < 0.05$) in fermented treatments than that of non-fermented treatment. Treatment P2 (14 days of fermentation) had the highest values, while treatment P3 had a lower percentage compared to that of P0, but higher than that of P1. The rise in the amount of CP content calculated during fermentation may be related to microbial biomass whose growth yields single-cell protein and hence raises the nitrogen concentration (Santi et al., 2025) in feed materials.

Fungal fermentation not only led to further microbial biomass proliferation, but also enhanced the breakdown of recalcitrant lignocellulosic bonds that had previously restricted protein digestibility in the feed matrix. The lignin and hemicellulose structures are broken down and more peripherally located protein fractions become available for detection in proximate analysis. Studies conducted by Puastuti et al. (2024) found that the addition of *Indigofera zollingeriana* into rice straw-based feed significantly affected rumen fermentation parameters and increased feed material protein content.

The most promising results came from the fermentation period of 14 days, when microbial activity was yet at optimum growth stage. However, at 21 days of fermentation (P3), the CP slightly decreased due to the protein being degraded into ammonia and other volatile nitrogen compounds. This result suggests that very-extended fermentation may hinder nitrogen utilization improvement of feedstuffs. These findings align with Asyidiqy et al. (2024), where it was shown that fermentation duration and related microbial activity throughout the fermentation period had a very pronounced influence on the protein quality of fermented feed.

Crude Fat

The results of the study indicated that crude fat content increased significantly ($P < 0.05$) in the fermented treatments compared with the control treatment. The highest crude fat content was observed in treatment P2. The increase in crude fat content during fermentation was presumably associated with changes in nutrient component proportions as well as lipid synthesis by fermentative microorganisms throughout the fermentation process (AOAC, 2016).

Microbial activity during fermentation is capable of producing secondary metabolites in the form of fatty acids and other lipid compounds, which contribute to the increased crude fat content. Furthermore, the reduction of fiber fractions and certain organic matter components during fermentation caused the proportion of crude fat to become relatively higher. Previous studies on the fermentation of agricultural by-product-based feed materials have demonstrated that fermentation may increase crude fat content as a consequence of changes in the chemical composition of the substrate (Ervinta et al., 2021).

Nevertheless, the increase in crude fat content remained within the normal range and was therefore considered safe for use as ruminant feed. Excessively high fat levels may interfere with rumen microbial activity; however, in the present study, crude fat values remained at levels capable of supporting dietary energy supply without negatively affecting rumen fermentation processes (Puastuti et al., 2024).

Ash Content

The ash content decreased significantly ($P < 0.05$) during the fermentation process. Treatments P2 and P3 exhibited lower ash content compared with the non-fermented treatment. The reduction in ash content during fermentation was presumably associated with changes in the proportion of organic and inorganic components resulting from microbial metabolic activity.

Fermentation may cause certain minerals to dissolve and undergo structural transformations during the anaerobic fermentation process. In addition, the increase in microbial biomass and the degradation of organic matter contributed to a relative decrease in ash proportion. Previous studies have also reported that the fermentation of rice straw based feed materials alters mineral composition due to the fermentative activity of microorganisms (Islam et al., 2022).

The decrease in ash content indicates that fermentation was capable of improving the organic quality of the feed material. This condition suggests that compositional changes occurring during fermentation were more dominant in the organic fraction than in the inorganic fraction. Therefore, fermentation may enhance the efficiency of nutrient utilization in agricultural by-product-based feed resources.

Nitrogen-Free Extract (NFE)

The nitrogen-free extract (NFE) content increased significantly ($P < 0.05$) in the fermented treatments compared with the control treatment. The highest NFE values were observed in treatments P2 and P3. NFE represents the readily soluble carbohydrate fraction that serves as a primary energy source for ruminant livestock.

The increase in NFE Contents during fermentation was ascribed to the degradation of complex fiber fractions into low MW soluble carbohydrates. The fermentation process displayed some enzymatic activity of microorganisms that improved polysaccharide degradation into simple sugars, also NFE fraction from the feed material was increased (Zayed, 2018).

In general, NFE values were higher indicating an improvement in energy quality. More readily available energy promotes rumen microbial activity and improves the efficiency of nutrient utilization in livestock. Consequently, 14 days of fermentation period was treated as the best optimum treatment because it could effectively increase NFE content well along with increased crude protein and reducing crude fiber contents at one time.

b. Phytochemical Profile of the Experimental Fedd Material

Table 2. Phytochemical Profile of Rice Straw and *Indigofera zollingeriana* Mixture under Different Fermentation Periods

Parameters	P0 (0 Days)	P1 (7 Days)	P2 (14 Days)	P3 (21 Days)
Tannins (%)	2.45 ± 0.10 ^c	2.20 ± 0.08 ^b	1.88 ± 0.07 ^a	1.95 ± 0.06 ^a
Saponins (%)	1.12 ± 0.05 ^a	1.35 ± 0.06 ^b	1.58 ± 0.07 ^c	1.50 ± 0.05 ^{bc}
Flavonoids (%)	0.88 ± 0.03 ^a	1.12 ± 0.05 ^b	1.45 ± 0.06 ^c	1.40 ± 0.05 ^c
Alkaloids (%)	0.45 ± 0.02 ^a	0.52 ± 0.03 ^b	0.60 ± 0.03 ^c	0.58 ± 0.02 ^{bc}
Total Phenolics (%)	1.25 ± 0.04 ^a	1.48 ± 0.05 ^b	1.76 ± 0.06 ^c	1.70 ± 0.05 ^c

Notes: Values followed by different superscripts within the same row indicate significant differences ($P < 0.05$).

Tannin Parameter

The study results indicated that the fermentation length had an important effect ($P < 0.05$) on rice straw and *Indigofera zollingeriana* mix tannin content. Tannin was significantly

reduced according to the period of fermentation, where P2 and P3 produced the lowest values regarding tannin compared with all other treatments. The decrease in tannins during fermentation was related to microbial activity that is able to degrade elaborate phenolic compounds into easier and more soluble forms (Makkar, 2016).

Reducing anti-nutritional compounds is an important aspect of fermentation through microbial enzymatic action, for instance, tannase enzymes which hydrolyze bonds in tannins. Higher levels of tannin can lead to reduced palatability and inhibit rumen microbes from properly utilizing proteins, so a decrease in tannin content is considered an important evidence for the improvement of feed quality (Rahman et al., 2022). Ampapon et al. (2019) also noticed that fermentating leguminous forages effectively reduced the tannin content and consequently improved the nutritional quality of feed materials.

Among the three time points, P2 (the 14-day fermentation treatment) showed the most reduction in tannin content. Meanwhile, tannin reduction remained relatively constant under the treatment of fermentation duration (21 days) (P3), and showed no significant difference with P2. However, like the tannin dynamics observed during fermentation points us to believe that after 14 days of fermentation this route had already reached an optimal point where further time do not promote meaningful changes. This condition is in accordance with the study performed by Suharlina et al. (2016) remarked about the degradation of phenolic compounds that it usually happens at a good-level during the active growth phase of fermentative microorganisms.

Saponin Parameter

The saponin content significantly increased ($P < 0.05P$) in the fermentation period. The content of saponin in treatment P2 was found to be the greatest among other treatments. The rising level of saponin throughout fermentation was perhaps connected with the damage of plant cells walls it enabled some release easy that could be captured by plant bioassays (Makkar, 2016).

Saponins are glycosidic compounds belonging to a group of secondary metabolites, which have substantial biological activities in the digestive tract of ruminant livestock. Saponins have the ability to reduce rumen protozoal populations at certain concentrations which can eventually enhance protein utilization efficiency and reduce methane gas production (Patra & Saxena, 2015). So, the fermentation can also improve rumen fermentation efficiency and livestock productivity through increasing saponin content.

Thus, these results suggest that the highest increase in saponin content found for treatment P2 shows a fermentation period of 14 days as the best condition to improve their availability. However, treatment P3 showed a small decrease in saponin content which seems to be due to the degradation of bioactive compounds arising from prolonged microbial activity. Extended fermentation may destabilize certain plant secondary metabolites, as previous studies have also reported (Rahman et al., 2022)

Flavonoid Parameter

Results indicated that, total flavonoids content was significantly increased ($P < 0.05$) with the increase in duration of fermentation. Treatments of P2 and P3 had much higher yield than the others. During fermentation damaged lignocellulosic structures released flavonoid compounds from the plant cell wall matrix making them more readily extractable and quantifiable (Suharlina et al. 2016).

Flavonoids are natural antioxidants that can scavenge free radicals and protect the body's cells against oxidative damage. Flavonoids have been proven to be beneficial for the health of the animal digestive tract and stable rumen microflora in animal feed (Ampapon et

al., 2019). These results support that fermentation improves nutritional quality and gives an insight into the functional value of feed materials due to the increase in flavonoid content caused by fermentation.

In this study, the 14-day fermentation treatment produced the highest flavonoid content and was considered the optimum period. Although flavonoid content in treatment P3 remained relatively high, its value was slightly lower than that of P2. This reduction was presumably caused by partial degradation of flavonoid compounds during prolonged fermentation. Islam et al. (2022) reported that phenolic and flavonoid compounds may decline if fermentation is conducted for excessively long periods due to oxidation and degradation of secondary metabolites by microorganisms.

Alkaloid Parameter

Fermented treatments all resulted in the highest alkaloid levels ($P < 0.05$) compared to non-fermented treatment). The greatest rise was found in the case P2 treatment. The increase of alkaloid content in the fermentation period was presumably related to chemical transformations of the plant materials making the antioxidant compounds more available and detectable during laboratory analyses (Harborne, 1998).

Alkaloids are important biologically active secondary metabolites, with potential natural antimicrobial and antioxidant properties. A long history of consumption related to many polygenic traits (Patra & Saxena, 2015). Alkaloids may act at appropriate concentrations inhibit their growth against pathogenic microorganisms that reside in the digestive tract of livestock ruminants and can improve animal health. Very high alkaloid concentrations can also demonstrate toxic effects, thus the necessity for careful management of their inclusion rates.

In the present study, 14 days of fermentation produced relatively optimal alkaloid content without excessive increases. This finding suggests that fermentation can be utilized as a controlled method for enhancing bioactive compound content. Recent studies have demonstrated that fermentation processes may enhance the biological activity of alkaloid compounds through modifications in the chemical structure of plant secondary metabolites (Rahman et al., 2022).

Total Phenolic Parameter

In the fermented treatments compared to the control treatment total phenolic content increased significantly ($P < 0.05$). Among all treatments, treatment P2 was the most productive one giving the highest total phenolic content and the results were significantly different from that of other treatments. The increase in total phenolics during fermentation was likely due to the microbial enzymes that broke down these complex connections between phenolic compounds and plant cell wall components (Makkar, 2016).

Total phenolics are a significant predictor of feed materials antioxidant activity. Phenolic compounds have been proven to prevent lipid oxidation and enhance the shelf-life of feed raw materials (Suharlina et al., 2016). Phenolic compounds serve a function of regulating specific microbial activities by improving fermentation efficacy and livestock health in the rumen system.

In this study, the highest increase in total phenolic content was obtained after 14 days of fermentation. However, under the 21-day fermentation treatment, total phenolic content slightly decreased, although it remained higher than the control treatment. This decline was presumably caused by the degradation of phenolic compounds due to prolonged microbial activity. These findings indicate that a 14-day fermentation period represents the optimum

duration for enhancing phenolic compound content without causing excessive degradation of the feed material's bioactive compounds.

Conclusion

The study reveals that different fermentation periods had a significant influence ($P < 0.05$) on the nutritional composition and phytochemical profile of rice straw and *Indigofera zollingeriana* mixture. Fermented feed showed better quality with increases in crude protein, crude fat, nitrogen-free extract (NFE) and bioactive compounds like flavonoids, saponins, alkaloids, total phenolics. In contrast, dry matter, crude fibre, ash and tannin contents were decreased during fermentation. The increase in the quality and nutritional value of feed material for ruminant livestock can be indicated by reduction in crude fiber and tannin contents after fermentation.

The second objective was to determine the best fermentation treatment by evaluating the increment of crude protein, flavonoids, total phenolics and saponins in addition to examining the reduction of crude fiber and tannin contents in fermented *C. spectabilis* raw leaves with different fermentation times (e.g., 2, 7, and 14 days). Based on these data, the 14-day fermentation treatment (P2) emerged as most beneficial due to its potent increases in crude protein, flavonoids, total phenolics, and saponins compared with other treatments whilst corresponding greater reductions in crude fiber and tannin contents were observed. Thus, we would state that the 14d no feed or fermentation fermentation improves the nutritional quality of ruminant feeds and bioactive compounds with this locally based feed processing technology.

References

- [1]. Abdullah, L. (2019). Herbage production and quality of shrub indigofera treated by different concentration of foliar fertilizer. *Media Peternakan*, 42(2), 85–92. <https://doi.org/10.5398/medpet.2019.42.2.85>
- [2]. Ampapon, T., Wanapat, M., Kang, S., & Phesatcha, K. (2019). Effects of phytonutrients on rumen fermentation, digestibility, and methane production in ruminants: A review. *Animal Bioscience*, 32(6), 857–867. <https://doi.org/10.5713/ajas.18.0405>
- [3]. AOAC. (2016). *Official Methods of Analysis of AOAC International* (20th ed.). Association of Official Analytical Chemists. Gaithersburg, MD.
- [4]. Asyidiqy, M. F., Hartutik, H., & Chuzaemi, S. (2024). The effect of fermentation duration on the nutritional quality of agricultural waste-based feed. *Jurnal Ilmu dan Industri Peternakan*, 10(1), 45–56. <https://doi.org/10.21776/ub.jiip.2024.010.01.05>
- [5]. Ervinta, R., Nurhaita, N., & Sari, M. (2021). Changes in crude fat content of fermented agricultural by-products as ruminant feed. *Jurnal Peternakan Indonesia*, 23(2), 145–153. <https://doi.org/10.25077/jpi.23.2.145-153.2021>
- [6]. Ghozali, I. (2018). *Aplikasi Analisis Multivariate dengan Program IBM SPSS 25* (9th ed.). Badan Penerbit Universitas Diponegoro. Semarang.
- [7]. Harborne, J. B. (1998). *Phytochemical Methods: A Guide to Modern Techniques of Plant Analysis* (3rd ed.). Chapman & Hall. London.

- [8]. Islam, S., Khan, M. J., & Hasan, M. R. (2022). Effect of fermentation on nutritional quality and fiber degradation of rice straw-based feed. *Journal of Animal Science and Technology*, 64(6), 1215–1227. <https://doi.org/10.5187/jast.2022.e94>
- [9]. Kirana, I. G. A., Bidura, I. G. N. G., & Candrawati, D. P. M. A. (2022). Nutritional quality improvement of rice straw silage with legume supplementation. *Majalah Ilmiah Peternakan*, 25(3), 189–198. <https://doi.org/10.24843/MIP.2022.v25.i03.p07>
- [10]. Makkar, H. P. S. (2016). *Animal Feed Science and Technology: Phytochemicals in Animal Nutrition*. Elsevier Science Publishers. Amsterdam. <https://doi.org/10.1016/j.anifeedsci.2016.01.001>
- [11]. Mayulu, H., Sunarso, S., & Christiyanto, M. (2018). Nutritional evaluation of fermented rice straw as ruminant feed. *International Journal of Science and Engineering*, 15(2), 95–101. <https://doi.org/10.12777/ijse.15.2.95-101>
- [12]. Nahrowi, N., Ridla, M., & Laconi, E. B. (2019). Improvement of agricultural waste quality through fermentation technology as ruminant feed. *Tropical Animal Science Journal*, 42(1), 60–68. <https://doi.org/10.5398/tasj.2019.42.1.60>
- [13]. Patra, A. K., & Saxena, J. (2015). Exploitation of dietary tannins to improve rumen metabolism and ruminant nutrition. *Journal of the Science of Food and Agriculture*, 95(1), 24–37. <https://doi.org/10.1002/jsfa.6752>
- [14]. Puastuti, W., Mathius, I. W., & Wina, E. (2024). Indigofera zollingeriana as a high-quality forage for ruminant feeding systems in tropical regions. *Animal Bioscience*, 37(1), 77–88. <https://doi.org/10.5713/ab.23.0152>
- [15]. Rahman, M. M., Hossain, M. A., & Islam, M. S. (2022). Fermentation effects on phytochemical compounds and anti-nutritional factors of forage plants. *Fermentation*, 8(9), 456. <https://doi.org/10.3390/fermentation8090456>
- [16]. Santi, R. K., Putra, D. A., & Wahyuni, T. H. (2025). Effect of fermentation duration on crude protein content of fermented feed ingredients. *Buletin Animal Agriculture Research*, 7(1), 12–20. <https://doi.org/10.24843/baar.2025.v07.i01.p02>
- [17]. Sari, M. L., Nurhaita, N., & Zain, M. (2020). Fermentation technology to improve the nutritional value of agricultural by-products as ruminant feed. *Advances in Animal and Veterinary Sciences*, 8(4), 381–387. <https://doi.org/10.17582/journal.aavs/2020/8.4.381.387>
- [18]. Steel, R. G. D., & Torrie, J. H. (2015). *Principles and Procedures of Statistics: A Biometrical Approach* (3rd ed.). McGraw-Hill Book Company. New York.
- [19]. Suharlina, S., Abdullah, L., Karti, P. D. M. H., & Astuti, D. A. (2016). Nutritional value and phytochemical content of Indigofera zollingeriana as forage for ruminants. *Media Peternakan*, 39(2), 121–127. <https://doi.org/10.5398/medpet.2016.39.2.121>
- [20]. Wahyuni, D., & Bijanti, R. (2021). Fermentation technology in improving feed palatability and nutritional quality. *Jurnal Nutrisi Ternak Tropis*, 4(2), 98–107. <https://doi.org/10.21776/ub.jnt.2021.004.02.4>

-
- [21]. Wina, E., Muetzel, S., & Becker, K. (2020). The impact of fermentation on fiber degradation and nutritional quality of fibrous feedstuffs. *Animal Feed Science and Technology*, 267, 114562. <https://doi.org/10.1016/j.anifeedsci.2020.114562>
- [22]. Zayed, A. A. (2018). Improvement of nutritive value of agricultural residues through biological treatments. *Agriculture & Food Security*, 7(1), 26. <https://doi.org/10.1186/s40066-018-0186-1>.