Research Article

Effect of Moringa Leaf Extract on Fiber Fractions' Content and Digestibility in Fermented Lemongrass Rations

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Abstract | This study aimed to evaluate the effect of Moringa leaf extract supplementation, as a natural antioxidant, in complete rations based on fermented citronella waste on the content and digestibility of fiber fractions. A Completely Randomized Design was employed with five treatments and three replications. The treatments consisted of different doses of Moringa leaf extract supplementation: P0 (no extract), P1 (0.125% extract), P2 (0.25% extract), P3 (0.375% extract), and P4 (0.5% extract). Parameters measured included the content and digestibility of Acid Detergent Fiber (ADF), Neutral Detergent Fiber (NDF), cellulose, and hemicellulose. The results demonstrated that increasing doses of Moringa leaf extract had no significant effect ($P > 0.05$) on the fiber fraction content; however, it significantly influenced ($P < 0.01$) their digestibility. The average NDF content ranged from 59.56% to 61.59%, ADF from 33.27% to 34.54%, cellulose from 29.93% to 32.08%, and hemicellulose from 26.85% to 28.32%. Further analysis revealed that the digestibility of NDF, ADF, cellulose, and hemicellulose in P1 (0.125%) was significantly higher than in the other treatments. Higher doses of Moringa leaf extract tended to reduce digestibility, possibly due to secondary metabolites in the extract.

Received | December 28, 2024; **Accepted** | April 10, 2025; **Published** | April 26, 2025
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Citation | Astuti, T., H. Tholib, S.A. Akbar, R.M. Sari and D.F. Basyirun. 2025. Effect of moringa leaf extract on fiber fractions' content and digestibility in fermented lemongrass rations. *Pakistan Journal of Agricultural Research,* 38(1): 46-52.
DOI | https://dx.doi.org/10.17582/journal.pjar/2025/38.1.46.52
Keywords | Moringa leaf extract, Fiber fractions, Lemongrass rations

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Introduction

The availability of quality feed is one of the main factors in increasing the productivity of ruminant livestock. However, the production of green fodder for livestock is becoming increasingly limited due to various challenges, including population growth, which necessitates land for settlements, the expansion of land for food production, and the development of other sub-sectors. Therefore, using agro-industrial waste as an alternative feed material is a sustainable approach to substituting green fodder and developing environmentally friendly feed. One example is the waste from the distillation of citronella (*Cymbopogon nardus*). This underutilized by-product of essential oil production includes both liquid waste (hydrosol) and solid waste (citronella stalks and leaves) This underutilized by-product of essential oil production includes both liquid waste (hydrosol) and solid waste (citronella stalks and leaves). According to the distiller, when asked directly in the field the distillation process produces only 0.8% essential oil, leaving behind substantial amounts of liquid waste (hydrosol) and solid waste (citronella stalks and leaves).

The study by Sukamto and Djazuli (2011) revealed that citronella waste conten 7% protein and 25.7% crude fiber. Similarly, Nurhayu and Warda (2018) reported that lemongrass waste has a dry matter content of 93.05%, crude protein of 6.92%, crude fiber of 33.71%, and Nitrogen-free extract of 44.56%. At the same time, feeding lemongrass distillation dregs at levels up to 40% improved the performance of Balinese cattle, with daily weight gains reaching 0.20 to 0.24 kg/head/day. This presents no significant difference compared to cattle-fed elephant grass. Additionally, research by Astuti *et al.* (2024) demonstrated that substituting field grass with citronella distillation by-products in a 50:50 green fodder-to-concentrate ratio resulted in digestibility values of 52.67% for dry matter, 56.58% for organic matter, and 67.55% for crude protein. The study by Elihasridas *et al.* (2021) reported that the contents of Neutral Detergent Fiber (NDF), Acid Detergent Fiber (ADF), neutral fiber carbohydrates, and lignin in citronella waste were 71.07%, 35.02%, 35.02%, and 2.8%, respectively. However, its use as an alternative source of green fodder is limited by the residual essential oil content, such as citronella and geraniol, which can interfere with rumen function (Usmiati *et al.*, 2012). In particular, lemongrass contains citronella oil with high concentrations of oxidants and phytochemicals yet low nutritional value. It makes feed processing technology essential before it can be effectively utilized as animal feed.

Fermentation is a feed processing technology designed to improve the quality of waste-based feed by utilizing the activity of microorganisms capable of reducing crude fiber, lignin, and anti-nutritional compounds. Astuti *et al.* (2023) reported that fermenting citronella waste using a rumen content bioactivator optimally increased crude protein and organic matter on the 20^{th} day of incubation. Accordingly, fermenting agricultural waste can enhance nutrient availability and improve the quality of crude fiber in rations. Meanwhile, research by Siska (2023) asserted that the contents of ADF, NDF, cellulose, and hemicellulose in citronella waste fermented with *T. reesei* were 49.81%, 63.68%, 31.23%, and 13.88%, respectively.

Moringa leaves are known for their numerous nutritional and health benefits, including antioxidant properties and their potential as a supplement in livestock feed. Moringa leaves (*Moringa oleifera*) are a natural ingredient rich in bioactive compounds such as flavonoids, tannins, and saponins. Kantja *et al.* (2022) reported that Moringa leaf meal contains 24.14% crude protein and 11.44% crude fiber. Furthermore, Moyo *et al.* (2011) added that dried Moringa leaves have an ADF content of 8.89%, NDF of 11.40%, cellulose of 4.01%, lignin of 1.8%, tannin of 3.12 mg/g, and total polyphenols of 2.02%. In addition, Moringa leaves also contain amino acids such as serine (1.087%), glycine (1.533%), alanine (3.033%), and tyrosine (2.650%). Rizkayanti *et al.* (2017) stated that Moringa leaves contain bioactive compounds such as alkaloids, flavonoids, and phenols, which are rich in antioxidant activity.

Based on this data, Moringa leaves can be utilized as a natural antioxidant and a supplemental feed ingredient when applied in appropriate dosages. Correspondingly, the combined use of Moringa leaf extract and fermented citronella waste is expected to provide dual benefits as a nutritional source and enhance fiber quality in livestock feed. This study aimed to evaluate the effect of Moringa leaf extract supplementation, as a natural antioxidant, in complete rations based on fermented citronella waste on the content and digestibility of fiber fractions.

Materials and Methods

This experimental research project utilizes a complete feed consisting of forage and concentrates in a 50:50 ratio. The forage is the solid waste from citronella oil distillation, comprising citronella leaves and stalks. Meanwhile, the concentrate comprises tofu waste, rice bran, palm kernel cake, premix, and salt. The citronella waste is fermented using a local bioactivator produced by incubating cattle rumen content with the addition of necessary materials. The fermented citronella waste is then supplemented with varying doses of Moringa leaf extract, which serves as the treatment in this study.

Experimental design

This study was analyzed using a completely randomized design with five treatments and three replications for each treatment. Data were obtained through Van Soest analysis to determine fiber fraction content and the *in vitro* Tilley and Terry method (1963) to evaluate the digestibility coefficients of fiber fractions. The treatments in this study involved varying doses of Moringa leaf extract supplementation in a complete feed based on fermented citronella waste.

The doses of Moringa leaf extract used were:

- P0: No Moringa leaf extract supplementation (0%)
- P1: Supplementation with 0.125% Moringa leaf extract
- P2: Supplementation with 0.25% Moringa leaf extract
- P3: Supplementation with 0.375% Moringa leaf extract
- P4: Supplementation with 0.50% Moringa leaf extract

The parameters measured included the content of NDF, ADF, cellulose, and hemicellulose with Van Soest method analysis (Van Soest, 1982). Duncan's Multiple Range Test (DMRT) was conducted as a post-hoc analysis if the statistical results indicated significant differences ($P < 0.01$), (Steel and Torrie, 1995).

Production of local rumen bioactivator and fermentation of citronella waste

The local bioactivator was produced using cattle rumen content obtained from slaughterhouse waste in Solok city. The rumen content was mixed with tofu wastewater and molasses in a ratio of 8:1:1. Additionally, 10% palm frond extract was added as an energy source to stimulate enzyme production by the microorganisms present in the rumen content. The mixture was then incubated for at least seven days (Astuti *et al*., 2022).

The citronella waste, consisting of stems and leaves leftover from citronella oil distillation, was sourced from oil distillation facilities, where it is typically discarded as waste. This waste was chopped into smaller particles using a chopper and then fermented using the rumen-based bioactivator that had been incubated for at least seven days. The fermentation process was conducted for 20 days of incubation.

Production of moringa leaf extract

Moringa leaves were collected from the surrounding environment and air-dried at room temperature. The dried leaves were then extracted using methanol in a 1:5 ratio of leaves to solvent. The extraction process employed a sonicator for 1 hour at 50°C to optimize the extraction of compounds with the aid of sonographic waves. After sonication, the mixture was soaked for 24 hours. The resulting dark green solution was filtered to separate the leaf residues, leaving the extract ready for use.

Formulation of ration and moringa leaf extract supplementation

The citronella waste, fermented for 20 days, is mixed with concentrate feed ingredients (rice bran, tofu waste, palm kernel cake, salt, and premix). Moringa leaf extract is used as a natural antioxidant supplement in the complete feed at specified doses. Each dose of Moringa leaf extract is diluted in equal proportions using distilled water (aqueous). For every 1 kg of ration, 30 ml of Moringa leaf extract is added. Consequently, the mixture is ready for laboratory analysis according to the predetermined parameters.

Results and Discussion

The content of ADF, NDF, cellulose, and hemicellulose in complete feed

The analysis of variance indicated that the complete feed based on fermented citronella waste, supplemented with different doses of Moringa leaf extract, exhibited no significant effect ($P > 0.05$) on the content of NDF, ADF, cellulose, and hemicellulose. This may be due to the Moringa leaf extract being unable to affect the structural components of the fiber fractions ADF, NDF, cellulose, and hemicellulose which are relatively stable components of the plant cell wall. Although Moringa leaf extract contains bioactive compounds such as flavonoids, tannins, and saponins, which have the potential as natural antioxidants, they may not significantly impact the structural fiber content in this case.

Notably, the higher the NDF level in the feed, the more crude fiber it contains. The data in Table 1 suggest that the average ADF content ranged from 32.27% to 34.54%, while the NDF content ranged from 59.59% to 61.59%. According to Ramli (2018), NDF content is considered high if it exceeds 63%. The NDF content in this study is still below that threshold. According to Riswandi (2016), the lower the NDF and ADF fractions, the higher the digestibility of the feed.

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Table 1: *Average content of ADF, NDF, cellulose, and hemicellulose in complete rations based on fermented lemongrass waste supplemented with moringa leaf extract.*

Treatments	ADF	NDF	Cellulosa	Hemicellolusa
P0	33.27	61.59	29.33	28.32
P1	33.81	61.26	30.75	27.50
P2	34.54	60.01	30.42	25.46
P3	34.23	59.56	31.00	25.33
P4	33.71	60.59	32.08	26.85
SE	1.55	1.55	1.24	2.68

Note: The treatment no effect (P>0.05). P0 = no extract, P1=0.125% extract, P2=0.25% extract, P3= 0.375% extract, and P4 = 0.5% extract.

According to Wati *et al.* (2012), NDF is influenced by the fiber content in the feed. Thus, although there were increases and decreases in the NDF content with different doses of Moringa leaf antioxidant supplementation, these differences were not statistically significant enough to conclude a real effect of Moringa leaf antioxidant supplementation on the NDF content in the ration. The results of this study are consistent with those of Akmal *et al.* (2023), who noted that the decrease in NDF content was due to the breakdown of lignocellulosic bonds during fermentation and the activity of developing microbes. According to Van Soest (1982), the ADF component consists of cellulose, which is easily digestible, and lignin and silica, which are difficult to digest due to their complex bonding. According to Biyatmoko (2014), ADF content is part of the crude fiber, consisting of lignin and silica. Essentially, the higher the crude fiber content in a plant, the higher the ADF content in the feed. ADF content represents the accumulation of fiber fractions in the form of cellulose and lignin (Nelson and Suparjo, 2011).

Kurutas (2015) said that the antioxidants are generally used to protect cells from oxidative damage and do not directly participate in the synthesis or degradation of cellulose. The cellulose content in feed ingredients can influence livestock performance . However, it is not directly related to antioxidant supplementation. Nevertheless, the ADF and NDF content in the complete feed in this study was higher compared to the study by Rahmawati and Hikmah (2022), which used Moringa leaves as a concentrate forage, with values ranging from 24.3% to 26.7% for ADF and 40.8% to 44.7% for NDF.

The digestibility of ADF, NDF, cellulose, and hemicellulose of complete feed

Table 2 provides data on the average NDF digestibility of complete feed based on fermented lemongrass waste and citronella waste supplemented with Moringa leaf extract.

The statistical analysis reveals that adding Moringa leaf extract to the complete feed significantly affects ($P < 0.01$) the digestibility of ADF, NDF, cellulose, and hemicellulose. This is attributed to Moringa leaves being rich in protein, vitamins, minerals, and bioactive compounds that can influence rumen microbial activity. At the same time, compounds such as saponins and tannins in Moringa leaves can modulate microbial populations, which play a role in fiber fermentation in the rumen. This indicates that the dose of Moringa leaf extract used can affect the functioning system within the rumen. Furthermore, the DMRT test results reveal that the optimal NDF digestibility was reported in the P1 treatment (52.26%), supplemented with 0.125% Moringa leaf extract, significantly different from the P0, P2, P3, and P4 treatments. In addition to containing antioxidants, Moringa leaf extract can activate secondary metabolites such as tannins, total phenols, and flavonoids, which stimulate activity within the rumen. However, if the dosage is inappropriate, these compounds can become anti-nutritional factors and reduce the total microbial population in the rumen.

Table 2: *Average values of NDF, ADF, cellulose, and hemicellulose digestibility.*

Treatments	NDF	ADF	Cellulosa	Hemicellulosa
P0	51.18^{b}	53.34^{b}	60.69^{b}	63.67^{a}
P1	52.26^{a}	54.73^{a}	62.11^{a}	64.76^{a}
P2	50.71^{b}	51.89^{c}	61.67^{a}	64.16^{a}
P3	49.61^{c}	50.58^{d}	58.78^{c}	59.74^{b}
P4	48.93^{d}	50.69^{d}	58.81^{c}	56.96^{c}

Note: Superscripts (a, b, c, and d) in the same columns indicate a highly significant effect ($P < 0.01$). P0 = no extract, P1=0.125% extract, P2=0.25% extract, P3= 0.375% extract, and P4 = 0.5% extract.

This can be observed in Table 2, where increasing the dose of Moringa leaf extract in the complete feed based on fermented citronella waste (0.25%, 0.375%, and 0.5%) caused a decrease in NDF digestibility. According to Theodorou *et al.* (1994), flavonoid supplementation can enhance rumen fermentation in dairy cattle. Notably, the increase in Moringa leaf extract dose in the feed ingredients is suspected of reducing rumen microbial populations, affecting ammonia concentration and pH levels. In contrast, low ammonia concentration can inhibit the growth and activity of microbes involved in feed digestion in the rumen, leading to a decrease in NDF digestibility. According to Rahalus *et al.* (2014), poor rumen microbial development results in suboptimal feed digestion, causing reduced fiber digestibility.

The results of the post hoc test using DNMRT in Table 2 suggest that supplementation of 0.125% Moringa leaf extract significantly differs from the other treatments and can increase the digestibility of ADF in the complete feed based on fermented citronella waste compared to the treatment without Moringa leaf extract supplementation (P0). Therefore, increasing the dose of Moringa leaf extract to 0.25%, 0.325%, and 0.5% decreased ADF digestibility of the complete feed by up to 5.2% in the P3 treatment (0.375% dose). According to Leitanthem *et al.* (2022), secondary metabolites found in Moringa leaves, including tannins, flavonoids, phenolics, and saponins, can improve nutrient digestibility. Abdel-Raheem and Hassan (2021) stated that rumen microbes can utilize these compounds as an energy source without negatively affecting rumen fermentation. As per Min *et al.* (2003), tannins, as part of secondary metabolites in low quantities, can improve rumen microbial performance by reducing excessive protein degradation in the rumen, providing more protein available for microbes. This condition makes rumen microbes more active due to the nutrients available for their survival and enables them to produce the enzymes needed for metabolic processes in the rumen. This aligns with Rahmawati and Hikmah (2022), who noted that protein supply could promote better rumen microorganism growth and increase cellulase and lignocellulose enzymes needed to digest fibrous components like NDF and ADF. This leads to improved feed digestibility. However, at higher doses, tannins can act as anti-nutritional factors by inhibiting the activity of microbial enzymes. This statement is consistent with the findings that increasing the dose of Moringa leaf extract reduces ADF digestibility. This is due to anti-nutritional substances, which can disrupt digestive enzyme activity in livestock.

The results of the post hoc test (DNMRT) suggest that in the P1 treatment, supplementation of Moringa leaf extract at a dose of 0.125% (P1) significantly differs from the P0, P3, and P4 treatments regarding the cellulose digestibility coefficient. The data in Table 2 indicate an increase in the cellulose digestibility of the complete feed based on fermented citronella waste supplemented with 0.125% Moringa leaf extract (P0: 60.69% vs P1: 62.11%). When the supplementation dose of Moringa leaf extract was increased to 0.25%, the digestibility coefficient of the complete feed decreased to 61.67%, although this was not significantly different from P1. Moreover, there was a 3.15% decrease in the cellulose digestibility coefficient of the complete feed in the P3 treatment (0.375% dose). This is likely due to the presence of phytochemicals in Moringa leaves, which, when used in large amounts, can have anti-nutritional effects. This aligns with the studies of Moyo *et al.* (2011), which stated that Moringa leaves contain several anti-nutritional substances, such as tannins, flavonoids, polyphenols, oxalic acid, and phytic acid. However, these anti-nutritional substances may disrupt the activity of digestive enzymes in livestock.

Conclusion

Based on the results of this study, it can be concluded that the addition of Moringa leaf extract supplementation as a natural antioxidant into the complete feed based on fermented citronella waste did not have a significant effect ($P>0.05$) on the fiber fraction contents (NDF, ADF, cellulose, and hemicellulose). However, it had a highly significant effect ($P<0.01$) on the digestibility of the fiber fractions. Nonetheless, the use of Moringa leaf extract at the dosage of P1 (0.125%) was optimal in increasing the digestibility of the fiber fractions, while increasing the dosage of supplementation led to a decrease in the digestibility of the fiber fractions. Overall, the phytochemical compounds such as flavonoids, tannins, and saponins positively influence the rumen microbial system at the appropriate dosage.

Acknowledgments

The authors extend their gratitude to the Directorate General of Higher Education, Research, and Technology, Ministry of Education, Culture, Research, and Technology, Indonesia for providing funding for basic research.

Novelty Statement

The novelty of this study was highlighting the role of natural antioxidants of Moringa leaf extract in complete feed based citronella.

Author's Contribution

Tri Astuti: Research ideas and designing research
Syahro Ali Akbar: Data processing
Harizal Tholib: Data collection in the field
Fajri Basyiru: Research administration

Conflict of interest
The authors have declared no conflict of interest.

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