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Original Article

Effects of Ammoniation–Fermentation Methods and Fermentation Duration on Nutrient Degradability, Gas Production, and Methane Emission of Rice Straw

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Abstract

Objective: This study aimed to evaluate the effect of Ammoniation-fermentation (Amofer) methods and fermentation duration on the nutritional quality, digestibility, and methane production of rice straw.
Methods: Two Amofer methods were compared: direct (A1) and indirect (A2), combined with four fermentation periods (0, 7, 14, and 21d).
Results: The results showed that both factors significantly affected in vitro dry matter degradability (IVDMD), in vitro organic matter degradability (IVOMD), and gas production ($P<0.05$). The indirect Amofer method (A2), which involved ammoniation prior to microbial fermentation, produced higher DMD (43.10%) and OMD (44.52%) than the direct method (A1). The best results were obtained from A2B2 (indirect Amofer, 14d), with the highest DMD (55.96%) and OMD (59.37%), as well as efficient gas production (103.7mL/g DM) and lower methane emission (1.48mL CH_4/g DM). Improved digestibility under A2B2 was associated with the breakdown of lignocellulosic bonds during ammoniation and enhanced microbial activity during fermentation. In contrast, extended fermentation (21d) decreased digestibility and increased methane output.
Conclusions: These findings indicate that the indirect Amofer method with 14d of fermentation optimizes rice straw utilization by enhancing feed digestibility, stimulating rumen microbial efficiency, and reducing methane emissions, contributing to sustainable ruminant nutrition systems

Keywords: Rice straw; ammoniation-fermentation; degradability; fermentation characteristic; methane mitigation

INTRODUCTION

The ruminant livestock sector in Indonesia still faces serious challenges related to the availability of forage, which fluctuates with the seasons. During the dry season, limited availability of fresh forage reduces livestock productivity, while during the harvest season, abundant agricultural by-products such as rice straw are often underutilized. In fact, national rice straw production is estimated to exceed 90 million tons per year [1[L1.1]], making it a highly potential and sustainable alternative feed source. However, rice straw has limitations due to its high crude fiber and lignin content, coupled with low protein levels and digestibility, which make it insufficient as a sole feed to meet the nutritional requirements of ruminants [2,3].

Various rice straw treatment methods have been developed to improve its quality, including ammoniation, fermentation, and their combination (amofer). Ammoniation with urea can break lignocellulosic bonds, thereby increasing the availability of fiber fractions, while fermentation with microbial inoculants helps reduce crude fiber content and enhance nutrient availability [4].

The combination of ammoniation and fermentation (amofer) is considered more effective because it integrates the advantages of chemical and biological treatments, leading to improvements in crude protein content and reductions in lignocellulosic fractions of low-quality roughages. Recent studies have demonstrated that ammoniation followed by fermentation significantly enhances digestibility and nutritional value in agricultural by-products such as citronella waste and rice straw [5,6,7,8]. Furthermore, urea-based ammoniation has been shown to alter microbial composition and metabolic activity in treated crop residues, thereby facilitating subsequent microbial degradation during fermentation [9,10]. In practice, the application of ammoniation-fermentation (amofer) technology among farmers is carried out in two different ways: (1) ammoniation and fermentation performed simultaneously from the beginning, and (2) ammoniation conducted first, followed by fermentation. These two methods are believed to produce different outcomes in feed quality due to variations in lignocellulose breakdown and microbial dynamics during each treatment phase. Nevertheless, the effectiveness of amofer remains influenced by the application technique (direct or indirect) and the fermentation duration, which determine microbial activity levels and ultimately affect the final feed quality.

The main problem is the lack of clear information regarding the most appropriate amofer method and the optimal fermentation duration to produce rice straw of the best quality. Improving rice straw quality is not only associated with dry matter and organic matter digestibility but also with gas and methane production in the rumen. Gas production serves as an indicator of feed fermentability, while methane reflects both energy utilization efficiency and the potential greenhouse gas emissions [5]. Therefore, research on rice straw processing through amofer methods is crucial, not only to improve livestock performance but also to mitigate environmental impacts.

Based on this background, this study aims to evaluate the effectiveness of two amofer methods—direct amofer (simultaneous addition of urea and inoculant) and indirect amofer (ammoniation followed by fermentation)—combined with different fermentation durations to improve the quality of rice straw. This study is expected to provide scientific information regarding the most suitable combination of method and fermentation period, as well as a tangible contribution to the utilization of agricultural by-products as high-quality ruminant feed. Furthermore, the findings of this research align with the achievement of the Sustainable Development Goals (SDGs), particularly SDG 2 (Zero Hunger) by improving food security, SDG 12 (Responsible Consumption and Production) by promoting the use of agricultural residues, and SDG 13 (Climate Action) by reducing methane emissions from the livestock sector.

MATERIALS AND METHODS

Place and Time of Research

This research was conducted at the Laboratory of Animal Nutrition and Feed Science, Department of Animal Science, Universitas Syiah Kuala, Indonesia. The in vitro digestibility and gas production analyses were carried out at the Ruminant Nutrition Laboratory under controlled laboratory conditions. The study was conducted over a four-month period, from May to August 2025, including substrate preparation, treatment application, incubation, laboratory analysis, and data processing.

Materials

The main substrate used in this study was rice straw obtained from local farmers in Bireuen, Aceh Province harvested after the rice cropping season. The straw was air-dried and chopped into approximately 3–5 cm lengths prior to treatment.

The chemical treatment utilized 4% urea (based on dry matter content) as the ammoniation agent. The biological treatment employed EM4 (Effective Microorganisms-4), a commercial microbial inoculum containing lactic acid bacteria, photosynthetic bacteria, yeast, and actinomycetes, as the fermentation starter.

Additional materials included distilled water, buffer solution, rumen fluid collected from fistulated cattle, CO_2 gas for maintaining anaerobic conditions, and standard laboratory reagents for proximate and in vitro analysis.

Equipment used comprised airtight plastic containers for ammoniation and fermentation, analytical balance, oven (105°C), furnace (550°C), incubator (39°C), water bath shaker, gas-tight syringes for total gas measurement, methane analyzer, and glassware for in vitro procedures.

Research Methods

This study employed an experimental method using a Completely Randomized Design (CRD) arranged in a 2 × 4 factorial pattern. The first factor (Factor A) was the Amofer method: A1: Ammoniation followed by fermentation (Direct Amofer); A2: Simultaneous ammoniation and fermentation (Indirect Amofer). The second factor (Factor B) was fermentation duration (days): B0: 0 (control); B1: 7; B2: 14; B3: 21.

Rice straw was treated with 4% urea (based on dry matter). For A1, ammoniation was conducted first in airtight conditions for 21 days, followed by fermentation using EM4 according to the assigned fermentation duration. For A2, urea and EM4 were applied simultaneously at the beginning of the treatment and incubated according to the designated fermentation period. Each treatment combination was replicated three times, resulting in 24 experimental units (2 methods × 4 fermentation durations × 3 replications). The in vitro degradability and gas production were determined using the modified in vitro gas production technique [11]. Samples were incubated at 39°C for 24 hours under anaerobic conditions using buffered rumen fluid.

Observed Variables

The observed variables were divided into degradability parameters and fermentation characteristics:

1. In Vitro Dry Matter Degradability (IVDMD) (%)

Measured as the percentage of dry matter loss after 24-hour incubation, calculated by the difference between initial dry matter and residue dry matter after incubation and oven drying.

2. In Vitro Organic Matter Degradability (IVOMD) (%)

Calculated based on the loss of organic matter after combustion at 550°C, representing the proportion of organic matter degraded during incubation.

3. Total Gas Production (mL/200 mg DM)

Measured using calibrated gas-tight syringes after 24-hour incubation. Total gas production reflects the extent of substrate fermentation by rumen microorganisms.

4. Methane (CH_4) Production (mL)

Methane concentration was determined from total gas samples using a methane analyzer. Methane production was expressed both as total volume and as a proportion of total gas production, indicating fermentation efficiency and environmental impact.

Data Analysis

The data obtained were subjected to analysis of variance (ANOVA) according to the Completely Randomized Design (CRD) factorial model (2 × 4). The statistical model included the main effects of Amofer method (A), fermentation duration (B), and the interaction between factors (A × B). If significant differences ($P < 0.05$) were detected, mean comparisons were further analyzed using Tukey's Honestly Significant Difference (HSD) at a 1% level of significance [12].

RESULTS

In vitro dry matter degradability (IVDMD). In vitro dry matter degradability (IVDMD) is an important parameter in evaluating the nutritional value of feed, as it indicates the fraction of dry matter that can be digested and utilized by rumen microbes and the host animal. The higher the IVDMD value, the greater the availability of energy and nutrients that can be supplied to support animal performance [13,14]. The effects of Amofer method and fermentation duration on IVDMD are presented in Table 1.

Table 1. Effect of amofer method and fermentation duration on in vitro digestibility and methane production

Amofer Method	Fermentation (days)	IVDMD (%)	IVOMD (%)	Methane (mL/g DM)
A1	0	41.93±0.25[a]	42.10±0.66[de]	0.91±0.02[a]
	7	27.61±0.06[b]	26.50±0.85[b]	2.12±0.07[d]
	14	37.08±0.70[c]	38.11±0.55[c]	2.02±0.07[cd]
	21	29.00±0.91[f]	27.10±0.82[b]	2.05±0.25[d]
A2	0	41.60±0.63[a]	41.93±0.25[d]	0.88±0.06[a]
	7	52.80±0.65[d]	57.35±1.03[e]	1.92±0.02[c]
	14	55.96±0.02[e]	59.37±0.59[e]	1.48±0.02[b]
	21	22.06±0.77[g]	19.41±1.17[a]	2.13±0.12[d]
SEM		0.34	0.45	0.05
p-value				
Amofer (A)		0.0021	0.003	0.0012
Duration (B)		<0.0001	<0.0001	<0.0001
A × B		0.0014	0.0022	0.0007

Note: A1: Direct Amofer; A2: Indirect Amofer; B0: 0 days; B1: 7 days; B2: 14 days; B3: 21 days.
Values are mean ± SEM (n = 3).
Means within each column followed by different superscripts differ significantly at P < 0.01 according to Tukey's Honestly Significant Difference (HSD).
** indicates significant at P < 0.01.
SEM = standard error of the mean.

The results of this study showed in Table 1 that the Amofer method had a significant effect (P<0.05) on the IVDMD of rice straw. Regarding the method factor, treatment A2 produced a higher average value (43.10%) compared to A1 (33.91%).

In vitro organic matter degradability (IVOMD)

In vitro organic matter degradability (IVOMD) indicates the proportion of organic fractions (carbohydrates, proteins, and fats) in the feed that can be degraded by rumen microbes and absorbed as an energy source. This parameter is closely related to the availability of metabolizable energy that can be utilized by livestock for growth, milk production, and reproductive performance. A high IVOMD value reflects increased efficiency in the utilization of rice straw as an energy source.

The results of Table 1 showed a significant difference (P<0.05) between Amofer methods as well as fermentation duration on the in vitro organic matter degradability (IVOMD) of rice straw. The highest IVOMD value was obtained in treatment A2B2 at 59.37%, while the lowest was observed in A2B3 at 19.41%. On average, A2 (44.52%) was higher than A1 (33.45%).

Methane Gas Production

The results of Table 1 showed that the treatment of the amofer method and fermentation duration had a significant effect on methane (CH_4) production from rice straw. The average CH_4 emission values ranged from 0.88 to 2.13 mL CH_4/g DM. The lowest value was obtained in treatment A2B0 at 0.88 mL CH_4/g DM, while the highest value was recorded in A2B3 at 2.13 mL CH_4/g DM.

Gas Production

The results of the study in Figure 1 showed that in vitro gas production increased with incubation time and fermentation duration, both in the direct (A1) and indirect (A2) amofer methods. The highest gas production was recorded in treatment A2B2 (indirect method with 14 days of fermentation), reaching 103.7 mL/g DM at 24 hours of incubation, while the lowest value was found in A1B0 (direct method without fermentation), at 71.8 mL/g DM.

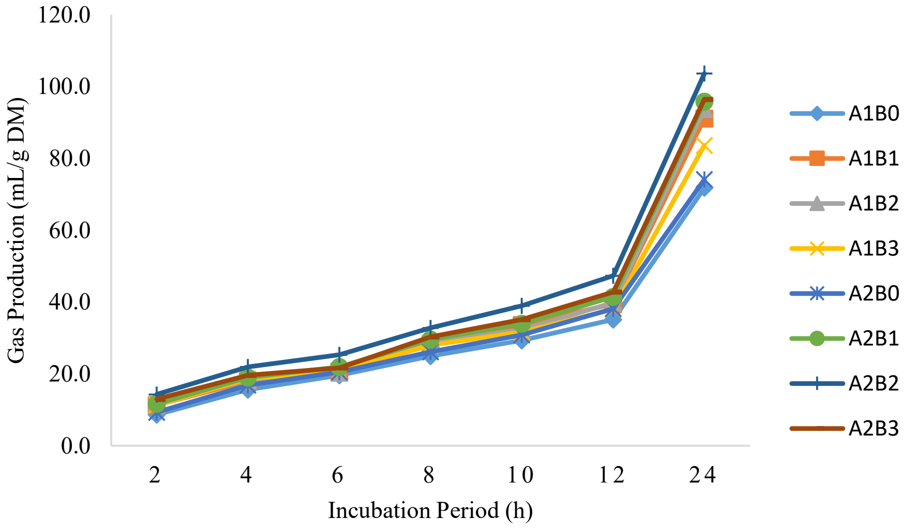

Figure 1. 24-hour incubation gas production

DISCUSSION

In vitro dry matter degradability (IVDMD)

The results of this study showed that both the Amofer method had a significant effect on the IVDMD of rice straw. This can be explained by differences in mechanism: in the direct Amofer method (A1), urea and EM4 were applied simultaneously, so that ammoniation and fermentation occurred at the same time. Meanwhile, in the indirect Amofer method (A2), the straw was first ammoniated for 7 days before being inoculated with EM4. This initial ammoniation process was able to loosen the lignocellulose bonds, making the fiber fraction more accessible to fermentative microbes [15]. Recent studies reported that urea ammoniation disrupts ester linkages between lignin and hemicellulose, increases cellulose accessibility, and enhances subsequent microbial colonization during fermentation [16]. These findings are consistent with [17,18,19] who reported that urea-alkali pretreatment improves lignocellulosic hydrolysis, reduces structural carbohydrate fractions, and enhances microbial degradation and in vitro degradability of rice straw.

In terms of fermentation duration, the highest IVDMD was obtained at 14 days (B2, 46.52c), which was significantly different from all other treatments. This condition indicates that microbial activity reached its optimal point during this period, with maximum degradation of structural carbohydrates. Observed peak cellulolytic activity and dry matter loss between 10–15 days of fermentation in urea-treated crop residues [20]. Conversely, 21 days of fermentation (B3, 25.53d) resulted in the lowest IVDMD value. This was presumably due to a decline in microbial activity after the peak fermentation phase and the accumulation of by-products that may inhibit further degradation [2, 21].

The interaction between the two factors reinforced these results. Treatment A2B2 (indirect Amofer, 14 days) produced the highest DMD value (55.96e), significantly different from all other combinations, while the lowest value was observed in A2B3 (indirect Amofer, 21 days, 22.06g). This pattern suggests that urea-treated rice straw undergoes improved dry matter degradability, particularly when incubation time and fermentation treatment are carried out optimally. Urea-treated rice straw has higher crude protein content and lower fiber fractions, which directly enhance both dry matter and organic matter degradability [22,23]. Thus, the findings of this study confirm that the application of the indirect Amofer method with 14 days of fermentation is the best combination for improving the DMD of rice straw.

In vitro organic matter degradability (IVOMD)

The increase in OMD with the indirect Amofer method (A2) is presumably due to its two-step process, namely ammoniation for 7 days, which functions to weaken lignocellulose bonds, followed by fermentation with EM4 inoculant. This process results in more effective fiber fraction degradation, making organic matter more digestible by rumen microbes. In contrast, in the direct Amofer method (A1), urea and inoculant were added simultaneously, leading to less optimal fiber degradation due to competition between enzymatic activities and incomplete ammoniation [13].

Fermentation duration also played an important role. At 14 days (B2), substrate conditions and microbial activity were at their optimum, leading to the breakdown of crude fiber and hemicellulose into simpler compounds. However, with longer fermentation (21 days, B3), OMD declined. This was likely due to the accumulation of metabolic by-products, reduced substrate availability, and the degradation of essential nutrients, which lowered degradability [24].

These findings are consistent with [4], who reported that a combination of chemical treatment (ammoniation) and biological treatment (fermentation with fungi or inoculants) improved rice straw degradability compared to a single treatment. Similarly [14] explained that ammoniation plays a role in breaking lignocellulose bonds, while fermentation complements it by enhancing microbial fiber-degrading enzyme activity. Studies on wheat straw have also shown a similar pattern, where combined chemical-biological treatments increased organic matter degradability by up to 55% [25]. Thus, it can be concluded that the indirect Amofer method (A2) is more effective in improving the OMD value of rice straw, particularly at 14 days of fermentation, compared to the direct Amofer method (A1).

Methane Gas Production

In general, the indirect amofer method (A2) produced slightly lower CH_4 emissions during the early stages of fermentation (B0–B2) compared to the direct amofer method (A1). This indicates that the preliminary ammoniation process can reduce methanogenic potential. Ammoniation is known to increase non-protein nitrogen content and alter the lignocellulosic structure, resulting in a decrease in indigestible fiber fractions (ADF and lignin) and shifting the fermentation pattern toward propionate production [26]. Propionate formation competes with the methane production pathway for hydrogen (H_2) utilization, thereby contributing to CH_4 emission reduction [5].

These findings are consistent with [27], who reported that rice straw treated with a combination of 5% urea and autoclave reduced CH_4 emissions by 5% compared to untreated straw at 48 h incubation. Fermentation duration significantly affected methane production. CH_4 emissions increased with longer fermentation time up to 21 days. The highest CH_4 values were observed in B3 (21 days) under both direct and indirect methods. This increase was likely due to enhanced methanogenic microbial activity resulting from a greater availability of degradable substrates at the advanced fermentation stage [28]. However, the lowest CH_4 value at 14 days of fermentation (A2B2 = 1.48 mL CH_4/g DM) suggests that during this period, hydrogen utilization by propionate and acetate producing microbes was most efficient, leading to reduced methane production. The methane reduction was also associated with a shift in the rumen microbial community, where Methanobrevibacter sp. populations declined while lactic acid bacteria increased [29]. Moreover, fermentation with starbio and molasses inoculum can suppress methanogenesis by enhancing populations of hydrogen-competing microbes such as Lactobacillus sp. and Propionibacterium sp [30]. Bio-fermentation reduced methane concentration in total gas volume without affecting the archaeal community, as part of the hydrogen was used for propionate formation instead of CH_4 conversion [31].

Interestingly, higher total gas production did not necessarily correspond with increased methane emission. Treatment A2B2 recorded the highest total gas volume (103.7 mL/g DM) but produced relatively low methane (1.48 mL CH_4/g DM). This indicates that fermentation proceeded more efficiently, with a greater proportion of non-methane gases such as CO_2 and volatile fatty acids [32]. Such a condition represents efficient low-emission fermentation, where feed energy utilization by the animal is optimized and energy loss in the form of CH_4 is minimized [33].

Gas Production

The increase in gas production indicates a higher fermentative activity of rumen microbes in degrading the organic matter of the feed substrate. In the indirect amofer method, the ammoniation step first breaks the lignin–cellulose bonds through the formation of ammonium carbonate from urea, which has alkaline properties that loosen the straw cell wall structure [16]. This condition enhances the accessibility of microbial cellulase enzymes to the substrate, improves crude fiber degradation, and consequently increases the total gas volume produced during in vitro fermentation. Rice straw treated with 5% urea and 9% corn steep liquor has higher gas than untreated straw [31]. The increase was associated with a reduction in lignin content and an increase in soluble carbohydrate fractions. Thus, this study confirms a consistent pattern that the combination of ammoniation and fermentation improves the quality of rice straw by enhancing degradability and rumen microbial activity.

Furthermore, the addition of Starbio inoculum and molasses during the fermentation process accelerated the colonization of cellulolytic and amylolytic microbes. Starbio contains a mixture of functional microorganisms such as Bacillus sp., Saccharomyces cerevisiae, and Aspergillus niger, which play essential roles in the decomposition of complex organic matter [34]. Starbio in rice straw fermentation increased gas production [35]. This demonstrates that the combination of inoculum and a simple energy source such as molasses can enhance fermentative activity in simulated rumen conditions.

However, the decrease in gas production after more than 14 days of fermentation (A2B3 = 96.5 mL/g DM) suggests that prolonged fermentation can reduce the availability of fermentable substrates. This may be due to excessive degradation of soluble carbohydrates and the accumulation of organic acids that can inhibit microbial activity18. The trend of increased gas volume up to 24 hours of incubation reflects optimal fermentation activity under the 14-day indirect amofer treatment. According [32], gas volume serves as an indicator of substrate fermentation efficiency and is positively correlated with organic matter degradability and volatile fatty acid (VFA) production. Therefore, the higher gas volume observed in A2B2 indicates that the 14-day indirect amofer process provides the best condition for rumen microbes to utilize soluble carbohydrate fractions.

The results of this study were higher than those reported [36], who obtained gas production of 25.53 mL/g DM from rice straw fermented with Trichoderma viride and Phanerochaete chrysosporium inoculant, and were comparable to [37] who achieved 93 mL/g DM using urea and supplementation of Paraserianthes falcataria and Sapindus rarak. This comparison confirms that the combination of the indirect amofer method and optimal fermentation duration can improve fermentation efficiency by 30–40% compared to untreated rice straw.

CONCLUSION

The indirect Amofer method combined with 14 days of fermentation produced the best results, yielding the highest dry and organic matter degradability and efficient gas production with low methane emission. This treatment effectively enhanced lignocellulose degradation and microbial fermentation efficiency, indicating that the indirect Amofer process for 14 days is the most optimal strategy to improve the nutritional quality and environmental performance of rice straw as ruminant feed.

CONFLICT OF INTEREST

The authors declare no conflict of interest with any financial organization regarding the material discussed in the manuscript.

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