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ORIGINAL PAPER

Fermentability, digestibility, and gas production of Garut sheep fed maize straw silage-based rations balanced with rice bran and rice husks (*in vitro*)

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ABSTRACT. This study was conducted to determine the effect of combining rice bran and rice husk as a substitute ingredient in maize straw silage-based rations on rumen fermentability. The study used a completely randomised design with different proportions of rice husks as a factor. Rice husk was a replacement for rice bran at levels of 0, 10, 20, 30, and 40% in a maize straw silage-based ration (dry matter (DM)). The forage-to-concentrate ratio was 60:40. Measurements included pH, ammonia, total volatile fatty acids, partial, methane and total gas production, conducted after 24 h of incubation, while dry matter digestibility and organic matter digestibility were measured after 48 h of incubation. Data were analysed using one-way ANOVA. The results showed that incrementing rice husk levels in the ration significantly reduced ($P < 0.01$) its quality. Fermentability, digestibility, total gas and methane production decreased linearly ($P < 0.01$) with increasing rice husk content. Our findings imply that the addition of rice husks (DM basis) to the ration should not exceed 20%, or the substitution of rice bran with rice husk should not exceed 50%.

Introduction

One of the objectives of the integrated farming system programme is to ensure the independence of village farms. Farmers are encouraged to meet animal feed needs using agricultural waste biomass, thereby reducing energy losses and converting waste into valuable food products. Agricultural waste biomass is increasingly being used to make valuable products in a sustainable manner (Siwal et al., 2021). Rice bran, rice husks, and maize straw are examples of lignocellulosic biomass from agricultural waste containing nutrients that can be utilised by ruminants (Dhanya, 2022). The gastrointestinal tract of ruminants is inhabited by microbes that can digest crude fibre, enabling the extraction of essential nutrients for body maintenance, growth, and reproduction, with protein and energy being the primary nutrients. If nutrition is not balanced to match specific production needs, the productivity of the animal declines (Oghenesuvwe et al., 2024).

There is a high demand for rice bran as feed for ruminants, and until now, its production has been sufficient to meet these requirements. However, rice bran is often mixed with other ingredients, most commonly with rice husks. Both rice bran and rice husks are used as mixing materials because they are by-products of processing grain into rice, producing approximately 20% rice husks and 10% rice bran as waste. Rice husks have a high lignin and silica content, which can reduce nutrient quality, feed digestibility, and livestock productivity. In addition, adulteration of rice bran with rice husks is a known issue (Rosani et al., 2024).

There is still little research on the use of rice husks as feed for ruminants. However, some studies have shown that rice husks have the potential to be applied as animal feed and can be chemically processed to increase their nutritional value (Rahat Naseer et al., 2017). The effects of rice husks and rice bran, in various forms, on rumen microbial fermentation has been previously reported, e.g., rice husks in the form of biochar (Van Dung et al., 2022), crude rice bran, and rice husks (Fidriyanto et al., 2020), ammoniated rice husks, or mushroomfermented rice husks (Begna et al., 2019).

Although rice bran and rice husk have been investigated by several researchers, there has been no reports on their use in maize straw silage and their effect on the fermentation process in the rumen and performance of Garut sheep. The objective of this study was to explore how adding rice bran and rice husk to maize straw silage-based feed affected rumen fermentation, digestion, and total gas and methane emissions. The study forms the basis for farmers and researchers regarding the use of rice bran containing rice husks as a concentrate for Garut sheep feed based on maize straw silage. We anticipate that adding rice husk to rice bran can affect the digestibility of the feed ration, underscoring the necessity for determining the maximum allowable limit of rice husk addition in maize straw silage-based rations.

Material and methods

Sample preparation

An excellent local variety of Banowati rice, obtained from the Sindanglaya regional rice factory located in Sindangsari village, Sukasari District, Sumedang Regency, was used to prepare rice bran and rice husks. Maize straw used for silage was derived from local sweet maize.

To prepare the silage, maize straw was laid out for 2 days, then was chopped into 2–3 cm fragments using a shredder. Subsequently, it was fermented in an airtight plastic drum. After 21 days, the container was opened and approx. 1 000 g was dried in the oven at 60 °C for 24 h as a sample for grinding.

Using a disc mill, rice bran, rice husks, and maize straw silage were ground separately until they all pass through a mesh 30. The sample was then filtered for 10 min using a sieve shaker. Rice bran, rice husks, and silage maize straw were then dried at 60 °C for 24 h. Next, samples of rice husks, rice bran and corn straw silage were weighed according to the treatment (the mixture was made as much as 50 g). To ensure homogeneity, the samples were stirred (Lacorte et al., 2012), and once uniformity of the texture and colour was attained, the samples were transferred into a clean glass or stainless steel container. Any non-representative materials, such as stones, wood chips or other plant debris were removed to prevent contamination. Following homogenisation, the material was stored in a sealed container (Rosani et al., 2024).

Rumen fluid was collected from adult male and female Garut sheep at the Jatinangor slaughter house (RPH Jatinangor, Sumedang, Indonesia). The rumen fluid was pre-filtered through a 3-layer gauze to remove feed particles, and then transferred into a heat-insulated container. In the laboratory, the rumen fluid from male and female sheep was filtered again and combined. For the *in vitro* experiment, the mixed rumen fluid was diluted with McDougall buffer solution (Laboratory of Ruminant Livestock Nutrition and Feed Chemistry, Bandung-Indonesia) at a ratio of 1:4. The nutrient content of rice bran, rice husk, and maize straw silage was determined using proximate analysis (Table 1).

Table 1. Nutrient content of rice bran, rice husk, and maize straw silage based on proximate analysis, %

Nutrients	Rice bran	Rice husk	Maize straw silage
Dry matter	10	10	20.39
Crude protein	13.8	3.8	14.16
Crude fibre	11.6	43.3	27.16
Ether extract	14.1	1.5	3.62
Ash	11.6	19.7	10.48
Nitrogen free extract	81	31.7	44.58
TDN (energy)	86.97	43.08	53.75
Lignin	3.84	19.57	4.6**
Silica	0.71*	34**	20.97**

TDN – total digestible nutrient; *(Satter et al., 2014), **(Maftu'ah and Nursyamsi, 2015)

Experimental design

The research was carried out at the Laboratory of Ruminant Livestock Nutrition and Feed Chemistry, Faculty of Animal Husbandry, Padjadjaran University.

The study employed a complete randomised design (CRD) to investigate the effect of rice husks on pH, ammonia, total volatile fatty acids (VFA), partial VFA, dry matter digestibility (DMD), organic matter digestibility , total gas, and methane content in an *in vitro* settings. The experiment included six replicates for each treatment, with five different levels of rice husks added to the maize straw silage-based ration (Table 2).

Table 2. Diet composition, %

Ingredients	Treatments				
	RH0	RH1	RH2	RH3	RH4
Rice bran	40	30	20	10	0
Rice husk	0	10	20	30	40
Maize straw silage	60	60	60	60	60

Inoculum preparation and incubation

In vitro assays were carried using two methods: *in vitro* ruminal fermentation for assessing fermentability, digestibility, gas production and *in vitro* ruminal fermentation (Yanza et al., 2018).

In vitro ruminal fermentation: gas production

Rumen fluid was mixed in equal proportion, filtered through four-layered cheesecloth, and promptly transported to the laboratory. Samples from each treatment were added to serum bottles in 0.5 g weighted amounts. Then, 50 ml of rumen McDougall buffer (Laboratory of Ruminant Livestock Nutrition and Feed Chemistry, Bandung-Indonesia) solution (1:4 v/v) was purged with oxygen-free CO_2 and added to each bottle. Serum bottles were sealed with a butyl rubber stopper and an aluminium crimp seal, and transferred to an incubator at 39 °C. Gas production was measured at 2, 4, 6, 8, 10, 12, and 24 h of incubation. Gas samples were collected using a syringe for methane analysis (Yanza et al., 2018).

In vitro ruminal fermentation: fermentability and digestibility

The same procedure was followed to measure fermentability and digestibility. After 24 h of incubation, samples were taken for parameter measurements (Yanza et al., 2018).

Ammonia, VFA concentration, pH, and fatty acid profile analysis

Rumen fluid pH was measured using a pH meter, and ammonia concentration was determined using the Conway microdiffusion technique. Partial VFA analysis was conducted using gas chromatography (Bruker SCION 436-GC, SCION Instruments, Munich-German) at the Poultry and Miscellaneous Livestock Instrument Standard Testing Centre (BPSI-UAT), Bogor, Indonesia (Yanza et al., 2018).

In vitro feed degradability measurement

Incubation was continued by mixing 50 ml of pepsin-HCl (Merck, Darmstadt-Germany) with the substrate (precipitate) collected after centrifugation. Samples for determining DMD and organic matter digestibility (OMD) were taken after a 48-h incubation. Thereafter, the substrate was separated by vacuum filtration using Whatman™ filter papers No. 41 (GE Healthcare UK Limited, Buckinghamshire, UK). Dry matter (DM) and OMD were calculated based on the amount of DM and organic matter (OM) that disappeared from the initial weight put into the tube (Yanza et al., 2018).

Total gas production and methane analysis

We measured the total amount of gas produced every 2 h during 24 h. Methane content in the total gas was analysed using a Shimadzu GC 14B chromatograph (Shimadzu, Ennevelin-France) at the Agricultural Environmental Instrument Standard Testing Centre (BSIP) (Yanza et al., 2018).

Statistical analysis

All data were analysed using one-way ANOVA and significant differences were further evaluated using the Duncan test. Pearson correlation analysis was performed to determine the effect of rice husks on parameters under study. All recorded data were analysed using IBM SPSS statistical software (version 25).

Results and discussion

The study investigated the impact of substituting rice bran with rice husks in maize straw silage-based rations on nutrient content, fermentability, digestibility, as well as total gas and methane production in laboratory settings. The addition of rice husks, which are rich in crude fibre, particularly lignin and silica, was expected to inhibit the digestive processes in the rumen. The following sections describe findings of our study.

Nutrient composition of the ration

The addition of rice husks can affect the nutrient content of the ration, necessitating a detailed evaluation through laboratory analysis. The ration is formulated to meet the nutritional needs of sheep during the fattening growth period (Kearl, 1982). Table 3 illustrates the impact of rice husk addition on the nutrient content of each treated ration. Variant RH0 had a more optimal nutrient content compared to variant RH4. We found that higher levels of rice husks resulted in reduced crude protein, crude fat, nitrogen-free extract, and TDN contents, but increased crude fibre and ash contents.

Table 3. Nutrient content of diets, % dry matter basis

Nutrients	Treatments				
	RH0	RH1	RH2	RH3	RH4
Crude protein	14.02	13.02	12.02	11.02	10.02
Crude fibre	20.94	24.11	27.28	30.45	33.62
Ether extract	7.81	6.55	5.29	4.03	2.77
Ash	10.93	11.74	12.55	13.36	14.17
Nitrogen free extract	59.15	54.22	49.29	44.36	39.43
TDN (energy)	67.04	62.65	58.26	53.87	49.48
Lignin	4.30	5.87	7.44	9.02	10.59

TDN – total digestible nutrient; RH0 – 40% rice bran+0% rice husk+60% maize straw silage, RH1 – 30% rice bran+10% rice husk+60% maize straw silage, RH2 – 20% rice bran+20% rice husk+60% maize straw silage, RH3 – 10% rice bran+30% rice husk+60% maize straw silage, RH4 – 0% rice bran+40% rice husk+60% maize straw silage

Similarly, other study showed that adding rice husks to rice bran could alter the composition of crude protein, nitrogen-free extract materials, and ether extracts, while significantly raising the ash and crude fibre content (Fidriyanto et al., 2020).

The differences in nutrient content of rations are caused by the raw materials (and their origin) of which the ration is composed, even if the same ingredients are used. For example, rice bran contains 42–45% carbohydrates, 23–27% fat, 12–14% protein, and 7–11% crude fibre. The exact composition may depend on factors such as the rice variety or efficiency of the milling system (Fidriyanto et al., 2020; Sapwarobol et al., 2021; Manzoor et al., 2023).

Rumen fermentation

Table 4 illustrates the impact of rice husks on rumen fermentability. The rumen pH significantly increased linearly ($P < 0.01$) with the addition of rice husks, while the concentration of ammonia and total volatile fatty acids (VFA) in the rumen fluid showed the opposite trend ($P < 0.01$) compared to group RH0. The concentrations of volatile fatty acids (acetate, propionate, isobutyrate, and N-butyrate) are affected by the rice husk. Statistical analysis indicated that the addition of rice husks did not affect the levels of iso-valeric acid, N-valeric acid, or the acetic acid-to-propionic acid ratio, yielding values that were not significantly different. Although not significant, iso-valeric acid, N-valeric acid, and the acetate-to-propionate ratio decreased linearly with increasing proportions of rice husks in the ration. The ratio of acetate to propionate did not exhibit a linear response ($P > 0.05$) with increasing proportion of rice husks in the ration.

Table 4. Effect of rice husks on fermentability, mM

Parameters	RH0	RH1	RH2	RH3	RH4	*P*-value	Contrast linear
pH	6.92^{a}	7.12^{ab}	7.23^{ab}	7.42^{b}	7.42^{b}	0.040	0.003
Ammonia	7.85^{d}	6.75^{c}	6.32^{bc}	5.87^{b}	4.02^{a}	0.000	0.000
VFA total	108.18^{c}	78.78^{b}	70.49^{b}	57.43^{ab}	34.37^{a}	0.000	0.000
VFA profile							
C2 (acetic acid)	53.22^{c}	42.03^{bc}	38.44^{bc}	30.41^{ab}	16.77^{a}	0.000	0.000
C3 (propionic acid)	41.25^{c}	25.69^{b}	20.81^{ab}	17.43^{ab}	9.94^{a}	0.000	0.000
iC4 (iso-butyric acid)	2.19^{b}	1.72^{ab}	1.62^{ab}	1.38^{a}	1.19^{a}	0.039	0.002
nC4 (N-butyric acid)	7.97^{b}	5.93^{ab}	5.85^{ab}	4.58^{a}	3.29^{a}	0.023	0.001
iC5 (iso-valeric acid)	1.56	1.29	1.30	1.18	0.95	0.131	0.013
nC5 (N-valeric acid)	0.55	0.45	0.48	0.40	0.30	0.113	0.013
acetic acid:propionic acid	1.43	1.68	1.99	2.05	1.94	0.737	0.240

VFA – volatile fatty acid; RH0 – 40% rice bran+0% rice husk+60% maize straw silage, RH1 – 30% rice bran+10% rice husk+60% maize straw silage, RH2 – 20% rice bran+20% rice husk+60% maize straw silage, RH3 – 10% rice bran+30% rice husk+60% maize straw silage, RH4 – 0% rice bran+40% rice husk+60% maize straw silage; ^{abc} means within a column with different superscripts are significantly different at $P < 0.05$

Ruminant animals require a stable rumen environment, and rumen pH, molar concentrations of ammonia, and VFA are important indicators of this stability, reflecting the state of rumen fermentation processes (Tomczak et al., 2019). The content of carbohydrates in the ration and the concentration of organic acids in the rumen determine the pH, and consequently, the pH of the rumen fluid effectively reflects the fermentation status of rumen microorganisms.

In this study, rumen pH (Table 4) in all treatments ranged from 6.92 to 7.42, which fell within the normal range of rumen ecology (pH 6.2–7.0). This indicates that increasing the level of rice husks in the silage-based rations helped maintain an appropriate pH level in the rumen. This process was supported by maize straw, which provided favourable environmental conditions. These findings are consistent with previous studies, which reported pH values ranging from 6.50 to 6.67 (Kyawt et al., 2024).

Our research showed that adding up to 40% rice bran and rice husk to the feed as a concentrate was safe for the rumen environment, as it did not lower the pH, and ensured a conducive environment for microbial growth and efficient nutrient absorption. Previous research has demonstrated that high levels of concentrates in ruminant diets often lead to a decrease in rumen pH due to the accumulation of VFAs produced by rumen microbial fermentation (Zhang et al., 2017; Sun et al., 2018).

It has been established that pH is an important determinant of ammonia absorption in the rumen epithelium, with an increase in rumen pH enhancing the rate of ammonia absorption (Abdoun et al., 2006). In this study, a decrease in rumen pH indicated reduced ammonia absorption through the rumen epithelium. In addition, low rumen pH is unfavourable for rumen microbial fermentation, as it prevents microorganisms from protein synthesis from ammonia. This reduced microbial activity results in lower ammonia utilisation, as evidenced by a lower pH and total VFA concentration. The pH value of rumen fluid is significantly negatively correlated with foam production and its persistence, and a lower pH of foamy rumen, increases the risk of bloating. This is consistent with a higher incidence of rumen bloat observed with higher ratios of feed concentrates (Tan et al., 2024). The application of a low-protein diet is an effective strategy to prevent rumen bloat (Tan et al., 2024).

In these tests, the rumen pH of the animals in the intensive feeding group was lower than that in the naturally grazed group. This could be attributed to the higher intake of non-structural carbohydrates by the intensively fed group, which were rapidly fermented in the rumen, resulting in increased VFA production. The ammonia concentration in the intensively fed group was significantly higher compared to the naturally grazing group, possibly due to the high content of soluble protein, easily digestible carbohydrates, and non-protein nitrogenous substances in the concentrate, as well as the slow degradation rate, which favours ammonia synthesis. Other authors reported that an increase in fibre content in dairy cows' feed resulted in a decrease in rumen fluid ammonia concentration, possibly due to the presence of carbohydrate structures in fibre that decreased ammonia production.

The rumen is a unique digestive organ of ruminants that plays an important role in providing energy to the body. It hosts a diverse community of microorganisms that decompose carbohydrates into VFA (Tang et al., 2019). After feed consumption, the rumen rapidly ferments the feed, resulting in the production of VFA and ammonia. The concentration and proportion of VFA in the rumen are primarily influenced by feed composition (Sun et al., 2020). Feeds high in fibre content contain high amounts of cellulose and haemicellulose, while starches easily fermented and soluble sugars are present in low amounts. Available sugars in these types of feeds are mostly structural carbohydrates that are difficult to ferment, resulting in low VFA production (He et al., 2024).

In this study, ammonia levels decreased with increasing proportion of rice husks in the ration. Specifically, ammonia concentration decreased from 7.85 mM to 4.02 mM, with an increase in rice husk proportion from 0 to 40%. This reduction in ammonia concentration in the rumen suggested that lignin present in rice husks could inhibit the breakdown of dietary proteins (Tang et al., 2019). The values obtained in the present study were lower than those reported in previous works, which obtained ammonia concentrations ranging from 8.60 to 9.70 mM with various levels of concentrate (Oghenesuvwe et al., 2024). This may be attributed to variations in the composition and quantity of the concentrate materials used. Nevertheless, ammonia concentration in treatment RH2 was still within the normal range for rumen ammonia levels, i.e. typically between 6 and 21 mM for optimal microbial protein synthesis.

Consistent with the trend in ammonia concentration, VFA also decreased as the proportion of rice husks in the ration increased. Total VFA levels in this study ranged 34.37–108.18 mM, acetate 16.77–53.22 mM, propionate 9.94–41.25 mM, and butyrate 4.48–10.16 mM. The addition of rice husks up to 20% yielded a VFA concentration of 70.49 mMol, which was a normal value suitable for rumen microorganisms. The typical range for effective VFA production that supports rumen microbial growth is between 70–150 mM and 70–130 mM. The decrease in VFA concentration was most likely due to the utilisation of VFA by microorganisms as a carbon source.

Previous studies reported total VFA concentrations ranging from 55.40 to 78.30 mM, with acetic acid between 29.00 and 41.00 mM, propionic acid between 19.00 and 21.70 mM, and butyric acid also between 19.00 and 21.70 mM (Oghenesuvwe et al., 2024). Additionally, studies involving different herbal extracts reported total VFA levels between 78 and 85.2 mM, acetate levels ranging from 57.8 to 61.6 mM, and propionate levels between 21.4 and 23.6 mM (Jo et al., 2022).

The acetate-to-propionate ratio is often used as an indicator of feed quality and utilisation efficiency in the rumen, with a lower A/P ratio suggesting higher feed efficiency and greater glucose synthesis in ruminants. In this study, although the A/P ratio tended to increase with higher levels of rice husks in the ration, the differences were not statistically significant. This implies that rice husks did not contribute to glucose production in livestock, which was also consistent with the observed reductions in ammonia and VFA contents at higher rice husk levels. The A/P ratio observed in this study ranged from 1.43 to 1.94, which is considered acceptable, as an efficient balance is indicated by a ratio below 3. Previous studies have reported A/P ratios ranging from 1.44 to 2.23 (Rathert-Williams et al., 2023) and 2.27 to 3.16 (Cattaneo et al., 2023).

Digestibility

Table 5 shows the impact of rice husks on DM and OMD. The results indicate a significant decrease ($P < 0.01$) in both DM and OMD with increasing levels of rice husks in the ration compared to the control (RH0). Specifically, DMD ranged from 27.44% to 52.74%, while OMD ranged from 38.92% to 63.07%. These results were consistent with an earlier *in vitro* study, which demonstrated that adding rice husks to rice bran led to higher acetic acid levels but reduced gas production potential, gas production rate, and both DM and OMD (Fidriyanto et al., 2020).

The decrease in ration digestibility observed with increasing levels of rice husks is attributed to the higher crude fibre content, particularly lignin and lignocellulose found in rice husks. Rice husks have low digestibility due to their high fibre content and low protein content (Roba et al., 2022). The digestibility value of rations with 10% (RH1) and 20% (RH2) rice husks can still be considered acceptable for ruminant livestock, as their digestibility remained within the recommended range. However, rations with 30% (RH3) and 40% (RH4) rice husks were unsuitable for ruminant feeding due to inadequate digestibility. Previous research has indicated that ruminant livestock requires feed with a minimum digestibility value of 60%. If digestibility falls between 40 and 60%, then urea treatment is necessary, while if it is below 40%, it is typically utilised as fuel or fertiliser (Preston, 1998). We recommend that the addition of rice husk as a substitute for rice bran should not exceed 50% (RH2), as its average digestibility of dry matter and organic matter is 42.16%, provided that other high-quality feed ingredients are included.

Figure 1 illustrates the regression pattern for the relationship between rice husk levels and the digestibility of DM and OM. The linear regression analysis indicated that increased levels of rice husks significantly reduced both types of digestibility. For DMD, the regression equation was as follows: $y = -0.6534x + 51.886$ with $R^2 = 97.47\%$, which meant that every 1% increase in rice husk content decreased DMD by 0.65%. Likewise, the regression equation is for OMD was: $Y = -0.6279x + 62.174$ with R2 = 96.19%. This indicated that every 1%

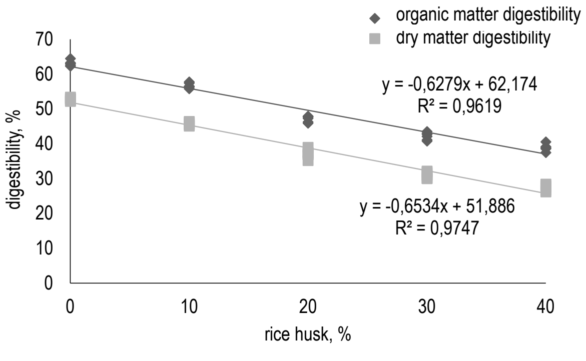

Figure 1. Relationship between digestibility and rice husk levels

Table 5. Effect of rice husks on digestibility, %

Parameters	RH0	RH1	RH2	RH3	RH4	*P*-value	Contrast linear
Dry matter digestibility	52.74^{e}	45.76^{d}	37.15^{c}	31.01^{b}	27.44^{a}	0.000	0.000
Organic matter digestibility	63.07^{e}	56.71^{d}	47.16^{c}	42.22^{b}	38.92^{a}	0.000	0.000

RH0 – 40% rice bran+0% rice husk+60% maize straw silage, RH1 – 30% rice bran+10% rice husk+60% maize straw silage, RH2 – 20% rice bran+20% rice husk+60% maize straw silage, RH3 – 10% rice bran+30% rice husk+60% maize straw silage, RH4 – 0% rice bran+40% rice husk+60% maize straw silage; $^{a-e}$ means within a column with different superscripts are significantly different at $P < 0.05$

increase in rice husk content reduced the digestibility of OM by 0.63%. Our findings demonstrate that rice husks with a high content of lignin and silica, which are not degradable by microbes, decrease the ration's digestibility by 0.65%. This result implies that the use of rice husks in maize straw silage based rations is limited. The findings were in line with previous research showing that an increase of 1% in the silica content of feed materials could decrease digestibility by 2-3% in ruminants (Van Soest, 2018).

The effect of rice husks on DMD can vary depending on specific factors, such as treatment, fermentation, or animal species. Previous research showed that sheep fed a feed based on treated or untreated rice husks had significantly higher intakes of DM and OM, which indicated positive effects on digestibility and growth performance (Roba et al., 2022). Other studies, however, revealed that substituting rice husks with 10% rice bran could significantly reduce DMD (Fidriyanto et al., 2020). In addition, the high fibre and lignin content of rice husks makes them more difficult to digest, particularly for monogastric animals. However, in ruminants, microorganisms in the rumen can break down rice husks, though this process may require extended digestion time (Syafrudin et al., 2020).

Although rice husks have limited nutritional value and low digestibility, several studies have explored methods to enhance their utility as animal feed. Fermentation and processing methods, such as the use of molasses and yeast, have been shown to improve the nutrient content and palatability of rice husks, potentially impacting their digestibility (Syafrudin et al., 2020). Additionally, biological treatments, such as fermentation with *Pleurotus ostreatus* (Beg et al., 1986), or supplementation with phytase, have been investigated to further improve the nutritional value and digestibility of rice husks.

Gas and methane production

The relationship between potential substrate degradation rates and *in vitro* gas production has been well documented (Menke et al., 1979) and is considered a reliable tool for feed evaluation. Table 6 presents the *in vitro* gas production profile after 24 h of incubation. The generation of total gas and methane was significantly influenced ($P < 0.01$) by the levels of rice husks. There was a clear linear decrease in both total gas and methane production with increasing rice husk levels ($P < 0.01$). While the rice husk levels significantly affected the overall gas production potential (a+b), they did not significantly influence the rate of gas production over time (c).

Gas generation over 24 h ranged from 28.63 to 51.08 ml, with the highest production in the control ration at 51.58 ml, and the lowest in RH4 at 28.63 ml. The increase in rice husk levels resulted in a linear decrease in total gas production. These values were lower than those obtained in an earlier study, which reported gas production in rations supplemented with various herbal extracts ranging from 116 to 122 ml (Jo et al., 2022). The discrepancy can be attributed to the differing composition of rations, particularly the variation in easily digestible carbohydrate content. In addition, lignocellulose sources were also shown to affect the production of total gas and methane (Kyawt et al., 2024).

Figure 2 presents the rate of gas production during 24 h of incubation. The results of this study showed that gas continued to be produced throughout the 24-h incubation period across all treatments. This phenomenon suggests that fermentable substrate was still available for up to 48 h of incubation. Additionally, Figure 2 also shows that increasing the proportion of rice husks could reduce gas production.

Consistently with total gas production, methane concentrations ranged from 9416.92 to 18161.34 ppm, with the lowest concentration recorded in RH4 at 9416.92 ppm and the highest in control rations at 18161.34 ppm. Methane emission decreased by approximately 48% at the highest level of rice husks (40%). This result is lower compared to previous

Table 6. Effect of rice husks on gas production and methane content, ml

Parameters	RH0	RH1	RH2	RH3	RH4	*P*-value	Contrast linear
Total gas production	51.08^{e}	44.80^{d}	39.65^{c}	33.78^{b}	28.63^{a}	0.000	0.000
a+b	52.86^{b}	45.36^{ab}	44.28^{ab}	41.27^{ab}	28.86^{a}	0.130	0.026
c	0.02	0.01	0.02	0.01	0.01	0.502	0.397
Methane content, ppm	18.161.34^{c}	16.207.82^{bc}	14.017.75^{b}	10.965.52^{a}	9.416.92^{a}	0.000	0.000

a+b – potential gas production, c – gas production rate; RH0 – 40% rice bran+0% rice husk+60% maize straw silage, RH1 – 30% rice bran+10% rice husk+60% maize straw silage, RH2 – 20% rice bran+20% rice husk+60% maize straw silage, RH3 – 10% rice bran+30% rice husk+60% maize straw silage, RH4 – 0% rice bran+40% rice husk+60% maize straw silage; $^{a-e}$ means within a column with different superscripts are significantly different at $P < 0.05$

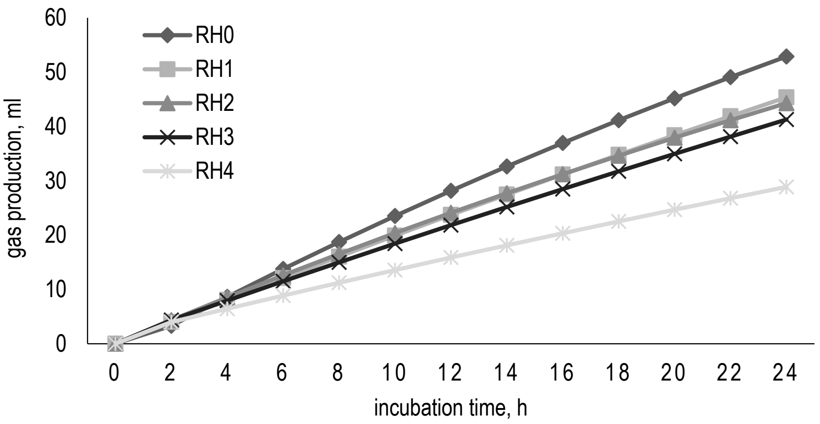

Figure 2. Gas production rate during 24 h of incubation

RH0 – 40% rice bran+0% rice husk+60% maize straw silage, RH1 – 30% rice bran+10% rice husk+60% maize straw silage, RH2 – 20% rice bran+20% rice husk+60% maize straw silage, RH3 – 10% rice bran+30% rice husk+60% maize straw silage, RH4 – 0% rice bran+40% rice husk+60% maize straw silage

studies, which reported methane concentrations in rations supplemented with various herbal extracts ranging from 11 000 to 18 600 ml (Jo et al., 2022).

The methane concentration in the control ration was higher than in rations containing rice husks. This is because the control ration contained 100% rice bran, a carbohydrate that is easily degraded by rumen microbes. These results were align with previous studies, where gas generation in the water-soluble fraction was statistically higher than in rice bran, ethanol, and water-insoluble rice bran (Manlapig et al., 2024). Similar findings were also reported for forage millet and sorghum silage, where higher gas production was observed in varieties rich in water-soluble, easily fermentable carbohydrates such as glucose, galactose, xylose, or mannose (Liu et al., 2017).

Figure 3 illustrates the regression pattern for the relationship between methane production and rice husk levels. The data indicate that the influence of rice husks could reduce methane emission in a linear manner. The effect of rice husk addition can be expressed by the following equation: $y = -227.31x + 18300$ with R2 = 70.82%, which means that every 1% increase in rice husks results in a decrease in methane levels by 227.3 ppm.

In contrast to the negative impact of lignification on digestibility described above, several studies reported that lignin in ruminant feed positively affected greenhouse gas emissions. Ruminant animal production is one of the most important sources of anthropogenic methane gas emission, the second most important greenhouse gas after carbon dioxide (Karakurt et al., 2012). Methane is produced in the rumen during anaerobic fermentation of organic matter and is subsequently released into the atmosphere (Bodas et al., 2012). When pure lignin is added to sheep feed in varying amounts, it reduces

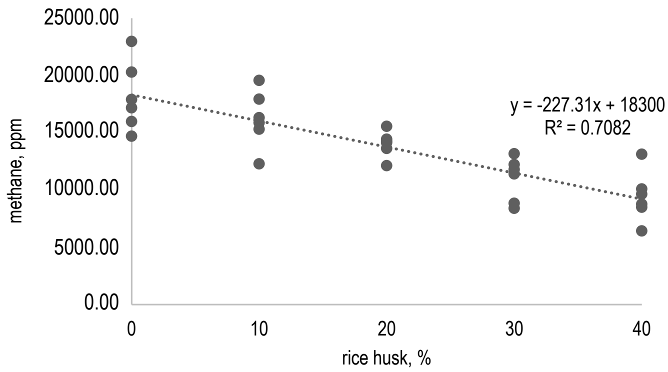

Figure 3. Relationship between methane production and rice husk levels

RH0 – 40% rice bran+0% rice husk+60% maize straw silage, RH1 – 30% rice bran+10% rice husk+60% maize straw silage, RH2 – 20% rice bran+20% rice husk+60% maize straw silage, RH3 – 10% rice bran+30% rice husk+60% maize straw silage, RH4 – 0% rice bran+40% rice husk+60% maize straw silage feed intake but does not affect growth performance. However, this decreases methane release during *in vitro* incubation of sheep feed formulations (Wang et al., 2009). Similarly, high-lignin feeds show relatively low methane release during *in vitro* incubation in rumen fluid obtained from cows compared to high-sugar feeds (Hindrichsen et al., 2004). In addition, when different types of fibres were incubated in buffalo inoculum, a negative correlation was found between lignin content and methane release (Singh et al., 2011).

The mechanism by which rice husks influence methane production in the rumen is not yet fully understood and requires further research. However, research shows that the addition of rice husks to ruminant feed can affect rumen fermentation capacity, potentially leading to changes in methane production (Fidriyanto et al., 2020; Van Dung et al., 2022). Moreover, methane production is influenced by various factors, including the quality and quantity of additional organic matter, soil redox potential, soil carbon content, and rice growth. Therefore, these factors may also alter the impact of rice husks on methane production in the rumen. Overall, the present study demonstrates the positive role of rice husk lignin in reducing ruminant methane emissions.

Based on the results of the above research, we believe that the information concerning substituting rice bran with rice husks in ruminant rations and its effects on rumen fermentation is novel. These findings suggest that adding rice husks to ruminant rations may impact the rumen's fermentation ability, as well as the overall digestion and metabolism of animals. Our results imply that the addition of rice husks (DM basis) to the ration should be limited to a maximum of 20%, or the substitution of rice bran with rice husk should not exceed 50%. This result provides a basis for limiting the amount of rice husks in the ration. The use of agricultural waste such as maize straw, rice bran, or rice husks can serve as a primary energy source for ruminants, thereby reducing energy losses in the form of decomposed lignocellulosic biomass and methane, resulting in high economic value for farmers.

Conclusions

This study shows that the addition of up to 50% rice husk to replace rice bran in maize straw-based rations remains within acceptable limits and can effectively reduce total gas and methane production. Further *in vivo* research is necessary to validate these findings and better understand the impact of rice husks on the performance of Garut sheep.

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Conflict of interest

The Authors declare that there is no conflict of interest.

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