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Effect of forage nutrient on sheep gas emission in different multi-species cropping system

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Highlights

- Authors chose in site ration system (IRS) to supply continuous high-quality forage.
- The research on different feeding ratios of five plant species in one pasture area.
- Evaluation of the effect of *I. zollingeriana* supplementation in varying levels.
- *I. zollingeriana* supplement improved dry/organic matter digestibility, total VFA.
- A balanced ration using several grasses and legumes could meet the sheep needs.

Abstract: The in site ration system (IRS) is an innovative forage production strategy designed to optimise livestock nutrition by integrating multiple forage species with complementary agronomic and nutritional characteristics. This research aimed to evaluate the effect of a balanced forage ration using IRS on sheep nutritional quality, digestibility, fermentability, and gas emission. The research was conducted at the Jonggol Animal Science, Teaching, and Research Unit, IPB University, where *Urochloa* hybrid cv. 'Mulato II' (*U. ruziziensis* × *U. decumbens* × *U. brizantha*), *Pennisetum purpureum* cv. 'Thailand', *P. purpureum* cv. 'Mott', and *Indigofera zollingeriana* were cultivated. The research evaluated the impact of varying levels of *I. zollingeriana* supplementation (0, 5, 10, 15, and 20%) on *in vitro* digestibility, ruminal fermentation parameters, gas production, and methane production. Results demonstrated that increasing *I. zollingeriana* supplementation significantly improved *in vitro* dry matter digestibility (*IVDMD*) and *in vitro* organic matter digestibility (*IVOMD*), with the highest values recorded in the 20% *I. zollingeriana* ration (70.4 and 69.4%, respectively). Additionally, total volatile fatty acid (*VFA*) concentrations increased proportionally with *I. zollingeriana* levels, although led to increased gas and methane production, with the 20% *I. zollingeriana* ration showing the highest methane output at 2.35 cm^3. These findings highlight the potential of IRS to enhance forage quality and nutrient utilisation through several grasses and legume species cultivation in a location that increased ruminal digestibility and fermentation efficiency. Further research is needed to mitigate the potential forage that minimises gas and methane production.

Keywords: digestibility, fermentability, forage quality, gas emission, *Indigofera zollingeriana*, in site ration system (IRS), sheep nutrition

INTRODUCTION

The in site ration system (IRS) is an innovative approach to providing forage material for livestock, including sheep. This concept was carried out by Telleng *et al.* (2016) and Ernawati *et al.* (2023a) that develop the intercropping system of different elephant grass varieties cultivated with *Indigofera zollingeriana*. The selection of planting systems can be adjusted to land conditions, environmental conditions, technology availability, and farmer needs. Intercropping enhances biodiversity by growing multiple crops together, improving pest control (Mwani *et al.*, 2021), and reducing chemical use (Rani and Kammara, 2024). The focus of the IRS system is the provision of multi-species green forage that allows optimal fulfilment of livestock nutrition.

The selection of multiple plant species in this system is based on the need to ensure a more balanced nutrient availability (Martinez, Gathorne-Hardy and Smith, 2024). The use of species such as *Urochloa* hybrid cv. 'Mulato II' (*U. ruziziensis* × *U. decumbens* × *U. brizantha*) (Marques *et al.*, 2017), *Pennisetum purpureum* cv. 'Thailand' (Ernawati *et al.*, 2023b; Herfan, Umar, and Husni, 2023), *P. purpureum* cv. 'Mott' (Osak, Anis, and Rumambi, 2018; Putra *et al.*, 2024), and *I. zollingeriana* aims to optimise biomass production with high nutritional quality. *I. zollingeriana* is essential in the IRS concept as an affordable protein source to meet sheep's nutritional needs (Abdullah, 2010; Abdullah and Suharlina, 2010; Suharlina *et al.*, 2016). The supplementation of *I. zollingeriana* leaf extract could reduce 19.28% methane gas production on sheep (Suhartati, 2020) while 30% fresh leaf would reduce 10% (Afzalani *et al.*, 2021). The combination of various forage species in IRS has the potential to produce rations with more complete nutritional content for sheep. High forage percentage in animal feed ration could produce a lower total gas (Vargas *et al.*, 2023) through different forage ration (Boadi *et al.*, 2004) that affected on rumen pH (Rosa *et al.*, 2024). On the other hand, forage composition and growing season could decrease CH_4 production (Wang *et al.*, 2005) and the recent research reported that forage genotype influenced the gas emissions (Lombardi *et al.*, 2022). Thus, a balanced feed formulation can reduce methane emissions, improve feed efficiency, and lower the livestock sector's environmental impact (Króliczewska, Pecka-Kiełb and Bujok, 2023). This research aimed to evaluate the effect of a balanced forage ration using IRS on sheep nutritional quality, digestibility, fermentability, and gas emission.

MATERIALS AND METHODS

FORAGES CULTIVATION

The forage crops cultivated in this research included *Urochloa* hybrid, *P. purpureum* cv. 'Thailand', and *P. purpureum* cv. 'Mott', while *I. zollingeriana* was already established at the research site. All forages were planted in 28 uniformly sized plots measuring 6 × 5 m for each species in Area 1 and 2 (Fig. 1). The planting distances were as follows: *Urochloa* hybrid at 60 × 60 cm, *P. purpureum* cv. 'Thailand' at 100 × 50 cm, and *P. purpureum* cv. 'Mott' at 75 × 75 cm. All plots were treated with a basal fertiliser application of 10 Mg·ha^{-1} of manure (a mixture of 60% cattle manure and 40% poultry manure), 200 kg·ha^{-1} of nitrogen, and 150 kg·ha^{-1} each of phosphorus and potassium. The plants were first pruned 75 days after planting, followed by subsequent harvesting 40 days after pruning (DAP).

FEED TREATMENTS PREPARATION

The feed ingredients used consisted of *Urochloa* hybrid, *P. purpureum* cv. 'Thailand', *P. purpureum* cv. 'Mott', *I. zollingeriana*, and molasses. The experimental feed used was the difference in the percentage of *I. zollingeriana*, including feed without *I. zollingeriana* (control), feed with 5% *I. zollingeriana* (R1), feed with 10% *I. zollingeriana* (R2), feed with 15% *I. zollingeriana* (R3), and feed with 20% *I. zollingeriana* (R4). The nutrient content of the feed ingredients was analysed using the Association of Officiating Analytical Chemists (AOAC) proximate method (Horwitz and Latimer Jr, 2005), including dry matter, ash, crude protein (*CP*), ether extract (*EE*), crude fibre (*CF*), and nitrogen free extract (*NFE*). The feed ingredients were also analysed for neutral detergent fibre (*NDF*), acid detergent fibre (*ADF*), hemicellulose, cellulose, lignin, calcium (Ca), and phosphorus (P). The feed treatment was formulated based on feed ingredients nutrient content and adjusted to the nutritional needs of breeding sheep (NRC, 1985) (Tab. 1).

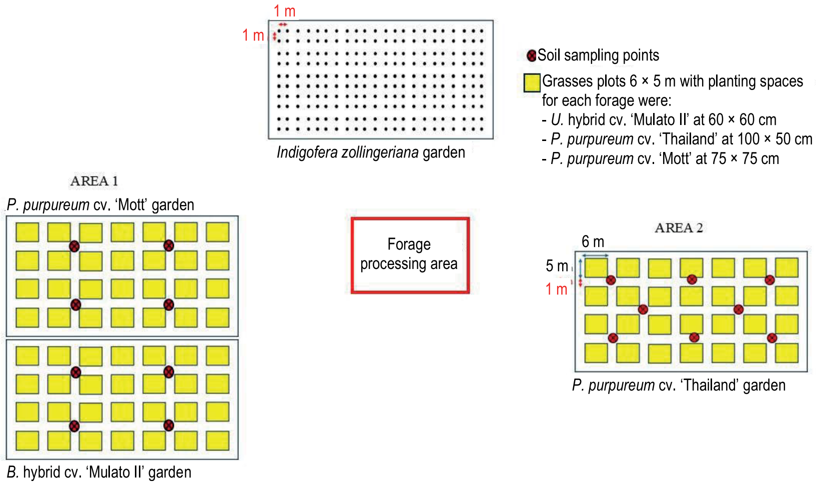

Fig. 1. Sampling plots area; source: own study

Table 1. Composition and nutrient content of feed treatments

Specification	Feed treatment				
	R0	R1	R2	R3	R4
Feed ingredients (%)					
I. zollingeriana (60 *DAP*)	0.0	5.0	10.0	15.0	20.0
Urochloa hybrid cv. 'Mulato II' (40 *DAP*)	35.0	24.5	30.0	32.0	36.5
Urochloa hybrid cv. 'Mulato II' (60 *DAP*)	14.0	24.5	30.0	29.0	32.5
P. purpureum cv. 'Thailand' (30 *DAP*)	20.5	15.0	13.0	6.0	1.5
P. purpureum cv. 'Thailand' (40 *DAP*)	5.5	13.0	3.5	7.5	1.0
P. purpureum cv. 'Mott' (30 *DAP*)	12.0	10.0	5.0	2.0	2.5
P. purpureum cv. 'Mott' (40 *DAP*)	8.0	3.0	3.5	3.5	1.0
Molasses	5.0	5.0	5.0	5.0	5.0
Nutrient content (%)					
Dry matter	92.5	92.1	93.1	92.7	92.8
Ash	9.61	9.34	8.94	8.81	9.08
Crude protein	12.4	10.9	13.2	11.1	12.7
Ether extract	17.7	17.5	16.9	17.5	16.2
Crude fibre	3.87	3.43	3.07	3.32	3.24
NFE	48.9	50.9	51.0	52.0	51.5
NDF	56.8	55.6	49.2	48.3	47.8
ADF	32.5	31.2	30.4	29.5	28.0
Hemicellulose	24.3	24.4	18.8	18.8	19.8
Cellulose	19.2	25.7	25.5	19.5	16.4
Lignin	10.9	4.04	3.17	9.57	9.17
Calcium	2.10	1.66	1.17	2.22	2.03
Phosphorus	0.75	0.94	0.95	1.17	1.04
Gross energy	4,232	4,219	4,218	4,170	4,279
Total nitrogen	1.98	1.75	2.12	1.78	2.04
C-organic	22.9	38.8	28.8	28.4	29.8
C–N ratio	11.6	22.2	13.6	16.0	14.7

Explanations: *DAP* = days after pruning, *NFE* = nitrogen free extract, *NDF* = neutral detergent fibre, *ADF* = acid detergent fibre, R0 = control, R1 = 5% *I. zollingeriana*, R2 = 10% *I. zollingeriana*, R3 = 15% *I. zollingeriana*, R4 = 20% *I. zollingeriana*.
Source: own study.

Composite soil samples were collected, homogenised, and sampled for detailed chemical analysis. Soil analysis was performed using potentiometry to understand the soil acidity, spectrophotometry for organic carbon and potential phosphorus, Kjeldahl method for nitrogen total, atomic absorption spectrometry (AAS) for potential potassium, and titrimetry for cation exchange capacity, and exchangeable acidity of aluminium and hydrogen.

IN VITRO ANALYSIS

In vitro, fermentability and digestibility measurements were carried out using the Tilley and Terry (1963) method that was conducted at standard temperature (38°C) and incubation time (48 h). The rumen fluid was collected from eight live sheep using stomach tube in the amount of 200 $cm^3 \cdot head^{-1}$ and pooled in one thermostat jar, the replicated based on the time collection. The animal usage received ethical approval under the reference number 284–2024 IPB. The pH measurements were carried out using a pH meter on the supernatant fluid. The NH_3 analysis was carried out using the Conway Microdiffusion technique (Masterson, 2014). The duration of the fermentation period for gas measurement were 2, 4, 8, 12, and 24 hours. Gas production and methane were collected for 10 replications using the method by Theodorou *et al.* (1994). Methane production was measured using gas chromatography with a SHIMADZU GC-14B gas chromatograph.

EXPERIMENTAL DESIGN AND DATA ANALYSIS

The research consists of two designs based on the stages where there was forage quality production and its effect on gas production (*in vitro* study). On the forage quality production, its species were cultivated based on the in site ration system (IRS) design in two areas as shown in Figure 1, each study area was divided into rectangular cells. The soil sampling was designed using a randomised along the valley slope where the forage was planted using an auger-boring instrument at the depths of 0–15 cm depth (topsoil) to take 1 kg of soil in 8 sampling points for each area (Fig. 1). The production data collection was designed by harvesting all plants within the plot, excluding the border area. The harvested biomass was assessed for fresh weight, dry weight, leaf weight, stem weight, and leaf-to-stem ratio.

In the second stage, in order to study the effect of forage on gas production a randomised complete block design (RCBD) has been used with five groups as replications. The grouping was done based on the time of rumen fluid collection. The data obtained from this research were analysed using analysis of variance (ANOVA). The Duncan multiple range test (DMRT) was followed for data with a significant effect. Pearson correlation analysis was used to measure the degree of relationship between each variable (pH, ammonia production, feed digestibility, and total volatile fatty acid (*VFA*). Data processing was carried out using the IBM SPSS Statistics 25 program.

RESULTS AND DISCUSSION

RESULTS

The pH values indicated that the soil in the research plots ranged from moderately acidic (5.50) to neutral (6.90) (Tab. 2). However, pH N KCl values confirmed the trend of slightly acidic conditions, with the soil in area 2 demonstrating a more acidic environment compared to area 1. Area 1 demonstrated higher organic carbon (3.04%), total nitrogen (1.48%), and cation exchange capacity (44.28 $cmol(+) \cdot kg^{-1}$). In contrast, area 2 had higher potential potassium (10.12 $mg \cdot (100\ g)^{-1}$) and slightly higher exchangeable acidity for H^+ (0.64 $cmol(+) \cdot kg^{-1}$). These

Table 2. Chemical characteristics of research plots soil

Parameter	Research plot	
	area 1	area 2
pH (H_2O)	6.90	5.50
pH (N KCl)	5.80	4.00
C-organic (%)	3.04	1.84
N-total (%)	1.48	0.18
P_2O_5 potential (mg·(100 g)$^{-1}$)	19.91	25.37
K_2O potential (mg·(100 g)$^{-1}$)	9.56	10.12
Cation exchange capacity (cmol(+) kg^{-1})	44.28	31.77
Exchangeable acidity Al^{3+} (cmol(+) kg^{-1})	<0.04	0.18
Exchangeable acidity H^+ (cmol(+) kg^{-1})	0.14	0.64

Explanations: area 1 = research plots for *Urochloa* hybrid and *P. purpureum* cv. 'Mott', area 2 = research plot for *P. purpureum* cv. Thailand.
Source: own study.

findings highlighted the distinct chemical characteristics of the research plots, with differences in nutrient availability and soil acidity potentially influencing plant growth and development.

The production characteristics of *Urochloa* hybrid, *P. purpureum* cv. 'Thailand', and *P. purpureum* cv. 'Mott' in Table 3 emphasised their potential as forage resources in the in site ration system (IRS). *Urochloa* hybrid had the highest fresh yield (41.8 Mg·ha^{-1}), dry yield (6.63 Mg·ha^{-1}), and leaf yield (34.1 Mg·ha^{-1}), while *P. purpureum* cv. 'Mott' showed the highest leaf-to-stem ratio (8.58), suggesting superior forage quality.

The dry matter (*DM*) content of forage in Table 4 ranged from 9.77 to 23.2% (as is). Among the forage types, *Urochloa* hybrid showed the lowest crude protein (*CP*) content. Additionally, the crude fibre content of grasses was significantly higher than that of *I. zollingeriana*, with grasses also showing the highest levels of neutral detergent fibre (*NDF*) and acid detergent fibre (*ADF*).

Table 3. Production of *Urochloa* hybrid cv. 'Mulato II', *P. purpureum* cv. 'Thailand', and *P. purpureum* cv. 'Mott' on 40 days after pruning (mean ±*SD*)

Production	*Urochloa* hybrid cv. 'Mulato II'	*P. purpureum* cv. 'Thailand'	*P. purpureum* cv. 'Mott'
Fresh yield (Mg·ha^{-1})	41.8 ±3.64	33.5 ±4.45	34.0 ±4.27
Dry yield (Mg·ha^{-1})	6.63 ±0.80	5.18 ±0.66	3.48 ±0.22
Leaf yield (Mg·ha^{-1})	34.1 ±2.69	17.0 ±1.59	30.4 ±3.92
Stem yield (Mg·ha^{-1})	7.71 ±1.01	16.4 ±3.00	3.54 ±0.39
Leaf-to-stem ratio	4.45 ±0.27	1.05 ±0.13	8.58 ±0.54

Explanations: *SD* = standard deviation.
Source: own study.

The R4 ration revealed a significantly higher rumen pH ($p < 0.005$) compared to the other ration treatments (Tab. 5). The pH value was slightly above the normal range, which ranged from 7.11 to 7.33. The R2, R3, and R4 rations showed the highest NH_3 values ($p < 0.05$), at 142, 147, and 141, respectively. The lowest and highest digestibility were obtained in the R0 and R4 rations, respectively. The results indicated that the R4 ration significantly increased in vitro dry matter digestibility (*IVDMD*) ($p < 0.01$), reaching a value of 70.4%. In the Table 5, it can be observed that *I. zollingeriana* supplementation significantly increased total volatile fatty acids (*VFA*) ($p < 0.01$), with the highest concentration observed in the R4 ration, reaching 154 mmol·dm^{-3}.

Table 4. Nutrient contents of feed ingredients

Nutrient content	Feed ingredient						
	Urochloa hybrid cv. 'Mulato II'		*P. purpureum* cv. 'Thailand'		*P. purpureum* cv. 'Mott'		*I. zollingeriana*
	40[1)]	60[1)]	30[1)]	40[1)]	30[1)]	40[1)]	60[1)]
Dry matter (%)	23.2	28.6	9.77	13.4	12.3	15.7	16.2
Ash (% *DM*)	9.81	9.54	10.4	7.18	12.0	10.6	10.4
Crude protein (% *DM*)	11.3	10.4	20.3	13.9	15.5	15.2	25.3
Ether extract (% *DM*)	2.78	3.50	3.15	3.48	3.72	3.49	5.00
Crude fibre (% *DM*)	21.2	25.0	27.4	28.2	24.4	24.8	15.7
NFE (% *DM*)	54.9	52.5	38.8	47.3	44.4	45.9	43.6
NDF (% *DM*)	52.7	55.1	61.1	70.0	63.2	66.2	39.1
ADF (% *DM*)	28.3	33.1	34.9	40.7	36.1	35.9	19.2
Hemicellulose (% *DM*)	24.4	22.0	26.2	29.3	27.1	30.4	19.9
Cellulose (% *DM*)	18.2	20.0	29.2	34.0	29.5	25.9	12.2
Lignin (% *DM*)	8.10	9.56	5.00	5.61	4.79	7.76	6.50
Calcium (% *DM*)	0.69	0.76	0.59	0.48	0.70	0.63	0.33
Phosphorus (% *DM*)	0.51	0.58	0.40	0.33	0.44	0.41	0.19

[1)] Days after pruning.
Explanations: *DM* = dry matter, *NFE* = nitrogen free extract, *NDF* = neutral detergent fibre, *ADF* = acid detergent fibre.
Source: own study.

Table 5. Ruminal pH, ammonia, digestibility rates, and total volatile fatty acids (*VFA*) of in site ration system with different forage compositions (mean ±*SD*)

Variable	Feed treatment					*p*-values
	R0	R1	R2	R3	R4	
pH	7.14 ±0.14^{B}	7.13 ±0.07^{B}	7.20 ±0.10^{AB}	7.11 ±0.12^{B}	7.33 ±0.16^{A}	0.005
NH_3 (ppm)	123 ±19.4^{b}	135 ±25.0^{ab}	142 ±21.0^{a}	147 ±14.3^{a}	141 ±16.4^{a}	0.014
IVDMD (%)	68.3 ±1.52^{B}	69.4 ±0.97^{AB}	69.5 ±1.36^{AB}	68.7 ±1.44^{B}	70.4 ±0.71^{A}	0.003
IVOMD (%)	67.7 ±1.69^{b}	68.9 ±1.10^{ab}	68.7 ±1.54^{ab}	67.8 ±1.48^{b}	69.4 ±0.83^{a}	0.035
Total *VFA* (mmol·dm^{-3})	120 ±16.1^{C}	135 ±11.85^{B}	139 ±15.8^{AB}	144 ±21.7^{AB}	154 ±14.0^{A}	0.000

Explanations: *IVDMD* = in vitro dry matter digestibility, *IVOMD* = in vitro organic matter digestibility, *SD* = standard deviation; a, b in the same row indicate a significant effect ($p < 0.05$) between treatments; A, B, C in the same row indicate a very significant effect ($p < 0.01$) between treatments.
Source: own study.

In Table 6, gas production at fermentation times between 4 and 12 hours has been shown to increase significantly ($p < 0.01$) with higher levels of *I. zollingeriana* supplementation. The R0 ration always showed the lowest data, while R4 ration was the highest. Methane production showed a significant effect ($p < 0.05$) at the 12-hour measurement time but not at other measurement times. The R4 ration treatment showed the highest methane production, producing 2.35 cm^3. In Table 7, it can be observed that dry and organic matter digestibility showed a significant positive correlation with the total *VFA* concentration.

Table 6. Kinetics of ruminal gas and methane production of in site ration system with different forage compositions (mean ±*SD*)

Time (h)	Feed treatment					*p*-values
	R0	R1	R2	R3	R4	
Gas production (cm^3)						
2	18.0 ±4.03	19.1 ±5.81	19.9 ±6.10	21.7 ±7.27	22.0 ±3.94	0.214
4	19.9 ±6.14^{B}	22.1 ±7.98^{AB}	23.8 ±8.88^{A}	25.1 ±6.71^{A}	23.8 ±8.38^{A}	0.002
8	37.7 ±10.7^{C}	40.8 ±9.16^{BC}	39.9 ±7.78^{BC}	43.3 ±8.23^{B}	47.5 ±7.80^{A}	0.000
12	58.9 ±12.0^{B}	62.6 ±10.5^{B}	62.5 ±10.8^{B}	63.2 ±12.9^{B}	68.4 ±11.9^{A}	0.000
24	115 ±13.3	118 ±15.0	119 ±13.0	117 ±12.3	121 ±10.9	0.193
Methane production (cm^3)						
2	0.10 ±0.06	0.09 ±0.06	0.07 ±0.05	0.10 ±0.05	0.10 ±0.07	0.211
4	0.28 ±0.17	0.36 ±0.28	0.30 ±0.18	0.25 ±0.16	0.32 ±0.21	0.237
8	0.74 ±0.58	0.80 ±0.42	0.92 ±0.65	0.70 ±0.41	0.83 ±0.48	0.522
12	1.88 ±0.10^{ab}	1.48 ±0.97^{b}	1.79 ±1.06^{b}	1.99 ±1.20^{ab}	2.35 ±1.01^{a}	0.024
24	6.03 ±1.82	4.89 ±2.17	5.09 ±2.38	6.22 ±2.37	5.58 ±2.74	0.405

Explanations: *SD*; a, b; A, B, C as in Tab. 5.
Source: own study.

Table 7. Correlations between ruminal pH, ammonia, digestibility rates, and total volatile fatty acids

Variable	pH	NH_3	*IVDMD*	*IVOMD*	Total *VFA*
pH	–				
NH_3	0.203	–			
IVDMD	0.363	0.035	–		
IVOMD	0.291	−0.014	0.982**	–	
Total *VFA*	0.367	0.370**	0.372*	0.310*	–

Explanation: *IVDMD*, *IVOMD*, *VFA* as in Tab. 5; **correlation is significant at the 0.01 level (2-tailed), * correlation is significant at the 0.05 level (2-tailed).
Source: own study.

DISCUSSION

Forage production in an in site ration system

The production of forage in IRS design was influenced by soil chemical properties. The soil in areas 1 and 2 was classified within the medium pH range, with high cation exchange capacity (*CEC*) in area 2 and very high *CEC* in area 1 (Landon, 1991). These soil conditions were considered suitable for agricultural crop cultivation. However, both areas exhibited low organic carbon (C-organic) content, which plays a significant role in crop production (Dang *et al*., 2024).

The selection of the four plant species was based on their resilience and adaptability to the environmental conditions at the research site. *Urochloa* sp. was known for its high tolerance to marginal soil conditions (Mutai, Njuguna and Ghimire, 2017; Worthington *et al*., 2021), shade (Torres-Lugo *et al*., 2022) and drought (Cheruiyot *et al*., 2018), while *P. purpureum* optimised carbon assimilation and reduced shoot dry matter under water stress, making it ideal for drought-prone regions (Cardoso *et al*., 2015). *I. zollingeriana* was served as a protein source in the ration formulation and provided agronomic benefits by enriching soil nitrogen content (Nohong, Baba and Yusuf, 2019). This reduced the N fertilisation need for other grass species (Dubeux Jr *et al*., 2024).

Ruminal pH, digestibility rates, and total volatile fatty acids

The IRS ration was rich in fibre which increased cellulolytic microorganisms' activity to digest the fibre content (Kim and Sung, 2022). The difference in *I. zollingeriana* percentage increased rumen pH in the *in vitro* experiment that was slightly above the normal range, which is generally between 6.0 and 7.0 (Perez *et al*., 2024). Rumen pH conditions were increased due to *I. zollingeriana* being a legume the increased the rumination time resulting in more saliva production, decreased acid production and higher pH (Schroeder *et al*., 2003).

Increasing the use of *I. zollingeriana* in the ration showed a positive relationship with increasing NH_3 concentrations in rumen fluid due to the high protein content (Ali *et al*., 2023). The NH_3 produced in this experiment were within the normal range, between 85 mg·dm^{-3} and 300 mg·dm^{-3} (McDonald *et al*., 2022). High NH_3 reflected faster rumen microorganism growth, which increased the process of converting carbohydrates into energy (Gunun *et al*., 2023). This phenomenon was explained by the significant positive relationship between increasing NH_3 concentrations and total *VFA* values, as shown in Table 7.

The percentage of *in vitro* digestibility in the IRS ration showed a synergistic relationship between *IVDMD* and *IVOMD*. The *IVDMD* and *IVOMD* in this research showed a good response, with digestibility values above 60% (Tab. 5). Badarina, Dwatmadji and Rapelino (2023) reported that the level of digestibility of dry matter and organic matter in the ration is related to the level of use of *I. zollingeriana* and *P. purpureum* cv. 'Mott', ranging from 60.56% to 70.46% and from 60.01% to 65.99%, respectively. The R4 ration had an *IVDMD* value of 70.37%, higher than the other rations. The reason was that high legume levels reduced the ash content in the feed, thereby increasing the proportion of organic matter could increase feed digestibility (Dewhurst, 2013; Phelan *et al*., 2015). Kaca *et al*. (2021) reported a positive correlation between the organic matter content in the feed and the digestibility of organic matter.

This research's *IVDMD* and *IVOMD* values showed a significant positive correlation with the total *VFA* concentration (Tab. 7) due to the total *VFA* determined by the amount of organic matter in feed ingredients that can be digested by rumen microorganisms (Noersidiq *et al*., 2020). The total *VFA* with the highest concentration shown by the R4 ration due to the high soluble dietary fibre fraction in *I. zollingeriana* (Gunun *et al*., 2023) was fermented faster and produced more *VFA* than the insoluble fraction (Urriola, Shurson and Stein, 2010; Jha *et al*., 2015).

Gas and methane production

Higher gas production was implied by the higher degradability value in *I. zollingeriana* due to legumes having higher ruminal dry matter (*DM*) degradation than grasses (Srinivas and Singh, 2010). The R4 ration treatment increased methane production at 12 hours of fermentation which shows that increased gas production also causes an increase in methane production (Foggi *et al*., 2024). The rumen generated methane as the end product of anaerobic feed fermentation, and the process utilizes dihydrogen and carbon dioxide during ruminal fermentation, enabling the metabolism of (*VFA*) (Karekar and Ahring, 2023). Adding *I. zollingeriana* increased the content of soluble fractions, which affected the rumen gas and methane production kinetics (Jha *et al*., 2015).

CONCLUSIONS

A balanced forage ration could be developed using in site ration system (IRS) through several grasses and legume species cultivation in a location. The different forage compositions could meet the sheep's nutritional requirements with the legume supplementation (*I. zollingeriana*) enhances *in vitro* feed digestibility and modulates rumen fermentation dynamics. The supplementation of 20% *I. zollingeriana* in the ration increased the highest ruminal digestibility and fermentation efficiency but also contributed to increased gas and methane production. In order to reduce gas and methane production, more research needs to mitigate the potential forage that minimises environmental impacts.

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CONFLICT OF INTERESTS

All authors declare that they have no conflict of interests.

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