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RESEARCH ARTICLE

Supplementation of feed blocks to the basal diet of native forage improves digestibility and ruminal fermentation in late-gestation sheep

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Abstract

The nutrient content of available native fodders for sheep under tropical conditions is low, while the nutrient requirements of sheep, particularly during late pregnancy, are high. This study aimed to enhance the nutritive value of various indigenous fodders using formulated feed block supplementation to address nutrient insufficiency in late-pregnant crossbred *Batur* sheep. Five basal diets were formulated from native fodders with and without feed blocks supplementation (FS). Treatments were arranged in a 5 × 2 factorial design, with the first factors being five basal diets and the second factors being supplementation with and without feed block. Each treatment had six replicates. The results showed significant interactions effect ($p < 0.01$) between the basal diets and supplementation on in vitro digestibility, ruminal pH value, NH_3-N, total volatile fatty acids (VFA) concentration, total gas production, total bacterial and protozoa population. The highest response of FS in terms of increased digestibility and total VFA production was observed in the basal diet comprising a 1:1 ratio of dwarf elephant grass and *Galinsoga* (*Galinsoga parviflora*) (P4). Feed block supplementation decreased protozoa and bacterial populations in most treatments but tended to increase methane emissions ($p = 0.6947$). The protozoa population decreased sharply in the P2 basal diet (native grass, carrot leaves, and white hoarypea (*Tephrosia candida* (Roxb.) DC), while the bacterial population increased significantly in the P4 basal diet. In conclusion, feed block supplementation to native fodder mixture basal diet improves feed digestibility and rumen fermentation to overcome nutrient insufficiency in late-pregnant crossbred *Batur* sheep.

Keywords: Batur sheep; digestibility; feed supplement; forage; microbe; rumen-fermentation.

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1. Introduction

Batur sheep, well-suited to the cold and humid conditions of the upland areas in Banjarnegara, are the predominant breed in the region. This sheep originating from crossing between local breeds such as the Fat and Thin Tailed Sheep and the Garut sheep, with an imported breed known as the Merino (Prayitno et al., 2008), these sheep have been developed by farmers since 1974. Recognizing its significance and uniqueness, the Indonesian Ministry of Agriculture officially designated the Batur sheep as one of the country's local sheep breeds through Decree No. 2916/Kpts/OT.140/6/2011. However, the population of Batur sheep in Indonesia has significantly decreased, with reports from Banjarnegara Regency indicating a decline of 15% per year in recent years, reaching only about 8,000 in 2019 (Department of Agriculture Fisheries and Animal Husbandry, 2019). Without improved practices, this decline will likely continue, negatively impacting the genetic and phenotypic quality of Batur sheep in the region. A study demonstrated that adult Batur sheep reared by smallholder farmers had a smaller body size than the standard body size set the Ministry of Agriculture of the Republic of Indonesia (Ibrahim et al., 2020). Although this study did not investigate the impact of body size on productivity, improving nutritional

management particularly during pregnancy could enhance the suitability, productivity and reproductive performance of Batur sheep according to their genetic potential (Behrendt et al., 2019; Nurlatifah et al., 2022; Schoonmaker & Eastridge, 2013; Silva et al., 2024).

In our previous study, we found that smallholder farmers in Batur District only fed Batur sheep with indigenous fodder from surrounding area (Tresia et al., 2021). However, Batur District, the primary area for Batur sheep farming in Banjarnegara Regency, has an abundance of various potential feed sources, including cultivated grass in crop beds, native forages, and biomass from horticultural crops such as cabbage and carrots. These fodder options possess good nutritional value and have the potential to meet the maintenance requirements (3% of body weight) of Batur sheep. Nonetheless, several studies have indicated that relying solely on forage might only fulfill some of their nutritional needs, leading to suboptimal growth (Delgadillo et al., 2020; Idamokoro & Masika, 2017). In sheep breeding, a deficiency in nutrient supply can adversely affect the optimal reproductive performance (Asín et al., 2021).

Nutrition during pregnancy plays a vital role in the viability and body composition of lambs at birth (Peñagaricano et al., 2014). The nutritional state of the ewes during early pregnancy significantly influences embryo attachment, resilience, placental development, fetal growth and organ formation (Roberti et al., 2018). In the later stages of pregnancy, energy demands increase due to the rapid growth of the fetuses and the preparation for lactation. Feed supplements are necessary during these critical periods. In late pregnancy, the combination of rapidly growing fetuses and the physical bulk of roughage, which limits feed intake, creates a challenge in maintaining nutritional balance for animals (Hanoğlu Oral & Yıldız, 2025). Multi-nutrient blocks containing crude protein, minerals, and energy-rich contents, particularly when fed low-quality fibrous feeds, improve nutrient utilization and productivity (Mobashar et al., 2023; Murillo-Ortiz et al., 2019; Ramos et al., 2019; Sankar et al., 2020). Studies have reported that dietary supplementation during early, middle and late of gestation increased the rate of lambing, litter size, and lamb birth weight (Genfors et al., 2023; Khotijah et al., 2022; Tulu et al., 2023). Considering these factors, the current study aimed to evaluate potential indigenous fodder mixtures and the effects of addition feed block supplementation on *in vitro* digestibility and fermentation characteristics.

2. Methodology

Location of the study

The feeding trial was conducted at the "Batur" sheep breeding and rearing center in Central Java, Indonesia. The term "Batur" is a given name to sheep resulting from the crossbreeding of local thin-tail sheep with the superior Merino breed, developed by smallholder farmers in the Batur district for higher meat production. The Batur district is situated at an elevation 1614 – 2069 masl (m above sea level) with an average temperature of 15 °C and a mean annual rainfall of 1280 mm.

Diet preparation

Eight types of fodder were collected from the field at Batur sheep farming area. The fodder types included native grass, *gewor*/tropical spidewort (*Commelina benghalensis* L.), *sudamala*/white hoarypea (*Tephrosia candida* (Roxb) DC), carrot leaves (*Daucus carota*), cabbage (*Brassica oleracea*), dwarf elephant grass (*Pennisetum purpureum* cv. Mott), *jangkung*/*Galinsoga* (*Galinsoga parviflora*), fermented water spinach (*Ipomoea aquatica*).

The ration treatment was formulated based on feeding trial conducted in collaboration with smallholder farmers. Thirty-three *Batur* sheep in late gestation were provided with a mixture of different proportion of selected forage types for 11 weeks. Feed was given *adlibitum*, with sheep consuming approximately 9.62 kg of fresh forage per head per day. The food was offered as a combination of various fodder types. The most frequently used combination, which showed the highest palatability, was evaluated for their nutritive value through an *in vitro* study. The amount of fresh forages consumed were converted to a dry matter basis.

For the evaluation of nutritive value, the fodder and feed block were dried in an oven at 60 °C for three days, then ground and passed through a 1 mm sieve. The treatment diet for *in vitro* study was formulated to meet nutrient requirements of late gestation ewes. The formulation was based on dry matter of the ingredients, calculated from the fresh feed consumed by the ewes. Basal diet was supplemented with one feed block per head per day (540 g). The feed block consisted of a mixture of cassava flour (38%), molasses (25%), premix (1%), urea (3%), lime (3%), DCP (2%), salt (3%), and indigofera leaves meal (25%). The nutrient content of the feedstuff is shown in Table 1, while the nutrient content of the diet treatments is shown in Table 2. The diet treatments were formulated as follows:

Table 1
Nutrient content of forage and feed supplement

Feedstuff (Local name/scientific name)	DM (%)	Dry matter/DM (%)									
		Crude protein	Ether extract	Gross energy	Crude fiber	Ash	Ca	P	NDF	ADF	NFE	TDN
Native grass	18.29	22.86	2.56	4,177.34	27.88	14.68	0.65	0.41	54.57	39.42	32.02	60.49
Gewor (*Commelina benghalensis* L.)	13.68	25.11	2.46	4,029.52	25.19	18.61	1.29	0.43	42.55	39.14	28.63	58.57
Sudamala (*Tephrosia candida* (Roxb) DC)	20.55	27.48	2.40	4,026.37	18.49	18.34	1.27	0.53	51.68	47.10	33.29	60.58
Carrot leaves (*Daucus carota*)	18.61	19.42	1.72	3,802.28	21.22	20.11	1.93	0.35	38.21	38.09	37.53	56.97
Cabbage (*Brassica oleracea*)	11.97	22.74	3.67	3,890.11	15.92	20.97	3.43	0.33	28.14	26.43	36.70	61.79
Dwarf elephant grass (*Pennisetum purpureum* cv. Mott)	20.02	27.48	2.40	4,026.37	18.49	18.34	1.27	0.53	51.68	47.10	33.29	60.58
Jangkung (*Galinsoga parviflora*)	15.85	27.78	2.29	4,011.13	21.41	19.24	2.84	0.46	41.03	38.95	29.27	58.75
Fermented water spinach (*Ipomoea aquatica*)	61.68	10.95	1.87	3,957.86	39.23	16.31	1.16	0.30	65.07	57.41	31.63	53.64
Feed supplements (FS)	72.77	12.96	0.86	3,676.24	10.17	17.27	4.04	0.50	31.42	23.92	58.74	69.65

NDF = Neutral detergent fiber, ADF = Acid detergent fiber, NFE = Nitrogen free extract, TDN = Total digestibility nutrient.

Table 2
Nutrient content of treatment rations

Nutrient contents (% DM)	Ration									
	P1	P2	P3	P4	P5	FS-P1	FS-P2	FS-P3	FS-P43	FS-P5
Dry matter	90.97	91.15	90.88	90.72	92.64	91.75	91.94	91.57	91.65	92.93
Ash	17.14	19.10	17.61	17.70	17.18	17.16	18.78	17.56	17.62	17.19
Crude protein	25.26	23.67	24.72	23.67	12.90	23.25	21.83	23.07	21.68	12.90
Crude fiber	23.50	19.92	21.31	26.22	35.10	21.32	18.25	19.75	23.24	32.78
Ether extract	1.75	2.09	2.75	2.33	1.84	2.21	1.87	2.48	2.05	1.74
Nitrogen free extract (NFE)[a]	31.63	35.23	33.61	30.08	32.99	36.06	39.26	37.14	35.40	35.39
Ca	1.06	1.57	1.54	1.45	1.34	1.55	1.99	1.89	1.93	1.59
P	0.46	0.44	0.44	0.36	0.31	0.47	0.45	0.45	0.39	0.33
Neutral detergent fiber	50.29	45.49	47.36	54.90	58.90	47.20	43.08	45.12	50.54	56.34
Acid detergent fiber	42.52	42.80	39.78	41.79	52.97	39.48	39.56	37.55	38.47	50.27
Hemicellulose [b]	7.76	2.69	7.58	13.11	5.92	7.72	3.52	7.57	12.07	6.07
Total digestibility nutrient	60.03	58.91	60.83	58.40	54.41	61.59	60.74	62.06	60.48	55.82
Gross Energy (Kcal/kg)	4,079.48	3,925.24	4,050.01	4,042.68	3,922.13	4,013.60	3,882.51	3,997.49	3,974.64	3,899.23

[a] NFE is based on the formula NFE= 100 – Dry matter – Crude protein – Crude fat – Crude fiber; [b] Hemicellulose is based on the formula of Van Soest (1991).

P1 = fodder mixture of native grass: *gewor*/tropical spidewort: *sudamala*/white hoarypea = 2:2:1 ratio
P2 = fodder mixture of native grass: carrot leaf: sudamala/ white hoarypea = 2:1:1 ratio
P3 = fodder mixture of native grass: cabbage: sudamala/ white hoarypea = 2:1:1 ratio
P4 = fodder mixture of dwarf elephant grass : *jangkung*/ *Galinsoga* =1:1 ratio
P5 = fodder mixture of carrot leaf :fermented water spinach = 1:1 ratio
FS-P1 = P1+ feed supplements
FS-P2 = P2 + feed supplements
FS-P3 =P3 + feed supplements
FS-P4 = P4 + feed supplements
FS-P5 = P5 + feed supplements

In vitro gas production

The digestibility of diet treatments was evaluated using *in vitro* study following the procedure of Theodorou & Brooks (1990) with a slight modification in the buffer media and rumen fluid ratio. In this study, the ratio was 2 : 1. Two sets of *in vitro* experiments were carried out: one set was designed to measure the amount of gas produced during 72 hours of fermentation, and the other set measured feed digestibility during 24 hours of incubation. Briefly, the *in vitro* procedure involved adding a 0.75-gram sample, 50 mL of McDougall buffer, and 25 mL of cattle rumen fluid to an anaerobic bottle (100 mL). The bottles were placed in a shaker water bath (Memmert-Germany, WNB 22 SC L518.0703) and incubated at 39 °C.

For digestibility measurement, the pH was measured immediately after a 24-hours incubation. The supernatant was then collected to determine the number of protozoa and bacteria. Next, 2-3 drops of $HgCl_2$ were added to the remaining media and sample to kill the microbes.

Finally, the supernatant was taken to measure the concentration of total volatile fatty acid (VFA) and ammonia (NH_3). The contents of the bottles were filtered onto pre-weighed Whatman Grade 41 (Cytiva, Marlborough, MA, USA, Cat. No. 1441-090 , 90 mm diameter) using a vacuum pump (Millipore XX5522050). The filters were then folded and dried at 105 °C using an oven (B-ONE, model OV-30) for 24 hours to determine the dry matter (DM) content. Subsequently, the samples were ashed at 450 - 600 °C for 8 hours in furnace to determine OM digestibility. The difference in weight between the initial sample and the dry and organic matter the residues were used to calculate digestibility.

For gas production measurement, the gas produced from feed fermentation was recorded at 0, 2, 4, 6, 8, 12, 24, 36, 48, and 72 hours of incubation using a glass syringe. After reading the gas produced, it was collected in vacuum tubes and analyzed for methane concentration using gas chromatography. Gas production kinetics were calculated using the equation $p = a + b\ (1 - e^{-ct})$ (Ørskov & McDonald, 1979), "where a= gas production from the immediately fermentable fraction (mL/200 mg DM), b= the gas production from the slowly fermentable fraction (mL/200 mg DM), (a+b) = the potential gas production (mL/200 mg DM); c = the gas production rate constant.

Chemical analysis

The dry matter, ash, extract and crude protein, calcium, and phosphorus content of each feed sample were determined by proximate analysis (AOAC, 1995). Gross energy was analyzed using a PARR 6400 Bomb Calorimeter. Neutral detergent fiber (NDF) and acid detergent fiber (ADF) contents were determined using the methods of van Soest et al. (1991). The total digestible nutrient (TDN) content was estimated according to Hartadi et al. (1980). For *in vitro* fermentation parameters, gas chromatography–mass spectrometry (Bruker Scion 436 GC) equipped with a BR-Wax fame capillary column (wall-coated open tubular fused silica, 30 m × 0.32 mm image diagnosis) was used to measure the concentration of volatile fatty acids (VFAs), and pH was measured using pH meter (Lamotte, pH Plus (5-1936). The NH_3-N concentration was determined using the method described by Conway & O'Malley (1942). The number of bacteria and protozoa was determined following the procedures of Oginomoto & Imai (1981).

Statistical analysis

Data were analyzed using analysis of variance as a 2 × 5 factorial in a randomized complete design, with supplementation as one factor and five different diets as the other factor, and 6 replicates per treatment. Significant differences among treatments ($p < 0.05$) were tested using Duncan's Multiple Range test using SAS software version 9.0 and the correlogram visualization was created using R Studio version 4.2.0.

3. Results and discussion

Chemical Composition

In the present study, the levels of crude protein (<2% DM), crude fiber (<3% DM), NDF (<5% DM), ADF (<5% DM), and GE (<70 Kcal/kg) were slightly higher in the ration without feed supplementation compared to the supplemented ration (Table 2). However, nitrogen free extract (NFE) and total digestible nutrients were higher in ration contain feed supplements this could be attributed to the feed block supplement being formulated with energy sources such as cassava flour and molasses.

In vitro digestibility

There was significant interaction between basal diet (BD) and feed block supplementation (FS) on IVDMD and IVOMD (Table 3). Feed block supplementation increased the *in* vitro digestibility of all basal diets, with the highest increase observed in FS-P4 treatment, which was not significantly different from FS-P1 and FS-P2 treatments. Feed supplementation (FS) in fodder basal diet for Batur sheep diets significantly improved the *in vitro* digestibility of both dry matter (IVDMD) and organic matter (IVOMD), total volatile fatty acid (TVFA), and NH_3. These findings regarding nutrient composition which energy, protein, mineral and essential nutrient contained in feed supplement can support microbial growth and improve fermentation activity. Previous studies have shown that feed supplements, such as feed block or multi-nutrient blocks, improved digestibility and rumen fermentation characteristics (Mobashar et al., 2023; Murillo-Ortiz et al., 2019; Sankar et al., 2020). Murillo-Ortiz et al. (2019) explained that supplementation of multi-nutrients blocks improved the rumen microbial population along with improvements in the fermentative parameters and feed intake in steers fed on low nutritive quality forages.

Table 3
In vitro digestibility (%) of different basal diet with or without feed block supplementation

Feed block supplement (FS)	Basal Diet (BD) type	IVDMD	IVOMD
0	P1	50.78c	45.95c
	P2	51.99b	47.58bc
	P3	46.67e	41.49e
	P4	52.61b	48.65ab
	P5	46.92e	41.99e
FS	P1	53.91a	48.77ab
	P2	53.89a	49.49a
	P3	49.40d	44.10d
	P4	54.20a	50.32a
	P5	50.55c	46.15c
SEM		0.36	0.42
Effects		p-values	
FS		<.0001	<.0001
BD type		<.0000	<.0000
FS x BD		<.0001	<.0001

Note: Means in the same column with different letters differ significantly.
P1: fodder mixture of native grass: gewor/tropical spidewort: sudamala/white hoarypea = 2:2:1 ratio.
P2: fodder mixture of native grass: carrot leaf :sudamala/ white hoarypea = 2:1:1 ratio.
P3: fodder mixture of native grass: cabbage: sudamala/ white hoarypea = 2:1:1 ratio.
P4: fodder mixture of dwarf elephant grass : jangkung/ Galinsoga =1:1 ratio.
P5: fodder mixture of carrot leaf :fermented water spinach = 1:1 ratio.
IVDMD: in vitro dry matter digestibility; IVOMD: in vitro organic matter digestibility.

Feed supplement contained a great concentration of molasses (25%) that are fermentable soluble carbohydrates components, which can help to explain the greater degradation of FS supplemented ration (FSP1–FSP5). The addition of molasses to diet has been found to increase the levels of volatile fatty acids in the rumen (Osman et al., 2020), optimize the provision of amino acids through microbial protein synthesis (Yeo et al., 2006), and promote gluconeogenesis in animals fed low-quality forage-based diets (Khalili, 1993). In addition, feed block supplement contained Indigofera leaf meal as protein source. Increasing the nitrogen content in the diet based on tropical grass (*Axonopus catarinensis*) by adding legumes has been shown to improve rumen digestive parameters (Dal Pizzol et al., 2017). Nurjannah et al. (2022) reported that the inclusion of *Indigofera sp.* in the feed supplement contributes positively to digestibility. The authors found that inclusion of 15% *Indigofera sp.* in diet based on *P. purpureum* cv. Mott resulted in similar effect to *P. purpureum* cv. Mott (70%) based diet and concentrate on DM, OM, CP, digestibility (69.95%, 70.72%, and 64.95% respectively). Suharlina et al. (2016) also demonstrated that concentrate feed containing 20% *Indigofera zollingeriana* has a high *in vitro* degradability due to its low content of crude fiber and high content of crude protein.

This study observed significant increase on IVDMD and IVODM due to feed block supplementation (Table 3). The FS-P1, FS-P2 and FS-P4 had higher IVDMD and IVODM. However, the highest increase due to FS supplementation in IVDMD and IVOMD was in P5 (mixture of carrot leave and fermented water spinach at 1:1 ratio) basal diet in which the IVDMD and IVOMD increased by 8.1% and 10.2% respectively. However, the IVDMD and IVOMD of P5 supplemented by FS (FS-P2) is still lower compared to P2 (native grass, carrot leaf, sudamala/ white hoarypea mixture at 2:1:1 ratio) basal diet without supplementation. The results of this IVDMD and IVOMD shows that feeding P2 without supplementation is more practical in the field because it has similar IVDMD and IVOMD other fodder mixture basal diet with FS supplementation. Even though FS supplementation increased IVDMD and IVOMD, the digestibility of the ration in our study remained relatively low, which is less than 60%. Even though CP content in the fodder mixture basal diet was high but energy supplementation from feed block could not balance the higher CP content of the basal diet. In addition, energy available in form of structural carbohydrates could not be used efficiently for microbial synthesis. Low digestibility is caused by high fibrous fractions including ADF (39.74% – 52.93%) and NDF (43.07% – 58.84%) content in rations this study. It is known that the ADF and NDF are good as feed for ruminant; however, it should be noted that high fiber decreases digestibility of diet whereas the

non-fiber carbohydrate (NFC) was positive correlation with digestibility. Most studies suggested that the NDF and ADF content in roughage feed has a negative correlation with digestibility in ruminants (Lang et al., 2022; Raffrenato et al., 2017; Riaz et al., 2014; Widodo et al., 2023; Yang et al., 2018; Zhao et al., 2016).

Rumen fermentation characteristics

Rumen fermentation characteristics of diet treatments are given in Table 4. The interaction between BD types and FS significantly increased ruminal pH, NH_3-N and total VFA concentration. The ruminal pH of ration ranged from 6.22 to 6.91. The effect of feed block supplementation varied with different basal diets. In diet P2 and P4, feed block supplementation significantly decreased ($p < 0.01$) ruminal pH, while in basal diet P3 and P5, it significantly increased ($p < 0.01$) ruminal pH. For NH_3-N concentration, it increased in ration P1, P3, P4 and P5 when supplemented with feed block ($p < 0.01$) but in P2 was decreased. The highest NH_3-N concentration was observed in FS-P4 (16.62 mM), followed by FS-P1 (15.69 mM), and FS-P2 (14.92 mM). Total VFA concentration in fodder diets supplemented with FS varied from 65.23 to 84.19 mM (Table 4) with the highest value of 84.19 mM shown by FS-P4 that increased by 19.54% compared to its corresponding non-supplemented basal diet (P4). Total VFA concentration in P1, P2, and P4 supplemented FS were significantly higher than P3 and P5 supplemented FS basal diets. Total VFA production in P2 ration did not increase due to feed block supplementation, indicating FS was unable to increase VFA, or P2 basal diet did not required supplementation. Proportion of acetic acid and butyric acid was affected by feed block supplementation (FS). Proportion of acetic acid decreased due to FS supplementation (from 48.71% to 47.33%), while propionic acid was affected by basal diet type (BD) the proportion of propionic acid in the range of 31.50% - 35.08%. No interaction was found between FS and BD for proportion of propionic acid, butyric acid, isovaleric acid and the ratio C2:C3.

The ruminal VFAs concentration in the current study was positively correlated with ruminal digestibility such the higher of IVDMD and IVOMD would in line with higher VFA production (Figure 2). The mean total VFA in the current study ranged from 68.13 to 84.19 mM which these values, except for P3 (68.13 mM) and P4 (69.66 mM), were the lowest levels advised for optimum rumen to support microbial for optimal rumen support of microbial growth. According to McDonald et al. (2022), the range is 70 - 150 mM. The addition of FS supplies soluble carbohydrates, which increases carbohydrate degradation and VFA concentration in the rumen. In line with Murillo-Ortiz et al. (2019) that reported inclusion multi-nutrient block increased TVFA from 95.1 mM to 99.6 mM. As the low of digestibility and concentration of total VFA in our study indicates that a higher concentration of FS may be required in the diet to enhance nutrient content and further rumen fermentation products.

Table 4
Rumen fermentation characteristic of different basal diet with or without feed block supplementation

Feed block supplement (FS)	Basal Diet (BD) type	pH	NH_3-N mg/dL	Total VFA (mMol)	VFA partial (%)						C2:C3
					C2	C3	iC4	nC4	iC5	nC5	
0	P1	6.82ab	14.33bc	72.94bc	50.09a	33.26	2.26e	8.96b	3.52	1.91bcd	1.51
	P2	6.79b	15.33ab	81.96a	47.76ab	32.20	5.27a	9.83ab	2.92	2.02abcd	1.48
	P3	6.42e	12.29de	65.23d	47.55ab	34.90	3.66c	9.44ab	2.82	1.62cd	1.36
	P4	6.86a	14.78b	72.10bc	50.46a	32.09	2.12e	9.10b	3.46	2.77a	1.58
	P5	6.59d	11.15e	69.89c	47.70ab	32.88	3.27dc	10.22ab	3.41	2.51ab	1.47
FS	P1	6.83ab	15.69ab	80.92a	47.61ab	31.57	4.55b	10.94a	3.45	1.87bcd	1.51
	P2	6.33f	14.92b	81.04a	47.78ab	30.80	4.99ab	10.88a	3.56	1.98bcd	1.57
	P3	6.58d	12.67d	74.79b	44.64b	35.26	3.21dc	10.36ab	4.18	2.35abc	1.27
	P4	6.29g	16.62a	84.19a	47.32ab	33.93	4.94ab	9.00a	3.32	1.49d	1.40
	P5	6.69c	13.01dc	73.63bc	49.31a	31.83	2.94d	10.28a	3.75	1.89bcd	1.55
SEM		0.03	0.25	1.09	0.38	0.37	0.21	0.18	0.12	0.09	0.03
Effects		p-values									
FS		<.0001	0.0015	<.0001	0.0433	0.5724	<.0001	0.0178	0.1087	0.1129	0.6662
BD type		<.0001	<.0000	<.0001	0.0629	0.0354	<.0001	0.0936	0.9303	0.7222	0.0819
FS x BD		<.0001	<.0001	0.0007	0.1136	0.4563	<.0001	0.2279	0.3612	0.0064	0.3938

Note: Means in the same column with different letters differ significantly.
P1: fodder mixture of native grass: gewor/tropical spidewort: sudamala/white hoarypea = 2:2:1 ratio.
P2: fodder mixture of native grass: carrot leaf :sudamala/ white hoarypea = 2:1:1 ratio.
P3: fodder mixture of native grass: cabbage: sudamala/ white hoarypea = 2:1:1 ratio.
P4: fodder mixture of dwarf elephant grass : jangkung/ Galinsoga =1:1 ratio.
P5: fodder mixture of carrot leaf :fermented water spinach = 1:1 ratio.
C2: acetic acid; C3: propionic acid; nC4: butyric acid; iC4: isobutyric acid; iC5: isovaleric acid; nC5: valeric acid.

It is also indicated that the addition of FS promoted changes in the proportion of acetic acid and N-butyric acid. These alterations may be attributed to differences in dietary carbohydrate composition. Furthermore, the addition of FS tended to increase isobutyric acid which is often associated with extensive ruminal protein degradation. McDonald et al. (2022) explained that, apart from acetic acid, propionic acid and butyric acid, other fatty acids were produced in the rumen by deamination of amino acids. These include isobutyric acid from valine, valeric acid from proline, 2-methyl butyric acid from isoleucine, and 3-methyl butyric acid from leucine. In agreement with these results, NH_3-N concentrations were greater ($p < 0.01$) for addition FS (FS-P1–FS-P5).

The concentration of NH_3-N in our study was 11.04–16.62 mM, which falls within the optimum concentration range to support the growth of rumen microbes. According to a review by Schwab & Broderick (2017), depending on the diet and fermentation conditions, ruminal ammonia concentrations of 5 to 11 mM are required to optimize flows of microbial N from the rumen. The high concentration of NH_3-N in the current study was due to protein and non-protein nitrogen (NPN) derived from the ingredients of the feed supplement, especially urea, which are easily dissolved and degraded in the rumen.

The availability of NH_3-N, in conjunction with fermentable carbohydrates, supports the growth and proliferation of rumen microbes. This synergistic effect enhances microbial biomass production, which is subsequently available to the host animal as a high-quality protein source upon digestion. In the rumen, urea will be hydrolyzed by bacterial urease into NH_3 and CO_2 that are used for amino acid biosynthesis or gastric tract dwelling bacteria (Hailemariam et al., 2021). In addition, the increase in ruminal pH and NH_3-N concentration implies enhanced protein degradation and fermentation processes, which can affect nutrient utilization and overall rumen function. The variations observed in NH_3-N concentration further highlight the influence of diet composition, indicating that the interaction between FS and specific forage ratios can modulate ruminal ammonia levels.

Population of bacteria and protozoa

Total bacteria and protozoa population is presented in Table 5. Bacterial and protozoal population significantly affected by the interaction of type basal diet and feed block supplementation. Feed block supplementation was only able to increase total bacteria population in diet P4. While other diets their bacteria population were not affected by types of fodder basal diet nor supplementation (without and with feed supplement). The highest population of bacteria was observed in P2 ($6.47x10^9$/mL). Supplementation of FS to basal diet significantly decreased ($p < 0.05$) protozoa population exclude in basal diet P1. The highest population of protozoa ($p < 0.05$) was observed in P2, with a value of 4.07 $x10^5$/mL), while the lowest population was recorded in FS - P3, with a value of 1.80 $x10^9$/mL.

Table 5
Bacterial and Protozoal population of different basal diet with or without feed block supplementation

Feed block supplement (FS)	Basal Diet (BD) type	Bacteria (x 10^9/mL)	Protozoa (x10^5/mL)
0	P1	5.37b	3.27cd
	P2	6.47a	4.07a
	P3	3.61e	2.83ef
	P4	2.57f	3.77ab
	P5	4.20d	2.77ef
FS	P1	5.10bc	3.40cd
	P2	4.70c	2.60f
	P3	3.73e	1.80g
	P4	4.93bc	3.53bc
	P5	4.13d	3.07de
SEM		1.46	0.24
Effects		p-values	
FS		0.0078	<.0001
BD type		<.0001	<.0001
FS x BD		<.0001	<.0001

Note: Means in the same column with different letters differ significantly.
P1: fodder mixture of native grass: gewor/tropical spidewort: sudamala/white hoarypea = 2:2:1 ratio.
P2: fodder mixture of native grass: carrot leaf :sudamala/ white hoarypea = 2:1:1 ratio.
P3: fodder mixture of native grass: cabbage: sudamala/ white hoarypea = 2:1:1 ratio.
P4: fodder mixture of dwarf elephant grass : jangkung/ Galinsoga =1:1 ratio
P5: fodder mixture of carrot leaf :fermented water spinach = 1:1 ratio.

Ruminal pH value in the present work was 6.29 to 6.86 that was within the normal range of 5.5 – 7.0 considered optimal for microbial digestion activity (Chen et al., 2019). The results also indicate that the inclusion of FS tends to alter microbial population in the rumen. There might be a synergistic or antagonistic mechanism between specific components in local fodder and FS content affecting bacterial populations. This may be attributed to the availability of nutrients essential for optimal microbial growth, including crucial microbial growth factors such as nitrogen availability. For example, bioactive compounds, i.e., saponins and tannins, are present in *Indigofera zollingeriana* in the feed supplement. However, in this study, we did not analyze the bioactive compounds in the feed supplement. Numerous studies have found that saponins have

different impacts on bacterial numbers, with some indicating a decrease (Muetzel, 2003; Wang et al., 2000), an increase (Gunun et al., 2022; Kim et al., 2023), or no effect (Gunun et al., 2019; Liu et al., 2019). Other studies have found that tannins can changes bacterial diversity (Costa et al., 2018; Silva de Sant'ana et al., 2022) and others research found no detectable effect (Díaz Carrasco et al., 2017; Salami et al., 2018). The mechanism by which saponins and tannins promote and inhibit the growth of some bacterial species in pure cultures is unclear. The chemical structure of tannins, as well as the bacterium species (Vasta et al., 2019), dose, stereochemistry, polymer size, and intermolecular lingkages (Kelln et al., 2021), and microbial adaptation to tannins (Patra & Saxena, 2011) determine their mode of action.

The population of ruminal protozoal appeared significant variation following incubation with various substrates, wherein FS demonstrated can decrease in some treatments FS (P2, P3, P4, P5) as well as increase the protozoal population (FS-P1). There were suggestions that maybe some content in local fodder could have antagonistic or synergistic effects when combined with FS content, particularly bioactive compounds that potentially contribute to protozoa reduction. *Indigofera zolingeriana* contains 3% – 7% tannin and 0.8% – 1.2% saponin (Antari et al., 2022). In the review by Kholif (2023), it was reported that ruminal protozoa are highly sensitive to saponins present in plants or plant extracts. Widyarini et al. (2021) found that administering saponins from *Nigella sativa* L. at concentrations of 0.2%, 0.4%, and 0.6% DM reduced protozoal populations *in vitro*. Additionally, there was a reduction in holotrich and entodiniomorph protozoa numbers with an increasing level of leaves of *Acacia mearnsii* containing condensed tannins in the ration. A similar decrease in protozoa numbers (-21%) was noted by Salami (Salami et al., 2018) when 4% of both condensed tannins (mimosa, gambier) or hydrolysable tannins (chestnut, tara) were included in the lamb's diet.

In vitro gas production

In vitro gas production and gas production kinetics parameters are presented in Table 6 and fitted total gas production is shown in Figure 1. Total gas production and kinetics were significantly affected by interactions of type fodder basal diet (BD) and feed block supplementation (FS) ($p < 0.01$). The FS supplementation significantly increased total gas production (mL/200 mg DM) of all BD diet with FS-P2 that showed to have the highest total gas production (52.88 mL/200 mg) compared to other diets. However, the highest increase in total gas production due to FS supplementation occurred in FS-P3 which increased by 19.72%, nevertheless its total gas production was still lower compared to diet FS-P1, FS-P2, and FS-P4. FS supplementation only increased the gas production from soluble fraction (fraction *a*) in FS-P2 diet, but this value was not significantly different to P1, P5 and FS-P1.

Table 6
Total gas production dan gas production kinetics of different basal diet with or without feed block supplementation

Feed block supplement (FS)	Basal Diet (BD) type	Total gas production (mL 200 mg^{-1})	a	b	C	a + b	CH_4 production (mL 200 mg^{-1})	Proportion of CH_4 (%)
0	P1	48.76c	3.37abc	48.81abc	0.041ab	52.18b	3.26	6.71
	P2	44.56e	1.61c	46.59bcd	0.038b	48.19c	3.21	7.19
	P3	40.52f	1.69c	42.45de	0.037b	44.14d	2.33	5.80
	P4	44.39d	1.83c	46.21bcd	0.037b	48.04c	3.39	7.78
	P5	45.77e	2.75abc	44.26de	0.047ab	47.00c	3.16	6.95
FS	P1	51.35b	4.70a	49.55ab	0.040ab	54.26ab	3.78	7.41
	P2	52.88a	4.43ab	50.46a	0.044ab	54.89a	4.08	7.84
	P3	48.51c	2.02c	50.55a	0.038b	52.57b	3.68	7.57
	P4	50.89b	1.49c	51.92a	0.043ab	53.42ab	3.36	6.67
	P5	47.19d	2.58bc	45.74cde	0.050a	48.32c	3.51	7.52
SEM		0.48	0.24	0.50	0.001	0.49	0.15	0.29
Effects					p-values			
FS		<.0001	0.061	<.0001	0.1576	<.0001	0.0663	0.4404
BD type		<.0001	0.0043	<.0001	0.1233	<.0001	0.7559	0.9483
FS x BD		<.0001	<.0001	<.0001	<.0001	<.0001	0.6947	0.7317

Note: Means in the same column with different letters differ significantly
P1: fodder mixture of native grass: gewor/tropical spidewort: sudamala/white hoarypea = 2:2:1 ratio
P2: fodder mixture of native grass: carrot leaf :sudamala/ white hoarypea = 2:1:1 ratio
P3: fodder mixture of native grass: cabbage: sudamala/ white hoarypea = 2:1:1 ratio
P4: fodder mixture of dwarf elephant grass : jangkung/ Galinsoga =1:1 ratio
P5: fodder mixture of carrot leaf :fermented water spinach = 1:1 ratio.

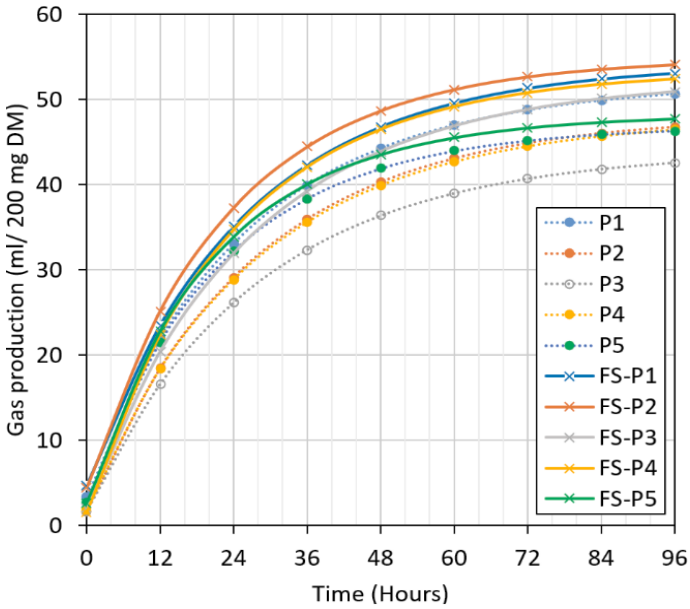

Figure 1. Cumulative total gas production of experimental diets during 72 h incubation. P1: fodder mixture of native grass: gewor/tropical spidewort: sudamala/white hoarypea = 2:2:1 ratio. P2: fodder mixture of native grass: carrot leaf :sudamala/ white hoarypea = 2:1:1 ratio. P3: fodder mixture of native grass: cabbage: sudamala/ white hoarypea = 2:1:1 ratio. P4: fodder mixture of dwarf elephant grass : jangkung/ Galinsoga = 1:1 ratio. P5: fodder mixture of carrot leaf :fermented water spinach = 1:1 ratio.

The gas production from fermentable but insoluble fraction (*b* fraction) in P2, P3 and P4 was significantly ($p < 0.05$) increased due to FS supplementation. The increase in b value of FS-P2, FS-P3 and FS-P4 was in the range 6.3% - 18.8%. Rate of gas production (*c* value) in FS-P5 was significantly ($p < 0.05$) faster/higher than FS-P3, P1, P2 and P4. Potential gas production (a + b) was significantly ($p < 0.05$) increase in basal diet (P1-P4) excluding P5, due to FS supplementation. The highest potential gas production was observed in FS-P2 (54.89 mL), which was significantly different from FS-P1, FS-P3, and FS-P4. Whereas methane gas (CH_4) production was not affected by any treatment factor neither by interaction of FS and BD, nor by main factor effect of feed block (FS) supplementation or type of fodder basal diet BD. The range of methane production was 2.33 - 4.08 mL/200 mg.

Results showed that cumulative gas production varied in accordance with various mixes of local fodder (Table 4). This can be explained by the different chemical compositions of the incubated substrates and the carbohydrate fraction content of each forage (Gemeda & Hassen, 2015). Moreover, our results suggest that supplementing FS significantly enhanced the total gas production at 72 hours in the *in vitro* fermentation. In this work showed that the cumulative gas production was positively correlated with ruminal digestibility, such that higher values of IVDMD and IVOMD were associated with higher cumulative gas production (Figure 2). A possible explanation of higher gas production from FS might be related to the fermentation of soluble carbohydrates, a rapidly fermentable fractions of the substrate as explained by Lanzas et al. (2007). In agreement with these results, potential gas production variable (a + b) concentrations (mL/200 g DM) were greater ($p < 0.01$) for addition FS (FSP1–FSP5). Similarly, Zhang et al. (2015) observed increased total gas production at 24 hours when they elevated the starch component by decreasing the fiber fraction in *in vitro* experiments. Baffa et al. (2023) demonstrated forages with higher potentially degradable fraction (aNDFom) content and lower lignin in fiber organic matter (6.691 g/kg) experienced the highest asymptotic cumulative gas production, the phase where cumulative gas production decreases up to zero. Furthermore, the availability of crude protein in the FS diet (FS-P1, FS-P5) may have contributed to the increased cumulative gas production, as it promotes microbial activity (Baffa et al., 2023). According to Oliveira et al. (2022) and Bach et al. (2005), crude protein levels below 70 g/kg may limit microbial activity due to the lack of nitrogen. This also implies that the P5 and FS-P5 (CP: 12.90%) diet included enough protein to support microbial activity.

The addition of FS, indeed, did not have a significant effect on CH_4 production in this current

study. Methane gas production ranged from 5.3 to 8.97 mL; however, the inclusion of FS in the diet tended to increase these variables by 0.42 - 2.69 mL, excluding FS-P4, which was related to the increase in cumulative gas production. This works found methane production was positively correlated with cumulative gas production at 72 hours (Figure 2).

Correlation of in vitro digestibility and rumen fermentation parameters

The correlations among the chemical composition, and *in vitro* digestibility of local fodder basal diet supplemented feed supplement are shown in Figure 2. Our findings suggest that IVDMD has negative correlation with ADF ($p < 0.05$), meanwhile IVOMD has no correlation with the nutritive value of diet treatments. However, the cumulative gas production at 72 hours was mainly attributed to a significant increase ($p < 0.01$) in IVDMD, IVODM, and NH_3. Similarly, TVFA and C_2 showed a positive correlation ($p < 0.0001$) with IVDMD, IVODMD, NH_3, and gas production. However, TVFA, C_3, and $_iC_4$ are negatively correlated with CF and NDF. There is a negative correlation between NH_3 with CF, ADF, as well as $_nC_4$ and NDF. Negative correlations ($p < 0.01$) were found between ether extract content with gas production 72h and C_2. Additionally, methane demonstrated a positive correlation ($p > 0.05$) with gas production.

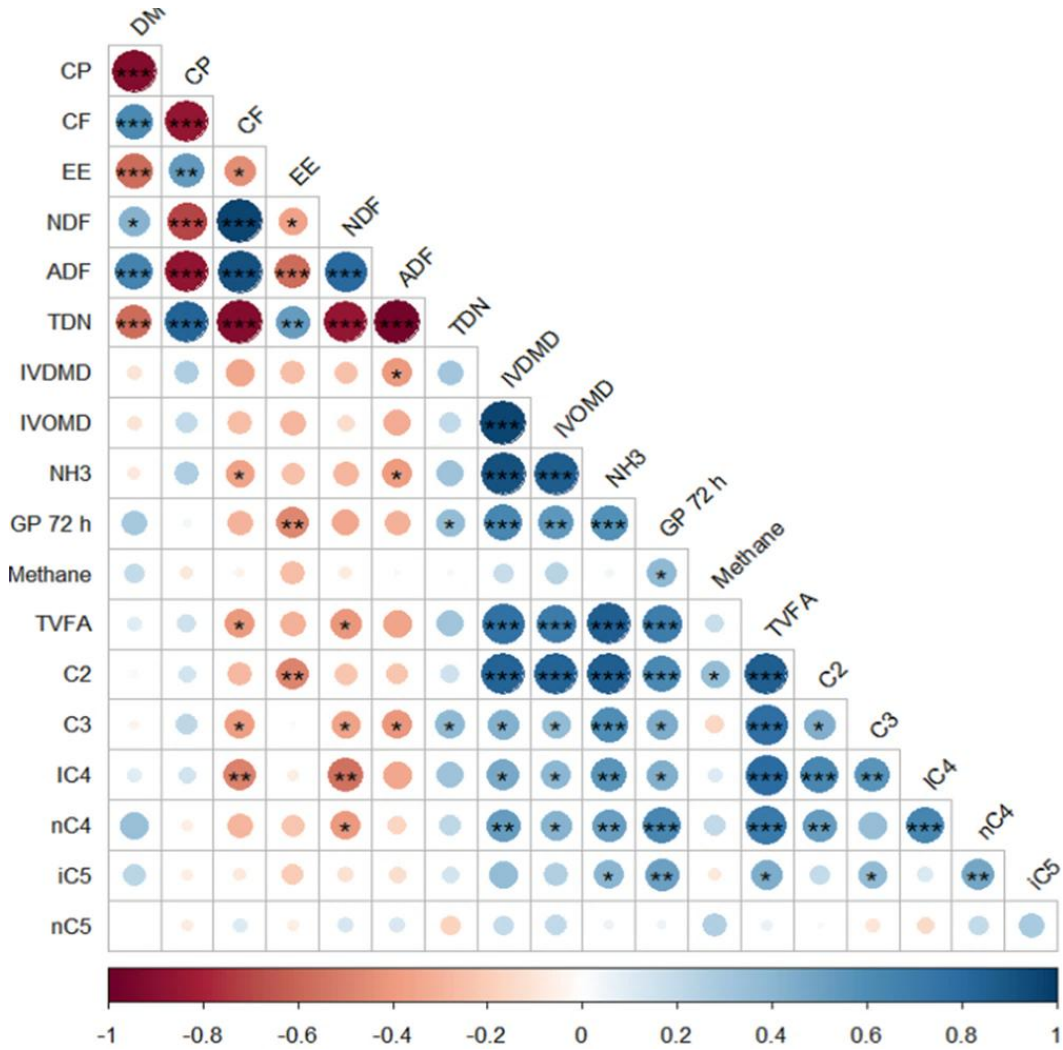

Figure 2. Correlation plot between chemical composition, tested in vitro digestion and fermentation parameters (n = 30). Positive and negative correlation coefficients are displayed in blue and brown scale, respectively. Significance level (*** $p < 0.001$, ** $p < 0.01$, and * $p < 0.05$). DM = dry matter; CP = crude protein; CF = crude fiber; EE = ether extract; NDF = neutral detergent fiber; ADF = acid detergent fiber; TDN = total digestible nutrients; IVDMD = *in vitro* dry matter digestibility; IVOMD = *in vitro* organic matter digestibility; GP72h = total gas production at 72 h; TVFA = total volatile fatty acids; C2=acetic acid; C3=propionic acid; nC4=butyric acid; iC4=isobutyric acid; iC5=isovaleric acid; nC5=valeric acid.

4. Conclusions

Supplementation of feed block in a diet based on a indigenous fodder mixture resulted in slight shifts in the ruminal fermentation pattern. The most effective applications of FS from five fodder mixtures basal diets to improve ruminal fermentation (digestibility, NH_3, VFA) and gas production *in vitro* were observed in a diet consisting a mixture of fresh forage from native grass, (*Tephrosia candida* (Roxb) DC), carrot leaves (*Daucus carota*), at ratio of 2:1:1 respectively (FS-P2), as well as a fresh forage from dwarf elephant grass and jangkung (*Galinsoga parviflora*) at a ratio of 1:1 (FS-P4). However, the digestibility rate and total VFA were still lower than the suggested values, which is an undesirable result. Thus, an improved approach is required to adjust these shortcomings, and in vivo studies are recommended to confirm these results.

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