Research Article

Optimizing Concentrate Supplementation for Madura Cattle: An *In vitro* Fermentation Study of Native Grass

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Abstract | Generally, the feed given to Madura cattle by small-holder farmers in Madura Island consists only of local forages and low-quality agricultural waste. Concentrate supplementation is paramount for improving nutrients intake and growth performance at secure level as high-concentrate rations can cause metabolic disorders. This study aimed to evaluate the effects of supplementing concentrate into native grass at different levels on *In vitro* fermentation characteristics. The treatments applied were: T0 (Control): 100% Native grass; T1: Native grass 60% + concentrate 40%; T2 : Native grass 50% + concentrate 50%; T3: Native grass 40% + concentrate 60%. A randomized block design was used to test four treatment diets that were formulated to represent different ratios of field grass to concentrate. The variables measured were *In vitro* gas production, ammonia concentration, efficiency of microbial protein synthesis (EMPS), dry matter (DM) and organic matter (OM) digestibility. Statistical analyses showed that the treatments significantly affected ($p<0.01$) gas production, ammonia concentration, EMPS, DM and OM digestibility. The increased level of concentrate in the diet in line with an increase in gas production, ammonia concentration, EMPS, DM and OM digestibility values. Microbial protein synthesis efficiency value was highest for the 40% field grass and 60% concentrate diet (T3) treatment (47.53 g N/kg FOM), but was not different to T2 (50% native grass + 50% concentrate). It can be concluded that the concentrate supplementation at 50% is more recommended for use by farmers to fattening Madura cattle as an implementation of the *in vivo* results.

Keywords | Complete feed, IVGP, NH_3 concentration, EMPS, IVDM-OMD, Madura cattle

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INTRODUCTION

In vitro techniques, such as those developed by Tilley and Terry (1963) and refined by Makkar *et al.* (1995), have been adopted for evaluating feed quality due to their efficiency, repeatability, and lower cost compared to *in vivo* methods. These techniques enable rapid assessment of fermentation characteristics, digestibility, and microbial protein synthesis, providing valuable insights for ruminant nutrition research and feed formulation.

Ruminant livestock production in tropical regions, including Southeast Asia, often faces challenges related to the quality and availability of feed resources. Diets are typically based on fibrous forages with low digestibility and protein content, especially during dry seasons, leading to suboptimal growth and reproductive performance (Suharti *et al.*, 2009). To address these nutritional gaps, concentrate supplementation commonly supplied to enhance protein and energy sources is needed. In this regard, a proper amount of concentrate supplementation that can be provided without disturbing rumen pH and microbial balance is a major issue for evaluation.

Balancing forage and concentrate in ruminant diets is essential to support efficient fermentation, maintain rumen health, and reduce environmental impacts. Fibrous components play a role in sustaining rumen motility and microbial stability, while readily degradable carbohydrates from concentrates contribute to higher energy availability and improved feed utilization (Klevenhusen, 2021). As Darwin *et al.* (2023) noted, while high concentrate levels increase degradability, they must be managed to avoid acid accumulation and microbial imbalance.

These general challenges are particularly evident in Indonesia, where indigenous cattle breeds such as Madura cattle are primarily raised under smallholder systems. Farmers in Madura Island typically rely on native grasses and agricultural by-products, resulting in low dietary protein and energy availability. This feeding pattern contributes to low productivity, especially during periods of forage scarcity (Kuswati *et al.*, 2022). Bridging this global issue to the local context, strategies that incorporate locally available concentrate sources are increasingly important to enhance nutrient supply without depending on imported or expensive feed ingredients.

Although several studies have examined concentrate supplementation in tropical ruminant diets (Kusmartono *et al.*, 2023), research specifically targeting Madura cattle using field-collected feed ingredients remains limited. This study aims to evaluate the effects of different levels of concentrate supplementation into native grass on ruminal fermentation characteristics using an *in vitro* approach. The native grass used in this study reflects typical forages provided by Madura cattle farmers in Bangkalan Regency, East Java, and the concentrate formulation represents ingredients accessible and affordable in the local context.

By investigating *in vitro* gas production, digestibility, ammonia concentration, and microbial protein synthesis efficiency (EMPS), this study seeks to provide practical insights into optimizing feed formulation for smallholder-based cattle systems in tropical environments. While the results offer promising preliminary data, they are intended as a precursor to future *in vivo* validation under field conditions.

MATERIALS AND METHODS

Experimental design and dietary treatments

This study utilized native grass as the primary fiber source, representing the typical forage fed to Madura cattle in smallholder systems. The concentrate was formulated using locally available feed ingredients copra meal, palm kernel meal, cassava, and dried tofu waste sourced from small farmers in Bangkalan Regency, Madura Island. The nutritional composition of these ingredients is presented in Table 1, which highlights the notably low crude protein (CP) content of the native grass used.

Table 1: Nutrient contents of the feed ingredients during experimental.

Ingredients	Nutrient contents (% DM)					
	DM	OM*	CP*	EE*	CF*	NFE*
Native grass	35.38	73.61	4.98	0.32	34.87	33.44
Copra meal	87.27	90.11	22.8	1.84	20.79	44.68
Palm kernel meal	92.48	95.99	15.52	8.66	20.79	51.02
Cassava wastes	88.97	81.58	1.48	0.66	14.9	64.54
Dried tofu wastes	88.35	96.32	14.53	13.59	23.89	44.31
Concentrate	88.86	89.27	12.55	4.83	19.34	10.82

The concentrate was formulated to contain approximately 12.55% crude protein to reflect common feeding practices and economic considerations in the region. This level was chosen based on surveys of local feed usage and farmer affordability, ensuring that the experimental diets were both nutritionally realistic and practically applicable. To assess the effect of increasing levels of concentrate supplementation, four dietary treatments were prepared:

- T0: 100% native grass (control)
- T1: 60% native grass + 40% concentrate
- T2: 50% native grass + 50% concentrate
- T3: 40% native grass + 60% concentrate

A Randomized Block Design (RBD) was employed to minimize variability introduced by the rumen fluid inoculum, which was identified as a potential confounding factor. Rumen fluid was obtained from three different beef cattle at a local slaughterhouse (limosin crossbreed), each of which served as a "block" in the experimental design. This approach was chosen to control for differences in microbial population, pre-slaughter diet, and animal physiology, all of which could influence fermentation outcomes.

Each dietary treatment was replicated three times, corresponding to the three independent rumen fluid sources. Although three replicates per treatment are at the lower limit for achieving strong statistical power, this design was constrained by the availability of fresh, high-quality rumen fluid and the logistical challenges associated with sample handling under anaerobic conditions. This limitation is acknowledged, and caution should be exercised when generalizing the results.

Research methods

In vitro procedures

In vitro gas production was measured using the method described by Makkar *et al.* (1995), while *In vitro* digestibility of dry matter (DMD) and organic matter (OMD) was assessed following the protocol of Tilley and Terry (1963). Ammonia (NH_3) concentration was determined using the Conway diffusion method (Conway, 1957), and the efficiency of microbial protein synthesis (EMPS) was calculated according to the procedure outlined by Blümmel *et al.* (1997). The gas production measurements were recorded at time intervals of 2, 4, 6, 8, 12, 16, 24, 36, and 48 hours of incubation. The cumulative gas volume was fitted to the exponential model described by Makkar *et al.* (1995):

$$Y=a+b(1-e^{(-ct)})$$

Where: Y= Cumulative gas production (ml) at time t, a= Gas production from the soluble fraction (ml), b= Potential gas production (mL/500 mg DM), c= Gas production rate constant (per hour), t= Incubation time (hours), and e= Base of the natural logarithm.

Rumen fluid source and inoculum preparation

Rumen fluid was collected from three freshly slaughtered, healthy beef cattle at a licensed local slaughterhouse (one beef cattle in each replication). To minimize potential bias introduced by pre-slaughter stress or diet variability, only animals that were fasted for less than 12 hours and exhibited normal physiological conditions were selected. The rumen fluid was pooled, strained through four layers of cheesecloth, and maintained under CO_2 flushing to preserve anaerobic conditions during transport and analysis. Pooling samples from three donor animals helped reduce individual animal variability and allowed for greater consistency across treatments. Each of the three pooled samples served as a block in the experimental design.

Feed formulation and chemical analysis

Feed formulations for the treatments were based on native grass (as forage) and a locally formulated concentrate consisting of copra meal, palm kernel meal, cassava, and dried tofu waste. The proportions of each ingredient in the concentrate are provided in Table 2.

Four treatment diets (complete feeds) were prepared as follows:

- T0: 100% native grass
- T1: 60% native grass + 40% concentrate
- T2: 50% native grass + 50% concentrate
- T3: 40% native grass + 60% concentrate

The chemical composition of the experimental diets was determined, and the results are summarized in Table 3. Analyses encompassed proximate components namely: dry matter (DM), organic matter (OM), crude protein (CP), crude fiber (CF), and ether extract (EE), as well as the detergent fiber fractions, neutral detergent fiber (NDF) and acid detergent fiber (ADF). These fiber fractions (NDF and ADF) were included to better interpret the digestibility results and their association with fiber composition, as recommended in digestibility literature (Van Soest et al., 1991).

Table 2: The proportions of feed ingredients of concentrate.

No	Ingredients	Proportion (%DM)
1.	Cassava waste	35
2.	Copra meal	30
3.	Palm kernel meal	15
4.	Dried Tofu waste	20
Total		100

Table 3: Nutrient contents of the feed treatment.

No	Treat-ment	Nutritional content (% DM)					
		DM (%)	OM*	CP*	EE*	CF*	NFE*
Native grass		35.380	73.610	4.980	0.320	34.870	33.440
Concentrate		88.860	89.270	12.550	4.830	19.340	52.550
1	T0	88.460	73.610	4.980	0.320	34.870	33.440
2	T1	88.620	79.870	8.010	2.120	28.660	41.080
3	T2	88.660	81.440	8.770	2.570	27.100	43.000
4	T3	88.700	83.010	9.530	3.020	25.550	44.910

Experimental design

A Randomized Block Design (RBD) was employed, with the three rumen fluid sources serving as blocks to control for biological variability. Each of the four dietary treatments was replicated three times (once per rumen fluid source).

Analyses of data

The data were subjected to analysis of variance (ANOVA) using the General Linear Model (GLM) procedure in Minitab® Version 17.1.0. The following statistical model is applied:

$$Yij= \mu + \beta j + \delta i + \varepsilon ij$$

Where Yij is the dependent variable, μ was the overall mean, βj was the effect of the block, δi is the effect of the treatment, and εij is the random error.

Differences among treatment means were compared using Tukey's Honest Significant Difference (HSD) test at a significance level of $p < 0.05$.

RESULTS AND DISCUSSION

Digestibility

In vitro DMD, OMD and TDN of complete diet with different roughage and concentrate ratios are presented in Table 4. Statistical analyses revealed that the treatments were significantly different (p<0.01) in DMD, OMD and TDN. The higher concentrate level the higher DMD, OMD and TDN. The highest DMD, OMD and TDN were observed in T3 treatment that complete diet with the highest concentrate level. Meanwhile the lowest DMD, OMD and TDN are observed in T0 where the complete diet only contained roughage.

Table 4: *In vitro* DMD, OMD and TDN of feed with different roughage and concentrate ratios.

Treatments	DMD (Dry matter digestibility) (%)	OMD (Organic matter digestibility) (%)	TDN (Total digestible nutrients) (%)
T0	56.60±3.96[a]	45.35±0.51[a]	47.62±0.53[a]
T1	61.79±3.79[b]	49.51±0.67[b]	51.98±0.70[b]
T2	67.07±3.12[c]	55.57±2.01[c]	58.34±2.11[c]
T3	69.22±2.89[d]	55.98±0.35[c]	58.78±0.36[c]

Note: Total Digestible Nutrient (TDN) (%) = 1.05 X OMD (Kearl, 1982). In the same column with different superscripts (a, b, and c) differ significantly at p<0.01

Table 4 showed that *In vitro* DMD and OMD of the diet increased with increasing concentrate levels and this is in accordance with Kumar *et al.* (2022) who reported that high concentrate in feed contributes to high soluble substrates which will increase the digestibility of DM and OM. In this study, *in vitro* DM and OM reached the highest level at the 60% concentrate level, indicating that increasing the concentrate up to 60% in the diet could improve nutrient digestibility.

The increase in *in-vitro* DMD and OMD with increasing levels of concentrate can be related to the nutritional content of each treatment as presented in Table 3. It can be seen that the higher the concentrate level in the treatment, the CF content decreased and the CP content increased. High CF levels, particularly components such as acid detergent fiber (ADF) and neutral detergent fiber (NDF), are negatively correlated with both DMD and OMD. This is because fiber fractions are less fermentable, making them more resistant to microbial breakdown in the rumen (Beck *et al.*, 2013). Furthermore, the CP content of feed significantly affects digestibility, where complete diet with higher CP levels generally enhances feed digestibility due to improved microbial activity in the rumen, as protein provides nitrogen for microbial growth. Beyihayo *et al.* (2015) conducted research on the effect of concentrate levels to hay of Brachiaria Mulato on *in vitro* DMD and OMD. It was concluded that *in vitro* DMD and OMD of diets increased with increase in crude protein levels.

The improved DMD in diets with higher concentrate proportions can be linked to enhanced fermentability of available carbohydrates and reduced structural barriers within the feed matrix. Concentrates often offer starch and readily soluble nutrients that foster microbial colonization and enzymatic breakdown (Karami *et al.*, 2021). By contrast, fully forage-based diets (as in T0) can hinder microbial attachment due to high neutral-detergent fiber (NDF) content and lignification (Van Soest *et al.*, 2020).

Furthermore, the TDN values in this study were in line with the *in vitro* DMD and OMD results, where the higher the level of concentrate in the complete diet the higher the TDN results. The low TDN values (47.62%-58.78%), which reflect the *in vitro* DMD and OMD in this study, are due to the poor quality of native grass obtained from small holder farmers.

In vitro rumen fermentation characteristics

In vitro rumen fermentation characteristics in term of gas production, gas production potential (b value), gas production rate (c value), as well as *In vitro* DM and OM Ruminal degradability of feed with different roughage and concentrate ratios are presented in Table 5.

Table 5: *In vitro* rumen fermentation characteristics of feed with different roughage and concentrate ratios.

Treatment	Gas production (ml/500 mg DM)	b value (ml/500 mg DM)	C value (ml/hour)	DM ruminal degradability (%)	OM ruminal degradability (%)
T0	89.48±0.82[a]	101.69+4.92[a]	0.035+0.008[a]	41.94+1.00[a]	45.67+0.69[a]
T1	112.40±2.60[b]	133.15+4.62[b]	0.038+0.002[b]	47.72+1.02[b]	50.67+0.16[b]
T2	124.86±0.44[c]	154.18+4.91[c]	0.040+0.003[c]	54.18+1.96[bc]	55.88+1.41[c]
T3	133.27±0.77[d]	156.56+3.14[c]	0.043+0.002[d]	56.44+1.37[c]	57.98+1.48[c]

Note: Different superscripts (a,b, c and d) in the same column significantly different (p<0.01). b value (asymptotic gas volume): The maximum volume of gas (usually in mL or mL/g DM) that can be produced from the fermentation of the insoluble but degradable portion of the feed. It reflects the potential extent of fermentation. c value (rate constant): A measure of the speed or rate at which gas is produced from the degradable fraction. It indicates how rapidly microbes ferment the substrate, typically expressed as a per hour rate (e.g., h^{-1}).

Statistical analyses showed that treatments were significantly different ($p<0.01$) in gas production, b value and c value as well as DM and OM Ruminal degradability. The higher concentrate level the higher gas production, b value and c value, DM and OM Ruminal degradability. The highest *in vitro* rumen fermentation characteristics were observed at T3 treatment that feed with the highest concentrate level. Meanwhile the lowest *in vitro* rumen fermentation characteristics were observed in T0 where the feed only contained native grass (Table 5).

Cumulative gas production, recorded at regular intervals (2, 4, 8, 12, 24, 36, and 48 h), exhibited considerable variation among treatments ($p < 0.01$). Treatment T3 exhibited the highest cumulative gas volumes (133.27 mL/500 mg DM), while T0 had the lowest gas production (89.48 mL/500 mg DM). Treatments T1 and T2 showed intermediate values of gas production, implying that moderate concentrate inclusion can sustain a desirable balance between fermentable substrates and ruminal buffering (Baber *et al*., 2020; Chen *et al*., 2021).

The higher gas production in T3 may be attributed to a consistent supply of readily available carbohydrates (e.g., starch), promoting robust microbial growth and fermentation activity (Wang *et al*., 2019). By contrast, the high-fiber matrix in T0 hinders fermentation kinetics, translating to reduced gas evolution. The cumulative gas production curves as shown in Figure 1 also suggests that T3 reached a plateau earlier than T1 and T2, reflecting more rapid substrate utilization by rumen microbes (Dong *et al*., 2019).

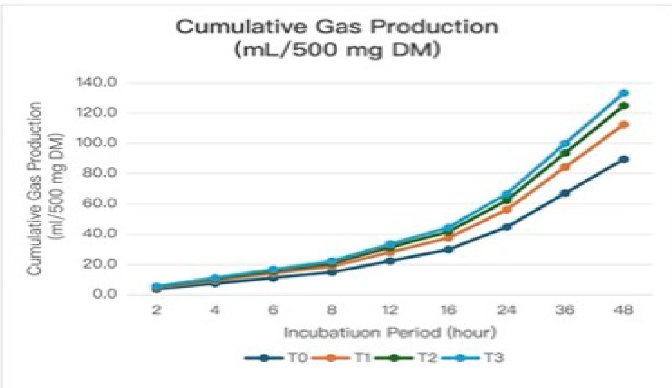

Figure 1: Cumulative gas production (ml/500 mg DM).

Fitting the exponential gas production model (Makkar *et al*., 1995) revealed that the fermentation rate constant (c) was significantly elevated ($p < 0.01$) for T3 compared to T0. The lag phase in T3 was shorter, potentially because concentrates accelerate microbial attachment and proliferation (Chen *et al*., 2021). Nevertheless, caution is advised regarding excessive concentrate inclusion, as overly rapid fermentation can predispose cattle to subacute ruminal acidosis (Karami et al., 2021).

The increased in gas production, b and c values as well as DM and OM ruminal degradability with increasing levels of concentrate might have been related to the nutritional content of each treatment as presented in Table 3 where the higher the concentrate level the CF content decreased and the CP content increased. Positive relationship between concentrate level in feed and gas production has also been confirmed by previous reports (Bannink *et al*., 2000). The higher energy available for microbial populations supplied by the higher concentrate could offer an explanation for this observation. Increasing concentrate means decreasing fibre or NDF content, which is a factor into increasing of gas production (Rodríguez *et al*., 2010). In present study, *In vitro* DM and OM ruminal degradability increase significantly ($P<0.01$) as concentrate ratio increase and the highest at level 60% concentrate in diet treatment.

Jayanegara *et al*. (2009) reported from their results of the research that there is a correlation between the chemical composition of feed such as CP and CF on rumen fermentation. Gas production was significantly ($P<0.05$) influenced positively by CP ($r2= 0.81$) and negatively by ADF content ($r2= 0.72$). This is because protein is a component that is very easily degraded in the rumen, while ADF consists of ligno-cellulose and silica which are very difficult to degrade in the rumen (Fonnesbeck *et al*., 1981). The decreased in CF content along with the increased in concentrate in the treated feed maybe one of the reasons why *In vitro* rumen degradation of DM and OM values also increased. Sumadi et al. (2017) stated that CF content is a limiting factor of digestion process, thereby affecting digestibility and ultimately reducing the level of digestibility.

Ruminal pH is considered the main factor that influences microbiome and the degradability of DM and OM, the concentration of NH3 (Li et al., 2014; McCann *et al*., 2016). The optimal range of pH rumen was 6.2 to 7.0, where the ecological environment of rumen microorganisms could be relatively stable, which could ensure normal rumen fermentation (Weng, 2013) In this study the rumen pH value is ideal, ranged from 6.41-6.84, indicated that the increased level of concentrate was safe and did not induce acidosis.

Ammonia (NH_3) concentration and efficiency of microbial protein synthesis (EMPS)

Ammonia concentration and EMPS based on *In vitro* gas production of feed with different roughage and concentrate ratios are presented in Table 6. Statistical analyses showed that the treatments had a significant effect ($p<0.01$) on NH_3 concentration and EMPS. A higher concentrate level resulted in a higher NH_3 concentration and EMPS. The highest NH_3 concentration and EMPS were observed in the T3 treatment, which received the highest concentrate level. Meanwhile, the lowest NH_3 concentration and EMPS were observed in T0, where the feed consisted only of roughage (Table 6).

Table 6: NH_3 concentration and EMPS based on *In vitro* gas production of feed with different roughage and concentrate ratio.

Treat-ments	NH^3 concentration (mg N-NH^3/l)	EMPS (gN/kg FOM)	pH
T0	56.10±1.12[a]	31.96±3.18[a]	6.84
T1	68.14±1.49[b]	40.18±2.44[b]	6.56
T2	78.06±1.29[c]	44.46±1.53[c]	6.62
T3	80.18±0.88[c]	47.53±2.53[c]	6.41

Note: Different superscripts (a,b,and c) in the same column significantly different ($p<0.01$).

There was no significant difference ($P>0.05$) between treatment T2 and T3. These observations suggest that a 50% concentrate in feed may be recommended from the perspective of *in vitro* fermentation. Crude protein content plays a role in the production of NH_3 compounds produced from rumen microbial activity. The high NH_3 concentration value of T3 treatment as presented in Table 6 was mainly due to the higher CP content in the T3 treatment (9.53%) than other treatments. By contrast, the pure roughage diet in T0 showed NH_3 concentrations 56.10 mg N-NH_3/L, which was on the lower threshold needed for optimal microbial protein synthesis (Chen *et al.*, 2021).

Hong *et al.* (2015) reported that carbohydrates play a role in providing energy for rumen microbes to carry out fermentation activities. The soluble nature of carbohydrates and their limited availability can reduce the rate of microbial fermentation activity, resulting in low NH_3 production. In this study, the higher the concentrate level, the higher the OM content, while the CF content decreased. Increasing concentrate levels in feed that were followed by increasing CP content could raise the NH_3-N concentration Ammonia nitrogen (NH_3-N) concentration and microbial CP production increased significantly ($p<0.05$) by increasing CP levels and with increasing concentrate levels in diet as well (Dung *et al.*, 2014).

The recorded ammonia concentrations (56–80 mg/L) in Table 6 are below the optimal range (85–300 mg/L) required for efficient microbial protein synthesis (EMPS) in the rumen, suggesting a potential nitrogen-energy imbalance that may limit microbial growth, particularly of fiber-digesting bacteria (Pazla *et al.*, 2022). This deficiency could lead to reduced microbial protein yield and feed digestibility, ultimately impacting animal productivity. Pazla *et al.* (2022) stated that the optimum standard for the concentration of NH_3 available in rumen fluid range 85-300 mg N-NH_3/l. McDonald *et al.* (2002) stated that the optimal concentration of ammonia in rumen fluid range 84.04-247.37 N-NH_3/l. This is in line with the fact that the quality of all treatment feed is low, particularly in terms of CP content. Although the observed NH_3 concentrations did not surpass typical optimal ranges reported elsewhere, the results strongly suggest that feeding balanced rations can sustain adequate rumen ammonia to meet microbial demands (Baber *et al.*, 2020; Montegiove *et al.*, 2021). Insufficient NH_3 levels impair microbial protein synthesis, whereas excessively high levels risk nitrogen wastage and potential environmental concerns (Wang *et al.*, 2019).

EMPS describes the amount of OM that is degraded in the rumen to be used as a substrate for microbial protein production. Microbial protein plays a role in providing 40-80% of the protein needs of ruminant livestock (Tanuwiria, *et al.*, 2017). Microbial protein synthesis activity can occur optimally if the carbohydrate supply is sufficient. Bach *et al.* (2005) concluded from their study that optimum bacterial growth in the rumen occurs when EMPS is 29 g of bacterial N/kg of fermented organic matter. In addition NRC (1996) recommended that EMPS that must be around 148 g/kg FOM truly digested (around 23.68 g of bacterial N/kg of fermented organic matter) in the rumen. The EMPS value obtained in this study ranging from 31.96–47.53 g N/kg FOM. These findings suggest that the EMPS values obtained in this study not only exceed the threshold for optimal microbial growth as proposed by Bach *et al.* (2005), but also surpass the NRC (1996) benchmark, indicating a potentially efficient microbial protein synthesis under the tested dietary conditions, particularly when low-quality roughage is strategically supplemented with concentrate sources (Van Soest, 1994).

These findings confirm that protein availability in conjunction with fermentable carbohydrates fosters robust microbial anabolism (Chen *et al.*, 2021; Karami *et al.*, 2021). In practical feedlot industry, feeding high-concentrate diets to ruminants is a common strategy to meet the high energy requirements and improve cost efficiency (Chen *et al.*, 2021).

Compared with low-concentrate diets, high-concentrate diets can be rapidly fermented by ruminal microorganisms to produce short-chain fatty acids (SCFAs), especially propionate and butyrate, which are conducive to enhancing growth performance and improving feed efficiency

CONCLUSION

The study results indicate that the feed ratio between native grass and concentrate significantly affects gas production, fermentation characteristics, ammonia concentration, microbial protein synthesis, as well as *in vitro* dry matter (DM) and organic matter (OM) digestibility. Concentrate supplementation enhances microbial activity and protein breakdown, positively impacting nutrient digestibility. Microbial protein synthesis efficiency value was highest for the 40% field grass and 60% concentrate diet (T3) treatment (47.53 g N/kg FOM), but was not different to T2 (50% native grass + 50% concentrate). It can be concluded that the concentrate supplementation at 50% is more recommended for use by farmers to fattening Madura cattle as an implementation of the *in vivo* results.

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NOVELTY STATEMENT

This study highlights a practical and cost-effective approach to enhancing ruminal fermentation by supplementing native grass with locally available concentrates like copra meal, tofu waste, and cassava, reflecting the real feeding practices of Madura cattle farmers in Indonesia. Through *in vitro* analysis, it provides region-specific insights that support improved feed efficiency without relying on imported inputs, offering valuable contributions to both scientific understanding and sustainable livestock nutrition in resource-limited tropical settings.

AUTHOR'S CONTRIBUTION

Mashudi contributed to data management, formal analysis, methodology development, and manuscript drafting. Meanwhile, Kusmartono, Poespitasari Hazanah Ndaru, Aprilia Dwi Kartika and Muhammad Johan Futohar were involved in shaping the conceptual framework, carrying out investigations, supervising the process, validating findings, and revising and editing the manuscript. All authors have read and approved the final version submitted to the journal.

Generative AI and AI-assisted technology statement

The generative artificial intelligence tools was used for language refinementsuch as improving grammar, enhancing readibility and clarity of expression. All ideas, interpretations have been reviewed, edited, and verified to ensure accuracy, originality and compliance with academic integrity standards. The final decisions remain the responsibility of the author.

Conflict of interest

The authors have declared no conflict of interest.

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