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ROSELLE CALYX POWDER CAN INCREASE TOTAL GAS PRODUCTION WITHOUT AFFECTING pH, METHANE, AND DRY MATTER AND ORGANIC MATTER DEGRADABILITY

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Abstract
Roselle calyx powder (RCP) can act as a natural dietary additive in a concentrate. A completely randomized design was employed in this study to determine the impact of 6 different doses of RCP supplementation (0, 0.1, 0.2, 0.4, 0.8, and 1.6%) in a rice straw-based diet using 6 replicates (n = 6) on in vitro dry matter degradability (IVDMD), in vitro organic matter degradability (IVOMD), pH, total gas production 8 hours (TGP8), total gas production 16 hours (TGP16), total gas production 24 hours (TGP24) and methane. RCP supplementation did not affect the degradability, pH, and methane. The lower dose of RCP-0.1 supplementation obtained greater TGP than the higher doses, increased TGP can be a sign of increasing rumen fermentation process. However, higher doses of RCP are not recommended, as the bioactive compounds may act as antinutrients, inhibiting the fermentation process.
Keywords: In Vitro, IVDMD, IVOMD, Sheep

TEPUNG ROSELA DAPAT MENINGKATKAN PRODUKSI GAS TOTAL TANPA MEMENGARUHI pH, METANA, SERTA KECERNAAN BAHAN KERING DAN BAHAN ORGANIK

Abstrak
Tepung rosela bermanfaat sebagai pakan aditif alami dalam konsentrat. Penelitian ini menggunakan rancangan acak lengkap untuk mengevaluasi pengaruh 6 level suplementasi RCP (0, 0,1, 0,2, 0,4, 0,8, dan 1,6%) dalam ransum berbasis jerami padi dengan 6 ulangan (n = 6) terhadap degradabilitas bahan kering secara in vitro (IVDMD), degradabilitas bahan organik secara in vitro (IVOMD), pH, produksi gas total 8 jam (TGP8), produksi gas total 16 jam (TGP16), produksi gas total 24 jam (TGP24), dan metana. Suplementasi RCP tidak berpengaruh terhadap kecernaan, pH, dan produksi metana. Suplementasi RCP pada level dosis rendah (0,1%) menghasilkan produksi gas total yang lebih tinggi dibandingkan dosis yang lebih tinggi. Peningkatan produksi gas total ini dapat menjadi indikator meningkatnya proses fermentasi di dalam rumen. Namun, penggunaan RCP dalam dosis tinggi tidak direkomendasikan, karena senyawa bioaktif yang terkandung di dalamnya berpotensi bertindak sebagai antinutrisi yang dapat menghambat proses fermentasi.
***Kata Kunci:In Vitro,* IVDMD, IVOMD, Domba**

INTRODUCTION

The prohibition of the use of AGPs in Indonesia is stated in Article 16 of the Ministry of Agriculture Regulation No. 14/2017, which is in accordance with Law No. 41/2014 in conjunction with Law No. 18/2009 concerning Animal Husbandry and Animal Health. As a response to this regulation, the government encourages farmers to apply alternative strategies to maintain animal health and productivity. These alternatives include organic acids, probiotics, enzymes, essential oils, high-value feed additives, strategies for biosecurity, better quality feed, and natural feed additives. One of the appropriate alternatives is Roselle-(*Hibiscus sabdariffa* L.) due to the presence of its bioactive tanin and saponin showing antioxidant activity, which prevents oxidations taking place during metabolism (Tsado et al., 2019).

The content of flavonoids in dried roselle flower calyces ranges from 419 ± 2 to 2260 ± 70 mg of quercetin per 100 g (Borrás-Linares et al., 2015) while the contents of tanin and saponin about 51.90 ± 3.89 mg/100g and

102.56 ± 6.89 mg/100 g, respectively (Tsado et al., 2019). Flavonoids represent a class of polyphenols with high anti-inflammatory and antioxidant activities and are particularly useful in maintaining health under stress conditions. Some studies have shown that flavonoid-containing compounds have the potential to reduce methane gas production in vitro and lower the acetate-to-propionate ratio (Olagaray & Bradford, 2019).

In poultry, incorporating roselle meal into feed improves productivity, as evidenced by higher growth rates, better feed conversion ratios, reduced mortality, and enhanced immunity (Faris et al., 2023). Additional benefits of roselle extract include improved metabolic functions, blood biochemistry, intestinal health, antioxidant activity, immune status, and increased ω-3 fatty acid content in breast muscle (Amer et al., 2022). Additionally, research indicates that roselle extract is non-toxic and plays a significant role in improving broiler performance throughout the finisher phase (Omolade et al., 2015). Notwithstanding these encouraging results, there exists a paucity of information regarding the nutritional benefits and rumen degradation characteristics of roselle calyces powder (RCP) when utilized as an additive in ruminant feed. Present investigations mainly concentrate on the impact of anthocyanins. Therefore, this study aims to investigate whether RCP can enhance total gas production and nutrient degradability, particularly in paddy straw-based diets.

Recent advancements in feed additive research have primarily focused on plant-derived bioactives with multifunctional properties that can enhance ruminant nutrition while mitigating environmental impacts. Despite the promising findings on anthocyanins and flavonoids from roselle, limited studies have explored their specific role in modulating rumen fermentation dynamics and fiber degradability in tropical forage-based diets. Given the challenges associated with low-quality roughage such as paddy straw, incorporating RCP as a dietary intervention presents a novel approach to improving digestibility and microbial efficiency. This study extends current knowledge by assessing the effects of RCP on gas production kinetics, nutrient degradation, and fermentation profiles, providing critical insights into its potential as a sustainable alternative in ruminant nutrition.

MATERIALS AND METHODS

Ethical approval

The Research Ethics Committee of Padjadjaran University (1097/UN6.KEP/EC/ 2024) approved all animal-handled protocols used in the current study.

Experimental design

This study was conducted using six doses of roselle petal powder in a completely randomized design with six replicates. The treatments applied were as follows:

T0: Control diet (CT), consisted of 60% commercial concentrate and 40% fermented rice straw.
T1: CT + 0.1% Roselle Calyx Powder
T2: CT + 0.2% Roselle Calyx Powder
T3: CT + 0.4% Roselle Calyx Powder
T4: CT + 0.8% Roselle Calyx Powder
T5: CT + 1.6% Roselle Calyx Powder

Feed Ingredients and Sample Preparation

Each feed ingredient was ground in such a way that it could pass through a 1 mm sieve using a sample mill (Ossel FFC-15). The concentrate consisted of (%) sugar beet pulps (26), a mixture of barley and wheat (26), soybean meal (22), maize distillers' grain (15), molasses (8), and a mineral mixture (3). The concentrate was made in a feedmill at PT. Lintas Nusa Pratama, Tasikmalaya. Fermented rice straw was made by incubating chopped rice straw waste and adding 1,5% molasses for 14 days. The fermented rice straw was made at Paddy Farm, Garut.

Freshly mature roselle (*Hibiscus sabdariffa* L.) calyces were obtained from Sidoarjo, East Java. The calyces were directly taken from the plants and manually separated from the seeds. The fresh calyces were initially sun-dried for 5 hours daily over three consecutive days. To ensure optimal dryness, they were then oven-dried at 60°C for 48 hours. Once dried, the calyces were ground using an Ossel FFC-15 grinder fitted with a 1 mm sieve. The processed samples were stored in plastic zipper bags for further use.

In Vitro Total Gas Production and methane

Approximately 300 mg of the substrate, prepared according to the treatment, was placed into a 100 mL glass syringe (Fortuna Optima). A piston lightly coated with vaseline was

inserted to ensure a proper seal. Afterward, 45 mL of RF-buffer inoculum was added to each syringe. The syringes were sealed with a 3-way stopcock and shaken thoroughly to mix the substrate with the RF-buffer inoculum. Then, the syringes were incubated in a water bath at 39°C for 24 hours. Total gas production was recorded every 2 hours, and the syringes were shaken during measurements to resuspend the sedimented substrate. After 24 hours of incubation, the percentage of LEL methane was measured using a Riken Keiki (GX-2012) gas analyzer by gas production on syringes, as described by Khan and Chaudhry (2010).

pH Level, Dry Matter, and Organic Matter Degradability

After incubation, the substrate was separated from the liquid using a centrifuge (Oregon LC-04S) at 3000 rpm for 15 minutes. The separated liquid can be analyzed for pH using the Eutech pH 700, the instrument properly set up and calibrated using standard buffer solutions, typically at pH 7.00, to enhance measurement precision. Before analysis, the electrode was rinsed with distilled water and gently dried to prevent contamination. The electrode was immersed in the sample, and the pH value is recorded once the reading stabilizes.

After that, substrate was oven-dried at 60°C for 48 hours. Once dried, the sample was weighed using a Mettler Toledo LA204E analytical balance with a capacity of 220 g and an accuracy of 0.1 mg. Weight before, and after drying, represents the dry matter lost through incubation and was subtracted to calculate the actual percentage of dry matter degradability.

IVOMD was determined after the IVDMD analysis. The dried substrate from the IVDMD procedure was placed in a furnace (B-One BFNC-7-1200), where the temperature was gradually increased to 550°C and maintained for 6 hours. The sample was then cooled in a desiccator and weighed. Organic matter degradability was calculated as the percentage difference between the organic matter in the original sample and the residue (Ramdani et al., 2014).

Statistical Analysis

Data on the effects of roselle calyx powder dosages across various treatments were analyzed using the one-way ANOVA procedure in MINITAB 21.4 statistical software. Statistical significance was determined at $P < 0.05$. For significant results, a Tukey test was conducted to compare the means.

RESULTS AND DISCUSSION

Roselle calyx powder acts as an antioxidant in ruminant metabolism, with its bioactive anthocyanin content playing an important role in capturing free radicals and protecting cell membranes from oxidative damage. Additionally, its antioxidant properties may enhance the activity of certain enzymes in the rumen (Anam et al., 2024). In this study, administering various levels of roselle calyx powder as feed additive in fermented rice straw-based sheep diets increased in vitro total gas production (Figure 1). Adding roselle calyx powder showed high total gas production at a 0.1% dose, with RCP generally resulting in higher total gas production compared to the control. The assessment of fermentability through in vitro gas production was represented by the kinetic parameters of gas production (Sun et al., 2013). Gas production results from fermentation, which was influenced by microbial activity within the rumen and indicated the quantity of digested organic material (Anggraeni et al., 2020; Sofyan et al., 2017).

However, at higher doses, RCP reduced total gas production, as excessive feed additive inclusion may elevate bioactive compounds like tannins that can inhibit rumen fermentation, The high-dose RCP contained a significant amount of total tannins. Administering RCP in large quantities may potentially act as an anti-nutrient, inhibiting the fermentation process. Balgees et al. (2013) stated that total gas production is negatively influenced by high levels of NDF, ADF, and tannins, which can impair digestibility at elevated doses.

Table 1. Nutrient Compositions

	Fermented rice straw	Commercial concentrate	Roselle Calyx Powder
Dry matter (%)	34.67	89.33	88.39
Organic matter (%)	22.47	9.85	6.43
Crude Protein (%)	4.18	7.48	7.88
Ether Extract (%)	1.79	4.11	0.92
NDF (%)	34.37	40.86	25.16
ADF (%)	56.71	44.75	21.53

Table 2. Influence of adding roselle calyx powder on pH, dry matter & organic matter degradability and methane production.

Measurement	T0	T1	T2	T3	T4	T5	SEM	P-Value
Dry Matter Degradability	47.1	42.73	37.44	37.1	49.6	46.12	2.811	0.730
Organic Matter Degradability	65.15	65.34	62.33	75.84	68.22	69.17	1.186	0.083
pH	6,9	6,85	6,85	6,87	6,87	6,83	0,009	0,396
Methane Production	40,7	19	49,7	31,2	25,67	65,5	4,723	0,083

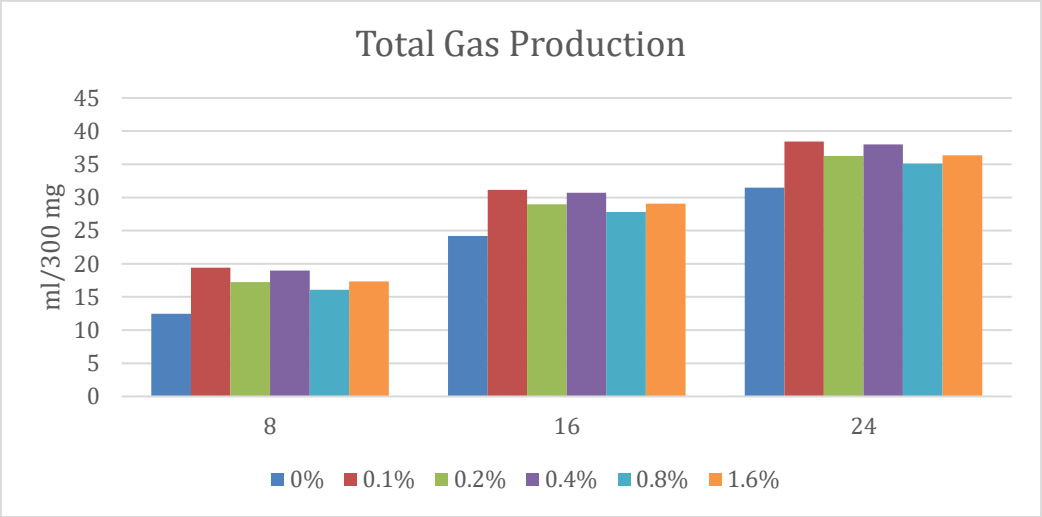
Mean values were not significantly different at $p > 0.05$ and highly significantly different at $p < 0.001$;
n = Number of replicates;
SEM = Standard error of mean;
n/a = Not available.

Table 3. Influence of adding roselle calyx powder on total gas production.

Treatments (tr)	Time (hours)			Fitted mean	SEM	p-value (time)
	8	16	24			
T0	12,48	24,21	31,48	22,72^{b}	0,576	<0,000
T1	19,41	31,14	38,41	29,65^{a}		
T2	17,25	28,99	36,25	27,50^{ab}		
T3	18,98	30,71	37,98	29,22^{a}		
T4	16,09	27,82	35,09	26,33^{ab}		
T5	17,34	29,08	36,34	27,59^{ab}		
Fitted mean	16,92^{c}	28,66^{b}	35,92^{a}			
SEM		0,982				
p-value (tr)		0,010				

Mean values were not significantly different at $p > 0.05$ and highly significantly different at $p < 0.001$;
n = Number of replicates;
SEM = Standard error of mean;
n/a = Not available.

Figure 1. Total gas production (ml/300 mg) at 12 and 24 hours.



The supplementation of RCP in treatments T1, T3, and T4 was found to reduce methane production, although the reduction was not statistically significant. Conversely, methane levels increased in treatments T2 and T5, indicating a possible variation in substrate availability or microbial adaptation that favors methanogenic pathways (Lazalde-Cruz et al., 2021).

The supplementations RCP in the diet had insignificant effects on IVDMD and IVOMD (Table 1). This outcome was attributed to the interaction of bioactive compounds in RCP, which did not negatively impact feed digestion but may have enhanced antioxidant activity in sheep. Adding saponin to the diet also did not show a significant effect on IVDMD, but adding it as a meal to the diet showed significant effects on IVOMD (Muhartatik et al., 2024). Abdillah et al. (2024) reported that administering nutmeg (*Myristica fragrans* Houtt.) essential oil (NEO) did not have a significant effect on IVDMD and IVOMD. The secondary compounds, such as polyphenol- and tannin-rich have beneficial nutritional properties and boost antioxidant activity in sheep diets without adverse effects on animal well-being and performance (Diaz-Medina et al., 2021).

CONCLUSIONS

Roselle Calyx Powder at a 0.1% dose had a more significant effect on total gas production but did not affect IVDMD and IVOMD. Therefore, RCP has the potential to increase gas production in ruminant diets without impacting rumen function. However, based on total gas production results, higher doses are not recommended, as the bioactive compounds may act as antinutrients, inhibiting the fermentation process.

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