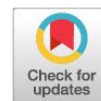


Research Article



Innovative Strategies for Methane Mitigation: The Role of Durian Seed Nanoemulsions (DSN) in *In vitro* Studies

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Abstract | The livestock sector, particularly ruminants, contributes to global warming through methane emissions, which are a significant source of greenhouse gases. These emissions also have a reciprocal impact on the ruminant sector, as climate change and unstable weather conditions affect the availability and quality of forage for livestock. This research, conducted using the Theodorou method on an *in vitro* scale with rumen fluid from cattle obtained via fistula, aims to explore the potential of *Durio zibethinus* seed waste in the form of nanoemulsion preparations for reducing methane gas emissions. Qualitative analyses confirmed the presence of tannins, flavonoids, alkaloids, saponins, and steroids in the Durian Seed Nanoemulsion (DSN). Quantitative assessments revealed flavonoid content at 4.2 mg RE/g dry weight, total phenolic content at 9.1 mg GAE/g dry weight, and total tannin content of 7.6%, with 4.4% as condensed tannins and 3.2% as hydrolysable tannins. *In vitro* gas production experiments showed that while the incorporation of DSN did not significantly affect gas production kinetics from soluble or potentially degradable feed fractions ($p > 0.05$), methane production was significantly reduced with increasing DSN levels ($p < 0.05$). These findings highlight DSN's potential as an effective additive for mitigating methane emissions in ruminant feed, suggesting that the active compounds in *Durio zibethinus* seed could play a key role in promoting more sustainable livestock practices.

Keywords | Feed additive, Methane, Reduction, *Durio zibethinus* seeds, Ruminant

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INTRODUCTION

The ruminant farming sector is a major source of greenhouse gas emissions, particularly methane (CH₄), which significantly contributes to global warming. Methane has a global warming potential approximately 23 times greater than carbon dioxide (Terefe *et al.*, 2024). Rising global temperatures pose a critical environmental

challenge (Richardson *et al.*, 2023). Omeme and Mona (2025) stated that methane possesses a significantly higher warming potential, being 21 times more effective at trapping heat in the atmosphere than carbon dioxide, thereby accelerating global warming. Additionally, Debbarma *et al.* (2023) identify various sources of methane, with emissions from methanogenic bacteria during the digestion process in ruminants being a significant contributor.

Baihaqi *et al.* (2023) demonstrated that Carica fruit seeds can effectively reduce methane emissions, control *Haemonchus contortus*, and are safe for rumen fermentation in ruminants. Additionally, research by Baihaqi *et al.* (2020a), (2020b), (2020c), along with studies by Sakti *et al.* (2024); Rokana *et al.* (2024); Prasetyo *et al.* (2024); Lokapirnasari *et al.* (2024); Prayudi *et al.* (2023); Lisnanti *et al.* (2023) and Baihaqi *et al.* (2024a), (2024b), highlighted that various active compounds derived from plant-based agro-industrial waste have been successful as alternative treatments, including bio-anthelmintics, bio-antibacterials, methane-reduction agents, and enhancers of livestock productivity.

The selection of durian seeds for this study is based on the fact that the waste from these seeds causes environmental pollution and is abundant in quantity (Gamay *et al.*, 2024). Strategies to reduce excessive methane emissions can involve the use of active plant compounds (Baihaqi *et al.*, 2023). Gamay *et al.* (2024) stated that durian seed waste contains active plant compounds that are largely underutilized. Zhao *et al.* (2024) reported that flavonoid compounds inhibit populations of methanogenic bacteria and protozoa in the rumen, improving organic matter digestibility and increasing populations of *Fibrobacter succinogenes*. Nascimento *et al.* (2021) noted that tannins promote microbial protein synthesis by forming protein-tannin complexes that inhibit methanogenic bacteria. Rira *et al.* (2019) suggested that plant bioactives could function as feed additives to reduce CH₄ production and greenhouse gas emissions. Furthermore, the formulation of nanoemulsion preparations enhances the solubility of active substances, facilitating their absorption and bioavailability in the gastrointestinal tract (Singh *et al.*, 2009). Dhakal *et al.* (2024) advocate for using plant bioactives as feed additives to reduce CH₄ emissions and overall greenhouse gas output. Therefore, this research aims to investigate the potential of compounds from *Durio zibethinus* seed nanoemulsions in mitigating methane emissions.

MATERIALS AND METHODS

LOCATION AND MATERIAL

Research on the analysis of *Durio zibethinus* seed nanoemulsions for methane reduction was conducted at the National Research and Innovation Agency. The *Durio zibethinus* seeds were collected from community gardens in Kediri Regency, Indonesia, and were used for the extraction process..

PREPARATION OF DURIO ZIBETHINUS SEEDS NANOEMULSIONS

The *Durio zibethinus* seeds were collected during the durian fruit season in June 2024 through direct collection, then oven-dried at 55°C and ground into powder. The powder

was extracted using ethanol as a solvent. The nanoemulsion formulation consisted of the ethanol extract of *Durio zibethinus* seeds, Tween 80, and phosphate buffer. The preparation of this formulation followed the method outlined in previous research by Harmi (2014). The extract of *Durio zibethinus* seeds at concentrations of 5% and 10%, along with 3 ml of Tween 80, was dissolved in the buffer solution and homogenized to a final volume of 100 ml. The extract mixture, along with the other ingredients, was blended until homogeneous using a centrifuge for 30 minutes at a speed of 10,000 rpm, with temperatures ranging from 10°C to 30°C.

IDENTIFICATION OF BIOACTIVE COMPOUNDS IN NANOEMULSIONS

The bioactive components of the *Durio zibethinus* seeds nanoemulsions were qualitatively identified using the method outlined by Adeyemi *et al.* (2017). Quantitative analysis of the total phenolic content in fruit peel waste was performed using the Folin-Ciocalteu method and expressed in mg of gallic acid. Concurrently, the total flavonoid content in fruit peel waste was analyzed using a colorimetric method, with results expressed in mg of rutin, following the procedure by Sari *et al.* (2023).

PREPARE FOR IN VITRO GAS PRODUCTION

Bali cattle rumen fluid samples were collected from Faculty of Animal Science, Universitas Gadjah Mada (Yogyakarta, Indonesia). This research used a feed ingredient design consisting of complete feed and the addition of *Durio zibethinus* seeds nanoemulsions with percentages of 3%, 5% and 7%. The results of the proximate analysis of the feed ingredients used are presented in Table 1.

Table 1: Chemical composition of complete feed.

Feed name	DM %	Percentage Based on Dry Matter (DM) (%)					
		Ash	CP	CF	CFiber	NFE	TDN
Complete feed	89.91	4.21	14.32	4.83	26.71	59.60	65.11

Note: CP= Crude Protein; CF = Crude Fat; Cfiber = Crude Fiber; NFE= Nitrogen-Free Extract; TDN= Total Digestible Nutrient .

The fermentation substrate used in each treatment was 300 mg. All samples were put into a syringe filled with rumen buffer fluid in triplicate. Then, according to Theodorou methods (Theodorou *et al.*, 1994), incubation using a water bath. Gas production was measured at 0, 2, 4, 6, 8, 12, 24, 36, and 48 hours and the gas collection for methane analysis was conducted at hour 24.

STATISTICAL ANALYSIS

The research design in this study is divided into 4 treatments, consisting of a control treatment and 3 different

concentrations of Durian Seed Nanoemulsion (DSN). One treatment in this study consists of 7 replications. The research results related to rumen fermentation characteristics were analyzed using a two-way analysis of variance and Duncan's multiple range test. Significant research results were stated with $p < 0.05$. The analyses were performed using IBM Statistical Package for the Social Sciences version 20 (IBM Corp., Chicago, USA). Data were presented as mean $\pm$ standard deviation (SD).

RESULTS

The results of qualitative observations (Table 2) of plant bioactivity showed that *Durio zibethinus* seeds tested positive for the presence of tannins, flavonoids, alkaloids, saponins, and steroids.

Table 2: Analyses of qualitative phytochemicals of *Durio zibethinus* seeds (DSN).

Secondary metabolite	DSN
Tannin	+
Flavonoid	+
Alkaloid	+
Saponin	+
Steroid	+

The results of the quantitative analysis of bioactive compounds in this study, including flavonoids, total phenols, and tannins, are presented in Table 3. The flavonoid content of the *Durio zibethinus* seed nanoemulsion was 4.2 mg RE/g dry weight (dw), the total phenolic content was 9.1 mg GAE/g dw, and the total tannin content was 7.6%, with condensed tannins accounting for 4.4% and hydrolysable tannins at 3.2%.

As shown in Table 4, statistical analyses revealed that incorporating DSN did not significantly affect gas production kinetics from soluble feed fractions (a), potentially degradable feed fractions (b), the fractional rate of gas production (c), or total gas production (a+b) ($p > 0.05$). However, DSN significantly reduced methane production, with a notable decrease in methane emissions observed when DSN was administered at higher levels ($p < 0.05$).

DISCUSSION

The results of this quantitative plant bioactive study are consistent with the research found by Aisyah *et al.* (2024), who studied the same plant parts, specifically the durian seeds. Baihaqi *et al.* (2023) stated that *Carica pubescens* seeds contain bioactive compounds both qualitatively and quantitatively, with details of flavonoid content at 3.1 mg RE/g dw, total phenol at 9.5 mg GAE/g dw, and total tan-

nin at 5.4% have successfully provided a significant methane reduction effect, positively causing death to worms in the abomasum of ruminant livestock, and also did not disrupt rumen fermentation characteristics. Króliczewska *et al.* (2023) stated that plant parts containing active plant compounds have the potential to be utilized as herbal medicine, aiding in methane emission reduction and serving various sectors.

Table 3: The quantification of *Durio zibethinus* seeds.

Mate-rial	Flavonoids content (mg RE/g dw)	Total phenolic (mg GAE/g dw)	Tannin total (%)	Con-densed tannin, CT (%)	Hydro-lyzed tannin, HT (%)
DSN	4.2	9.1	7.6	4.4	3.2

Table 4: In vitro assessment of rumen gas production following the addition of DSN.

Parameter	Treatment group			
	DSN 0%	DSN 3%	DSN 5%	DSN 7%
Gas production from soluble feed fraction (a; ml/300 mg/dry matter) ^{ns}	11.27 $\pm$ 0.31	11.26 $\pm$ 0.30	11.24 $\pm$ 0.29	11.23 $\pm$ 0.28
Gas production from potentially degraded feed fractions (b; ml/300 mg/dry matter) ^{ns}	114.01 $\pm$ 0.30	113.99 $\pm$ 0.28	113.97 $\pm$ 0.27	113.95 $\pm$ 0.26
Fractional rate of gas production (c; ml/hour)	0.05 $\pm$ 0.00	0.05 $\pm$ 0.00	0.05 $\pm$ 0.00	0.05 $\pm$ 0.00
Potential gas production (a+b; ml/300 mg dry matter) ^{ns}	125.28 $\pm$ 0.38	125.27 $\pm$ 0.36	125.25 $\pm$ 0.34	125.24 $\pm$ 0.32
CH ₄ (ppm)	15.31 $\pm$ 0.32 ^a	11.15 $\pm$ 0.49 ^b	8.37 $\pm$ 0.18 ^c	6.21 $\pm$ 0.14 ^d

a-d: Different superscripts in a row indicate significant difference ($p < 0.05$) between means.

Table 4 may demonstrate these effects of DSN supplementation on rumen gas production kinetics. The findings of the recent study align with prior research conducted by Acosta-Lozano *et al.* (2023), which stated that the addition of *Acacia mearnsii*, which contains tannins, did not significantly affect gas production parameters but resulted in a significant decrease in methane production along with the tannin content in the plant. This effect is likely due to a reduction in methanogenic archaea and ruminal protozoa and the formation of tannin complexes. Additionally, Baihaqi *et al.* (2023) added that the addition of *Carica pubescens* seeds also did not significantly change gas production but could significantly reduce methane production. In contrast to the findings of Liu *et al.* (2023), who found that the isolated silibinin from the fruit of *Silybum marianum* (chrysanthemum plant) resulted in a decrease in total gas production and methane in line with the high silibinin content.

The active compounds in DSN aqueous extract successfully provided an effect for methane reduction. The findings were in line with those reported by previous researchers, Martins *et al.* (2024), who conducted a meta-analysis of various references, stated that several methane mitigation interventions using β -cyclodextrin, *Leptospermum pater-soni*, *Salix caprea*, *Heracleum* spp., nitroglycerin, *Fructulus Ligustri*, and *Sesbania grandiflora*. According to Lambo *et al.* (2024), they highlighted the regulatory mechanisms of medicinal plants (MP) and emphasized the critical gaps that need addressing to enhance their efficacy. MP has the potential to decrease enteric methane production by 8-50% through regulating rumen fermentation pathways, shifting hydrogen towards propionogenesis, and modifying the diversity, structure, and population of methanogens and protozoa in the rumen.

Lins *et al.* (2018) noted that the inclusion of Moringa seeds containing tannins did not significantly affect ($p>0.05$) total gas production. In vitro gas testing serves as a parameter for evaluating rumen microbial conditions and digestibility. This study demonstrated that administering DSN effectively reduced methane emissions, particularly as the percentage of DSN increased. These outcomes align with previous research suggesting that certain active plant compounds can lower methane production. For instance, tannic acid, salicylic acid, and quercetin have been shown to decrease rumen methane formation after 48 hours of incubation, exhibiting a significant reduction compared to the control using corn silage (Nørskov *et al.*, 2023). Furthermore, the addition of tannin extract from *Acacia mearnsii* has been proposed as a strategy for methane reduction, although it did not affect the production of short-chain fatty acids, despite the decrease in rumen CH₄ levels (Junior *et al.*, 2022).

CONCLUSIONS AND RECOMMENDATIONS

The results of the exploration using nanoemulsion from *Durio zibethinus* seeds (DSN) significantly reduced methane levels, likely due to the active compounds in the durian seeds. Further research is needed to determine the optimal concentration for direct application to ruminant livestock (in vivo).

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This study pioneers the use of Durian Seed Nanoemulsions (DSN) as a cutting-edge nanomaterial-based approach for methane mitigation in in vitro conditions. Unlike conventional methods, DSN harnesses nanotechnology to enhance bioavailability, reactivity, and targeted efficacy, offering a highly efficient, plant-derived, and sustainable alternative. By optimizing nanoparticle properties, this research provides breakthrough insights into methane reduction mechanisms, paving the way for next-generation, nano-based strategies in sustainable livestock production and environmental conservation.

AUTHOR'S CONTRIBUTIONS

ER, ZAB, AD, FT: Designed the study and collected samples and performed examinations. All authors have drafted and revised the manuscript. All authors have read, reviewed, and approved the final manuscript.

CONFLICT OF INTEREST

The authors declare that they have no competing interests.

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