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Lauric acid mitigates heat stress-induced methane emissions and fermentation inefficiency by modulating microbial populations in the rumen of Bach Thao Goat

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ABSTRACT

This study investigated the effects of lauric acid (LA) supplementation on methane emissions, fermentation characteristics, and microbial dynamics in Bach Thao goat rumen fluid under non-heat stress (non-HS) and heat stress (HS) conditions. Methane yield progressively increased in all treatments, with the highest levels observed under HS conditions. Methane emissions were significantly affected by LA or HS from 12 to 72 hours ($P < 0.05$). LA supplementation significantly reduced methane levels under non-HS and HS conditions, demonstrating its anti-methanogenic properties and potential to mitigate HS-induced methane production. *In vitro* Dry matter degradability (DMD) and organic matter degradability (OMD) were significantly lower under HS conditions compared to non-HS conditions ($P < 0.05$). While LA had no significant impact on DMD and OMD under non-HS conditions, it effectively restored these parameters to near-normal levels under HS conditions ($P < 0.05$). Ammonia concentrations were significantly elevated under HS conditions but were reduced by LA supplementation under both conditions ($P < 0.05$). However, pH values remained unaffected by LA under both conditions ($P > 0.05$). Microbial analysis revealed that HS significantly increased the populations of *Streptococcus* and *Methanobrevibacter*, key contributors to digestion and methane production, respectively. LA supplementation effectively reduced these microbial populations under HS conditions, supporting microbial balance and improving fermentation efficiency ($P < 0.05$). The findings highlight the potential of LA to reduce methane emissions and maintain rumen fermentation efficiency by modulating key microbial populations.

Keywords: methane, lauric acid, ruminants, rumen, protozoa.

INTRODUCTION

In recent years, increasing debate has suggested that the primary cause of global climate change lies in the agricultural sector, particularly livestock farming, which is widely regarded as a significant source of greenhouse gas emissions. HS is a primary factor adversely affecting animal performance (Bernabucci et al., 2010). Additionally, HS disrupts goats' digestion and rumen fermentation patterns, leading to decreased production efficiency (Yadav et al., 2013). An animal's capacity to adapt and cope with climatic changes relies heavily on maintaining the proper functioning of its rumen and ruminal microbiota (Bernabucci et al., 2009). However, elevated temperatures can impair these processes, influencing methane emissions, particularly the rate and intensity of methane production in goats. Hence, reducing methane emissions from ruminants has become an urgent priority in modern agricultural systems.

HS-induced methane emissions increase under high-temperature conditions due to disruptions in rumen microbial balance (Bernabucci et al., 2010). HS alters the composition of rumen microorganisms by increasing populations of Streptococcus and Methanobrevibacter (Bernabucci et al., 2010; Baek et al., 2020). These microbes contribute to higher methane production while reducing feed digestibility efficiency. Additionally, methane-favoring bacteria like Streptococcus can

disrupt rumen fermentation by elevating rumen ammonia concentrations, which negatively impact digestibility and reduce overall production efficiency in livestock (Lee et al., 2024). Reducing the abundance of methane-producing bacteria can decrease methane emissions by altering rumen fermentation patterns, improving feed efficiency and nutrient digestion.

LA, the primary component of coconut oil, is a medium-chain fatty acid with robust antimicrobial characteristics known to be harmful to protozoa and methanogenic bacteria (Burdick et al., 2022). Coconut oil, a natural source abundant in LA (C12:0), has been shown to significantly reduce methane emissions during rumen fermentation while enhancing digestibility when included in ruminant diets. According to Faciola and Broderick (2014), supplementing dairy cattle diets with coconut oil inhibits methanogenic bacteria and improves fermentation efficiency. Similarly, Hristov et al. (2009) showed that coconut oil lowers methane emissions, modifies milk fatty acid composition, and improves digestion efficiency. Yabuuchi et al. (2006) also observed that LA-rich coconut oil markedly alters rumen microbial populations and reduces methane emissions when included in starch-rich diets. This evidence suggests that LA is a potential inhibitor of methane, which may regulate the balance of rumen fermentation by inhibiting methane-producing bacteria, leading to decreasing methane emission and increasing feed digestibility during HS conditions.

However, studies on the effects of LA in Bach Thao goats remain limited, particularly under non-HS and HS conditions. In this study, we explored the impact of LA supplementation on in vitro rumen microorganisms, fermentation characteristics, methane emissions, and nutrient digestibility using Bach Thao goat rumen fluid under non-HS and HS conditions.

MATERIALS AND METHODS

Animals, diets, and treatments

The study was conducted at the Non-Ruminant Production Technique Laboratory, Faculty of Animal Science, Can Tho University, Vietnam. The study was designed as a completely randomized design with four treatments and three replications. Base diet (Table 1) was supplemented with or without 50 g/Kg of LA (GRM7187-500G, Himedia, India) at 39.0 °C (Control, LA) or 41 °C (HS, HSLA) for 72 h.

Fresh rumen contents were collected from three Bach Thao goats (20.3 ± 0.8 kg) using a stomach tube before their morning feeding. The rumen contents were extracted through four layers of cheesecloth to remove solid particles and prepare the fluid for further experimental use. The components and nutrition composition of the goats' diet are detailed in Table 1. The fluid was stored in a thermal container at 39 °C and transferred to the laboratory within 1.5 hours to preserve accuracy (Akhter et al., 1998).

In vitro rumen fermentation procedure and analysis

The rumen fluid was combined with a buffer solution in a ratio of 1:2. The buffer solution was prepared using Menke and Steingass's method (1988). All procedures were performed under oxygen-free conditions by flushing with CO_2, ensuring completion within 30 minutes. Each 100 mL bottle was prepared with 0.3 ± 0.0010 g of feed sample and 30 mL of rumen fluid mixed with buffer, following the standard of Menke and Steingass (1988). The containers were processed at 39.0 ± 0.5 °C for non-HS conditions and 41.0 ± 0.5 °C for HS conditions during 72 hours. Eight hundred sixty-four bottles (4 treatments×3 individual samples×3 bottles per sample×3 runs x 8-time points) were prepared with 3 syringes as blanks without samples. Methane yield, pH, ammonia concentration,

Table 1. Ingredients and nutrition composition of diet

Items	Diet
Ingredients (% DM)	
Elephant grass	70
Concentrate	30
Nutrition composition	
DM (% FW)	90.4
OM (% DM)	91.6
CP (% DM)	12.6
EE (% DM)	4.15
NDF (% DM)	59.3
ADF (% DM)	30.7
Ash (% DM)	8.4

Note: DM – dry matter; FW – fresh weight; OM – organic matter; CP – crude protein; EE – ether extract; NDF – neutral detergent fiber; ADF – acid detergent fiber.

nutrient degradability, and microbial population were recorded at 3, 6, 9, 12, 24, 36, 48, and 72 hours. Methane yield was analyzed using a 100 mL syringe to extract headspace gas. Using a methane gas detector (SPD203, Total Metter, China). A pH meter (Orion Star™ A214, Thermo Fisher Scientific, USA) was used for pH measurement. For ammonia concentration, 1.5 mL of rumen fluid was mixed with 150 µL of 0.25% trichloroacetic acid (w/v) and centrifuged at 7.000 rpm for 10 minutes (Dlab D1008). The supernatant was stored at –20 °C and later analyzed for ammonia using a modified phenyl-hypochlorite method (Weatherburn, 1967) with a microplate reader at 650 nm. A 0-hour blank was used to calculate net ammonia production. The contents of the bottles from the first run were passed through four sheets of cheesecloth for inoculation with Columbia blood agar plates containing 5% sheep blood (M144, Himedia, India) incubated at 37 °C in 5% CO_2 for *Streptococcus* analysis. *Methanobrevibacter* was cultured, and analysis followed the method of Traore et al. (2019). The bottle contents were collected from the second incubation run into 50 mL falcon tubes for the third run to determine undigested dry matter (DM) and organic matter (OM). In vitro DMD and OMD were calculated based on DM and OM contents following the AOAC (1990) protocol. Degradability parameters were calculated with the DM and OM contents before and following 12 and 24 h of *in vitro* incubation. The residue was filtered, washed with distilled water, dried at 105 °C to determine IVDMD, and ashed at 550 °C to determine IVOMD. Degradability was determined using the following formula:

$$IVDMD\ (\%) = ((Initial\ dry\ weight - Residue\ dry\ weight)/Initial\ dry\ weight) \times 100 \quad (1)$$

$$IVOMD\ (\%) = ((Initial\ organic\ weight - Residue\ ash\ weight)/Initial\ dry\ weight) \times 100 \quad (2)$$

Statistical analysis

The experimental data were initially entered into Microsoft Excel and subsequently processed using a one-way analysis of variance within the General Linear Model framework, as implemented in Minitab software. Statistical differences between treatment means were assessed using Tukey's test, with statistical significance determined at $P < 0.05$.

RESULTS

Impact of LA on in vitro methane yield using Bach Thao goat rumen fluid under non-HS and HS conditions

Methane is a significant component of greenhouse gas emissions during HS in ruminants. Therefore, evaluating the effect of LA supplementation on methane yield is crucial to determining its potential to reduce methane emissions under high-temperature conditions (Figure 1). Methane yield increased progressively across all treatments, with the highest values observed in the HS group

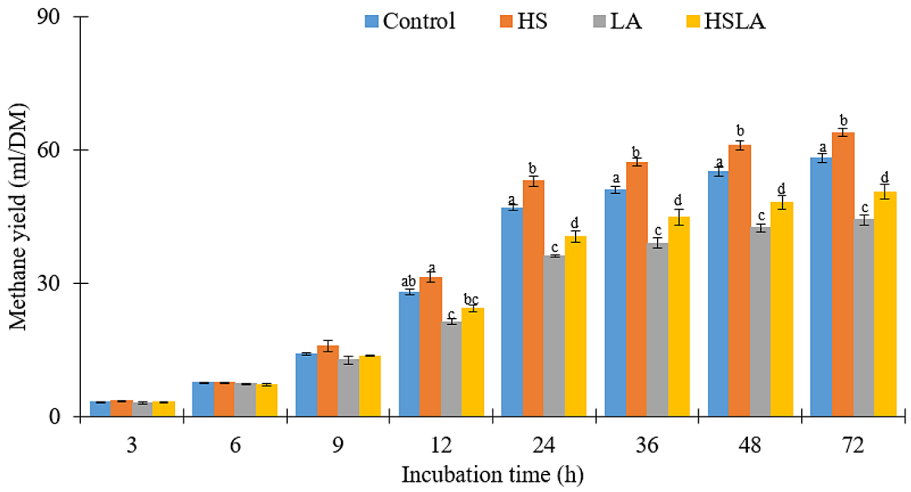

Figure 1. Effect of LA supplementation levels on in vitro methane yield during incubation times under non-HS and HS conditions. Rumen fluid was treated with or without 50 g/kg of LA at 39.0 °C (Control, LA) or 41 °C (HS, HSLA) for 72 h. Means with different letters in the same row (a–d) indicated a significant difference, according to the Tukey test at $P < 0.05$. SEM, standard error of the mean

at each incubation period. In vitro, methane emissions from Bach Thao goat rumen fluid had no significant effect by LA and HS during the first 9 hours ($P > 0.05$), but it was significantly influenced by both treatments from 12 to 72 hours ($P < 0.05$). In addition, methane emissions increased sharply after 24 hours of incubation, then stabilized without significant further increases while maintaining the influence patterns of LA and HS observed at the 12- and 24-hour time points. Therefore, we primarily focus on describing the effects of LA and HS at 12 and 24 hours to enhance clarity and avoid confusion in the results section. At 12 hours, compared to the control group, HS did not affect methane emissions with or without the LA supplementation group ($P > 0.05$). However, the LA supplementation group significantly reduced methane levels under non-HS conditions ($P < 0.05$). A clear pattern regarding the methanogenic suppressant ability of LA was observed at 24 hours. HS increased methane levels with or without the LA supplementation group when compared with the control ($P < 0.05$), and methane emissions were significantly lower in Bach Thao goat rumen fluid supplementation with LA than without LA ($P < 0.05$). The exact figure was observed in both groups under non-HS conditions, and LA significantly reduced methane yield compared to the control group ($P < 0.05$). These results indicate that LA is an antimethanogenic compound under non-HS and HS conditions after 12 to 24 hours of incubation. LA has the potential to mitigate methane production in ruminants exposed to HS.

Impact of LA on in vitro fermentation characteristics using Bach Thao goat rumen fluid under non-HS and HS conditions

After LA reduced methane production under both normal and HS conditions, we investigated whether LA supplementation mitigated the adverse effects of HS on rumen fermentation by analyzing degradability, pH, and ammonia (Table 2). DMD and OMD in the HS group were significantly lower compared to the Control group in both time points ($P < 0.05$). However, no significant effect of LA supplementation had a noticeable impact on DMD and OMD under non-HS conditions while significantly restoring these parameters to near-normal levels under HS conditions in Bach Thao rumen fluid ($P < 0.05$). However, LA supplementation did not significantly influence pH under either non-HS or HS conditions ($P > 0.05$). These data demonstrated that LA enhanced rumen fermentation in Bach Thao goats under both non-HS and HS conditions, particularly by mitigating the severe effects of HS on rumen fermentation after 72 hours of incubation.

Effect of LA on in vitro microorganism dynamic using Bach Thao goat rumen fluid under non-HS and HS conditions

Streptococcus and *Methanobrevibacter* are key rumen microorganisms, *Streptococcus* influencing pH stability through rapid carbohydrate fermentation and lactic acid production, and *Methanobrevibacter* facilitating methane

Table 2. Effect of LA supplementation levels on in vitro degradability, pH, ammonia at 12 and 24 h incubation under non-HS and HS conditions. Rumen fluid was treated with or without 50 g/kg of LA at 39.0 °C(Control, LA) or 41 °C (HS, HSLA) for 72 h

Items	Treatments				SEM	P
	Control	HS	LA	HSLA		
In vitro dry matter degradability, %						
12 h	59.8	50.5	59.0	58.2	1.590	0.011
24 h	65.6	54.3	64.7	63.8	2.050	0.015
In vitro organic matter degradability, %						
12 h	62.61^{a}	52.28^{b}	61.52^{a}	60.26^{a}	1.520	0.005
24 h	68.69^{a}	56.21^{b}	67.46^{a}	65.96^{a}	2.000	0.008
pH value						
12 h	6.92^{a}	6.27^{b}	6.91^{a}	6.22^{b}	0.014	< 0.001
24 h	6.92^{a}	6.25^{b}	6.92^{a}	6.22^{b}	0.017	< 0.001
Ammonia, mg/dL						
12 h	8.31^{a}	9.55^{b}	7.89^{c}	8.92^{d}	0.037	< 0.001
24 h	10.18^{a}	11.94^{b}	9.15^{c}	10.86^{d}	0.059	< 0.001

production by utilizing hydrogen and carbon dioxide during fermentation (Garsa et al., 2019; Singh et al., 2004). Measuring key microbial populations, such as Streptococcus and Methanobrevibacter, is essential due to their pivotal roles in understanding how LA supplementation influences rumen fermentation efficiency and methane production. The populations of *Streptococcus* and *Methanobrevibacter* were significantly affected by HS and LA supplementation, highlighting their critical roles in rumen fermentation. Under HS conditions, the overgrowth of *Streptococcus*, a lactate-producing bacterium associated with ruminal acidosis, was observed in the HS group (8.15×10^8 CFU/mL). Still, its population was significantly reduced in the HSLA group (7.34×10^8 CFU/mL, $P < 0.05$). The population of *Methanobrevibacter*, a key methanogen responsible for methane production, was also significantly elevated under HS conditions (7.70×10^7 CFU/mL). At the same time, LA supplementation effectively reduced methanogen populations in the HSLA group (7.42×10^7 CFU/mL, $P < 0.05$) after 12 and 24h incubations. These findings demonstrate the ability of LA supplementation to support microbial balance and enhance fermentation efficiency under HS conditions (Table 3).

DISCUSSION

LA has been shown to decrease methane emissions in dairy cattle (Yanza et al., 2021). Moreover, methane mitigation strategies can enhance organic matter degradation and balance rumen fermentation by altering the microbial ecosystem (Shinkai et al., 2023). In addition, HS significantly impacts rumen microorganisms, leading to alterations in organic matter degradation and overall rumen fermentation balance (Kim et al., 2022). Therefore, the current study investigated whether LA treatment modifies microorganisms responsible for reducing HS-induced methane production while maintaining digestive efficiency and rumen fermentation balance in Bach Thao goat rumen fluid.

In the present study, LA at a concentration of 50 g/Kg significantly increased methane emission in Bach Thao goat rumen fluid following 24 h of incubation. This result is similar to a previous in vitro study, in which Holstein cow's rumen fluid was treated with 50 g/Kg under non-HS conditions (Machmüller et al., 2002). In addition, an *in vivo* study demonstrated a reduction in methane levels when dairy cattle were treated with a higher dose of LA (125 mg/L) under normal temperature (Joch et al., 2023). LA exhibits antibacterial properties, especially against gram-positive bacteria. According to Hristov et al. (2009), LA suppresses the growth of methanogenic bacteria, which are strongly associated with methane-producing fermentation processes. LA achieves this by disrupting the cell membranes of bacteria through absorption and breakdown, leading to a decline in their population densities in the rumen (Hristov et al., 2009). Machmüller and Kreuzer (1999) also noted that LA directly affects gas-producing bacteria and alters the proportions and products of rumen fermentation, further contributing to the reduction in methane yield. In our results, methane production did not differ significantly between treatments in the initial 3–9 hours. It likely reflects an early incubation phase before LA exerted its full anti-methanogenic effect, possibly due to the time required for microbial populations to respond or adapt to LA. LA's mode

Table 3. Effect of LA supplementation levels on in vitro microbial population at 12 and 24 h incubation under non-HS and HS conditions. Rumen fluid was treated with or without 50 g/kg of LA at 39.0 °C (Control, LA) or 41 °C (HS, HSLA) for 72 h.

Items	Treatments				SEM	P
	Control	HS	LA	HSLA		
Streptococcus bovis, 10^8 CFU/mL of rumen content						
12 h	6.41[a]	8.15[b]	5.34[c]	7.34[d]	0.087	< 0.001
24 h	6.75[a]	8.58[b]	5.63[c]	7.73[d]	0.093	< 0.001
Methanobrevibacter, 10^7 CFU/mL of rumen content						
12 h	7.18[a]	7.70[b]	6.34[c]	7.42[d]	0.039	< 0.001
24 h	7.65[a]	8.19[b]	6.75[c]	7.91[d]	0.041	< 0.001

Note: means with different letters in the same row (a–d) indicated a significant difference according to the Tukey test at $P < 0.05$. SEM, standard error of the mean.

of action involves disrupting microbial cell membranes, which may not immediately eliminate methanogens or protozoa in the first few hours. After ~12 hours, however, a clear divergence emerged: LA-treated samples showed significantly lower methane than controls. This delayed response suggests that LA's methane inhibition becomes evident only after sufficient exposure and microbial adaptation. Such a pattern is consistent with reports that some feed additives require an adaptation period for sustained methane mitigation. For example, essential oils often show only transient effects as microbes adapt, whereas the methane-mitigating effect of LA persists after an initial lag (Klop, 2016). Therefore, the lack of effect in the first 9 hours can be attributed to the rumen microbiota needing time to adjust and for LA to inhibit methanogenic archaea selectively. Once this adaptation phase passed, LA's activity was evident in the significant methane reductions observed from 12 hours onwards. This interpretation is supported by the progressive nature of LA's impact on our study and others rather than by an immediate short-term suppression. Therefore, our results confirm that LA effectively reduced methane production under non-HS conditions and mitigated HS-induced methane emissions in Bach Thao goat rumen fluid.

In this study, the similarity in both *in vitro* of DMD and OMD at 12 h and 24 after incubation under non-HS and HS conditions could be attributed to the alteration of rumen microorganisms and increased stability of rumen fermentation facilitated by the LA in vitro degradation process. Methane is a byproduct of rumen fermentation, formed from Carbon dioxide and hydrogen through methanogenic activity (Boadi et al., 2004). Methane mitigation reduces hydrogen consumption by methanogens, allowing more hydrogen to be utilized by other microbial pathways that enhance OM degradation. The present study revealed a greater inhibition of methanogens like Methanobrevibacter and an enhancement of *Streptococcus* by LA. Therefore, improvement in DMD and OMD observed with methane mitigation strategies by LA may be due to direct inhibition of LA in methanogen.

The present study demonstrates that LA treatments did not significantly affect rumen pH under non-HS and HS conditions. According to Wanapat et al. (2014), pH levels between 6.5 and 7.0 are optimal for ruminal fermentation, microbial activity, and microbial growth. Previous studies have reported that HS can lower ruminal pH to 5.80 to 6.30, potentially disrupting fermentation and microbial efficiency (Park et al., 2022). In this study, lower fermentation (OM degradation) under heat stress would suggest less acid production and a higher pH. However, heat stress (HS) alters rumen fermentation patterns in ways that can lower pH despite reduced total VFAs. In our HS treatment, ruminal pH dropped to 6.22–6.28 compared to 6.92 in the non-HS control. Two factors likely explain this: (1) increased lactic acid production and (2) reduced buffering capacity. Under HS, the microbial community shifted toward more lactic-acid-producing bacteria (notably *Streptococcus*) (Zhao et al., 2019). Lactic acid is much stronger than the main VFAs; even a slight increase can depress pH disproportionately. Indeed, previous studies have reported that because lactate is not as readily absorbed or utilized, heat-stressed cows experience an accumulation of lactate in the rumen and a significant pH drop, even as total VFAs decrease (Russell and Rychlik, 2001; Zhao et al., 2019). In our study, HS likely induced a similar phenomenon (though we did not directly measure lactate, the elevated *Streptococcus* and low pH suggest it).

Secondly, heat stress reduces feed intake and alters rumination patterns, leading to less saliva secretion (Nardone et al., 2010). Saliva provides bicarbonate and phosphate buffers that usually neutralize rumen acids. HS animals pant and drool, lose saliva, and spend less time ruminating; consequently, buffering declines. The net result is that even if absolute VFA production is lower, the rumen's ability to neutralize acids is compromised. Additionally, the VFAs produced under HS may be produced over a shorter span (e.g., a rapid fermentation of available soluble carbohydrates by *Streptococcus* early on), leading to transient pH dips.

In summary, the quality of fermentation under HS (more lactate, less acetate) and reduced buffering outweighed the effect of lower quantity of fermentation. It explains the paradox of a lower pH despite lower OM degradation. Importantly, our observed pH under HS (~6.2) is within the range reported for subacute acidosis risk in heat-stressed ruminants. We have added an explanation in the manuscript to clarify that heat stress can lower rumen pH via lactate accumulation and diminished saliva buffering, even though total fermentative output is reduced (Russell and Rychlik, 2001; Nardone et al., 2010). These findings

suggest that although HS reduces rumen pH, it remains within a moderately functional range, and LA supplementation does not significantly alter this parameter under either condition.

In this study, ammonia concentrations at 12 and 24 hours after incubation were elevated under HS, consistent with findings reported by Kim et al. (2022) when cattle were exposed to HS. This increase may be attributed to disruptions in digestive processes caused by HS, including reduced rumen motility, which affects the rate of protein breakdown and ammonia absorption. Conversely, LA treatment effectively reduced ammonia levels under non-HS and HS conditions, aligning with previous studies (Hristov et al., 2009). The reduction in ammonia concentrations is likely due to LA's ability to decrease the rumen protozoa population. Hristov et al. (2011) observed that LA supplementation reduced the protozoa population in the rumen by approximately 96%, highlighting its role in mitigating ammonia production.

The results of this study demonstrated that LA decreased the populations of both *Streptococcus* and *Methanobrevibacter* under non-HS and HS conditions. This effect can be attributed to LA's ability to disrupt Streptococcus and Methanobrevibacter cell membranes, thereby reducing their viability (Zhou et al., 2018; Yabuuchi et al., 2006). These findings are consistent with Zhou et al. (2018) and Yabuuchi et al. (2006), who also reported reductions in the populations of *Streptococcus* and *Methanobrevibacter* when LA was included in the diet. Additionally, HS is known to exacerbate the presence of *Streptococcus* in goats, potentially leading to complications such as acidosis and reduced feed efficiency (Pragna et al., 2018). We acknowledge that *Streptococcus* species (e.g., *S. bovis*) play a role in rapid carbohydrate fermentation, producing lactic acid and other metabolites that can be converted to propionate by other microbes. In moderation, this contributes to rumen energy yield. However, under our experimental conditions (especially in heat stress), *Streptococcus* was over-abundant and likely contributing to an imbalance. Heat stress caused an overgrowth of *Streptococcus* (8.15×10^8 CFU/mL in the HS group) accompanied by accumulated lactic acid and depressed pH. Such an overgrowth can be detrimental: research on heat-stressed cows has shown that high *Streptococcus* abundance corresponds with increased ruminal lactate and lowered pH (Zhao et al., 2019). Excess lactic acid is a strong acid that, if not rapidly utilized, leads to suboptimal rumen conditions and can inhibit fiber-degrading bacteria (Russell and Rychlik, 2001). *S. bovis* is known as an initiator of ruminal acidosis when it blooms unchecked (Jin et al., 2021). In our study, LA supplementation tempered this overpopulation of *Streptococcus* (reducing it to 7.34×10^8 CFU/mL under HS) and prevented extreme lactic acid buildup, stabilizing pH. We did not observe any drop in overall carbohydrate fermentation efficiency due to *Streptococcus* reduction; on the contrary, organic matter degradability under HS was restored to a level close to the non-HS level with LA. It suggests that other rumen microbes (e.g., slower-growing fibrinolytic and lactate-utilizers like *Megasphaera* spp.) could ferment carbohydrates more efficiently once the excessive lactate production by *Streptococcus* was curtailed. We agree that *Streptococcus* contributes positively to starch breakdown, but the population was beyond the optimal range in our context. By moderating *Streptococcus* proliferation, LA maintained fermentation in a healthier balance – enough rapid fermenters to digest starch but not so many as to cause acid accumulation. This balance is supported by the maintained total VFA production (indicated by normal pH) and unimpaired digestibility in the LA-treated groups. Thus, the reduction of *Streptococcus* in our study is interpreted as a beneficial modulation that prevented adverse effects (lactate overload) without significantly hindering carbohydrate fermentation. We have clarified this point in the discussion, noting that while *Streptococcus* has beneficial functions, controlling its overgrowth under stress conditions can improve overall rumen stability and fermentation efficiency (Zhao et al., 2019). Furthermore, a higher population of *Methanobrevibacter* during HS exposure has been linked to increased methane production in ruminants (Sales et al., 2021). LA is known for its potency against many rumen microbes, especially Gram-positive organisms. However, our evidence suggests that LA's action in this study was selective rather than indiscriminately toxic. First, preserving normal digestibility and fermentation endpoints (as discussed in Comment 2) indicates that the overall microbial activity was not wiped out. If LA had poisoned the entire microflora, we would have seen severe drops in feed degradation and volatile acid production, which did not occur. Instead, LA specifically reduced certain microbial groups: methanogenic archaea (*Methanobrevibacter*) and

Streptococcus were significantly suppressed, while fiber digestion was maintained. It aligns with LA's known spectrum of activity: it strongly affects methanogens, protozoa, and many Gram-positive bacteria, but Gram-negative bacteria tend to be more resistant due to their protective outer membrane (Yanza et al., 2021). Many key fiber degraders (e.g. *Fibrobacter succinogenes* or *Prevotella* spp.) are Gram-negative and likely suffered less from LA exposure. Indeed, prior studies report that medium-chain fatty acids predominantly inhibit Gram-positive rumen bacteria and protozoa, with Gram-negatives less sensitive (Yanza et al., 2021). Additionally, LA's ability to lower ammonia accumulation in our study suggests it was not broadly toxic to all microbes. A likely explanation for lower ammonia is the suppression of protozoa (which release ammonia when they lyse bacteria). Hristov et al. (2011) observed LA reduced rumen ciliate protozoa by ~50%, which would reduce protein turnover and ammonia production. This protozoa-killing effect benefits methane mitigation (since protozoa symbiotically harbor methanogens) and nitrogen retention without indicating general microbiota collapse. We also note that similar doses of LA (around 5% of diet) have been used in vivo with manageable impacts on fermentation – for example, coconut oil at 50 g/kg diet reduced methane. Still, it did not cause acidotic pH or complete loss of fiber digestion in cattle. In summary, while LA is antimicrobial, it predominantly targeted the methanogens and excessive *Streptococcus* in our system. The remaining microbial community was sufficiently intact to ferment (evidenced by stable pH and digestibility). We have added clarification that LA's effects are selective – it supports fermentation efficiency by removing specific methanogen and protozoal populations rather than sterilizing the rumen (Yanza et al., 2021). This selectivity makes LA a promising mitigation agent: it can reduce methane yield without shutting down overall rumen function. Our data indicate that LA reduced methane without impairing overall fermentative activity. Notably, LA supplementation did not decrease dry matter or organic matter degradability under normal conditions (no significant difference in DMD/OMD vs. control), and it improved these metrics back toward normal levels under heat stress. Had LA indiscriminately inhibited the entire microflora, we would expect lower feed degradability; instead, digestibility was maintained or enhanced. Likewise, LA had no significant effect on total rumen pH, implying that volatile fatty acid production from fermentation was not broadly depressed. Literature reports frequently show that medium-chain fatty acids can reduce methane while maintaining or improving fermentation efficiency. For example, Faciola and Broderick (2014) observed that adding coconut oil (rich in LA) to dairy cow diets inhibited methanogens and improved fermentation efficiency, and Hristov et al. (2009) also found methane suppression accompanied by improved digestive efficiency. These findings mirror our results. Additionally, we measured ammonia-N as an indicator of protein fermentation: ammonia levels were reduced by LA (under both non-HS and HS conditions) relative to controls. It suggests a more efficient microbial nitrogen uptake or a reduction of hyper-ammonia-producing bacteria rather than a collapse of protein fermentation. Together, these points indicate that the methane mitigation observed with LA was due to the specific inhibition of methanogenic archaea (and associated hydrogen-transfer microbes) and not a result of general fermentation shutdown. LA-treated rumen fluid continued to ferment substrates effectively, as evidenced by sustained pH and digestibility, thereby ruling out a broad suppressive effect on total fermentation. Therefore, reducing these microorganisms by LA may help mitigate the harmful effects of HS by decreasing methane production and improving digestibility through altered rumen fermentation.

Our findings can inform practical strategies for mitigating enteric methane in Vietnam and similar tropical agricultural systems. One immediate application is the dietary inclusion of LA-rich supplements (such as coconut oil or coconut byproducts) for ruminants during hot climates. As a tropical country, Vietnam has ready access to coconut oil – a natural source containing ~45–50% lauric acid. Feeding trials could incorporate coconut oil at approximately 5% of the diet (50 g/kg dry matter), at which our in vitro study showed significant methane reduction. Incorporating this into rations for cattle, goats, or sheep could reduce methane emissions per unit of feed consumed. Importantly, our results suggest this can be done without sacrificing, and potentially even improving, animal productivity under heat stress since LA supplementation maintained feed digestibility under HS conditions. This dual benefit is especially valuable in tropical systems where heat stress and low feed efficiency often coincide. From a broader perspective, using LA as a feed

additive aligns with sustainable agriculture initiatives. It leverages a locally available resource (coconut) to tackle greenhouse gas emissions. For instance, smallholder dairy or goat farms in Vietnam could mix a small amount of coconut oil into the daily feed. This practice would modulate the rumen microbiome toward lower methane output, as demonstrated by our study and others. Over time, cumulative reductions in methane could contribute significantly to national climate change mitigation targets, given the large ruminant populations.

Furthermore, LA supplementation is relatively cost-effective and straightforward compared to technical solutions (like biogas capture), making it suitable for rural farming conditions. It's also worth noting that adding fat to ruminant diets in hot climates has additional advantages: fat is a high-energy, low-heat-increment feed. Replacing some fermentable carbs with LA-rich fat can reduce metabolic heat production, helping animals cope with heat stress while cutting methane emissions (Kang et al., 2019). Thus, farmers could incorporate LA specifically in the hot season as a nutritional and methane-mitigating strategy. In practice, extension programs in tropical countries could demonstrate the use of coconut oil or purified lauric acid in feed, highlighting findings such as ours – where methane was cut by roughly 20–30% with LA. These results and evidence from dairy cattle studies (e.g., LA reducing methane in cow diets) provide a strong case for policy and on-farm adoption. In summary, the outcomes of this research support integrating LA supplementation into feeding regimes as a practical greenhouse gas mitigation tool in Vietnam and other tropical regions, especially under conditions of heat stress where its benefits are maximized.

Our study of goats builds on a substantial literature on cattle and sheep. For example, Machmüller and Kreuzer (1999) reported that supplementing coconut oil (rich in LA) in sheep diets suppressed methane emissions significantly, highlighting that the underlying mechanism (inhibition of methanogens and protozoa) is not species-specific. LA has also proven effective in dairy cows: Hristov et al. (2009) showed that adding coconut oil to lactating cow rations lowered enteric methane output and even improved digestibility. More recently, an *in vivo* trial by Joch et al. (2023) used a purified LA supplement in dairy cattle and observed an apparent reduction in methane emissions under thermoneutral conditions. These studies collectively reinforce that medium-chain fatty acids like LA consistently reduce methanogenesis in the rumen of different host species. A 2021 meta-analysis (Yanza et al., 2021) encompassing both *in vitro* and *in vivo* experiments in cattle and sheep confirmed that dietary medium-chain fatty acids (including LA) robustly decrease methane production across studies. The consistency of the effect suggests that any ruminant with a foregut fermentation system can respond similarly to LA supplementation.

Of course, the practical application may differ slightly by species – for instance, dairy cows have been supplemented with 100–200 g/day of coconut oil in experiments, whereas sheep and goats, being smaller, receive lower absolute amounts (often 20–50 g/day in trials). However, on a dietary percentage basis, the inclusion levels (around 3–5% of diet dry matter) and outcomes (typically 20–40% methane reduction) are comparable. Our goat findings align with what has been seen in cattle and sheep at similar inclusion rates. This cross-species effectiveness is likely because LA's target (methanogenic archaea and certain bacteria/protozoa) are common to all ruminant rumens. We have noted in the manuscript that our results have broader relevance to ruminant livestock in general. Therefore, LA can be used in dairy cattle, sheep, and other ruminants as a methane mitigation strategy. Ongoing research in cattle and sheep continues to refine the optimal dosing and to monitor any species-specific side effects (such as milk fat depression in high-producing dairy cows at very high-fat inclusion). However, at moderate levels, LA supplementation has been effective and biologically appropriate across species. We are confident that the principle demonstrated in Bach Thao goats – that LA cuts methane emissions while maintaining fermentation – holds for other ruminants, which is supported by the cited studies in cows and sheep. We have added references to these studies to emphasize that our work aligns with findings in cattle and sheep, underlining LA's broad applicability as an enteric methane mitigator.

CONCLUSIONS

In conclusion, LA was shown to reduce methane emission under non-HS conditions by manipulating microorganisms and maintaining rumen fermentation on Bach Thao goat rumen fluid.

Furthermore, LA inhibited HS-induced methane emission and digestive inefficiency by reducing the population of Streptococcus and Methanobrevibacter, contributing to the rumen ammonia.

The findings presented in this study collectively demonstrate that LA has potential uses in regulating methane mitigation by reducing rumen microorganisms and maintaining rumen ammonia concentration.

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