**Research Article**

# Modification of *In Vitro* Methanogenesis and Rumen Fermentation by using Lauric Acid: A Meta Analysis

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**Abstract** | This study aimed to evaluate the effectiveness of lauric acid, either as pure lauric acid or lauric acid-rich oil, on rumen fermentation and methanogenesis by using a meta-analysis approach from published articles. A total of 89 data points from 24 articles were compiled in a database, consisted of various information, i.e., level of lauric acid, microbial population, rumen fermentation characteristics, total gas production, methane production, and digestibility. The data obtained were analyzed by using the mixed-model methodology. Results showed that the addition of lauric acid reduced methane production ($P < 0.001$). This decrease was followed by a tendency of methanogen population reduction ($P < 0.10$) and bacteria ($P < 0.10$) in the rumen. The addition of lauric acid did not alter total volatile fatty acids (VFA), acetate, propionate and butyrate, but tended to decrease ammonia concentration ($P < 0.1$). Lauric acid also reduced dry matter digestibility ($P < 0.05$). It is concluded that lauric acid is an effective agent for mitigating methanogenesis in the rumen.

**Keywords** | Lauric acid, Meta-analysis, Methane, Rumen

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## INTRODUCTION

Ruminants are a group of livestock that can utilize high-fibrous feeds with the help of various microbes through a fermentation process ocurring in the rumen. This fermentation process in the rumen produces volatile fatty acids (VFA) and several gases, including carbon dioxide ($CO_2$) and hydrogen ($H_2$). Furthermore, methanogens utilize $CO_2$ and $H_2$ to form methane has ($CH_4$) (Vlaming, 2008). Such fermentation in the rumen produces 80-95% of total methane gas, while the remaining 5-20% methane is produced in the large intestine (Martin et al., 2008). Around 27% of the total production of methane that impacts global warming is originated from the livestock sector (Haryanto and Thalib, 2008). Globally, methane emission from enteric fermentation and manure management currently is estimated to reach above 80 and 8 Tg, respectively, and the trend of emission continues to increase (Kumari et al., 2020). On the other hand, methane gas production is a form of energy loss of 8-14% of the total digested energy used by ruminants (Cottle et al., 2011).

Dietary management is apparently the best option for immediately mitigating methane emission from livestock at farm level, although breeding and livestock management seem to be better options in the long run (Kumari et al., 2020).

With regard to dietary management, various attempts have been performed to reduce methane production, including directly inhibiting the methanogenesis process, adding propionate precursors, and defaunation (Moss et al., 2000). In addition, efforts to effectively mitigate methane gas can be carried out by adding medium-chain fatty acids (MCFA) such as lauric acid, myristic acid and palmitic acid into diets. Generally, MCFA sources demonstrated a stronger effect in mitigating methane than those of long-chain fatty acids effect on methanogens (Yanza et al., 2020). Lauric acid is known as an antibacterial, antiprotozoal, antiviral, and antifungal agent (Enig, 1999). Machmuller et al. (2002) showed that pure lauric acid reduced the protozoa population and methane production by 76%. Furthermore, some oils high in lauric acid such as coconut oil, virgin coconut oil, palm kernel oil, and maggot oil, can also reduce methane production. Coconut oil contains lauric acid by 51-53% (Sui et al., 2007). According to Yabuuchi et al. (2006), adding coconut oil into a high grain feed reduced methane production and increased total VFA production and the proportion of propionate. Meanwhile, virgin coconut oil contains lauric acid by 46.9-48% (Marina et al., 2009) and this oil could reduce methane production by 18.39-29.7% and did not interfere with rumen microbial activity when given at 2-8% in feed (Sondakh et al., 2015). The lauric acid content in palm kernel oil is 46-52% (Alamu et al., 2008). Dohme et al. (2000) stated that palm kernel oil reduced protozoa and methanogens so that methane production decreased by 34%. According to Jayanegara et al. (2020), maggot oil contains 43.1% lauric acid and could reduce methane production without affecting VFA and ammonia production.

Data regarding the influence of lauric acid on methanogenesis and rumen fermentation require integration and analysis across various studies in order to generate a more robust conclusion. This study therefore aimed to evaluate the effectiveness of lauric acid supplementation, either pure lauric acid or lauric acid-rich oil, on rumen fermentation and methanogenesis by using a meta-analysis approach from published articles. All related parameters such as microbial population, rumen fermentation characteristics, total gas production, methane gas, and nutrient digestibility were evaluated in order to comprehensively determine the effects of adding lauric acid to diet in the *rumen fermentation system*.

## MATERIALS AND METHODS

### Database development

The database was developed from various journal articles that have been published. Data collection was done by searching for articles from various sources such as ScienceDirect, Google Scholar, and Scopus about the addition of lauric acid, either pure lauric acid (LA) or lauric acid-rich oil, such as coconut oil (CO), virgin coconut oil (VCO), palm kernel oil (PKO), and maggot oil (MO) on rumen fermentation and methanogenesis. The criteria used were as follow: (1) articles were published in English, and (2) the addition of lauric acid or lauric acid-rich oil on the parameters of rumen fermentation and methanogenesis. The keywords used for the search were lauric acid, coconut oil, virgin coconut oil, palm kernel oil, maggot oil, rumen, and/or methane.

The database consisted of 89 treatments from 24 articles as described in Table 1. Various parameters were recorded in a Microsoft Excel 2013 worksheet, such as the level of lauric acid (g/kg DM of substrate), protozoa population ($10^4$/ml), bacteria ($10^9$/ml), methanogens ($10^4$/ml), total volatile fatty acids (VFA, mmol/l), acetate (C2), propionate (C3), butyrate (C4), isoC4, valerate (C5), isoC5, C2/C3, ammonia (mmol/l), pH, total gas production (ml/g DM), methane (mmol/d or mmol/l), dry matter digestibility (%) and organic matter digestibility (%). After that, the data for each parameter were equated by converting them to a predetermined unit. The feed used was mostly hay, straw, grass, maize silage, and concentrate. The descriptive statistics of the database is presented in Table 2.

### Statistical analysis

The data obtained in the database were analyzed by using a meta-analysis approach based on the mixed model methodology (St-Pierre, 2001). The mixed model analysis (PROC MIXED) was performed with SAS software version 9.1 (SAS Institute Inc., Cary, NC, USA). The studies were taken as the random effects, while the level of lauric acid was taken as the fixed effect. The statistical model used is as follows:

$$Y_{ij} = B_0 + B_1X_{ij} + B_2X_{ij}^2 + s_i + b_iX_{ij} + e_{ij}$$

Where; $Y_{ij}$ was the dependent variable, $B_0$ was overall intercept across all experiments (fixed effect), $B_1$ was linear regression coefficient of Y on X (fixed effect), $B_2$ was quadratic regression coefficient of Y on X (fixed effect), $X_{ij}$ was the value of the continuous predictor variable (lauric acid addition level), $s_i$ was random effect of experiment i, and $e_{ij}$ was the unexplained residual error. The regression equations were also presented with Akaike information criterion (AIC). The model was considered to be significant at $P < 0.05$ and tended to be significant at $0.05 < P < 0.10$. Selection of the model (linear vs quadratic) was based on a lower AIC and/or significance of the model.

**Table 1:** The studies used in the database to evaluate the effectiveness of lauric acid on rumen fermentation and methanogenesis.

| No. | Reference | Method | Diet | Source | Lauric acid level (g/kg DM substrate) |
|---|---|---|---|---|---|
| 1 | Cieslak *et al.* (2016) | RUSITEC | Concentrate, hay | CO | 23.7 |
| 2 | Dohme *et al.* (1999) | RUSITEC | Hay, maize silage, concentrate | CO | 25.4 |
| 3 | Dohme *et al.* (2000) | RUSITEC | Hay, maize silage, concentrate | CO, PKO | 46.7-47.1 |
| 4 | Dohme *et al.* (2001) | RUSITEC | Hay, maize silage, concentrate | LA | 47.5 |
| 5 | Dong *et al.* (1997) | RUSITEC | Hay, wheat | CO | 46.6 |
| 6 | Jayanegara *et al.* (2020) | GBI | King grass, concentrate | MO | 21.6 |
| 7 | Jayanegara *et al.* (2021) | GBI | Elephant grass, concentrate | MO | 4.3-21.6 |
| 8 | Kang *et al.* (2016) | GBI | Straw, concentrate | CO | 9.2 |
| 9 | Kim *et al.* (2014a) | GVI | Alfalfa, maize, concentrate | LA | 47.5 |
| 10 | Kim *et al.* (2014b) | GVI | Grass, concentrate | CO | 14.1 |
| 11 | Klevenhusen *et al.* (2019) | RUSITEC | Straw, maize, wheat | LA | 47.5 |
| 12 | Kongmun *et al.* (2010) | Menke | Straw, concentrate | CO | 31.9 |
| 13 | Machmuller *et al.* (1997) | RUSITEC | Hay, maize silage, concentrate | CO | 25 |
| 14 | Machmuller *et al.* (2001) | RUSITEC | Hay, maize silage, concentrate | CO, LA | 18.4-52.9 |
| 15 | Machmuller *et al.* (2002) | RUSITEC | Straw, maize silage, concentrate | LA | 47.5 |
| 16 | O'Brien *et al.* (2014) | GPT | Grass, silage, barley | LA | 1.2-9.9 |
| 17 | Panyakaew *et al.* (2013a) | CBC | Straw, rice bran, soybean meal, molasses | CO | 16.1 |
| 18 | Panyakaew *et al.* (2013b) | GBI | Hay, concentrate | CO | 0.3-0.5 |
| 19 | Patra *et al.* (2012) | GBI | Maize silage, hay, alfalfa, concentrate | CO | 1.3-2.6 |
| 20 | Sitoresmi *et al.* (2009) | HGT | King grass, concentrate | CO | 6.4-19.1 |
| 21 | Soliva *et al.* (2004) | RUSITEC | Straw, concentrate | LA | 48.5 |
| 22 | Sondakh *et al.* (2015) | HGT | King grass, concentrate | VCO | 10.8-43.2 |
| 23 | Yabuuchi *et al.* (2006) | GBI | Hay, maize, concentrate | CO, PKO | 0.9 |
| 24 | Yang *et al.* (2016) | GVI | Alfalfa, maize silage, corn | VCO | 15.2 |

DM: dry matter; RUSITEC: rumen simulation technique; GBI: glass bottle incubation; GVI: glass vessel incubation; GPT: gas production technique; HGT: hohenheim gas test; CBC: batch culture; LA: lauric acid; CO: coconut oil; VCO: virgin coconut oil; PKO: palm kernel oil; MO: maggot oil.

## RESULTS AND DISCUSSION

### Microbial population

Rumen microbes are important factors in the fermentative digestion process in the rumen. The microbes in the rumen include bacteria, protozoa, and fungi. Bacteria are the largest biomass in the rumen. The bacterial population tended to decrease ($P < 0.1$) with the addition of lauric acid to the feed (Table 3). Adding fat can reduce the total bacterial population, cellulolytic bacteria, and amylolytic bacteria, because the bacteria cannot stick to the feed particles. The scanning electron microscope showed that there was no close bacterial colonization on the surface of forage-based feed given the addition of coconut oil (Dong et al., 1997). Apparently, this is because fat envelopes the fiber fraction of feed so that bacteria could not stick to the feed particles (Rodrigues et al., 2019; Irawan et al., 2021). Moreover, lauric acid can also interfere with the activity of particularly some gram-positive bacteria although it has less effect on gram-negative bacteria (Hovorkova et al., 2018). According to Yanza et al. (2020), the addition of MCFA *in vitro* had no effect on the bacterial population in the rumen. Meanwhile, according to Dong et al. (1997), adding coconut oil to feed reduced the population of total bacteria, cellulolytic bacteria, amylolytic bacteria, and methanogens in high-forage feeds.

The addition of lauric acid had no effect on protozoa population in the rumen. However, some other studies reported a negative relationship between oil and ruminal protozoa population. Fat may interfere with the growth and activity of rumen microbes, especially protozoa, because protozoa do not have lipase enzyme to break down the fat that covers it (Hristov et al., 2004). According to Klevenhusen et al. (2019), the addition of lauric acid in the form of esterification or monolaurin reduced the number of protozoa in the rumen. Several studies have shown that MCFA had a strong antiprotozoal effect, whether in pure or oil form (Newbold et al., 2015), and lauric acid is the most toxic MCFA to protozoa (Hristov et al., 2004). Machmuller et al. (2003) stated that protozoa play an important role in methanogenesis because about 20-37 % of methanogens are attached to the surface of the protozoa.

The addition of lauric acid tended to decrease methanogens ($P < 0.1$) in the rumen. Methanogens are a group of microorganisms in the rumen that produce methane. The formation of methane occurs by reducing carbon dioxide and hydrogen, catalyzed by enzymes produced by methanogens. Methanogens attach on the surface of the protozoa to obtain hydrogen supply, which is then used by the microbes to produce methane and simultaneously to prevent the accumulation of hydrogen in the rumen (Hegarty and Nolan, 2007). Therefore, the decrease in protozoa may indirectly reduce the number of methanogens and reduce the availability of hydrogen for methanogenesis (Jordan et al., 2006). In another study, Dong et al. (1997) observed that coconut oil supplementation reduced the production of methane which was associated with a decrease in methanogens. In addition, Dohme et al. (2000) found that palm kernel oil reduced methanogens so that the production of methane decreased by 34%.

**Table 2:** Descriptive statistics of the data used to evaluate the effectiveness of lauric acid on rumen fermentation and methanogenesis.

| Parameters | n | Mean | SD | Min. | Max. |
|---|---|---|---|---|---|
| **Microorganism** | | | | | |
| Protozoa ($10^4$/ml) | 52 | 12.1 | 33.8 | 0.00 | 150 |
| Bacteria ($10^9$/ml) | 32 | 6.09 | 6.97 | 0.35 | 31.0 |
| Methanogen ($10^4$/ml) | 8 | 79.5 | 165 | 0.10 | 475 |
| **Rumen fermentation** | | | | | |
| Total VFA (mmol/L) | 70 | 85.2 | 44.9 | 21.0 | 231 |
| C2 (%) | 79 | 53.1 | 12.5 | 11.7 | 73.8 |
| C3 (%) | 79 | 22.0 | 8.17 | 5.16 | 43.8 |
| C4 (%) | 79 | 12.8 | 5.39 | 2.76 | 24.8 |
| IsoC4 (%) | 34 | 0.84 | 0.67 | 0.20 | 2.45 |
| C5 (%) | 52 | 3.88 | 2.56 | 0.60 | 9.59 |
| IsoC5 (%) | 36 | 1.86 | 1.29 | 0.24 | 5.00 |
| C2:C3 | 75 | 2.67 | 0.89 | 1.10 | 5.04 |
| Ammonia (mmol/L) | 59 | 14.5 | 7.35 | 2.12 | 17.2 |
| pH | 74 | 6.70 | 0.33 | 5.68 | 7.81 |
| Total Gas (mL/g DMs) | 36 | 97.0 | 45.6 | 49.2 | 228 |
| $CH_4$ (mmol/d) | 43 | 6.02 | 3.39 | 0.49 | 15.5 |
| $CH_4$ (mmol/L) | 45 | 2.55 | 5.28 | 0.01 | 23.5 |
| **Digestibility (%)** | | | | | |
| DMD | 15 | 56.0 | 10.7 | 38.1 | 80.0 |
| OMD | 25 | 49.0 | 7.52 | 33.4 | 59.0 |

n: number of observations; SD: standard deviation; Min: minimum; Max: maximum; VFA: volatile fatty acids; C2: acetate; C3: propionate; C4: butyrate; IsoC4: isobutyrate; C5: valerate; IsoC5: isovalerate; $CH_4$: methane; DMD: digested dry matter; OMD: digested organic matter.

### Rumen fermentation characteristics

Volatile fatty acid (VFA) is the main energy source for ruminants. Adding lauric acid did not affect total VFA concentration. The amount of VFA formed is strongly influenced by the digestibility and quality of the feed. Generally, the addition of fatty acids to feed would reduce nutrient degradation and fermentation in the rumen and, in turn, cause VFA production to decrease (Aharoni et al., 2004). VFA generally consist of acetate, propionate, butyrate, and valerate. The proportion of acetate is the largest component of VFA about 65%, propionate 21%, butyrate 14%, isobutyrate 1%, valerate and isovalerate below 3% (Hungate, 2013). The composition of the resulting VFA will greatly affect the production of methane gas in the rumen because methanogens use hydrogen, which is a precursor to the formation of methane gas from the process of acetate and butyrate formation. Conversely, propionate formation will reduce methane gas production in the rumen because propionate uses hydrogen for its formation (Jayanegara, 2008).

The addition of lauric acid to the feed did not affect the proportion of acetate, propionate and butyrate. The addition of lauric acid can inhibit cellulolytic bacteria that produce acetate and butyrate from the fermentation process. According to Jayanegara (2008), acetate and butyrate are the main fermentation products of cellulolytic bacteria. Cellulolytic bacteria are the most sensitive bacteria to the addition of fat in the feed. Moreover, the addition of lauric acid also did not alter is butyrate and isovalerate. The deamination of protein forms isovalerate and isobutyrate. Protein deamination is the process of separating amino groups from amino acids to be synthesized into urea which in its formation also produces ammonia, valerate, and isobutyrate (Muchtadi, 2008). According to Moss et al. (2000), the decrease in methanogenic activity will result in the production of hydrogen, which is the result of fermentation used in the formation of propionate and isovalerate because propionate and isovalerate utilize hydrogen in their formation. Increasing the proportion of propionate results in a decrease acetate to propionate ratio. According to Machmuller et al. (2001), adding coconut oil to feeds with low structural carbohydrate content increased the proportion of propionate and decrease the proportion of isobutyrate, valerate, and isovalerate without affecting the proportion of acetate and butyrate.

Ammonia is the end product of the protein degradation process by rumen microbes. The ammonia concentration in the rumen tended to decrease ($P < 0.1$) with the addition of lauric acid. The decrease ammonia concentration in the rumen indicates a decrease in protein degradation by rumen microbes (Kondo et al., 2016). This has an effect because adding oil to the feed will affect the population

**Table 3:** Parameter estimates the effectiveness of lauric acid on rumen fermentation and methanogenesis based on linear relationship.

| Parameters | Parameter estimates | | | | | | AIC |
|---|---|---|---|---|---|---|---|
| | n | Intercept | SE Intercept | Slope | SE slope | P value | |
| **Microorganism** | | | | | | | |
| Protozoa ($10^4$/ml) | 52 | 22.9 | 12.7 | -0.085 | 0.063 | 0.185 | 415 |
| Bacteria ($10^9$/ml) | 32 | 7.80 | 2.40 | -0.067 | 0.034 | 0.060 | 197 |
| Methanogen ($10^4$/ml) | 8 | 223 | 79.3 | -6.59 | 2.56 | 0.062 | 88.7 |
| **Rumen fermentation** | | | | | | | |
| Total VFA (mmol/L) | 70 | 94.0 | 10.3 | -0.077 | 0.074 | 0.304 | 613 |
| C2 (%) | 79 | 53.0 | 2.80 | -0.041 | 0.028 | 0.142 | 537 |
| C3 (%) | 79 | 21.5 | 1.74 | 0.0075 | 0.028 | 0.793 | 521 |
| C4 (%) | 79 | 13.0 | 1.07 | -0.0015 | 0.017 | 0.931 | 441 |
| IsoC4 (%) | 34 | 0.74 | 0.21 | -0.0015 | 0.0010 | 0.139 | 17.1 |
| C5 (%) | 52 | 3.41 | 0.62 | 0.027 | 0.010 | 0.015 | 227 |
| IsoC5 (%) | 36 | 1.82 | 0.39 | -0.0009 | 0.0022 | 0.672 | 66.2 |
| C2:C3 | 75 | 2.69 | 0.18 | -0.0004 | 0.0042 | 0.928 | 201 |
| Ammonia (mmol/L) | 59 | 14.2 | 1.67 | -0.033 | 0.019 | 0.080 | 342 |
| pH | 74 | 6.64 | 0.08 | 0.0003 | 0.0006 | 0.679 | -42.9 |
| Total gas (mL/g DM) | 36 | 98.3 | 16.3 | -0.170 | 0.295 | 0.570 | 364 |
| $CH_4$ (mmol/d) | 43 | 7.79 | 0.88 | -0.073 | 0.012 | <0.001 | 196 |
| **Digestibility (%)** | | | | | | | |
| DMD | 15 | 61.1 | 4.24 | -0.309 | 0.122 | 0.030 | 105 |
| OMD | 25 | 49.8 | 2.04 | -0.044 | 0.082 | 0.601 | 173 |

The model is tended to be significant at $P < 0.10$ and significant at $P < 0.05$; *n:* number of observations; SE: standard error; RMSE: residual mean square error; $R^2$: coefficient of determination; VFA: volatile fatty acids; C2: acetate; C3: propionate; C4: butyrate; IsoC4: isobutyrate; C5: valerate; IsoC5: isovalerate; $CH_4$: methane; DMD: dry matter digestibility; OMD: organic matter digestibility.

and microbial activity in the rumen so that microbes cannot degrade feed optimally (Hidayah, 2014). According to Dohme et al. (2000), the addition of palm kernel oil reduced the concentration of ammonia in the rumen. The pH value is a main factor for the sustainability of the fermentation process for growth and microbial activity in the rumen. The addition of lauric acid to the feed did not affect the rumen pH and therefore is able to maintain the stability of rumen fermentation. In line with the result of this study, Soliva et al. (2004) reported that the addition of lauric acid did not affect the pH of the rumen fluid, with an average pH value of 6.8 in all treatments.

## Total gas, digestibility and methane production

Total gas production is the result of the feed fermentation process that occurs in the rumen. Total gas production has a relationship with the digestibility value. Adding lauric acid to the feed did not change the total gas production. In contrast to this study, total gas production at 72 hours of incubation decreased linearly with coconut oil (Kongmun et al., 2010). The dietary addition of black soldier fly (BSF) or its extracted oil reduced gas production, apparently because BSF oil has a high lauric acid content (Jayanegara et al., 2017; 2020). Moreover, according to a research by Hristov et al. (2009), the addition of coconut oil reduced dry matter and organic matter digestibility because coconut oil covered the fiber fraction of feed and thus rumen bacteria cannot degrade the fiber causing a decrease in total gas production. Such reduction of digestibility was also observed in the present study; lauric acid reduced DMD ($P < 0.05$) atlhough the effect was insignificant for that of OMD. The addition of fat may disrupt the population and microbial activity in the rumen, particularly the cellulolytic microbes (Patra and Yu, 2012). According to Machmuller et al. (1997), the addition of coconut oil reduced the digestibility of organic matter from 61.3% to 41.4%. In addition, the dietary supplementation of maggot oil reduced the digestibility of dry matter and organic matter (Jayanegara et al., 2021). Digestibility can also be affected by feed components, such as cellulose, hemicellulose, lignin, NDF, and ADF which are difficult to digest, and hence, reduce the digestibility value. In contrast, easily digestible carbohydrates such as starch may increase dry matter and organic matter digestibility (Deboever et al., 2005).

Methane gas production is the result of microbial fermentation in the rumen. The amount of methane produced is an indicator of the low efficiency of feed use by livestock because methane production is a form of feed energy loss of 8-14% of the total digested energy used for livestock productivity (Cottle et al., 2011). Reducing methane gas production is a strategy to reduce greenhouse gas emissions and increase feed efficiency (Martin et al. 2008). According to Tavendale et al. (2005), the mechanism of decreasing methane production in ruminants is divided into two, indirectly by inhibiting fiber digestion and directly by inhibiting the growth and activity of methanogens. Addition of lauric acid decreased methane production ($P < 0.001$). Methane is produced by methanogens in which some of them are attached to the protozoa. Therefore, the decrease in protozoa population by adding fat to the feed can reduce methane production (Dohme et al., 1999). Moreover, the addition of lauric acid-containing oil in ruminant feeds is very effective in competing with methanogens (Dohme et al., 2001). According to Sondakh et al. (2015), adding 2-8 % virgin coconut oil in feed reduced gas production by about 18.39-29.7 % and does not interfere with rumen microbial activity.

## CONCLUSIONS AND RECOMMENDATIONS

Lauric acid or lauric acid-rich oil is an effective agent for decreasing *in vitro* methanogenesis in the rumen. The addition of lauric acid also reduces bacteria, methanogens, ammonia concentration and dry matter digestibility. In practice, both lauric acid or lauric acid-rich oil (such as coconut oil) may be supplemented into ruminants' diets at large scale animal operations or small scale farms, respectively, and at appropriate levels. Such supplementation, apart from its beneficial effect to mitigate enteric methane emission, would also enhance energy density of the diets for further supporting the production performance of the animals. However, the addition at high or excessive level is not recommended due to its potential adverse effects on rumen fermentation and nutrient digestibility.

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## NOVELTY STATEMENT

Previously published data regarding the influence of lauric acid on methanogenesis and rumen fermentation require integration and analysis across various studies in order to generate a more robust conclusion.

## AUTHOR'S CONTRIBUTION

Study design and supervision: KGW, DE, AJ; Data collection and tabulation: AC, RKN; Data analysis: AC, RPH, AJ; Writing manuscript draft: AC, RKN, RPH; Revising manuscript draft: KGW, DE, AJ.

### CONFLICT OF INTEREST

The authors have declared no conflict of interest.

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