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Research article

Optimum level of *Lactobacillus plantarum* supplementation as probiotic on *in vitro* degradability and rumen fermentation products of total mixed ration

Nursaadah Syahro Fitriyah[1,2], Lukman Abiola Oluodo[1,2,4], Patipan Hnokaew[2], Siriporn Umsook[2], Prayad Thirawong[2,3], Trisadee Khamlor[2] and Saowaluck Yammuen-Art[2,*]

[1]Graduate Program in Faculty of Agriculture, Chiang Mai University Under the CMU Presidential Scholarship, Chiang Mai 50200, Thailand
[2]Department of Animal and Aquatic Science, Faculty of Agriculture, Chiang Mai University, Chiang Mai 50200, Thailand
[3]Department of Animal Science, Faculty of Agriculture at Kamphaeng Saen, Kasetsart University, Nakhon Pathom 73140, Thailand
[4]Outreach Department, Rubber Research Institute of Nigeria, Benin City 1069, Nigeria

Abstract

The objective of this study was to determine *in vitro* gas production kinetics, and rumen fermentation products of total mixed ration (TMR) supplemented with different levels of *Lactobacillus plantarum* supplementation using rumen liquor from Thai native cattle. The experimental design was Completely Randomized Design (CRD). The *in vitro* procedure was completed using 200 mg DM of TMR from four different concentrations of *L. plantarum*; CON (without supplementation of *L. plantarum*), T1 (10^7 CFU / mL of *L. plantarum* supplementation), T2 (10^8 CFU / mL of *L. plantarum* supplementation), and T3 (10^9 CFU / mL of *L. plantarum* supplementation). The samples were prepared in three replications and incubated for 96 hours for gas production. *In vitro* rumen degradation experiment was done in four replications and incubated for 24 hours. *In vitro* gas production for 96 hours and gas production from insoluble fraction (b) were improved in the T1 and T2 groups ($P < 0.05$). The *in vitro* dry matter and organic matter degradability, and true degradability were particularly enhanced in the T1 group compared to other treatments ($P < 0.05$). The pH and NH_3 in this study were significantly affected ($P<0.05$). The significant improvements in the *in vitro* rumen fermentation products and degradability endorse *L. plantarum* supplementation at a concentration of 107 CFU/mL as a probiotic candidate.

Keywords: Rabies-free community, Multisectoral collaboration, Local government, Public communication, Community engagement

***Corresponding author:** Saowaluck Yammuen-Art, Department of Animal and Aquatic Science, Faculty of Agriculture, Chiang Mai University, Chiang Mai 50200, Thailand. E-mail: saowaluck.y@cmu.ac.th.

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INTRODUCTION

Ruminants play an essential role in the human food supply. Among the frequently used breeds in Thailand's livestock industry, Thai native cattle were formerly used as labor animals in rice paddies due to their comparatively low production efficiency. However, their primary role in consumption has now evolved. There is potential for improvement in the efficiency and effectiveness of Thailand's livestock industry, particularly within the beef cattle industry. Antibiotic is renowned to boost the livestock efficiency as it battles the unfavourable microorganism at the same time help growing and generating high yield of the products. Nevertheless, the concerns are raised as antibiotic has residual effect which could induce resistance (Asredie and Engdaw, 2015). The utilization of live microorganisms, termed "probiotics," is an ideal option to improve the efficiency of ruminants. Probiotics are mostly viable, safe, and maintain the host well-being by antagonizing other harmful microbes (Uyeno et al., 2015). By modulating rumen microflora, probiotics enhance the fermentation process and nutrient absorption, increase microbial protein synthesis, cellulolytic activity, and lessen acidosis (Wahrmund et al., 2012).

To adapt and multiply, probiotics should ideally be a natural inhabitant of the host organism. It is necessary for it to be able to occupy and attach to the epithelial cells of the gastrointestinal tract (Adjei-Fremah et al., 2018). The most widely used probiotic strain is lactic acid bacteria (LAB), such as, *Bifidobacterium, Streptococcus, Lactobacillus*, and *Propionibacterium* (Seo et al., 2010). The *Lactobacillus* is a prospective probiotic strain because it is a resident in the rumen, can be found as predominantly LAB in ruminants gut system. *Lactobacillus* is designated as a non-pathogenic and genetically stable bacterium.

Lactobacillus plantarum is a LAB species suitable as a probiotic. This bacterium is native to rumen ecology and binds to the rumen epithelium as well (Stewart, 1992). The research conducted by Astuti et al. (2018b) using *in vitro* gas production technique and real-time PCR concluded that *L. plantarum* can survive in the rumen. Using 1.5×10^{8} CFU/mL commercial strain of *L. plantarum* in the rumen for *in vitro* fermentation enhances OMD and total VFA (Direkvandi et al., 2021). The utilization of 0.5×10^{7} CFU/mL *L. plantarum* isolated from ensiled *Elymus nutans* improves dry matter degradation (Zhang et al., 2023). Supplementation of 10^{9} CFU/mL *L. plantarum* isolated from rumen of Ongole Crossbred cattle produces higher gas (Ridwan et al., 2018). Incorporating *L. plantarum* on *in vitro* rumen fermentation efficiently increases individual VFA production, reduces acetate: propionate ratio, supplying higher energy for the host and reduce methane emission (O'Brien et al., 2013; Astuti et al., 2018a; Izuddin et al., 2018).

The determination of the optimal dosage of LAB for rumen fermentation is a critical factor influencing its effectiveness (Ellis et al., 2016). While the positive impact of *L. plantarum* supplementation on rumen fermentation is undeniable, there remains a limited understanding of the ideal dosage of *L. plantarum* for *in vitro* rumen fermentation. The lack of clarity regarding the dosage of *L. plantarum* required to enhance ruminant performance necessitates further investigation. Our hypothesis posits that optimum concentration of *L. plantarum* will result in enhancement of rumen fermentation products and *in*

vitro degradability. Therefore, this study aims to determine the *in vitro* gas production kinetics and fermentation parameters of a total mixed ration (TMR) supplemented with different levels of *L. plantarum* using rumen liquor from Thai native cattle.

MATERIALS AND METHODS

Production of *L. plantarum*

The *L. plantarum* was isolated from the ruminal fluid of a goat (Oluodo et al., 2023). This experiment has been permitted by the Chiang Mai University Institutional Biosafety Committee (CMUIBC0666001, Approval No. A666001). A single colony of *L. plantarum* was picked from a 15-hour 10^9 CFU / mL MRS agar plate then put into the tube containing 10 mL of MRS B. This tube was incubated for 15 hours then picked in the amount of 1 mL into 100 mL Erlenmeyer flask containing MRS B. The suspension was centrifuged at 10000 rpm for 5 minutes after 15 hours incubation with Allegra X-22R Benchtop Centrifuge, Beckman Coulter, USA. To produce 10^8 CFU / mL of *L. plantarum*, 20 mL of 10^9 CFU/mL bacteria suspension was mixed with NaCl then centrifuged. The same procedure was done to prepare 10^7 CFU / mL bacteria using dilution of 10^8 CFU / mL. All the pellet containing bacteria was kept in -4^{0}C for preservation. *L. plantarum* was added to the syringes by vortex mixing it with buffered mineral solution.

In vitro degradation of TMR supplemented with *L. plantarum*

In vitro gas production method

The degradation of TMR was performed using the gas production technique developed by Menke et al. (1979). Rumen fluid was compiled prior to morning feeding, from 4 fistulated Thai native cattle (BW 250 ± 15 kg) from the Chiang Mai University Laboratory Animal Centre under the ethics license no. 2565/AG-0001. *Ad libitum* corn silage and 2 kg of concentrate per day were fed to the cattle. The TMR was formulated including the ingredient as Table 1. The treatments were:

CON : TMR without *L. plantarum* supplementation
T1 : TMR + 10^7 CFU / mL of *L. plantarum* supplementation
T2 : TMR + 10^8 CFU / mL of *L. plantarum* supplementation
T3 : TMR + 10^9 CFU / mL of *L. plantarum* supplementation

The TMR was weighed 230 ± 5 mg in three replications. The samples were placed in plunger-equipped 100 mL syringes. Blanks were empty syringes then filled with rumen-medium mixture. The rumen fluid and buffered mineral solution were mixed in a ratio 1:2, then put into each prewarmed syringe (39^{0}C). The syringes were put in the rotor, speed rate 30 rpm, and incubated in water bath. Gas production measurements were recorded at 2, 4, 6, 8, 10, 12, 24, 48, 72, and 96 hours during the incubation period using the scale on the glass syringe plunger.

Table 1 Component and proximate analysis of total mixed ration (TMR)

Items	Proportion (%)
Component of TMR	
Napier Pak Chong 1	60.00
Maize cob and husk	10.80
Corn meal	15.00
Rice bran	10.00
Urea	1.20
Oil palm meal	1.00
Dicalcium phosphate	0.80
Soybean meal	0.50
Sesame meal	0.50
Premix[1)]	0.10
Chemical composition, DM (%)	
Dry matter	41.77
Organic matter	91.80
Crude protein	15.89
Ether extract	5.66
NDF	57.53
ADF	20.84
ADL	2.97
Hemicellulose	36.69
Cellulose	17.87

[1)]Premix per 1 kg = 5.4 g manganese, 14.2 g iron, 1.0 g copper, 2.9 g zinc, 3.9 g sodium, 19.0 mg iodine, 0.9 mg potassium, 1.1 mg cobalt; ADF = Acid detergent fiber; ADL= Acid detergent lignin; DM = Dry matter; NDF = Neutral detergent fiber.

The syringes were categorized into separate sets with four replications to determine *in vitro* substrate degradation. The mixture of rumen-buffered mineral solution and 500 mg of feed were moved to Buchner funnel lined with Whatmann filtrate paper no. 41 for filtering following 24 hours incubation. The filtrate paper covered by the residue were put in the 100^{0}C oven overnight. The *in vitro* substrate degradability was calculated with following formula:

$$\text{IVDMD (\%)} = \frac{(\text{dry matter initial weigh} - \text{dry matter final weigh})}{\text{dry matter initial weigh}} \times 100$$

$$\text{IVOMD (\%)} = \frac{(\text{organic matter initial weigh} - \text{organic matter final weigh})}{\text{organic matter initial weigh}} \times 100$$

The microbial biomass yield (MBY) was determined according to Blümmel et al. (1997). The samples were weighed 500 mg then combined with rumen-medium mixture and incubated for 24 hours as described on the *in vitro* degradability methodology. Upon the 24 hours incubation period, the samples were filtered then put in the 100^{0}C oven overnight. The difference between weight obtained after the filtration-incubation process and the initial weight is considered as the apparently degraded substrate. Following the overnight oven, the filtrate papers were rinsed in the Beaker glass with neutral detergent solution (NDS). This method was done following the NDF by Van Soest et al. (1991). The difference between the weight of sample after washing with NDS and the initial weight was regarded as the truly degraded substrate. The MBY was determined with the formula by Blümmel et al. (1997) :

$$\text{MBY (mg/500mgDM)} = \frac{\text{truly degraded substrate} - \text{apparently degraded substrate}}{\text{truly degraded substrate}}$$

The exponential model was used to calculate the gas kinetic parameters based on accumulated gas production data (Orskov and Mcdonald, 1979) : $Y = a + b\ (1 - e^{-ct})$, where Y = volume of gas produced at time "t" (mL), a = production of gas from the soluble fraction (mL), b = production of from the insoluble fraction (mL / 200 mgDM), c = rate of gas production from insoluble fraction (%/h), |a| + b = potential extent of gas production, and t = incubation time.

The CP and EE (%DM) contents of the feed, and the data of gas production at 24 hours were applied to find out the values of metabolizable energy (ME, MJ / kg DM) and net energy lactation (NEL, MJ / kg DM) reported by Menke and Steingass (1988): ME_{TMR} (MJ / kg DM) = 0.72 + 0.1559GP + 0.0068CP + 0.0249EE (R = 0.95) and NEL_{TMR} (MJ / kg DM) = -0.61 + 0.1138GP + 0.0046CP + 0.0150EE (R = 0.95), where GP = 24 hours net gas production (mL / 200 mg DM), CP = crude protein (% DM), and EE = ether extract (% DM).

***In vitro* ruminal pH, ammonia (NH_3), and volatile fatty acids (VFA)**

The rumen fluid pH was metered after 24 hours of incubation with a portable pH meter. The phenol-hypochlorite method was used for ammonia (NH_3) analysis (Weatherburn, 1967). For the VFA analysis, the 1.5 mL rumen fluid was centrifuged using the Eppendorf tubes at 10000 rpm for 5 minutes. The supernatant was carefully filtered through a non-pyrogenic filter sized 0.45 µm and placed toward the 1 mL tapered vial. The 20 µL of samples were inserted into an HPLC system (E2695 Aminex®, Alliance Waters, USA) and eluted through the HPX-87H Ion Exclusion Column (Bio-Rad, USA, 9 µm, 7.8 × 300 mm). The mobile phase was 0.02 N H_2SO_4 at UV-Vis was recorded at 210 nm, and a flow rate of 1 mL / min.

Statistical analysis

The *in vitro* gas production was carried out in three replicates, whereas *in vitro* substrate degradability was performed in four replicates. The experiment data was analyzed with IBM Statistic SPSS version 26.0. The ANOVA was employed to evaluate the data based on the completely randomized design (CRD). Shapiro-Wilk and Levene's tests were carried out to assess the homogeneity and normality on the data. The mathematics model used is linear model for CRD: $Y_{ij}=\mu+\alpha_i+\varepsilon_{ij,}$ where $Y_{ij\,=}$ observation value, µ = general mean, α_i = treatments effect, ε_{ij} = experimental error. To assess the mean differences between treatments, the Duncan Multiple Range Test (DMRT) was performed (Steel and Torrie., 1980).

RESULTS

In vitro gas production at different time points was significantly affected by the addition of *L. plantarum.* At 2 and 6 hours of *in vitro* gas production, the treatment groups of T1 and T2 substantially produced the highest gas ($P < 0.01$) compared to CON and T3 groups. The addition of *L. plantarum* did not significantly affect the gas production at 4 hours. The gas production of T2 at the 8-hour incubation period was remarkably the highest ($P < 0.01$), while there was no significant difference between the CON and T1 groups, whereas T3 generated the lowest gas production. At 10-hour mark of the incubation, T1 and T2 groups significantly produced the highest gas ($P < 0.01$), while CON and T3 were categorized in the same group of lowest gas production. The gas production at 12 hours in the T1 group was remarkably highest ($P < 0.01$), but there was no significant difference to the CON group, followed by the T2, then T3 group. The gas production of T1 group at 24 hours was significantly highest, followed by T2, T3, and CON groups subsequently. The T1 and T2 groups significantly produced higher gas at 48, 72, and 96 hours ($P < 0.01$) than T3 and CON groups. The treatment groups, specifically T1 and T2, exhibited the highest gas production at different time periods during incubation compared to the group without supplementation. However, the T3 group that had the highest level of *L. plantarum* inclusion was associated with CON at several time points or even demonstrated the lowest gas production (Table 2).

Furthermore, the kinetics of gas production was significantly affected by the incorporation of *L. plantarum* in the *in vitro* rumen fermentation. The T1 and T2 groups had substantially greater ($P < 0.01$) insoluble fraction gas production (b) than T3 and CON groups. The T1 group gas production rate (c) was substantially highest ($P < 0.01$) among other treatments. Similarly, in the T1 group, there was a significant rise in ME and NEL ($P < 0.01$), followed by T2, then T3 and CON groups (Table 2).

Table 2 *In vitro* gas production of TMR with different concentration level of *L. plantarum* supplementation.

Variables	Treatments[1]				SEM	*P-value*
	CON	T1	T2	T3		
***In vitro* gas production (mL / 200 mg DM)**						
2 hours	1.85^{b}	4.15^{a}	4.15^{a}	1.85^{b}	0.66	<0.01
4 hours	7.38	6.46	6.46	6.46	0.23	0.438
6 hours	8.77^{b}	9.69^{a}	8.76^{a}	6.46^{c}	0.69	<0.05
8 hours	11.53^{ab}	12.46^{ab}	13.83^{a}	10.61^{b}	0.69	<0.05
10 hours	14.31^{b}	16.61^{a}	17.08^{a}	13.38^{b}	0.89	<0.01
12 hours	20.77^{ab}	23.07^{a}	19.85^{b}	16.61^{c}	1.34	<0.01
24 hours	34.16^{c}	51.96^{a}	46.15^{b}	36.00^{c}	4.17	<0.01
48 hours	78.47^{b}	99.22^{a}	96.92^{a}	81.22^{b}	5.31	<0.01
72 hours	85.85^{c}	106.14^{a}	104.31^{a}	92.30^{b}	4.86	<0.01
96 hours	90.00^{c}	111.68^{a}	110.77^{a}	95.99^{b}	5.41	<0.01
Kinetics of gas production						
b (mL / 200 mg DM)	112.03^{c}	136.78^{a}	138.12^{a}	124.52^{b}	6.10	<0.01
c (/hr)	0.02^{b}	0.03^{a}	0.02^{b}	0.02^{b}	0.00	<0.01
Estimated parameters						
ME (MJ/kg DM)	6.94^{c}	9.32^{a}	8.57^{b}	7.19^{c}	0.57	<0.01
NEL (MJ/kg DM)	3.86^{c}	5.54^{a}	5.01^{b}	4.03^{c}	0.40	<0.01

[1]Treatments were (1) TMR + rumen fluid + no *L. plantarum* suspension (CON), (2) TMR + rumen fluid + 10^{7} CFU / mL of *L. plantarum* (T1), (3) TMR + rumen fluid + 10^{8} CFU / mL of *L. plantarum* (T2), and (4) TMR + rumen fluid + 10^{9} CFU / mL of *L. plantarum* (T3). SEM = standard error mean; superscript a-d connote values among treatment groups are significantly different at $P < 0.05$; b = production of from the insoluble fraction; c = rate of gas production from insoluble fraction; ME = metabolizable energy; NEL = net energy lactation.

Inoculation of *L. plantarum* in the *in vitro* degradation substantially affects pH and NH_3 (mM). The pH of the CON, T1, and T2 groups was considerably lower ($P < 0.01$) compared to the T3 group. The T3 group disclosed a significantly ($P < 0.01$) high NH_3 concentration (mM), followed by the T2, T1, and CON groups. Furthermore, total VFA concentration in the rumen is significantly affected by the supplementation of *L. plantarum* with T3 generates highest total VFA concentration, followed by CON and T1, and T2. Specifically, the individual VFA concentrations, such as acetate ($P = 0.957$), propionate ($P = 0.218$), and butyrate ($P = 0.941$), among CON, T1, T2, and T3 groups were not substantially different. Subsequently, the addition of *L. plantarum* had a significant impact ($P < 0.01$) on lowering A:P ratios in the T3 group compared to the CON group (Table 3).

The addition of *L. plantarum* have significant effects in substrate degradability. The T1 groups showed significantly highest ($P < 0.01$) IVDMD (%), IVOMD (%), and true degradability, while the CON and T2 groups were not significantly different, then T3 group is the lowest. Following that, the T1 group had a notably lowest ($P < 0.01$) MBY (mg/500 mg DM) than that in the T3 group, CON and T2 groups subsequently (Table 3).

Table 3 *In vitro* ruminal fermentation of TMR with different concentration level of *L. plantarum* supplementation.

Variables	Treatments[1]				SEM	*P-value*
	CON	T1	T2	T3		
pH	7.76[b]	7.73[b]	7.73[b]	7.82[a]	0.02	<0.01
NH_3 (mM)	4.53[c]	5.35[b]	5.61[b]	6.50[a]	0.41	<0.01
Ruminal VFA (mmol)						
Total VFA	65.25[b]	65.47[b]	61.04[c]	68.82[a]	1.59	<0.01
Acetate	54.16	55.39	52.15	55.08	0.73	0.957
Propionate	5.83	6.00	5.88	8.34	0.61	0.218
Butyrate	5.38	4.29	4.19	5.31	0.32	0.941
A:P	9.71[a]	9.20[ab]	8.87[b]	6.01[c]	0.54	<0.01
Substrate degraded (%)						
IVDMD	50.86[b]	56.39[a]	49.79[b]	47.10[c]	1.95	<0.01
IVOMD	55.14[b]	61.34[a]	54.17[b]	51.24[c]	2.12	<0.01
True degradability	62.57[b]	66.36[a]	61.69[b]	57.42[c]	1.83	<0.01
MBY						
(mg/500 mg DM)	59.41[a]	49.87[c]	59.52[a]	51.58[b]	2.55	<0.01
(% of true degradable substrate)	22.75[b]	18.10[d]	23.11[a]	21.55[c]	1.14	<0.01

[1]Treatments were (1) TMR + rumen fluid + no *L. plantarum* suspension (CON), (2) TMR + rumen fluid + 10^7 CFU / mL of *L. plantarum* (T1), (3) TMR + rumen fluid + 10^8 CFU / mL of *L. plantarum* (T2), and (4) TMR + rumen fluid + 10^9 CFU / mL of *L. plantarum* (T3). SEM = standard error mean; superscript a-d connote values among treatment groups are significantly different at $P < 0.05$; IVDMD = *in vitro* dry matter degradability; IVOMD = *in vitro* organic matter degradability; MBY = Microbial Mass Yield.

DISCUSSION

The *in vitro* total gas production correlates positively with *in vitro* substrate degradability (Blummel and Becker, 1997; Muck et al., 2007). The supplementation of 10^7 CFU/mL *L. plantarum* (T1) improves the fermentation rate by generating more total gas production at 24-hours incubation as well as IVDMD (%), IVOMD (%), and true degradability (%) compared to control group. This optimal outcome observed at a concentration of T1 (10^7 CFU/mL *L. plantarum*) corresponds to the findings reported by Direkvandi et al. (2021). The high *in vitro* gas production at 24 hours and substrate degraded value in the T1 (10^7 CFU / mL) group compared to the control group could be corresponding with cellulolytic bacterial activity. Incorporating prebiotics such as *L. plantarum* prevents the buildup of lactic acid by degrading it into acetic acid (Nocek et al., 2002). This condition is favorable for the fiber-degrading bacteria, substantiated by increased activity of CMCase and β-glycosidase in the rumen fluid inoculated with *L. plantarum* (Guo et al., 2020). Additionally, these enzymes that break down complex carbohydrate into its simple form, consequently it becomes more susceptible to degradation (Lee et al., 2019). Hence, this scheme could boost rumen fermentation to generate higher gas production and substrate degradability compared to the control group. Similarly, the rise in net gas production correlates with higher values of estimated parameters in T1 group, such as ME (Mj / kg DM) and NEL (Mj / kg DM). Whilst net gas production has considerably positive correlation with degradability, it has inverse relationship with MBY as both related to substrate truly degradable (Blummel and Becker, 1997).

Furthermore, the T1 (10^7 CFU / mL) group had the lowest MBY and proportion of true degradability among other treatments. The capability of *L. plantarum* to create a favorable environment for cellulolytic bacteria is associated with the MBY. Cellulolytic bacteria in the rumen produce enzymes, such as cellulases and hemicellulases, which break down polysaccharides into simpler carbohydrates, making them more accessible to other rumen microorganisms. The increased concentration of fermentable substrates supports microbial growth, leading to an enhancement in MBY.

The NH_3 concentration and ruminal pH are closely related. In this experiment, *L. plantarum* supplementation modulates the rumen pH and NH_3 concentration. The T3 (10^9 CFU / mL) group produces the highest ammonia concentration among other treatments, which correlates with the high pH level on this treatment compared to the group without supplementation. The pH level generated from this experiment is considered as protein engorgement. A pH level lower than 5.2 is marked as an acidosis symptom (Owens et al., 1998) whilst alkaline pH level of 8.0-9.2 is appraised as a sign of ammonia toxicity (Grünberg et al., 2009). Aside from the high NH_3 concentration, the increase in ruminal pH of this experiment induced by *L. plantarum* supplementation correlates with its ability to maintain rumen pH by converting lactic acid into other chemical compounds such as acetic acid, propionic acid, butyric acid, and formic acid (Punia Bangar et al., 2022). These metabolites have a less acidic, detrimental effect on rumen microbes and could increase the pH of this experiment. Additionally, *L. plantarum* contributes in the deamination process where it converts excess ammonia into microbial protein. This mechanism assists in maintaining a proper balance of nitrogen compounds in the rumen and indirectly contributes to pH adjustment. Salivary contamination or rapid putrefaction of digested protein coupled to protracted rumen atony could also lead to an increase in the ruminal pH level (Grünberg et al., 2009).

The ruminal ammonia concentration generated from T1, T2, and T3 groups is within the range of 5.00-17.65 mM (McDonald et al., 2010). *L. plantarum* as a lactic acid bacteria utilize NH_3 to generate microbial protein. Aside from rumen undegradable protein, microbial protein is one of the most predominant protein sources for ruminants. The deamination process of amino acids from the degradable protein results in ammonia and ketoacids; afterwards, the bacteria synthesize both products into microbial protein. Simple diffusion of NH_3 into the ruminal epithelium could also be an additional source of NH_3 (Wang et al., 2017). This microbial protein synthesis is affected by the availability of energy sources from the diet.

The VFA is vital for ruminants as it is the main source of energy for growth and reproduction. Previous studies have confirmed that supplementation of *L. plantarum* could increase production of total VFA, acetic acid, butyric acid (Izuddin et al., 2018; Direkvandi et al., 2021), and propionic acid (Astuti et al., 2018a). In this *in vitro* experiment, individual VFAs, such as acetate, butyrate, and propionate in all treatments were not affected by the supplementation of *L. plantarum*, while total VFA concentration is positively affected by *L. plantarum*. The increase of VFA was caused by the ability of *L. plantarum* to build suitable conditions for fiber-degrading bacteria degrading complex polysaccharide (Guo et al., 2020). Additionally, the supplementation of *L. plantarum* lowered the A:P ratio on the higher level of supplementation group

than group without supplementation. This decrease on A:P ratio could lead to more efficient fermentation due to reduced methane gas. Furthermore, a study conducted by O'Brien et al. (2013) revealed that the use of *L. plantarum* decreased A:P ratio by inhibiting methane producing bacterium through the production of hydrogen peroxide.

CONCLUSIONS

The *L. plantarum* supplementation at concentration of 10^7 CFU/mL enhances gas production *in vitro* at 12 and 24 hours, increases rumen fermentation products and substrate degradability using rumen liquor compiled from Thai native cattle. These results are demonstrating its potential as a probiotic candidate. However, further examination by supplementing *L. plantarum* in the diet of Thai native cattle is required to be done as in determining the effect of this microorganism.

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AUTHOR CONTRIBUTIONS

Nursaadah Syahro Fitriyah: contributing to the commencing of the experiment, collecting and analyzing the data, and writing the manuscript.
Lukman Abiola Oloudo: contributing to the commencing the experiment and critical reviews of the manuscript.
Patipan Hnokaew: contributing to the commencing of the experiment and critical reviews of the manuscript.
Siriporn Umsook: contributing to the commencing the experiment and critical reviews of the manuscript.
Prayad Thirawong : contributing to the commencing of the experiment and critical reviews of the manuscript.
Trisadee Khamlor: contributing to the critical reviews of the manuscript.
Saowaluck Yammuen-Art: contributing to the designing, commencing the experiment, collecting and analyzing the data, and writing the manuscript.

CONFLICT OF INTEREST

We certify that there is no conflict of interest with any financial organization regarding the material discussed in the manuscript.

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