



Research article

Effect of *Lactiplantibacillus plantarum* with vegetable oil supplementation on rumen fermentation and lactation performance in dairy goats

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Abstract

This study was conducted to determine the effect of different concentrations of *Lactiplantibacillus plantarum* with vegetable oils on *in vitro* rumen fermentation and lactation performance in dairy goats. The *in vitro* rumen fermentation was divided into seven groups: CON (TMR without supplementation), LP6SB (TMR + 10⁶ CFU/mL of *L. plantarum* + 2% soybean oil), LP7SB (TMR + 10⁷ CFU/mL of *L. plantarum* + 2% soybean oil), LP8SB (TMR + 10⁸ CFU/mL of *L. plantarum* + 2% soybean oil), LP6SF (TMR + 10⁶ CFU/mL of *L. plantarum* + 2% sunflower oil), LP7SF (TMR + 10⁷ CFU/mL of *L. plantarum* + 2% sunflower oil), and LP8SF (TMR + 10⁸ CFU/mL of *L. plantarum* + 2% sunflower oil). The rumen fermentation parameters measured included ruminal pH, fatty acid profiles, and ammonia (NH₃) concentrations. The lactation performance experiment was divided into three groups: CON (without supplementation), LP (*L. plantarum* 70 mL of 10⁹ CFU/mL/head), and LPSF (*L. plantarum* 70 mL of 10⁹ CFU/mL/head + 2% sunflower oil). The performance parameters were feed intake, milk yield, and milk composition. *In vitro* gas production at 24 hours showed that LP8SB and LP8SF were the highest among other groups (58.87 mL and 59.30 mL) (P<0.001). However, ruminal pH at 24 hours for LP8SB and LP8SF was the lowest compared with other groups (6.74) (P<0.001). The LP8SB and LP8SF exhibited the highest acetic acid, propionic acid, butyric acid, and total VFA production (87.60 mmol/L and 79.51 mmol/L, respectively). The NH₃ levels at 24 hours revealed that LP8SB and LP8SF were the highest concentrations compared with other groups (24.13 and 24.08 mM, respectively) (P < 0.001). Roughage intake on days 60 and 84 for LPSF was higher than for LP (950 and 1000 vs. 820 and 890 g/d, respectively) but not different with control group. Milk fat content, milk protein content, lactose, and solid not fat content did not show significant differences among treatments (P > 0.05). It may be concluded that *L. plantarum* 10⁸ CFU/mL with both oil supplementations increased rumen fermentation concentrations while *L. plantarum* 10⁷ CFU/mL with sunflower oil supplementation increased roughage intake but milk composition were not affected by *L. plantarum* 10⁷ CFU/mL with sunflower oil supplementation.

Keywords: Dairy goat, Goat milk, *Lactiplantibacillus plantarum*, Probiotic, Vegetable oil.

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INTRODUCTION

Probiotic supplementation in ruminants increases milk production and milk composition. Probiotics can improve fiber degradation and fermentation in the rumen (Arawolo and He, 2018). Many species of lactic acid bacteria have been reported to possess probiotic properties. *Lactobacillus plantarum* (*L. plantarum*) is a species of lactic acid bacteria that has been recognized for its ability to increase efficiency in ruminants (Monteiro et al., 2021). *L. plantarum* supplementation increased volatile fatty acids (VFA) and major cellulolytic bacteria such as *Ruminococcus albus* and *Ruminococcus flavefaciens* (Oskoueian et al., 2021). A study conducted by Asturi et al. (2022) in fistulated Ongole breed cattle concluded that *L. plantarum* TSD10 increased total VFA, propionic acid, and NH_3 . However, acetic acid, isobutyric acid, total protozoa, and pH decreased after supplementation with *L. plantarum* TSD10. Additionally, the supplementation of *L. plantarum* has been shown to reduce methane production in the rumen, thereby minimizing the loss of gross energy intake due to methane emissions (Alazzeh et al., 2013). The different results when *L. plantarum* or lactic acid bacteria (LAB) were used as probiotics show that their effect depends on the type of strains, dose, and substrate (Jiao et al., 2017).

In addition to bacterial supplementation, fat supplements are incorporated into animal diets as a source of essential fatty acids and fat-soluble vitamins, while also serving as an additional energy source. Energy is important for milk production (Morand-Fehr and Sauvant, 2013). The addition of oil as the source of fat to ruminant feed can increase the energy in the diet without adding extra grains (Silva et al., 2011). Furthermore, the addition of oil in the diet can prevent ruminal acidosis, improve low-fat yield and provide essential fatty acids (Groehn et al., 1992). Vegetable oils, such as palm oil, canola oil, soybean oil, and sunflower oil, are the most common sources of lipids used in animal feeding due to their abilities to provide polyunsaturated fatty acids (Karami et al., 2013). Supplementation of 6% soybean oil in Murciano-Granadina goat increased monounsaturated fatty acid in milk higher than without supplementation (29.3% and 21.8%, respectively) and polyunsaturated fatty acid in milk of supplemented with 6% soybean oil higher than without supplementation (4.15% and 3.73%, respectively) (Bouattour et al., 2008). Moreover, stearic acid (C18:0) in the milk of Nubian goats was the highest in the 2% sunflower oil supplemented group (26.00) compared to the group without supplementation (24.80) (Abo EL-Nor and Khattab, 2012).

Accordingly, this study was conducted to determine the effect of different concentrations of *L. plantarum* isolated from goat rumen fluid with vegetable oil supplementation on gas production kinetics, rumen fermentation, and rumen fatty acid (FA) profile *in vitro*. Additionally, its impact on goat milk yield, milk composition, and milk fatty acids (FA) profile in dairy goats was assessed.

MATERIALS AND METHODS

The rumen fermentation experiment was conducted to assess the effects of different levels of *L. plantarum* and 2 types of vegetable oil on rumen fermentation by the incubation of experimental diets over 24 h (Experiment 1). Based on the results obtained in Experiment 1, the lactation performance experiment (Experiment 2) was conducted on goats to further test the rumen fermentation results.

Rumen fermentation (Experiment 1)

Bacteria preparation

L. plantarum in the experiment was approved by the Chiang Mai University Institutional Biosafety Committee (CMUIBC0666001, Approval No. A666002). *L. plantarum* of 10^9 CFU/mL concentration was cultured in MRS broth (1 mL of *L. plantarum* /100 mL MRS broth) at 37 °C. Bacteria were harvested after 18 h of

incubation and centrifuged at 10000 rpm for 5 min with an Allegra X-22R Benchtop Centrifuge (Beckman Coulter, USA). *L. plantarum* was diluted by 0.85 NaCl to produce 10⁸, 10⁷, and 10⁶ CFU/mL of *L. plantarum*. All bacterial samples were stored at 4°C for preservation.

Chemical composition analysis

The chemical composition of TMR was analyzed by the proximate and detergent methods. Samples were dried at 60 °C for 48 hours for chemical composition analysis, including dry matter (DM), organic matter (OM), crude protein (CP) and ether extract (EE) using the proximate method (AOAC, 1990). Neutral detergent fiber (NDF), Acid detergent fiber (ADF), and acid detergent lignin (ADL) were determined using the Van Soest method (Goering and Van Soest, 1970). The composition and ingredients listed in the TMR are presented in Table 1.

Table 1 Chemical compositions of the feed used in the *in vitro* rumen fermentation experiment

Item (%DM basis)	Experimental diets	
	TMR	
Feed ingredients (%)		
Corn silage	18.84	
Pangola hay	12.81	
Water	30.14	
Sweet corn husks	18.84	
Rice bran	4.52	
Corn meal	6.03	
Soybean meal	8.67	
Premix	0.15	
Chemical composition (%DM basis)		
Dry matter (%)	41.78	
Organic matter	92.02	
Crude protein	16.85	
Ether extract	4.08	
Ash	7.98	
NDF	66.04	
ADF	37.80	
ADL	10.72	
Hemi-cellulose	28.24	
Cellulose	27.08	

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, Hemi-cellulose = NDF-ADF, Cellulose = ADF-ADL

Fatty acid profile of feed analysis

The amount of 1 g of dried feed was placed in a fat extraction tube, then 0.7 mL of 10 N KOH in water, and 5.3 mL of MeOH were added to the tube. The tube was then kept at controlled temperature in a water bath at 55°C for 1.5 hours, with vigorous manual agitation for 5 seconds every 20 minutes. After cooling the tube with cold tap water, 0.58 mL of H₂SO₄ in water at a concentration of 12 M was added. The tube was mixed, and then re-incubated in a 55°C water bath for 1.5 hours, with manual agitation. The tube was cooled to below room temperature following the synthesis of FAME (fatty acid methyl esters). Subsequently, 3 mL of hexane was added, and the tube was vortex-mixed for 5 minutes (O'Fallon et al., 2007). The tube was then centrifuged for 5 minutes at 380 × g, and the hexane layer containing the FAME was transferred to a gas chromatography (GC) vial. The fatty acids (FA) were quantified by injecting 1 µL of the samples into a GC detector; FID @ 250 °C (GC-7820A, Agilent Technologies Inc.), according to the parameters described by Anzhany et al. (2023), using a CP-Sil 88 fused-silica capillary column (100 m length, 0.25 mm inner diameter, and 0.20 µm film thickness; Agilent Technologies). The quantification of FA was determined by comparing the retention

time of samples to the standard. The retention time of samples to the retention time of the food industry FAME mix standard (37 components; RESTEK Corporation, Bellefonte, PA) was compared to generate the FA concentration shown in [Table 2](#).

Table 2 Fatty acid composition of feed used in the *in vitro* rumen fermentation experiment

Item (%DM basis)	Experimental diets		
	TMR	Sunflower oil	Soybean oil
Fatty acid composition (% of total fat)			
C12:0	0.10	ND	0.01
C14:0	0.59	0.07	0.09
C15:0	0.09	0.01	0.01
C16:0	21.61	5.71	10.76
C16:1, cis-9	0.20	0.08	0.03
C17:0	0.17	0.01	ND
C18:0	3.09	ND	ND
C18:1, trans-9	0.06	2.40	2.17
C18:1, cis-9	33.22	34.34	25.33
C18:2, cis-9,12	36.71	56.75	54.62
C18:3, cis-9,12,15	3.62	ND	ND
C21:0	ND	0.62	7.00
C22:0 FAME	0.53	ND	ND

***In vitro* gas production method and experimental design**

In vitro gas production was performed using the gas production technique designed by [Menke et al. \(1979\)](#). The rumen fluid of two dairy goats was collected from a slaughterhouse (Hamza farm). Commercial vegetable oils (soybean oil and sunflower oil) manufactured by Thanakorn Vegetable Oil Products Co., Ltd. were used in this study. This trial was approved by the Animal Care and Use Committee, Faculty of Agriculture, Chiang Mai University, under ethics license no. AG01005/2566.

The experimental design used a complete randomized design. The treatments were divided into seven groups.

CON: TMR without supplementation;

LP6SB: TMR + 10⁶ CFU / mL of *L. plantarum* + 2 % soybean oil supplementation;

LP7SB: TMR + 10⁷ CFU / mL of *L. plantarum* + 2 % soybean oil supplementation;

LP8SB: TMR + 10⁸ CFU / mL of *L. plantarum* + 2 % soybean oil supplementation;

LP6SF: TMR + 10⁶ CFU / mL of *L. plantarum* + 2 % sunflower oil supplementation;

LP7SF: TMR + 10⁷ CFU / mL of *L. plantarum* + 2 % sunflower oil supplementation;

LP8SF: TMR + 10⁸ CFU / mL of *L. plantarum* + 2 % sunflower oil supplementation.

The chemical composition of TMR is listed in Table 1. The TMR was dried at 60°C and ground through a 0.1 mm sieve. A 0.23 g sample was then placed in 100 mL glass syringes in triplicate. The rumen fluid was mixed with a buffer solution in a 1:2 ratio ([Menke et al., 1979](#)). The buffer solution was prepared with water, macromineral solution, resazurine solution, and micromineral solution. The mixed rumen fluid was added to a 100 mL glass syringe containing TMR and incubated at 39°C in a thermostat bath.

Gas production and kinetics of gas production

The gas accumulation was collected at 2, 4, 8, 10, 12, 24, 48, 72 and 96 hours to calculate the decomposition values of the fermented material in the rumen.

The kinetics of gas production were determined according to the equation of Ørskov and McDonald (1979) using the following equation:

$$y = a+b(1-\exp^{-ct}),$$

where

y = gas production at time t ,

a = production of gas from the soluble fraction (mL),

b = production from the insoluble fraction (mL / 200 mgDM),

c = rate of gas production from the insoluble fraction (%/h),

$|a| + b$ = potential extent of gas production,

\exp = exponential,

t = time when data was recorded.

The gas production potential was calculated from the equation of Menke and Steingass (1988) using the following equation:

$$d = |a|+b,$$

where a = production of gas from the soluble fraction (mL),

b = production from the insoluble fraction (mL / 200 mgDM).

Estimated parameters calculation

Organic matter digestibility (OMD) was used to evaluate the energy from gas production at 24 hours, which included the metabolizable energy (ME), and the amount of short-chain fatty acids (SCFA) were calculated using the following equation by Ørskov and McDonald, (1979):

$$\text{OMD (\%)} = 15.38 + 0.8453Gv + 0.0595CP + 0.0675XA,$$

$$\text{ME (MJ/Kg DM)} = 2.20 + 0.136Gv + 0.0057CP,$$

$$\text{SCFA (mol)} = 0.0239Gv - 0.0601,$$

where CP = crude protein amount (g/kg DM)

CF = crude fiber amount (g/kg DM),

XA = ash amount (g/kg DM)

Gv = net gas volume produced in 24 hours, calculated from the following equation: $Gv (\text{ml}) = [(V24-V0-GPo) \times 200 \times [(Fh + Fc)/2]/W]$

Where V24 = volume of gas generated at 24 hours,

V_0 = volume of gas generated before incubation,

Gpo = mean value of gas generated in blank tube at 24 hours,

Fh = Roughage correction factor,

Fc = Concentrate correction factor,

W = sample weight (mg DM).

In vitro ruminal pH and ammonia-N (NH₃-N)

The pH of rumen fluid was measured with a portable pH meter model S-610L (Peak Instruments Inc.), after 24 hours of incubation. Furthermore, 1.5 mL of rumen fluid was collected, and then the filtered sample was centrifuged using an Allegra X-22R Benchtop Centrifuge (Beckman Coulter, USA) at 10,000 $\times g$ for 5 min. The amount of 50 μL supernatant was collected for ammonia analysis. A standard solution was prepared using 50 μL ammonium chloride and 50 μL distilled water in a 20 mL test tube, 1 mL phenol, 1 mL hypochlorite, and 8 mL distilled water. The solution was mixed and incubated for 10–15 min to allow the color to develop from clear to blue to dark blue. Standard solution was dissolved in distilled water with concentrations of 500, 1000, 1500, and 2000 μL , respectively. Samples were prepared for analysis by adding 50 μL of the supernatant into a 20 mL test tube, adding 50 μL of distilled water, 1 mL of phenol, 1 mL of hypochlorite, and 8 mL of distilled water. Subsequently, samples were left for 10–15 minutes for color development. The samples were measured using a UV-Vis Spectrophotometer model C30M (PG Instruments Ltd) at a wavelength of 625 nm. Data were retrieved and used to construct a linear equation and estimate the ammonia-N in the sample (Chaney and Marbach, 1992).

Ruminal volatile fatty acid (VFA)

The rumen fluids were transferred into centrifuge tubes after incubation. The residue samples were then centrifuged in an ultracentrifuge at 10,000 g for 5 min at 4 °C. The supernatant fraction was carefully filtered through a 0.45 µm non-pyrogenic filter into a tapered vial before analysis. Then 1 µL of the samples was injected into a GC detector; FID @ 250 °C (GC-7820A, Agilent Technologies Inc.) and eluted through a Zebron ZB-FAME column (30-meter x 0.25 mm x 0.20 µm). The quantification of VFA was determined by comparing the retention time of samples to the standard. Sample retention times were compared with those of the external standard.

Ruminal fatty acid profile analysis

The amount of 1 mL of rumen fluid was placed in a fat extraction tube, then 0.7 mL of 10 N KOH in water, and 5.3 mL of MeOH were poured into the tube. The tube was then kept at controlled temperature in a water bath at 55°C for 1.5 hours, with vigorous manual agitation for 5 seconds every 20 minutes. After cooling the tube with cold tap water, 0.58 mL of H₂SO₄ in water at a concentration of 12 M was added. The tube was mixed, and then re-incubated in a 55°C water bath for 1.5 hours, with manual agitation. The tube was cooled to below room temperature following the synthesis of FAME (fatty acid methyl esters). Subsequently, 3 mL of hexane was added, and the tube was vortex-mixed for 5 minutes (O'Fallon et al., 2007). The tube was then centrifuged for 5 minutes at 380 x g, and the hexane layer containing the FAME was transferred to a gas chromatography (GC) vial. The fatty acids (FA) were quantified by injecting 1 µL of the samples into a GC detector; FID @ 250 °C (GC-7820A, Agilent Technologies Inc.), according to the parameters described by Anzhany et al. (2023), using a CP-Sil 88 fused-silica capillary column (100 m length, 0.25 mm inner diameter, and 0.20 µm film thickness; Agilent Technologies). The quantification of FA was determined by comparing the retention time of samples to the standard. The retention time of samples to the retention time of the food industry FAME mix standard (37 components; RESTEK Corporation, Bellefonte, PA) was compared to generate the FA concentration, as shown in Table 2.

Lactation performance (Experiment 2)

The lactation performance experiment (Experiment 2) was conducted on goats to validate the findings from the rumen fermentation study. Supplementation with *L. plantarum* and 2% sunflower oil decreased the acetate-to-propionate (A:P) ratio and improved C18:2 cis-9,12 concentrations. However the optimum level of *L. plantarum* supplementation as a probiotic was 10⁷ CFU/mL or 70 mL of 10⁹ CFU/mL/head.

L. plantarum and vegetable oil preparation

L. plantarum was cultured in MRS broth at 37 °C for a 10⁹ CFU/mL concentration. Bacteria were harvested after 18 h of incubation and centrifuged at 10000 rpm for 5 min with an Allegra X-22R Benchtop Centrifuge (Beckman Coulter, USA). To prevent irritation when administered to animals, all pellet-containing bacteria should be washed twice with aquadest after centrifugation. Samples of bacteria were kept at 4°C for preservation. The concentration of bacteria was checked for 14 days under the same conditions as the farm. The sunflower oil used in this experiment was commercial grade (Thanakorn Vegetable Oil Products Co., Ltd.).

Animals and experimental design

The study was conducted on Boonboon dairy goat farm (120/5 Moo 3, Tung Fai Subdistrict, Mueang District, Lampang Province 18.374118474472706, 99.54526572121796) by selecting 15 crossbred Saanen goats, aged 2 ±0.5 years old, weight of 42.5 ±10.09 kg, parity 1-3 times, and the number of days in milk 37.2 ±28.7 days. All goats were tested for Brucellosis, vaccinated against foot and mouth

disease, swollen neck disease, blackleg disease, and dewormed before the experiment. The health of all animals was monitored weekly during the study period.

The experiment was approved by the Animal Care and Use Committee, Faculty of Agriculture, Chiang Mai University, under ethics license no. AG01005/2566. The experimental design was a randomized complete block design (RCBD) using parity as the block.

The experimental animals were divided into three groups as follows:

CON: without supplementation,

LP: supplementation with *L. plantarum* 70 mL of 10⁹ CFU/mL/head

LPSF: supplementation with *L. plantarum* 70 mL of 10⁹ CFU/mL/head and 2% sunflower oil

All groups were fed two kg of Pangola grass hay and one kg of commercial concentrate. The composition of each feedstuff is presented in Table 3. Feeding was done twice a day, daily morning (06.00) and afternoon (15.00). Each goat was placed in an individual pen with *ad libitum* drinking water.

Table 3 Chemical compositions of feed used in the lactation performance experiment

Item	Experimental diets	
	Pangola hay	Concentrate
Chemical composition (%DM basis)		
Dry matter	93.91	85.47
Crude protein	6.23	20.09
Ether extract	1.18	1.95
Ash	8.98	11.45
NDF	72.31	60.31
ADF	48.47	36.56
ADL	12.41	10.41
Fatty acid composition (% of total fat)		
C12:0	1.25	3.72
C14:0	1.54	3.15
C15:0	0.90	0.10
C16:0	34.91	44.15
C16:1, cis-9	0.61	0.14
C17:0	1.40	0.14
C18:0	4.69	4.71
C18:1, trans-9	0.30	0.04
C18:1, cis-9	8.41	29.78
C18:2, cis-9,12	21.82	13.00
C18:3, cis-9,12,15	18.75	0.79
C21:0	0.68	0.02
C22:0 FAME	4.75	0.25

NDF = neutral detergent fiber; ADF = acid detergent fiber; ADL = acid detergent lignin

Feedstuffs collection and analysis

The experiment was carried out for a period of 84 days. Feed intake was recorded once per day. One kilogram each of Pangola grass hay and commercial concentrate was collected on days 0, 28, 56, and 84 and then pooled for the chemical composition analysis. The feedstuffs utilized in this study were analyzed by the proximate (AOAC, 1990) and Van Soest methods (Goering and Van Soest, 1970). The composition of feedstuffs is presented in Table 4.

Milk collection and composition analysis

Milk yield was recorded once per day. Milk samples were collected on days 0, 14, 28, 42, 56, 70, and 84, separated into 2 sets. The first set of sample was analyzed for milk composition by MilkoScan FT2; (FOSS, Hillerød, Denmark) to determine the milk composition (fat, protein, lactose, total solids, and solid non-

fat). The 3.5% fat-corrected milk yield (3.5% FCM) and energy-corrected milk yield (ECM) were calculated using the formula by [NRC \(2001\)](#):

$$3.5\% \text{ Fat corrected milk (FCM)} = [0.4324 \times \text{milk (kg)}] + [16.218 \times \text{milk fat (kg)}],$$

$$\text{Energy corrected milk (ECM)} = (0.3246 \times \text{milk yield}) + (12.86 \times \text{fat yield}) + (7.04 \times \text{protein yield})$$

Fatty acid profile of milk analysis

The second set of samples was frozen and stored at -20 °C for fatty acid profiles analysis in milk. A total of 1 mL of milk was placed in a fat extraction tube, then 0.7 mL of 10 N KOH in water, and 5.3 mL of MeOH were added to the tube. The tube was then kept at controlled temperature in a water bath at 55°C for 1.5 hours, with vigorous manual agitation for 5 seconds every 20 minutes. After cooling the tube with cold tap water, 0.58 mL of H₂SO₄ in water at a concentration of 12 M was added. The tube was mixed and then re-incubated in a 55°C water bath for 1.5 hours, with manual agitation. The tube was cooled to below room temperature following the synthesis of FAME (fatty acid methyl esters). Subsequently, 3 mL of hexane was added, and the tube was vortex-mixed for 5 minutes ([O'Fallon et al., 2007](#)). The tube was then centrifuged for 5 minutes at 380 × g, and the hexane layer containing the FAME was transferred to a gas chromatography (GC) vial. The fatty acids (FA) were quantified by injecting 1 µL of the samples into a GC detector; FID @ 250 °C (GC-7820A, Agilent Technologies Inc.), according to the parameters described by [Anzhany et al. \(2023\)](#), using a CP-Sil 88 fused-silica capillary column (100 m length, 0.25 mm inner diameter, and 0.20 µm film thickness; Agilent Technologies). The retention time of samples to the retention time of the food industry FAME mix standard (37 components; RESTEK Corporation, Bellefonte, PA) was compared to generate FA concentration.

Statistical analysis

All data were analyzed using the IBM SPSS Statistics 26.0. Analysis of variance (ANOVA) was used for a complete randomized design (CRD) on the *in vitro* study. The mathematic model used was a linear model for CRD: $Y_{ij} = \mu + \alpha_i + \epsilon_{ij}$, where Y_{ij} = observation value, μ = general mean, α_i = treatment effect (treatments 1-7), and ϵ_{ij} = experimental error. Mean differences among treatments were determined using Tukey's HSD. The main effect was the analysis of a two-factor factorial design (level of bacteria and each oil) with interaction. The mathematic model is $Y_{ijk} = \mu + \alpha_i + \beta_j + (\alpha\beta)_{ij} + \epsilon_{ijk}$. Where: Y_{ijk} : the observed response for the k-th replication under level i of bacteria level and level j of oils type, μ : the overall mean of the response, α_i : the effect of the i-th level of bacteria level, β_j : the effect of the j-th level of oils type, $(\alpha\beta)_{ij}$: the interaction effect between bacteria level and oils type, ϵ_{ijk} : the random error term, assumed to be normally distributed with mean zero and constant variance. Mean differences among main effects were determined using Tukey's HSD.

The lactation performance study used analysis of covariance (ANCOVA) for Randomized Complete Block Design (RCBD) for the lactation performance study. The mathematic model is $Y_{ij} = \mu + \tau_i + \beta_j + b(X_{ij} - X) + \epsilon_{ij}$ where: Y_{ij} = observed value of the dependent variable (response) for the i-th treatment in the j-th block, μ = overall mean, τ_i = fixed effect of the i-th treatment ($i = 1-3$), β_j = effect of the j-th block ($j = 1-3$, day in milk), X_{ij} = covariate associated with Y_{ij} (data of day 0 and parity), X = overall mean of the covariate, b = regression coefficient of Y on the covariate, ϵ_{ij} = random error. Mean differences among treatments were determined using Bonferroni ([Holm, 1979](#)).

$Y_{ij} = \mu + \tau_i + \beta_j + b(X_{ij} - X) + \epsilon_{ij}$ where: Y_{ij} = observed value of the dependent variable (response) for the i-th treatment in the j-th block, μ = overall mean, τ_i = fixed effect of the i-th treatment ($i = 1-3$), β_j = effect of the j-th block ($j = 1-3$, day in milk), X_{ij} = covariate associated with Y_{ij} (data of day 0 and parity), X = overall mean of the covariate, b = regression coefficient of Y on the covariate, ϵ_{ij} = random error, assumed $\sim N(0, \sigma^2)$, independent

RESULTS

In vitro gas production

Gas production at 2 hours of *L. plantarum* 10⁸ CFU/mL + 2% sunflower oil and *L. plantarum* 10⁸ CFU/mL + 2% soybean oil exhibited the highest gas production (10.50 mL and 9.91 mL, respectively), significantly exceeding other treatments ($p < 0.001$). This trend continued at 4, 6, and 8 hours, where *L. plantarum* 10⁸ CFU/mL + 2% sunflower oil and *L. plantarum* 10⁸ + 2% soybean oil demonstrated the highest gas production ($P < 0.001$). At 24 hours, *L. plantarum* 10⁸ CFU/mL + 2% (59.30 mL) and *L. plantarum* 10⁸ CFU/mL + 2% soybean oil (58.87 mL) maintained the highest gas production, significantly surpassing the control and other treatments ($p < 0.001$). At 48, 72, and 96 hours, these two treatments continued to produce the highest gas volumes, with *L. plantarum* 10⁸ CFU/mL + 2% sunflower oil and *L. plantarum* 10⁸ CFU/mL + 2% soybean oil reaching 80.62 mL and 80.56 mL at 96 hours ($P < 0.001$), as shown in Table 4. The level of 10⁸ CFU/mL supplementation produced the highest gas among other levels ($P < 0.001$), as shown in Table 5. The *in vitro* gas production was not affected by oils type. There were no interactions between level and oils type for *in vitro* gas production.

Table 4 Gas production and fermentation parameters of total mixed ration supplemented with *L. plantarum* and sunflower oil by *in vitro* gas production techniques

Variable	Control	Soybean oil			Sunflower oil			SE M	P-value
		LP10 ⁶	LP10 ⁷	LP10 ⁸	LP10 ⁶	LP10 ⁷	LP10 ⁸		
<i>In vitro</i> gas production (mL)									
2 hours	3.71 ^c	6.21 ^{cb}	6.22 ^{cb}	9.91 ^a	4.96 ^{cb}	7.40 ^b	10.50 ^a	0.57	<0.001
4 hours	6.80 ^c	8.69 ^{cb}	10.57 ^b	16.11 ^a	7.43 ^c	10.49 ^b	16.06 ^a	0.82	<0.001
6 hours	8.67 ^d	9.93 ^d	13.06 ^c	22.31 ^a	9.91 ^d	12.34 ^c	20.39 ^b	1.14	<0.001
8 hours	11.14 ^d	13.03 ^{cd}	16.17 ^b	28.50 ^a	13.01 ^{cd}	14.81 ^{bc}	26.56 ^a	1.46	<0.001
10 hours	14.86 ^c	16.75 ^{bc}	19.27 ^b	35.94 ^a	16.72 ^{bc}	18.51 ^b	33.36 ^a	1.81	<0.001
12 hours	18.57 ^d	20.48 ^{bcd}	23.63 ^b	40.28 ^a	19.82 ^{cd}	22.83 ^{bc}	40.15 ^a	2.00	<0.001
24 hours	32.82 ^d	37.22 ^{bc}	41.04 ^b	58.87 ^a	36.54 ^{cd}	40.72 ^b	59.30 ^a	2.27	<0.001
48 hours	44.59 ^d	49.63 ^{bc}	53.47 ^b	72.50 ^a	48.30 ^{cd}	53.06 ^b	72.27 ^a	2.40	<0.001
72 hours	49.55 ^d	54.59 ^{bc}	58.44 ^b	78.08 ^a	53.26 ^{cd}	58.62 ^b	77.84 ^a	2.39	<0.001
96 hours	52.03 ^d	56.45 ^{bcd}	59.07 ^{bc}	80.56 ^a	55.11 ^{cd}	60.47 ^b	80.62 ^a	2.46	<0.001
Kinetics of gas production									
A (mL)	2.02	0.51	0.94	1.17	1.69	0.64	1.28	0.20	0.645
B (mL/0.2 g DM)	55.16 ^c	59.15 ^{bc}	61.14 ^{bc}	78.55 ^a	58.20 ^{bc}	62.01 ^b	78.43 ^a	2.10	<0.001
C (%/hr.)	0.04 ^b	0.04 ^b	0.04 ^b	0.05 ^a	0.04 ^b	0.04 ^b	0.05 ^a	0.00	<0.001
 a + b	57.18 ^b	59.66 ^b	62.08 ^b	79.71 ^a	59.89 ^b	62.65 ^b	79.72 ^a	2.11	<0.001
Fermentation parameters									
pH 24 h.	7.20 ^a	7.09 ^b	7.06 ^b	6.74 ^c	7.12 ^{ab}	7.08 ^b	6.74 ^c	0.04	<0.001
pH 48 h.	7.01 ^a	7.03 ^a	6.94 ^b	6.74 ^c	7.02 ^a	7.02 ^a	6.72 ^c	0.03	<0.001
NH₃ 24 h. (mM)	15.74 ^b	15.32 ^b	16.44 ^b	24.13 ^b	15.82 ^b	16.57 ^b	24.08 ^a	0.84	<0.001
NH₃ 48 h. (mM)	16.85 ^b	17.19 ^b	18.06 ^b	27.76 ^b	16.07 ^b	17.30 ^b	26.37 ^a	1.03	<0.001

Different superscripts a-b mean significant difference in treatments at $p < 0.05$ level.

Kinetics of gas production

Production from the insoluble fraction in supplementation with *L. plantarum* 10⁸ CFU/mL + 2% soybean oil, and *L. plantarum* 10⁸ CFU/mL + 2% sunflower oil was higher than other groups (78.55, and 78.43 mL/200 mgDM, respectively) ($P < 0.001$). The rate of gas production from the insoluble fraction in supplementation with *L. plantarum* 10⁸ CFU/mL + 2% soybean oil, and *L. plantarum* 10⁸ CFU/mL + 2% sunflower oil produced the highest rate compared to other groups (0.05 %/h) ($P = 0.001$). The potential extent of gas production in supplementation with *L. plantarum* 10⁸ CFU/mL + 2% sunflower oil, and *L. plantarum* 10⁸ CFU/mL + 2%

soybean oil was higher than other groups (79.72, 79.71, respectively) ($P<0.001$), as shown in **Table 4**. The gas production from an insoluble fraction, a rate of gas production from the insoluble fraction, and potential extent of gas production in supplementation with *L. plantarum* 10^8 CFU/mL was higher than other levels ($P < 0.001$). The kinetics of gas production was not affected by oils type., as shown in **Table 5**. There were no interactions between level and oils type for kinetics of gas production.

Table 5 Main effects of *L. plantarum* and sunflower oil on gas production and fermentation parameters by *in vitro* gas production techniques

Variable	Main effect					SEM	P-value			
	Level		Oil				Main effect	Level	Oil	
	LP10 ⁶	LP10 ⁷	LP10 ⁸	Soybean oil	Sunflower oil					
<i>In vitro</i> gas production (mL)										
2 hours	5.58 ^y	6.81 ^y	10.21 ^x	7.45	4.62	0.57	<0.001	0.770	0.254	
4 hours	8.06 ^z	10.53 ^y	16.09 ^x	11.79	11.33	0.82	<0.001	0.324	0.508	
6 hours	9.92 ^z	12.70 ^y	21.35 ^x	15.10	14.21	1.14	<0.001	0.113	0.349	
8 hours	13.02 ^z	15.49 ^y	27.53 ^x	19.23	18.13	1.46	<0.001	0.055	0.337	
10 hours	16.74 ^y	18.89 ^y	34.65 ^x	23.99	22.86	1.81	<0.001	0.134	0.341	
12 hours	20.15 ^z	23.23 ^y	40.22 ^x	28.13	27.60	2.00	<0.001	0.554	0.946	
24 hours	36.88 ^z	40.88 ^y	59.08 ^x	45.71	45.52	2.27	<0.001	0.868	0.919	
48 hours	48.96 ^z	52.27 ^y	72.38 ^x	58.53	57.88	2.40	<0.001	0.610	0.929	
72 hours	53.92 ^z	58.53 ^y	77.98 ^x	63.70	63.24	2.39	<0.001	0.742	0.894	
96 hours	55.78 ^y	59.77 ^y	80.58 ^x	65.36	65.40	2.46	<0.001	0.977	0.708	
Kinetics of gas production										
A (mL)	1.10	0.79	1.23	0.87	1.20	0.20	0.645	0.407	0.305	
B (mL/0.2 g DM)	58.68 ^y	61.58 ^y	78.49 ^x	66.28	66.22	2.10	<0.001	0.970	0.903	
C (%/hr.)	0.04 ^y	0.04 ^y	0.05 ^x	0.04	0.04	0.00	<0.001	0.574	0.723	
 a + b	59.78 ^y	62.37 ^y	79.72 ^x	67.15	67.42	2.11	<0.001	0.879	0.991	
Fermentation parameters										
pH 24 h.	7.10 ^x	7.07 ^x	6.74 ^y	6.96	6.98	0.04	<0.001	0.587	0.852	
pH 48 h.	7.02 ^x	6.98 ^y	6.73 ^z	6.90	6.92	0.03	<0.001	0.262	0.009	
NH₃ 24 h. (mM)	15.57 ^y	16.51 ^y	24.11 ^x	18.63	18.82	0.84	<0.001	0.567	0.788	
NH₃ 48 h. (mM)	16.63 ^z	17.68 ^y	27.07 ^x	21.01	19.91	1.03	<0.001	0.001	0.642	

Different superscripts x-z mean significant difference in the main effect of level at $p < 0.05$ level.

Fermentation parameters

At 24 hours, *L. plantarum* 10^8 CFU/mL + 2% sunflower oil and *L. plantarum* 10^8 CFU/mL + 2% soybean oil had the lowest pH value (6.74), while control group showed the highest value (7.20). A similar trend was observed at 48 hours, with *L. plantarum* 10^8 CFU/mL + 2% sunflower oil and *L. plantarum* 10^8 CFU/mL + 2% soybean oil remaining the lowest value (6.72 and 6.74, respectively), whereas the control group remained the highest value (7.01). Ammonia nitrogen concentrations after 24 hours differed significantly among treatments ($P < 0.001$). The highest NH₃-N concentrations were observed in *L. plantarum* 10^8 CFU/mL + 2% soybean oil (24.13 mM) and *L. plantarum* 10^8 CFU/mL + 2% sunflower oil (24.08 mM). Similarly, *L. plantarum* 10^8 CFU/mL + 2% soybean oil (27.76 mM) and *L. plantarum* 10^8 CFU/mL + 2% sunflower oil (26.37 mM) maintained significantly higher NH₃-N concentrations at 48 hours compared to other treatments ($P < 0.001$), as shown in **Table 4**.

Estimated parameters

Organic matter digestibility for supplementation with *L. plantarum* 10^8 CFU/mL + 2% sunflower oil, and *L. plantarum* 10^8 CFU/mL + 2% soybean oil was the highest value compared with other groups (81.45% and 81.08%, respectively) ($P<0.001$). Metabolizable energy for supplementation with *L. plantarum* 10^8 + 2%

sunflower oil, and *L. plantarum* 10⁸ CFU/mL + 2% soybean oil was the highest compared with other groups (11.28 and 11.22 MJ/Kg, respectively) (P<0.001). The amount of short-chain fatty acids for supplementation with *L. plantarum* 10⁸ CFU/mL + 2% sunflower oil and *L. plantarum* 10⁸ CFU/mL + 2% soybean oil was the highest compared with other groups (1.36 and 1.35 mol, respectively) (P<0.001), as shown in Table 6. The level of *L. plantarum* 10⁸ CFU/mL supplementation produced higher organic matter digestibility, metabolizable energy, and amount of short-chain fatty acids than other levels, while pH was the lowest value compared with other levels (P < 0.001), as shown in Table 7.

Table 6 Estimated parameters and ruminal VFA's of total mixed ration supplemented with *L. plantarum* and sunflower oil by *in vitro* gas production techniques

Variable	Control	Soybean oil			Sunflower oil			SEM	P-value
		LP10 ⁶	LP10 ⁷	LP10 ⁸	LP10 ⁶	LP10 ⁷	LP10 ⁸		
Estimated parameters									
OMD (%)	59.07 ^d	62.78 ^{bc}	66.01 ^b	81.08 ^a	62.21 ^{cd}	65.74 ^b	81.45 ^a	1.92	<0.001
ME (MJ/Kg)	7.68 ^d	8.27 ^{bc}	8.79 ^b	11.22 ^a	8.18 ^{cd}	8.75 ^b	11.28 ^a	0.31	<0.001
SCFA (mol)	0.72 ^d	0.83 ^{bc}	0.92 ^b	1.35 ^a	0.81 ^{cd}	0.91 ^{bc}	1.36 ^a	0.05	<0.001
Ruminal VFA's 24 h. (mM)									
Total VFA	54.77 ^b	56.63 ^b	57.13 ^b	87.60 ^a	51.86 ^b	52.10 ^b	79.51 ^a	2.04	<0.001
Acetic acid	37.57 ^b	38.19 ^b	37.45 ^b	58.52 ^a	36.19 ^b	35.81 ^b	52.12 ^a	0.68	<0.001
Propionic acid	13.74 ^b	14.45 ^{ab}	13.66 ^b	19.20 ^a	12.70 ^b	13.17 ^b	19.24 ^a	0.60	0.002
Butyric acid	3.47 ^{cd}	3.99 ^{cd}	6.02 ^{bc}	9.88 ^a	2.96 ^d	3.11 ^{cd}	8.16 ^{ab}	3.15	<0.001
A:P	2.73	2.63	2.89	3.05	2.89	2.74	2.72	0.08	0.915
Ruminal VFA's 48 h. (mM)									
Total VFA	53.37 ^c	50.27 ^c	54.25 ^{bc}	70.10 ^{ab}	44.10 ^c	48.80 ^c	74.25 ^a	1.60	0.001
Acetic acid	35.55 ^{ab}	32.08 ^b	35.85 ^{ab}	45.61 ^a	28.91 ^b	31.87 ^b	46.03 ^a	0.54	<0.001
Propionic acid	14.46 ^b	14.77 ^b	14.71 ^b	16.16 ^{ab}	12.88 ^b	13.82 ^b	19.77 ^a	0.55	0.002
Butyric acid	3.35 ^b	3.42 ^b	3.68 ^b	8.33 ^a	2.32 ^b	3.10 ^b	8.45 ^a	2.57	<0.001
A:P	2.46 ^{ab}	2.18 ^b	2.44 ^{ab}	2.83 ^a	2.24 ^b	2.31 ^b	2.32 ^b	0.06	0.017

Different superscripts a-b mean significant difference in treatments at p <0.05 level.

Ruminal VFA concentrations

Significant differences in total VFA concentrations were observed among treatments after 24 hours (P < 0.001). *L. plantarum* 10⁸ CFU/mL + 2% soybean oil exhibited the highest total VFA production (87.60 mmol/L), followed by *L. plantarum* 10⁸ CFU/mL + 2% sunflower oil (79.51 mmol/L), while the lowest values were recorded in *L. plantarum* 10⁶ CFU/mL + 2% sunflower oil (51.86 mmol/L) and *L. plantarum* 10⁷ CFU/mL + 2% sunflower oil (52.10 mmol/L). Acetic acid concentration was significantly greater in *L. plantarum* 10⁸ CFU/mL + 2% soybean oil (58.52 mmol/L) and *L. plantarum* 10⁸ CFU/mL + 2% sunflower oil (52.12 mmol/L) compared to other treatments (P < 0.001). Similarly, propionic acid levels were significantly elevated in *L. plantarum* 10⁸ CFU/mL + 2% soybean oil (19.20 mmol/L) and *L. plantarum* 10⁸ CFU/mL + 2% sunflower oil (19.24 mmol/L) compared with the control and other groups (P = 0.002). Butyric acid concentrations varied markedly among treatments (P < 0.001), with *L. plantarum* 10⁸ CFU/mL + 2% soybean oil showing the highest level (9.88 mmol/L), and *L. plantarum* 10⁸ CFU/mL + 2% sunflower oil presenting a relatively high value (8.16 mmol/L), while the lowest concentrations were found in *L. plantarum* 10⁶ CFU/mL + 2% sunflower oil (2.96 mmol/L) and *L. plantarum* 10⁷ CFU/mL + 2% sunflower oil (3.11 mmol/L). At 48 hours, similar trends were observed. Total VFA concentration was significantly higher in *L. plantarum* 10⁸ CFU/mL + 2% sunflower oil (74.25 mmol/L) and *L. plantarum* 10⁸ CFU/mL + 2% soybean oil (70.10 mmol/L) compared to the control (53.37 mmol/L) and other treatments (P < 0.001). Acetic acid levels remained significantly elevated in *L. plantarum* 10⁸ CFU/mL + 2% soybean oil (45.61 mmol/L) and *L. plantarum* 10⁸ CFU/mL + 2% sunflower oil (46.03 mmol/L) relative to the

other groups ($P < 0.001$). Propionic acid concentrations were the highest in *L. plantarum* 10^8 CFU/mL + 2% sunflower oil (19.77 mmol/L) and *L. plantarum* 10^8 CFU/mL + 2% soybean oil (16.16 mmol/L) and differed significantly across treatments ($P = 0.002$). Regarding butyric acid, *L. plantarum* 10^8 CFU/mL + 2% soybean oil (8.33 mmol/L) and *L. plantarum* 10^8 CFU/mL + 2% sunflower oil (8.45 mmol/L) demonstrated the highest concentrations at 48 hours, which were significantly greater than those observed in other treatments ($P < 0.001$). However, a significant difference was detected ($P = 0.017$) at 48 hours, as shown in Table 6. The level of 10^8 CFU/mL supplementation produced higher total VFA, acetic acid, propionic acid, and butyric acid than other levels and a pH value lower than other levels ($P < 0.001$). Supplementation with 2% sunflower oil resulted in a reduction of the A:P ratio at 48 hours, as shown in Table 7.

Table 7 Main effects of *L. plantarum* and sunflower oil on estimated parameters and ruminal VFA's by *in vitro* gas production techniques

Variable	Main effect					SEM	P-value		
	Level	LP 10^6	LP 10^7	LP 10^8	Oil		Main effect	Level	Oil
Estimated parameters									
OMD (%)	62.50 ^z	65.88 ^y	81.27 ^x	69.96	69.80	1.92	<0.001	0.870	0.919
ME (MJ/Kg)	8.23 ^z	8.77 ^y	11.25 ^x	9.43	9.40	0.31	<0.001	0.865	0.916
SCFA (mol)	0.82 ^z	0.92 ^y	1.35 ^x	1.03	1.03	0.05	<0.001	0.843	0.942
Ruminal VFA's 24 h. (mM)									
Total VFA	54.24 ^y	54.62 ^y	83.56 ^x	67.12	61.16	2.04	<0.001	0.014	0.776
Acetic acid	37.19 ^y	36.63 ^y	55.32 ^x	44.72	41.38	0.68	<0.001	0.053	0.407
Propionic acid	13.58 ^y	13.42 ^y	19.22 ^x	15.77	15.04	0.60	<0.001	0.467	0.747
Butyric acid	3.47 ^y	4.56 ^y	9.02 ^x	6.63	4.74	3.15	<0.001	0.002	0.325
A:P	2.76	2.82	2.88	2.86	2.78	0.08	0.881	0.703	0.488
Ruminal VFA's 48 h. (mM)									
Total VFA	47.19 ^y	51.53 ^y	72.18 ^x	58.21	55.72	1.60	<0.001	0.410	0.309
Acetic acid	30.50 ^y	33.86 ^y	45.82 ^x	37.85	35.60	0.54	<0.001	0.316	0.680
Propionic acid	13.82 ^y	14.27 ^y	17.97 ^x	15.21	15.49	0.55	0.02	0.731	0.032
Butyric acid	2.87 ^y	3.39 ^y	8.39 ^x	5.15	4.62	2.57	<0.001	0.146	0.361
A:P	2.21 ^y	2.38 ^y	2.57 ^x	2.48	2.30	0.06	0.019	0.049	0.060

Different superscripts x-z mean significant difference in the main effect of level at $p < 0.05$ level.

Fatty acid profile in rumen after incubation for 24 and 48 hours

The C18:1; trans-9 after 24 hours of incubation for supplementation with *L. plantarum* 10^6 CFU/mL + 2% sunflower oil was higher than the control group but not different from other groups (12.647, and 6.107 % of total fat, respectively) ($P < 0.005$). C18:2; cis9,12 after 24 hours of incubation for supplementation with *L. plantarum* 10^7 CFU/mL + 2% sunflower oil was the highest compared to other groups (10.571 % of total fat) ($P < 0.005$), as shown in Tables 8. C18:1; tran-9 after 48 hours of incubation for supplementation with *L. plantarum* 10^7 CFU/mL + 2% soybean oil, was higher than control but not different from other groups (12.773, and 5.700 % of total fat, respectively) ($P < 0.005$). Sunflower oil supplementation can increase C18:2; cis-9, trans-11 (CLA) compared with soybean oil supplementation at 24 hours (1.083 and 0.493, respectively) ($P = 0.033$), as shown in Tables 8, 9, 10 and 11.

Table 8 Fatty acid profile (C12:0 – C20:0) of rumen fluid supplementation with *L. plantarum* and sunflower oil after incubation for 24 hours by *in vitro* gas production techniques

Variable	Control	Soybean oil			Sunflower oil			SEM	P-value
		LP10 ⁶	LP10 ⁷	LP10 ⁸	LP10 ⁶	LP10 ⁷	LP10 ⁸		
Fatty acid profile 24 hours (% of total fat)									
C12:0	1.557	0.780	1.375	0.700	0.708	0.654	1.262	0.112	0.152
C14:0	3.303	2.962	4.144	2.530	2.105	2.277	3.917	0.658	0.364
C14:1; cis-9	2.348	6.272	4.522	5.637	5.079	2.110	4.746	0.694	0.748
C15:0	2.128	1.804	1.657	1.989	1.759	1.597	2.007	0.072	0.537
C16:0	29.045	24.680	26.948	24.490	21.520	21.646	25.868	0.800	0.160
C16:1; cis-9	1.809	1.155	1.525	1.335	1.171	1.143	1.350	0.075	0.258
C17:0	1.275	1.077	1.028	1.055	1.051	0.959	1.079	0.027	0.167
C18:0	31.244	32.157	31.866	33.355	35.303	32.332	32.809	0.790	0.917
C18:1; tran-9	6.107 ^b	12.188 ^a	10.962 ^a	12.438 ^a	12.647 ^a	9.839 ^{ab}	9.011 ^{ab}	0.615	0.022
C18:1; cis-9	11.210	11.117	11.271	10.475	12.040	15.079	11.463	0.500	0.392
C18:2; cis-9, tran-11	1.218	0.440	0.427	0.613	1.199	0.675	1.375	0.132	0.219
C18:2; tran-10, cis-12	0.734	0.440	0.377	0.462	0.688	0.416	0.833	0.059	0.218
C18:2; cis9,12	2.578 ^b	4.134 ^b	3.205 ^b	4.144 ^b	4.033 ^b	10.571 ^a	3.557 ^b	0.654	0.026
C20:0	0.821	0.795	0.694	0.780	0.698	0.702	0.725	0.030	0.924

Different superscripts a-b mean significant difference in treatments at p <0.05 level.

Table 9 Main effects of *L. plantarum* and sunflower oil on fatty acid profile (C12:0 – C20:0) after incubation for 24 hours by *in vitro* gas production techniques

Variable	Main effect					SEM	P-value		
	Level	Oil		Main effect	Level		Oil	Level * Oil	
	LP10 ⁶	LP10 ⁷	LP10 ⁸	Soybean oil	Sunflower oil				
Fatty acid profile 24 hours (% of total fat)									
C12:0	0.744	1.015	0.981	0.952	0.875	0.112	0.433	0.683	0.058
C14:0	2.534	3.211	3.223	3.212	2.766	0.658	0.493	0.435	0.090
C14:1; cis-9	5.676	3.316	5.191	5.477	3.978	0.694	0.550	0.399	0.936
C15:0	1.781	1.627	1.998	1.817	1.788	0.072	0.246	0.865	0.979
C16:0	23.100	24.297	25.179	25.372	23.011	0.800	0.397	0.097	0.147
C16:1; cis-9	1.163	1.334	1.342	1.338	1.221	0.075	0.553	0.468	0.550
C17:0	1.064	0.993	1.067	1.053	1.030	0.027	0.309	0.556	0.647
C18:0	33.730	32.099	33.082	32.459	33.481	0.790	0.740	0.545	0.609
C18:1; tran-9	12.417	10.400	10.725	11.862	10.499	0.615	0.152	0.139	0.178
C18:1; cis-9	11.578	13.175	10.969	10.955	12.861	0.500	0.314	0.111	0.535
C18:2; cis-9, tran-11	0.820	0.551	0.994	0.493	1.083	0.132	0.377	0.033	0.649
C18:2; tran-10, cis-12	0.564	0.396	0.647	0.426	0.646	0.059	0.256	0.079	0.528
C18:2; cis9,12	4.084	6.888	3.850	3.827	6.054	0.654	0.091	0.058	0.026
C20:0	0.746	0.698	0.752	0.756	0.708	0.030	0.822	0.518	0.850

Table 10 Fatty acid profile (C12:0 – C20:0) of rumen fluid supplementation with *L. plantarum* and sunflower oil after incubation for 48 hours by *in vitro* gas production techniques

Variable	Control	Soybean oil			Sunflower oil			SEM	P-value
		LP10 ⁶	LP10 ⁷	LP10 ⁸	LP10 ⁶	LP10 ⁷	LP10 ⁸		
Fatty acid profile 48 hours (% of total fat)									
C12:0	0.924	1.331	0.772	0.674	0.899	1.105	0.990	0.107	0.780
C14:0	5.501	3.776	3.194	2.794	3.175	4.189	3.717	0.284	0.337
C14:1; cis-9	2.927	3.235	1.279	2.431	2.504	2.156	3.364	0.726	0.324
C15:0	2.881	2.467	2.684	2.536	2.267	2.646	2.014	0.128	0.733
C16:0	29.184	28.897	26.875	25.971	25.851	27.350	25.231	0.527	0.374
C16:1; cis-9	1.469	1.547	1.133	1.226	1.015	1.067	1.129	0.069	0.324
C17:0	1.031	1.014	1.041	1.077	1.006	1.057	1.073	0.021	0.976
C18:0	34.041	31.734	32.051	32.683	35.280	32.727	31.653	0.690	0.839
C18:1; tran-9	5.700 ^c	10.887 ^{ab}	12.773 ^a	11.859 ^{ab}	12.462 ^a	8.278 ^{bc}	11.121 ^{ab}	0.619	0.017
C18:1; cis-9	9.510	11.082	10.788	9.198	9.891	10.834	9.863	0.351	0.778
C18:2; cis-9, tran-11	0.564	0.357	0.890	1.093	0.977	1.023	0.604	0.109	0.522
C18:2; tran-10, cis-12	0.550	0.318	0.726	0.621	0.529	0.692	0.512	0.135	0.197
C18:2; cis9,12	3.472	2.680	5.059	5.176	3.431	6.625	3.989	0.478	0.339
C20:0	0.991	0.675	0.737	0.752	0.712	0.736	0.760	0.027	0.124

Different superscripts a-b mean significant difference in treatments at p <0.05 level.

Table 11 Main effects of *L. plantarum* and sunflower oil on fatty acid profile (C12:0 – C20:0) after incubation for 48 hours by *in vitro* gas production techniques

Variable	Main effect					SEM	P-value		
	Level	LP10 ⁶	LP10 ⁷	LP10 ⁸	Soybean oil		Main effect	Level	Oil
Fatty acid profile 48 hours (% of total fat)									
C12:0	1.115	0.938	0.832	0.925	0.998	0.107	0.661	0.779	0.396
C14:0	3.476	3.691	3.255	3.255	3.693	0.284	0.805	0.429	0.417
C14:1; cis-9	2.870	1.717	5.843	2.952	4.001	0.726	0.122	0.511	0.626
C15:0	2.367	2.665	2.275	2.562	2.309	0.128	0.557	0.416	0.803
C16:0	27.374	27.112	25.601	27.247	26.144	0.527	0.404	0.343	0.450
C16:1; cis-9	1.281	1.178	1.100	1.302	1.071	0.069	0.573	0.118	0.337
C17:0	1.010	1.049	1.075	1.044	1.045	0.021	0.573	0.975	0.979
C18:0	33.507	32.389	32.168	32.156	32.220	0.690	0.785	0.536	0.545
C18:1; tran-9	11.674	10.526	11.490	11.840	10.620	0.619	0.576	0.218	0.61
C18:1; cis-9	10.487	10.811	9.530	10.356	10.196	0.351	0.453	0.851	0.662
C18:2; cis-9, tran-11	0.667	0.956	0.849	0.780	0.868	0.109	0.631	0.724	0.219
C18:2; tran-10, cis-12	0.424	0.566	0.709	0.555	0.578	0.135	0.192	0.853	0.538
C18:2; cis9,12	3.056	5.842	4.583	4.305	4.682	0.478	0.119	0.715	0.536
C20:0	0.693	0.737	0.756	0.721	0.736	0.027	0.606	0.782	0.951

Feed intake

The roughage intake during the first 30 days of the experiment was not different among the three groups, but the group supplemented with *L. plantarum*, and sunflower oil had the highest intake value on days 60 and 84, which was different from the group supplemented with *L. plantarum* (950 and 1000 vs. 820 and 890 g/d, respectively) but not different from the control group. The dry matter intake (g/d) of the supplemented group with *L. plantarum* and sunflower oil had the highest intake value, which was different from the group supplemented with *L. plantarum* but not different from the control group (1754 and 1798 vs. 1622 and 1690 g/d, respectively). However, it was not different in all three groups on days 30 and 84 of the experiment. The intake of concentrate, intake of dry matter (% BW) and DMI/BW^{0.75} were not different in all three groups on days 30, 60, and 84 of the experiment, as shown in Table 12.

Table 12 DMI of Saanen goats supplemented with *L. plantarum* and sunflower oil

Variable	Days	Treatments			SEM	P-value
		Control	<i>L. plantarum</i>	<i>L. plantarum</i> + sunflower oil		
Body weight (kg)		46.60 ±17.36	39.60 ±4.16	39.60 ±4.16		
Roughage Intake (g/d)	30	890	800	890	0.24	0.183
	60	890 ^{ab}	820 ^b	950 ^a	0.27	0.029
	84	900 ^{ab}	890 ^b	1000 ^a	0.20	0.030
Concentrate Intake (g/d)	30	1020	1000	1010	0.05	0.398
	60	1010	1000	1010	0.04	0.277
	84	1000	1000	1000	0.01	0.700
Total DMI (g/d)		1708	1604	1702	0.24	0.103
(%BW)	30	1704 ^{ab}	1622 ^b	1754 ^a	0.26	0.013
	60	1704 ^b	1690 ^b	1798 ^a	0.19	0.011
	84	3.94	4.10	4.19	0.17	0.743
(g/kg BW^{0.75})	30	117.25	123.00	129.12	8.53	0.872
	60	117.24	124.96	138.29	8.95	0.666
	84	119.90	137.04	146.37	9.77	0.525

Milk yield and milk composition

Milk yields on days 63 and 70 for the control group were higher than *L. plantarum* supplementation, but not different from *L. plantarum* with sunflower oil supplementation group. The 3.5% FCM and ECM on days 42 and 70 showed significant differences ($P < 0.05$), where the control group had a higher 3.5% FCM, and the ECM compared to the *L. plantarum* supplementation group. Total solids content on day 42 for the control group was higher than *L. plantarum* supplementation but not different from the *L. plantarum* with sunflower oil supplementation group. Milk fat content, milk protein content, lactose, and solid not fat content did not show significant differences among treatments ($P > 0.05$), as shown in Tables 13, 14, and 15.

Fatty acids (FA) profile of milk

The C12:0 content in milk on day 56 in the control group was higher than *L. plantarum* supplementation (6.976 and 3.273, respectively) ($P < 0.001$). The C20:2; cis-11,14 content in milk on day 84 in the control group was higher than *L. plantarum* supplementation (0.027, and 0.010, respectively) ($P < 0.001$). However, the supplementation had no detrimental impact on the profiles of other fatty acids ($P > 0.05$), as shown in Tables 16 and 17.

Table 13 Milk yield of Saanen goats supplemented with *L. plantarum* and sunflower oil

Variable	Day	Treatments			SEM	P-value
		Control	<i>L. plantarum</i>	<i>L. plantarum</i> + sunflower oil		
Milk yields (g/Days)	7	1211.15	1091.73	1149.18	133.75	0.267
	14	1369.33	1114.20	1273.12	131.40	0.084
	21	1370.46	1046.03	1281.17	128.68	0.088
	28	1335.67	997.59	1242.39	120.48	0.165
	35	1279.58	977.49	1241.65	118.42	0.242
	42	1239.29	950.61	1224.78	112.02	0.101
	49	1200.07	885.83	1229.05	113.96	0.137
	56	1208.55	756.81	1081.47	112.54	0.086
	63	1248.53 ^a	804.41 ^b	1091.55 ^{ab}	114.16	0.041
	70	1274.76 ^a	805.09 ^b	1084.44 ^{ab}	114.62	0.043
3.5%FCM (kg/day)	77	1300.73	771.28	1070.14	120.50	0.073
	84	1286.06	745.16	1076.12	122.70	0.087
	14	1.33	1.21	1.31	0.14	0.280
	28	1.38	1.10	1.25	0.13	0.136
	42	1.36 ^a	1.02 ^b	1.26 ^a	0.12	0.013
ECM	56	1.30	0.84	1.16	0.13	0.094
	70	1.28 ^a	0.83 ^b	1.10 ^{ab}	0.13	0.047
	84	1.30	0.84	1.13	0.13	0.110
	14	1.07	0.97	1.04	0.11	0.240
	28	1.10	0.88	0.99	0.11	0.138
	42	1.09 ^a	0.82 ^b	0.99 ^a	0.10	0.012
	56	1.04	0.68	0.91	0.10	0.094
	70	1.03 ^a	0.67 ^b	0.87 ^{ab}	0.10	0.043
	84	1.04	0.67	0.89	0.10	0.105

3.5%FCM= 3.5% fat corrected milk; ECM= energy corrected milk

Table 14 Milk fat, milk protein, and lactose of Saanen goats supplemented with *L. plantarum* and sunflower oil

Composition	Days	Treatments			SEM	P-value
		Control	<i>L. plantarum</i>	<i>L. plantarum</i> + sunflower oil		
Fat (%)	14	3.48	3.45	3.44	0.11	0.991
	28	3.85	3.63	3.31	0.15	0.364
	42	4.26	3.48	3.48	0.17	0.081
	56	3.75	3.77	3.73	0.14	0.990
	70	3.36	3.35	3.44	0.19	0.896
	84	3.35	3.99	3.65	0.20	0.256
Protein (%)	14	3.21	3.21	3.29	0.06	0.526
	28	3.31	3.10	3.34	0.07	0.352
	42	3.30	3.13	3.28	0.06	0.523
	56	3.18	3.08	3.24	0.08	0.635
	70	3.14	3.15	3.38	0.08	0.326
	84	3.20	3.34	3.41	0.11	0.740
Lactose (%)	14	4.49	4.37	4.39	0.06	0.419
	28	4.40	4.31	4.32	0.05	0.440
	42	4.37	4.34	4.36	0.05	0.868
	56	4.44	4.35	4.35	0.05	0.518
	70	4.32	4.25	4.28	0.04	0.595
	84	4.33	4.32	4.28	0.04	0.602

Table 15 Solid not fat and total solid of Saanen goats supplemented with *L. plantarum* and sunflower oil

Composition	Days	Treatments			SEM	P-value
		Control	<i>L. plantarum</i>	<i>L. plantarum</i> + sunflower oil		
Solid Not Fat (SNF) (%)	14	8.34	8.32	8.30	0.09	0.927
	28	8.33	8.19	8.28	0.09	0.482
	42	8.30	8.23	8.28	0.08	0.722
	56	8.27	8.22	8.26	0.09	0.942
	70	8.19	8.21	8.27	0.10	0.893
	84	8.40	8.59	8.40	0.12	0.746
Total Solid (TS) (%)	14	11.65	11.51	11.78	0.16	0.687
	28	12.06	11.58	11.62	0.19	0.348
	42	12.49 ^a	11.49 ^b	11.80 ^{ab}	0.19	0.047
	56	11.97	11.79	12.02	0.17	0.684
	70	11.41	11.38	11.74	0.27	0.520
	84	11.61	12.19	12.10	0.31	0.645

Table 16 Fatty acid profile (C6:0 - C18:1; cis-9) of Saanen goats supplemented with *L. plantarum* and sunflower oil

Fatty acids (% of total fat)	Days	Treatments			SEM	P-value
		Control	<i>L. plantarum</i>	<i>L. plantarum</i> + sunflower oil		
C6:0	28	0.732 ^a	0.081 ^b	0.099 ^{ab}	0.107	0.023
	56	0.217	0.303	0.303	0.083	0.947
	84	0.349	0.098	0.073	0.063	0.348
C8:0	28	1.874	1.368	1.988	0.147	0.217
	56	1.792	1.288	1.684	0.120	0.755
	84	1.711	1.667	1.579	0.084	0.941
C10:0	28	7.538	8.394	9.396	0.553	0.054
	56	10.086	4.813	8.371	0.599	0.251
	84	8.335	6.632	8.827	0.249	0.477
C12:0	28	5.423	5.063	6.931	0.366	0.491
	56	6.976 ^a	3.273 ^c	5.844 ^b	0.475	< 0.001
	84	4.960	4.405	5.722	0.165	0.585
C14:0	28	12.238	10.350	11.766	0.628	0.825
	56	12.782	11.918	11.839	0.438	0.706
	84	11.339	11.068	10.270	0.178	0.074
C14:1; cis-9	28	0.215	0.110	0.229	0.048	0.697
	56	0.272	0.040	0.067	0.020	0.200
	84	0.175	0.193	0.115	0.016	0.209
C16:0	28	37.814	39.474	37.371	1.483	0.775
	56	35.742	42.113	36.609	1.182	0.188
	84	34.903	36.871	34.981	0.982	0.742
C16:1; cis-9	28	0.626	0.579	0.764	0.051	0.742
	56	0.719	0.836	0.862	0.085	0.868
	84	0.819	0.994	0.656	0.065	0.439
C18:0	28	6.996	7.042	7.626	0.472	0.972
	56	8.142	7.715	7.210	0.549	0.937
	84	8.674	6.953	9.896	0.446	0.120
C18:1; trans-9	28	1.313	2.059	1.729	0.196	0.593
	56	1.714	1.752	1.451	0.243	0.918
	84	2.274	2.940	2.910	0.200	0.341
C18:1; cis-9	28	22.394	19.714	20.584	0.797	0.493
	56	18.158	24.023	22.264	0.716	0.194
	84	22.060	25.049	23.245	0.622	0.727

Table 17 Fatty acids profile (C18:2; trans-10, cis-12 (CLA) - C20:4; cis-5,8,11,14) of Saanen goats supplemented and *L. plantarum* with sunflower oil

Fatty acids (% of total fat)	Days	Treatments			SEM	P-value
		Control	<i>L. plantarum</i>	<i>L. plantarum</i> + sunflower oil		
C18:2; cis-9, trans-11 (CLA)	28	0.010	0.021	0.025	0.003	0.456
	56	0.005	0.013	0.012	0.001	0.476
	84	0.008	0.005	0.011	0.001	0.742
C18:2; trans-10, cis- 12 (CLA)	28	0.012	0.013	0.011	0.002	0.958
	56	0.009	0.009	0.008	0.001	0.973
	84	0.003	0.005	0.007	0.001	0.763
C18:2; trans-9,12	28	0.147	0.215	0.198	0.013	0.249
	56	0.204	0.250	0.259	0.027	0.886
	84	0.222	0.267	0.198	0.012	0.139
C18:2; cis-9,12	28	2.837	2.158	1.003	0.216	0.112
	56	2.156	1.823	1.633	0.061	0.180
	84	2.666	1.976	1.639	0.177	0.847
C18:3; cis-6,9,12	28	0.036	0.049	0.054	0.004	0.534
	56	0.031	0.033	0.036	0.003	0.936
	84	0.034	0.024	0.055	0.005	0.313
C18:3; cis-9,12,15	28	0.411	0.650	0.675	0.146	0.965
	56	0.178	0.304	0.737	0.146	0.755
	84	0.745	0.951	0.081	0.151	0.224
C20:0	28	0.108	0.153	0.191	0.019	0.638
	56	0.199	0.132	0.194	0.028	0.676
	84	0.237	0.152	0.191	0.025	0.218
C20:1; cis-11	28	0.092	0.106	0.119	0.018	0.974
	56	0.070	0.075	0.132	0.015	0.684
	84	0.116	0.158	0.114	0.013	0.370
C20:2; cis-11,14	28	0.013	0.023	0.004	0.005	0.108
	56	0.014	0.012	0.020	0.002	0.714
	84	0.027 ^a	0.010 ^c	0.021 ^b	0.003	< 0.001
C20:3; cis-8,11,14	28	0.030	0.034	0.014	0.003	0.687
	56	0.026	0.011	0.024	0.003	0.149
	84	0.032	0.007	0.037	0.003	0.252
C20:4; cis-5,8,11,14	28	0.269	0.269	0.311	0.026	0.313
	56	0.033	0.135	0.125	0.023	0.442
	84	0.174	0.029	0.063	0.032	0.618

DISCUSSION

Supplementation of *L. plantarum* could increase the amount of insoluble fraction (b) and total gas content, which is consistent with studies by [Astuti et al. \(2018\)](#), [Izuddin et al. \(2018\)](#), and [Ridwan et al. \(2018\)](#), which indicated that the increased digestibility is caused by higher gas production. Therefore, this demonstrates the positive relationship between gas production and digestibility ([Blummel et al., 1997](#); [Muck et al., 2007](#)). Similarly, the rise in net gas production was linked to higher values of ME (MJ/Kg DM) and NEL (MJ/Kg DM). However, the supplementation of LAB could decrease pH value ([Soriano et al., 2014](#)) and increase NH₃ in the rumen ([Contreras-Goveaa et al., 2013](#)). The range of NH₃ in the rumen is 5.00-17.65 mM ([McDonald et al., 2010](#)). Glutamine and ammonium chloride could be directly utilized by microorganisms, and ammonium ions can dissociate, increasing the NH₃-N concentration ([Geisseler et al., 2011](#)). Supplementation of *L. plantarum* prevents the accumulation of lactic acid by decomposing it to acetic acid ([Nocek et al., 2002](#)), which affects the degradation of fibrous material ([Guo et al., 2020](#)) due to *L. plantarum* has the ability to increase the activities of CMCase and β-glycosidase. The increase of propionic acid by rumen fermentation can improve growth performance ([Kenney et al., 2015](#)). *L. plantarum* supplementation could stimulate the rumen fermentation process by adjusting the microbial composition, which helps improve the digestion and fermentation processes in the rumen, increasing the number of cellulolytic bacteria, such as *Ruminococcus albus* and *Ruminococcus flavefaciens* ([Nocek et al., 2002](#);

Arawolo and He, 2018; Oskoueian et al., 2021), and reducing the numbers of methane-producing bacteria and protozoa (Nalla et al., 2022). This process increases the absorption of nutrients by animals.

The milk and milk composition were unaffected by supplementation, similar to findings by Lounglawan and Suksombat (2001). *S. bovis* and *Lactobacillus* spp. are predominant rumen bacteria under sub-acute ruminal acidosis (SARA) (McCann et al., 2016). Increased dietary fermentable carbohydrates stimulate starch-catabolizing bacteria such as *S. bovis*, leading to rapid accumulation of ruminal lactate (Ghorban et al., 1966). The overgrowth of lactic acid bacteria and subsequent lactate accumulation can induce microbial dysbiosis, epithelial damage, rumenitis, systemic inflammation, and metabolic complications such as liver accesses (Aschenbach et al., 2019). These adverse effects are likely associated with the observed reduction in milk yield. The milk fat content of all experimental groups decreased due to the inverse relationship between milk yield and milk fat. Milk fat content decreased due to the increased amount of milk passing through the mammary glands. In mammary cells, externally supplied fatty acids may compete with newly synthesized short-chain fatty acids for esterification, potentially inhibiting lipogenic enzymes through feedback mechanisms (Palmquist et al., 1993). Supplementation with *cis*9-18:1 has been shown to preferentially occupy the sn-2 position in milk fat triglycerides, replacing 16:0, which reduces its proportion while increasing *cis*9-18:1 (DePeters et al., 2001). Similar shifts in milk triglyceride profiles have been reported in cows fed diets high in linoleic acid (Christie, 1981; Palmquist et al., 1993). Therefore, increased uptake and incorporation of dietary and rumen-derived fatty acids likely contribute to the observed decrease in de novo fatty acid synthesis in cows receiving unsaturated fat supplements (Palmquist et al., 1993). The inconsistencies across studies may be due to various factors, as the experiments were conducted by different research groups under varying conditions. Differences in probiotic preparation, animal-related factors such as age, physiological state, health status, and feeding practices likely played a role. Additionally, the observed beneficial effects were probably dependent on the specific probiotic strains used. High milk yield was also associated with a higher rate of lipolysis in the mammary glands, resulting in reduced milk fat (Krnjaić et al., 2022). The meta-analysis by Oliveira et al. (2017) linked higher milk yield to an increase in dry matter intake (DMI) of 0.26 kg/day, a trend also noted in the current study. Lactose was not affected by the supplementation of *L. plantarum* with or without sunflower oil. The fatty acids profiles were generally unaffected by supplementation.

CONCLUSIONS

L. plantarum 10⁸ CFU/mL with soybean oil and *L. plantarum* 10⁸ CFU/mL with sunflower oil improves *in vitro* rumen degradability and fermentation. *L. plantarum* 10⁸ CFU/mL with soybean oil and *L. plantarum* 10⁸ CFU/mL with sunflower oil supplementation increases acetic acid, propionic acid, butyric acid, NH₃, and C18:1; *trans*-9 but decreases pH after incubating for 24 and 48 hours by the *in vitro* gas production technique. Supplementation with *L. plantarum* 10⁸ CFU/mL increases *in vitro* rumen degradability, rumen fermentation, acetic acid, propionic acid, butyric acid, NH₃. Supplementation with sunflower oil increases C18:2; *cis*-9, *tran*-11 (CLA) in rumen after incubation for 24 hours. During the lactation performance experiment, the groups supplemented with *L. plantarum* 10⁷ CFU/mL, and sunflower oil had the highest roughage intake on days 60 and 84 but not different from control group but milk composition were not affected by *L. plantarum* 10⁷ CFU/mL with sunflower oil supplementation



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CONFLICT OF INTEREST

The authors certify that there are no conflicts of interest with any financial organization regarding the material discussed in the manuscript.

AUTHOR CONTRIBUTIONS

Tanakorn Tanukarn: contributing to the commencing of the experiment, collecting and analyzing the data, and writing the manuscript.

Chayawat Sawangjaeng: contributing to the resource of the experiment.

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.

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