BIODIVERSITAS
Volume 26, Number 7, July 2025
Pages: 3423-3433

ISSN: 1412-033X
E-ISSN: 2085-4722
DOI: 10.13057/biodiv/d260733

Methane emission and nutritive evaluation of green, black, oolong, and white tea leaves

SRI WAHYUNI[1], ASEP SUDARMAN[1,♥], AHMAD SOFYAN[2], DESSY PERMATA[1], I NYOMAN GUNA DARMA[1], DIKY RAMDANI[3], NANIK HIDAYATIK[4], ANURAGA JAYANEGARA[1,♥♥]

[1]Department of Nutrition and Feed Technology, Faculty of Animal Science, Institut Pertanian Bogor. Jl. Agatis Kampus IPB Darmaga, Bogor 16680, West Java, Indonesia. Tel.: +62-251-8622841, ♥email: a_sudarman@yahoo.com, ♥♥email: anuraga.jayanegara@gmail.com
[2]Research Center for Animal Husbandry, National Research and Innovation Agency. Jl. Raya Jakarta-Bogor, Bogor 16911, West Java, Indonesia
[3]Department of Animal Production, Faculty of Animal Husbandry, Universitas Padjadjaran. Jl. Raya Bandung-Sumedang Km. 21, Sumedang 45363, West Java, Indonesia
[4]Division of Basic Veterinary Medicine, Faculty of Veterinary Medicine, Universitas Airlangga. Jl. Dr. Ir. H. Soekarno, Surabaya 60115, East Java, Indonesia

Manuscript received: 20 March 2025. Revision accepted: 20 July 2025.

Abstract. *Wahyuni S, Sudarman A, Sofyan A, Permata D, Darma ING, Ramdani D, Hidayatik N, Jayanegara A. 2025. Methane emission and nutritive evaluation of green, black, oolong, and white tea leaves. Biodiversitas 26: 3423-3433.* This study aimed to evaluate the impact of non-fermented and fermented leaves of green tea, black tea, oolong tea, and white tea on nutritive value, methane emission, and *in vitro* ruminal fermentation characteristics. Each type of tea leaves was prepared in both fresh (non-fermented) and fermented forms. Fermented tea leaves were treated with water in a 1:2 ratio. Each treatment was prepared in five replicates and incubated for 30 days. A completely randomized factorial design was applied, with tea type as the primary factor and fermentation status (non-fermented and fermented) as the secondary factor. Results revealed that the highest crude protein content was found in white tea (29.41%), followed by green tea (17.87%). There was a significant effect ($p<0.05$) of tea types on IVDMD and IVOMD, with fresh oolong tea exhibiting the highest value and white tea the lowest. Excellent fermentation profiles were observed among different tea types ($p<0.001$) regarding gas production after 48 hours, with fresh green tea producing the highest amount. The highest methane production was observed in fermented green tea (2.35%), while the lowest was in fermented oolong tea (0.89%). Fermented oolong tea as a feed additive in ruminant diets may reduce enteric methane production, potentially improving feed efficiency. In conclusion, fermentation is able to maintain the nutritional quality of tea leaves but the effect on methane emission is varied.

Keywords: Chemical composition, fermentation, in vitro rumen, methane emission, tea leaves

INTRODUCTION

The accumulation of various greenhouse gases, such as carbon dioxide (CO_2), methane (CH_4), and nitrous oxide (N_2O) has been considered as a primary factor responsible for global warming. Studies have indicated that the livestock sector significantly impacts environmental change (Rojas-Downing et al. 2017). Among livestock, ruminants, particularly dairy and beef cattle, contribute substantially to methane emissions, primarily through enteric fermentation in the rumen, with approximately 80% resulting from enteric fermentation and the remainder from manure management (Ouatahar et al. 2021). Methane from enteric fermentation accounts for 44% of the total greenhouse gas emissions from livestock (Kebreab et al. 2023). In addition, 2-5% of feed energy is lost due to CH_4 production in the livestock industry (Olijhoek et al. 2018).

To address this issue, various CH_4 mitigation strategies have been explored, including diet reformulation, animal breeding and management, improvement of forage quality, and rumen manipulation (Honan et al. 2021; Arndt et al. 2022; Beauchemin et al. 2022). Among these strategies, dietary interventions using plant-based feed additives rich in bioactive compounds, as anti-methanogenic, particularly natural polyphenols and tannins, has gained significant attention due to their ability to alter rumen microbial populations and reduce methanogenesis (Dijkstra et al. 2025). These additives, often administered in small quantities, have the potential to significantly reduce ruminal methanogenesis (Honan et al. 2021). For instance, a previous study by Molina-Botero et al. (2019) investigated the effect of polyphenols such as saponins and tannins on CH_4 production and rumen microbial populations. Moreover, Kondo et al. (2018) and Wang et al. (2022) observed plants rich in bioactive compounds as feed additives in ensiling diets, could alter rumen metabolism and inhibit methanogenesis.

Tea (*Camellia sinensis* (L.) Kuntze) is a rich source of bioactive compounds, including alkaloids, tannins, saponins, and catechins (Tshikhudo et al. 2019; Zeng et al. 2020). Tannins from tea leaves can inhibit protein degradation during ensiling and reduce CH_4 emissions by up to 30% (Cardoso-Gutierrez et al. 2021). Condensed tannins (CT) from tea have also been reported to reduce enteric CH_4 emissions in ruminants (Thang and Hiep 2020). Green and black tea leaves have also been reported to decrease methane production without affecting rumen degradability (Ramdani et al. 2022). Moreover, incorporation of various

tea leaves, including green, black, and vine tea leaves into ruminant diets enhances both ensiling performance and rumen fermentation characteristics (Lin et al. 2021; Zhang et al. 2021; Huang et al. 2022; Lin et al. 2022). Oolong tea is rich in bioactive compounds, including alkaloids, polyphenols (such as catechins, tannins, flavonols, flavonol glycosides), flavonoids, amino acids, protein, and polysacharides (Chen et al. 2003; Hosoda et al. 2003; Ng et al. 2018). Meanwhile, white tea primarily contains catechins such as (-)-epicatechin (EC), (-)-epigallocatechin (EGC), which are classified as flavonol monomers, along with (-) epicatechin 3 gallate (ECG) and (-)-epigallocatechin 3-gallate (EGCG) (Dias et al. 2013). Tea polyphenols, particularly catechin derivatives, have been widely recognized for their strong antioxidant and antimirobial activities.

Despite extensive research on the effects of green and black tea on ruminal fermentation and methane mitigation, the effects of oolong and white tea on methane mitigation remain largely unexplored. These types of tea undergo distinct processing techniques compared to green and black tea, which may significantly influence their bioactive compounds, which may consequently affect their methane mitigation properties. Moreover, fermentation can modify polyphenol composition and bioavailability (Yang et al. 2023). However, the comparative effects of fermented and non-fermented tea leaves on methane production and rumen fermentation characteristics have not been thoroughly investigated. This study aimed to evaluate the effects of fresh (non-fermented) and fermented leaves from four different tea types (i.e., green tea, black tea, oolong tea, and white tea) on methane emission, nutritive value, and in vitro rumen fermentation profiles. The findings from this research could support the development of innovative dietary strategies aimed at reducing methane emissions in ruminant livestock, thereby promoting sustainable and environmentally friendly livestock production systems.

MATERIALS AND METHODS

Ethical clearance approval

All procedures were in accordance with the standard of ethical procedure from the Commission of Ethical Clearance for Pre-clinical Experiment, Integrated Laboratory for Research and Testing (LPPT), Universitas Gadjah Mada, Yogyakarta, Indonesia (Approval No. 00004/04/LPPT/IV/2022).

Sample preparation

Tea leaves were obtained from the Indonesian Research Institute for Tea and Cinchona (PT. Perkebunan Nusantara III, Bandung, West Java, Indonesia; 7°08'35.5"S 107°30'55.9"E). The fresh tea leaves were initially plucked from *Camellia sinensis* var. *assamica* (J.W.Mast.) Kitam. plants from the same farm. Four different types of tea were selected and utilized in the present study, i.e., green tea, black tea, oolong tea, and white tea. The samples used in the research consisted of dried tea leaves, which were then processed into fermented and non-fermented (fresh).

These tea types were arranged in a factorial experimental design (2×4). The first factor was fermentation (F), consisting of non-fermented (N) and fermented (F) treatments. Meanwhile, the second factor was four tea types (P), which included green tea (G), black tea (B), oolong tea (O), and white tea (W) leaves. The full combined treatments were as follows: NGL, non-fermented green tea leaves; NBL, non-fermented black tea leaves; NOL, non-fermented oolong tea leaves; NWL, non-fermented white tea leaves; FGL, fermented green tea leaves; FBL, fermented black tea leaves; FOL, fermented oolong tea leaves; FWL, fermented white tea leaves. Data collection was carried out with five replications (N = 5). These tea leaves treatments are shown in Figure 1.

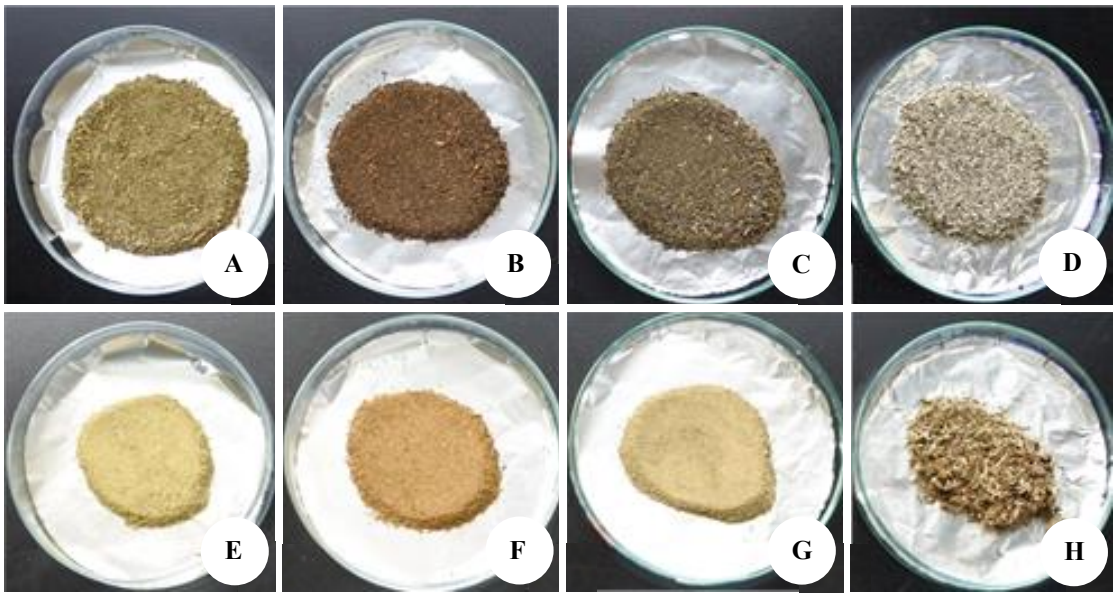

Figure 1. Tea leaves treatments after incubation for 30 days in the present study: A. NGL: Non-fermented Green tea Leaves, B. NBL: Non-fermented Black tea Leaves, C. NOL: Non-fermented Oolong tea Leaves, D. NWL: Non-fermented White tea Leaves, E. FGL: Fermented Green tea Leaves, F. FBL: Fermented Black tea Leaves, G. FOL: Fermented Oolong tea Leaves, H. FWL: Fermented White tea Leaves

Approximately 250 g of each tea leaf sample was vacuum-sealed and then packed into polyethylene plastic bags to remove air. In the treatment of non-fermented tea leaves (N), no water was added. Meanwhile, for fermented tea leaves (F), water was added in a 1:2 ratio being stored in the freezer (-20°C). Samples were stored in a closed container box lined with aluminium foil. Samples were ensiled under anaerobic conditions for 30 days. After 30 days of incubation, the plastic bags were opened, and each sample was divided into two parts with equal proportions. Then, samples were dried using freeze-dried and ground using a 1 mm sieve.

Rumen liquor collection

The Rumen Liquor (RL) for the in vitro trial was collected from two fistulated Bali cattle, each with a body weight of approximately 290 kg. They were around 3.5 years old and had been conditioned with a diet of 60% forage and 40% concentrate. After collection, the rumen contents were immediately filtered through two layers of cheesecloth over a funnel to obtain RL, transferred into a pre-warmed thermos, and brought to the laboratory. Rumen fluid was taken at approximately 250 mL per cow, resulting in a total of 500 mL of rumen fluid. Subsequently, a measured amount of each RL was transferred into a pre-warmed dark bottle containing a buffer solution while being kept in a water bath (39°C) to prepare buffered RL at a 1:2 ratio of RL to buffer solution. The bottles containing buffered RL were further purged with CO_2 to eliminate O_2 and sealed tightly.

Determination of nutritive value, methane emission and in vitro rumen fermentation

The chemical composition of the samples, including Dry Matter (DM), ash, Crude Protein (CP), and Crude Fiber (CF) was analyzed following the method of Association of Official Analytical Chemist (AOAC 2005). Chemical composition analysis was conducted using a composite method, in which 2 g were taken from each replication. The dry matter content was determined by drying approximately 1 g of sample at 105°C until a constant weight was achieved. The sample was burned at 600°C to determine the ash content. Crude protein was analyzed using the Kjeldahl method with a process of digestion, distillation, and titration, after which the protein content was calculated by multiplying the nitrogen content by a factor of 6.25.

The in vitro rumen fermentation procedure followed the method described by Theodorou et al. (1994). It is widely used in ruminant nutrition research to assess the fermentability and potential methane mitigation effects of various feedstuffs before advancing to in vivo trials. Approximately 0.6 g of each sample was weighed and placed into a 100 mL incubation bottle. Rumen fluid was obtained from two fistulated Bali cattle, filtered, and homogenized. After measuring the pH of the rumen fluid, it was mixed with McDougall's buffer at a 1:2 ratio, then flushed with CO_2. The buffer solution was prepared by mixing 5.7 g NA_2HPO_4, 6.2 g KH_2HPO_4, 0.6 g $MgSO_4$7.H_2O, 13.2 g CaCl2H2O, 10 g $MnCl_2$ 4.H_2O, 1 g $COCl_2$ 6.H_2O, 35 g $NaHCO_3$, 4 g $NaH_4.HCO_3$, 100 mg resazurin, 2 mL NaOH, 285 mg NA_2S 7.H_2O, and 1000 mL of distilled water. Subsequently, 50 mL of the buffer-rumen fluid mixture was added to each incubation bottle and flushed with CO_2. The incubation bottles were sealed with the rubber stoppers, metal caps, and stainless-steel clamps, and the samples were incubated in a waterbath at 39°C for 48 h.

After 48 h incubation, the liquor from each incubation bottle was centrifuged (Thermo Scientific, Heraeus-Megafuge) to separate the solid and supernatant fractions. The supernatant was analyzed for Volatile Fatty Acids (VFA) and ammonia (NH_3) content. The preparation for VFA analysis followed the method described by Luo et al. (2015). After 48 h incubation, the rumen liquid was centrifuged at 4,000×g for 10 minutes in 15 mL falcon tube. Then, 1 mL of supernatant that had been centrifuged was then added 0.2 mL of metaphosphoric acid and 40 µL of crotonic acid. The mixture samples were vortexed and incubated in 30 minutes at 4°C, then centrifuged at 12,000 rpm for 10 minutes. The samples was then filtered through 0.22 µm microporous membrane. A total of 1 mL of liquid was put into the GC-MS (Gas Chromatography-Mass Spectometry) vial and injected into GC-MS (Mega-Wax MS) for analysis.

Ammonia concentration was determined by using the Conway microdiffusion method (Galyean 1980). Vaseline was applied to the edges of the Conway plate and its lid. Rumen fluid, previously centrifuged at 3,000×g for 15 minutes, was placed in the Conway plate at a volume of 1 mL. An equal volume (1 mL) of NA_2CO_3 solution was added adjacent to the rumen fluid, then 1 mL of boric acid solution with indicator was placed in a small cup in the middle of Conway plate. The Conway plate is tightly closed and gently mixed so that the NA_2CO_3 solution with the supernatant is mixed. After 24 h at room temperature, the lid of the Conway was opened, and the boric acid solution was titrated with 0.05 N H_2SO_4 until the color changed to bright red. The ammonia concentration is calculated using a formula as follows: ammonia concentration (mM) = mL H_2SO_4 (results of titration) × N H_2SO_4 × 1.000. The protozoa population in the rumen fluid was determined by using a hemocytometer with MFS (Methyl Green Formalin) solution, following the method described by Ogimoto and Imai (1981). The MFS solution contained 35% formaldehyde solution (100 mL), 900 mL distilled water, 0.6 g methyl green, and 8 g of NaCl. A 1 mL sample of rumen fluid was mixed with 5 mL of MFS solution. The number of protozoa was counted using a sampling method, involving four squares, with each edge comprising 16 small squares in the hemocytometer. The number of protozoa per mL (N) was calculated as: N × 104 × dilution factor (Ogimoto and Imai 1981). Microbial protein content was analyzed according to Makkar et al. (1982). The pH of the rumen liquid was measured by using a pH meter that had been standardized with a buffer solutions of pH 4 and pH 7. To determine In Vitro Dry Matter Degradability (IVDMD) and In Vitro Organic Matter Degradability (IVOMD), the residue of the samples in the incubation bottles was dried in an oven at 105°C for 24 h and subsequently ashed in a furnace at 500°C to determine the dry matter and ash content, respectively.

Gas production was recorded at the hours of 0, 4, 8, 12, 24, 36, and 48 after incubation by using a syringe equipped

with a needle with a 50 mL volume (Jayanegara et al. 2019). After 24 h, measurement of methane production was performed by injecting the gas sample into a gas chromatography (GC-MS, Thermo Scientific). The data for gas and methane production were fitted using the exponential equation of Ørskov and McDonald (1979) as follows:

$$P = a + b\ (1\text{-}e^{-ct})$$

Where :
P : Cumulative gas production at time t (h)
a : Gas production from the immediate soluble fraction
b : Gas production from the insoluble fraction
c : Gas production rate constant for the insoluble fraction b
t : Incubation time (h)
(a + b) : The potential extent of gas production

Data analysis

All data of chemical composition and in vitro fermentability were organized in rows and columns in relevant spreadsheet of Microsoft Excel and carefully checked. Chemical composition parameters were analyzed based on composite samples (2 g from each of the five replications per treatment). All data from in vitro fermentability was analyzed using CoSTAT. The CoSTAT statistical program was used to investigate differences across mean treatments using a Two-Way Analysis of Variance (ANOVA) and posthoc Least Significant Difference (LSD) to compare the fermentation (F), the types of tea leaves (P) and their interaction from five replicates (Cohort 2022). Statistical significance between groups was marked at $p<0.05$ and tended to be significant at $p<0.1$.

RESULTS AND DISCUSSION

Chemical composition

The results of the proximate analysis are presented in Table 1, demonstrating that the fermentation process influenced the chemical composition of tea leaves. The Crude Protein (CP) in non-fermented (fresh) tea leaves ranged from 17.87% to 29.41% Dry Matter (DM), whereas in fermented tea leaves, ranged from 17.49% to 29.20% DM. Among the different tea types, the CP content was highest in white tea, followed by oolong tea, black tea, and green tea. Fermentation decreased the CP content in green tea, oolong tea, and white tea, except in black tea, where a slight increase was observed. Green tea had the highest CF content among all types of tea, reaching 16.24%. In contrast, oolong tea exhibited the lowest CF content in both non-fermented (fresh) and fermented treatments, with values of 12.41% and 9.96%, respectively.

In vitro rumen fermentation and methane emission

Table 2 presents the mean values of in vitro rumen measurement for non-fermented and fermented tea leaves of different types. The results indicate that the tea type factor (P) had a highly significant effect ($p<0.001$) on ruminal pH, whereas the fermentation factor (F) had no significant impact on ruminal pH. Fresh oolong tea exhibited the highest pH value, while fermented white tea had the lowest. In general, fermentation (F) decreased ruminal pH The tea type factor (P) also had a significant effect ($p<0.001$) on total gas production after 48 h of incubation. Among the different tea types, fresh green tea, black tea, and oolong tea exhibited higher total gas production compared to their fermented counterparts. However, in white tea, the fermented leaves produced more gas than the non-fermented (fresh) white tea. Among all tea types, green tea had the highest total gas production after 48 h of incubation, while white tea had the lowest.

Methane (CH_4) production was significantly affected by tea type factor (P) ($p<0.001$). The highest CH_4 production was observed in fermented green tea, whereas the lowest was recorded in fermented oolong tea (Figure 2). However, in vitro measurement indicate that fermentation (F) had no significant impact on total gas production, CH_4 production, In Vitro Degradability of Dry Matter (IVDMD), In Vitro Degradability of Organic Matter (IVOMD), ammonia concentration, and Volatile Fatty Acids (VFA). Although IVDMD and IVOMD were not significantly affected by fermentation factor (F), various tea types exhibited significant differences for both parameters ($p<0.05$). The ranking of tea types from the highest to the lowest IVDMD and IVOMD was as follows*:* oolong tea, green tea, black tea, and white tea. The highest dry matter and organic matter degradability values were observed in non-fermented (fresh) oolong tea, while the lowest values were recorded in fresh white tea.

Based on Table 2, pH fermentation had significant differences ($p<0.001$) influenced by both fermentation factor (F) and the tea type factor (P). Moreover, a highly significant interaction ($p<0.001$) was observed between fermentation (F) and tea type (P) on pH fermentation. This table indicates that fresh oolong tea had the highest pH value, whereas fermented white tea had the lowest. In general, the results show that fermentation factor (F) decreased ruminal pH across all tea types. The ranking of ruminal pH values, from the highest to the lowest, based on mean values, was as follows: oolong tea, green tea, white tea, and black tea.

Different tea types (P) had a highly significant effect ($p<0.001$) on propionic acid (C3) concentration and the acetate-to-propionate (C2:C3) ratio (Table 4). Acetic acid (C2) showed a significant effect ($p<0.05$) among different tea types. In addition, there was a significant effect ($p<0.05$) in the interaction between fermentation (F) and tea type (P) on the acetate-to-propionate ratio. The highest acetic acid (C2) concentration was observed in fermented oolong tea, while the lowest was found in fresh (non-fermented) black tea. However, fresh (non-fermented) black tea had the highest propionic acid (C3) concentration, whereas fresh oolong tea had the lowest. Regarding the acetic-to-propionate (C2:C3) ratio presented in Table 4, the highest ratio was observed in oolong tea, while the lowest was observed in black tea. This table also indicates that fermentation (F), tea types (P), and their interaction (F×P) had no significant effect on butyric acid concentration.

Table 1. Chemical composition of non-fermented and fermented various of tea leaves

Treatment	Component (%)			
	DM	Ash	CF	CP
NGL	87.07	5.67	16.24	17.87
NBL	85.07	5.63	13.31	22.30
NOL	89.14	5.66	12.41	25.23
NWL	87.00	4.94	13.11	29.41
FGL	33.62	5.47	14.89	17.49
FBL	26.29	4.96	10.87	22.49
FOL	26.38	4.66	9.96	24.86
FWL	29.94	4.12	12.18	29.20

Note: DM: Dry Matter, CF: Crude Fiber, CP: Crude Protein, NGL: Non-fermented Green tea Leaves, NBL: Non-fermented Black tea Leaves, NOL: Non-fermented Oolong tea Leaves, NWL: Non-fermented White tea Leaves, FGL: Fermented Green tea Leaves, FBL: Fermented Black tea Leaves, FOL: Fermented Oolong tea Leaves, FWL: Fermented White tea Leaves

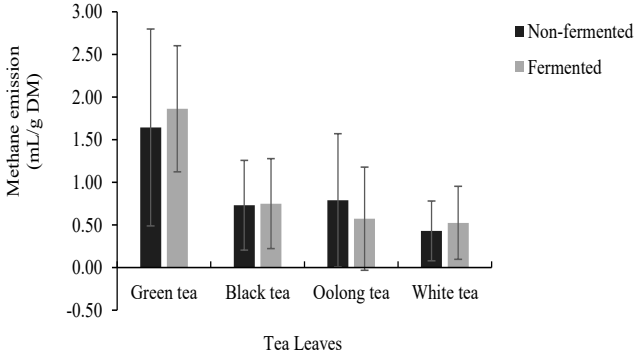

Figure 2. Methane emission of tea leaves treatments after incubation time

Table 2. Ruminal pH, total gas production, methane emission, and in vitro degradability of non-fermented and fermented tea leaves

Variable	Tea type				Mean ± SD	*p*-value		
	Green tea	Black tea	Oolong tea	White tea		F	P	F×P
Ruminal pH								
Non-fermented	6.8 ± 0.13^{b}	7.1 ± 0.11^{a}	6.8 ± 0.25^{b}	7.2 ± 0.19^{a}	5.6 ± 0.28	0,520^{ns}	<0.001	0,290^{ns}
Fermented	6.8 ± 0.15^{b}	7.2 ± 0.08^{a}	6.9 ± 0.16^{b}	7.1 ± 0.13^{a}	5.1 ± 0.24			
Mean±SD	6.8 ± 0.14	7.1 ± 0.10	6.9 ± 0.21	7.1 ± 0.16				
Total gas production (mL)								
Non-fermented	74.0 ± 18.65^{a}	48.6 ± 12.9^{b}	66.9 ± 17.68^{a}	38.4 ± 13.54^{b}	55.5 ± 2.88	0.530^{ns}	<0.001	0.873^{ns}
Fermented	71.9 ± 16.12^{a}	43.6 ± 8.46^{b}	57.8 ± 21.19^{a}	41.2 ± 14.89^{b}	54.0 ± 5.24			
Mean±SD	73.0 ± 17.39	46.1 ± 10.71	62.3 ± 19.43	39.8 ± 14.21				
CH_4 (%)								
Non-fermented	2.0 ± 0.95^{a}	1.3 ± 0.71^{b}	1.1 ± 0.87^{b}	1.0 ± 0.48^{b}	1.3 ± 0.82	0.636^{ns}	<0.001	0.881^{ns}
Fermented	2.4 ± 0.55^{a}	1.5 ± 0.81^{b}	0.9 ± 0.59^{b}	1.1 ± 0.66^{b}	1.5 ± 0.84			
Mean±SD	2.2 ± 0.75	1.4 ± 0.73	1.0 ± 0.71	1.0 ± 0.55				
IVDMD (%)								
Non-fermented	70.0 ± 5.53^{ab}	59.9 ± 7.11^{bc}	74.9 ± 13.18^{a}	52.0 ± 14.25^{c}	64.2 ± 13.40	0.409^{ns}	0.013*	0.588^{ns}
Fermented	69.7 ± 9.62^{ab}	64.7 ± 17.83^{bc}	72.2 ± 11.97^{a}	62.4 ± 5.21^{c}	67.2 ± 11.76			
Mean±SD	69.8 ± 7.40	62.3 ± 13.05	73.5 ± 11.96	57.2 ± 11.50				
IVOMD (%)								
Non-fermented	66.8 ± 3.11^{ab}	54.0 ± 5.61^{bc}	73.7 ± 13.70^{a}	45.3 ± 16.78^{c}	60.7 ± 14.83	0.490^{ns}	0.016*	0.377^{ns}
Fermented	64.6 ± 7.60^{ab}	59.9 ± 18.37^{bc}	68.8 ± 8.69^{a}	56.4 ± 4.82^{c}	62.4 ± 11.27			
Mean±SD	65.7 ± 5.60	56.9 ± 13.17	71.2 ± 11.12	51.5 ± 12.32				
Ammonia (mM)								
Non-fermented	29.4 ± 6.23	26.5 ± 4.30	34.0 ± 13.84	31.1 ± 3.54	30.2 ± 7.92	0.198^{ns}	0.264^{ns}	0.478^{ns}
Fermented	32.9 ± 7.38	22.0 ± 5.09	26.5 ± 10.93	25.9 ± 8.90	26.8 ± 8.65			
Mean±SD	31.2 ± 6.70	24.3 ± 5.04	30.3 ± 12.40	28.5 ± 6.94				
Total VFA (mM)								
Non-fermented	99.3 ± 102.11^{a}	78.7 ± 15.76^{ab}	77.5 ± 20.76^{ab}	80.7 ± 15.89^{b}	84.0 ± 20.24	0.522^{ns}	0.096^{ns}	0.684^{ns}
Fermented	104.9 ± 34.10^{a}	76.8 ± 33.17^{ab}	74.3 ± 5.72^{ab}	55.8 ± 6.80^{b}	78.0 ± 27.62			
Mean±SD	102.1 ± 28.53	77.8 ± 23.25	75.9 ± 13.73	68.2 ± 17.51				

Note: IVDMD: In Vitro Dry Matter Degradability, IVOMD: In Vitro Organic Matter Degradability, SD: Standard Deviation, F: Fermentation, P: Various types of tea leaves, F×P: Interaction between fermentation (F) and tea types (P), ^{abc}: Superscript with different letters indicates significant difference at p<0.05, ns: Non-significant

Table 3 shows that microbial protein content was significantly affected (p<0.001) by tea types (P). However, fermentation factor (F) had no significant effect on microbial protein content and protozoa population. Among the different tea types, the ranking of microbial protein content, from the highest to the lowest, was as follows: black tea, oolong tea, white tea, and green tea. The highest microbial protein content was observed in fermented black tea, while the lowest was observed in fermented green tea. Significant differences (p<0.001) were also observed among the tea types in the soluble fraction (a) of gas kinetics (Table 5). White tea exhibited the lowest rate in this parameter, whereas green tea had the highest. The potential degradable fraction (b) and total gas production kinetics (a+b) also

varied significantly ($p<0.001$) among tea types, with no significant effect observed for fermentation factor (F) or its interaction (Table 5). Black tea showed the lowest b kinetics, while white tea exhibited the lowest total gas production kinetics (a+b). However, oolong tea exhibited the highest b kinetics and total gas production (a+b).

Discussion

The fermentation process on tea leaves over a 30-day incubation period led to a decrease in Crude Protein (CP) content in green tea, oolong tea, and white tea. The present results are consistent with previous studies, which reported a reduction in CP content with the addition of different levels of green tea grounds (Xu et al. 2012) and a decline in CP content in green tea silage at inclusion levels of 5% and 10% (Kondo et al. 2018). However, in the present study, fermented black tea exhibited an increase in CP content (Table 1). This may be attributed to the length of the fermentation period, which aligns with the bacterial growth phase. Enhancing feed quality through fermentation technology can decrease fiber content while increasing protein levels. Fermentation changes the chemical structure of the substrate of complex compounds into simpler compounds, and eliminate anti-nutrient substances while improving the quality of the substrate (Suwignyo et al. 2015). The increase in CP content could be due to the secretion of extracellular enzymes (which are proteins by nature). In addition, as fermentation progresses, microbial biomass may increase, contributing to the observed rise in protein content. The CP content of fermented black tea increased from 22.30% to 22.49% after 30 days of incubation. These results support previous findings by Adejuwon et al. (2021) and Anyiam et al. (2023), who reported an increase in protein content upon the fermentation of fortified sweet potato and cassava.

Table 3. Microbial protein and protozoa population of non-fermented and fermented tea leaves

Variable	Tea type				Mean ± SD	*p*-value		
	Green tea	Black tea	Oolong tea	White tea		F	P	F×P
Microbial protein (mg/mL)								
Non-fermented	29.4 ± 4.98^{b}	51.2 ± 10.37^{a}	36.3 ± 8.20^{b}	24.2 ± 11.52^{b}	35.3 ± 8.77	0.813^{ns}	<0.001	0.158^{ns}
Fermented	23.9 ± 6.64^{b}	64.5 ± 17.59^{a}	25.2 ± 7.38^{b}	31.3 ± 4.54^{b}	36.2 ± 9.04			
Mean±SD	26.7 ± 5.81	57.9 ± 13.98	30.8 ± 7.79	27.8 ± 8.03				
Protozoa population (log/mL)								
Non-fermented	4.7 ± 0.07	4.8 ± 0.20	4.7 ± 0.28	4.6 ± 0.35	4.7 ± 0.22	0.486^{ns}	0.549^{ns}	0.346^{ns}
Fermented	4.7 ± 0.15	4.5 ± 0.29	4.7 ± 0.32	4.8 ± 0.12	4.7 ± 0.22			
Mean±SD	4.7 ± 0.11	4.6 ± 0.25	4.7 ± 0.30	4.7 ± 0.23				

Note: SD: Standard Deviation, F: Fermentation, P: Various types of tea leaves, F×P: Interaction between fermentation (F) and tea types (P), ^{abc}: Superscript with different letters indicates significant difference at $p<0.05$, ns: Non-significant

Table 4. pH, acetic acid (C2), propionic acid (C3), butiric acid (C3), and C2:C3 ratio of non-fermented and fermented tea leaves

Variable	Tea type				Mean ± SD	*p*-value		
	Green tea	Black tea	Oolong tea	White tea		F	P	F×P
pH								
Non-fermented	5.7 ± 0.04^{b}	5.2 ± 0.03^{b}	5.9 ± 0.03^{a}	5.6 ± 0.03^{c}	5.6 ± 0.03	<0.001	<0.001	<0.001
Fermented	5.1 ± 0.08^{b}	5.1 ± 0.31^{b}	5.2 ± 0.24^{a}	4.8 ± 0.12^{c}	5.1 ± 0.19			
Mean±SD	5.4 ± 0.06	5.1 ± 0.17	5.6 ± 0.13	5.2 ± 0.07				
C2 (%)								
Non-fermented	76.5 ± 0.36^{b}	75.7 ± 1.37^{c}	79.3 ± 0.73^{a}	79.0 ± 0.77^{ab}	77.6 ± 1.80	0.632^{ns}	0.030*	0.114^{ns}
Fermented	78.7 ± 1.21^{b}	76.1 ± 0.85^{c}	79.5 ± 2.60^{a}	77.2 ± 1.47^{ab}	77.9 ± 1.97			
Mean±SD	77.6 ± 0.79	75.9 ± 1.11	79.4 ± 1.66	78.1 ± 1.12				
C3 (%)								
Non-fermented	13.9 ± 1.55^{b}	15.8 ± 1.07^{a}	12.0 ± 0.24^{b}	12.2 ± 0.60^{b}	13.5 ± 1.80	0.800^{ns}	<0.001	0.650^{ns}
Fermented	12.3 ± 0.20^{b}	15.1 ± 0.68^{a}	12.5 ± 1.35^{b}	13.6 ± 0.80^{b}	13.4 ± 1.38			
Mean±SD	13.1 ± 0.87	15.4 ± 0.88	12.2 ± 0.80	12.9 ± 0.70				
C4 (%)								
Non-fermented	9.7 ± 1.33	8.6 ± 0.34	8.7 ± 0.70	8.8 ± 0.40	8.9 ± 0.81	0.648^{ns}	0.306^{ns}	0.636^{ns}
Fermented	9.0 ± 1.10	8.8 ± 0.67	8.1 ± 1.26	9.2 ± 0.68	8.8 ± 0.94			
Mean±SD	9.3 ± 1.21	8.7 ± 0.50	8.4 ± 0.98	9.0 ± 0.54				
C2:C3 (%)								
Non-fermented	5.6 ± 0.60^{a}	4.8 ± 0.42^{b}	6.6 ± 0.16^{a}	6.5 ± 0.39^{a}	5.9 ± 0.85	0.882^{ns}	<0.001	0.047*
Fermented	6.4 ± 0.18^{a}	5.1 ± 0.27^{b}	6.4 ± 0.86^{a}	5.7 ± 0.43^{a}	5.9 ± 0.74			
Mean±SD	6.0 ± 0.39	4.9 ± 0.34	6.5 ± 0.51	6.1 ± 0.41				

Note: SD: Standard Deviation, F: Fermentation, P: Various types of tea leaves, F×P: Interaction between fermentation (F) and tea types (P), ^{abc}: Superscript with different letters indicates significant difference at $p<0.05$; ns: Non-significant

Table 5. Dynamics of ruminal gas kinetics of non-fermented and fermented tea leaves

Variable	Tea type				Mean ± SD	*p*-value		
	Green tea	Black tea	Oolong tea	White tea		F	P	F×P
a (mL)								
Non-fermented	-2.8 ± 0.50^{b}	-0.5 ± 0.68^{a}	-2.1 ± 0.42^{b}	0.3 ± 0.73^{a}	-1.2 ± 0.58	0.607^{ns}	<0.001	0.443^{ns}
Fermented	-2.5 ± 0.91^{b}	-0.6 ± 0.34^{a}	-2.0 ± 1.77^{b}	-0.5 ± 0.42^{a}	-1.4 ± 0.86			
Mean±SD	-2.6 ± 0.71	-0.5 ± 0.51	-2.1 ± 1.09	-0.1 ± 0.57				
b (mL)								
Non-fermented	161.6 ± 34.44^{a}	72.2 ± 20.12^{b}	187.3 ± 44.16^{a}	79.8 ± 32.47^{b}	125.2 ± 32.80	0.041	<0.001	0.504
Fermented	134.7 ± 33.41^{a}	77.3 ± 10.04^{b}	138.3 ± 46.26^{a}	73.2 ± 23.65^{b}	105.9 ± 28.34			
Mean±SD	148.2 ± 33.92	74.8 ± 15.08	162.8 ± 45.21	76.5 ± 28.06				
c (mL)								
Non-fermented	0.0 ± 0.01	0.0 ± 0.00	0.0 ± 0.01	0.0 ± 0.01	0.0 ± 0.01	0.108^{ns}	0.182^{ns}	0.931^{ns}
Fermented	0.0 ± 0.01	0.0 ± 0.01	0.0 ± 0.01	0.0 ± 0.01	0.0 ± 0.01			
Mean±SD	0.0 ± 0.01	0.0 ± 0.01	0.0 ± 0.01	0.0 ± 0.01				
a+b (mL)								
Non-fermented	158.8 ± 34.14^{a}	86.2 ± 19.58^{b}	185.2 ± 43.94^{a}	80.1 ± 32.09^{b}	127.6 ± 32.44	0.037^{ns}	<0.001	0.502^{ns}
Fermented	132.3 ± 32.60^{a}	76.8 ± 9.75^{b}	136.3 ± 44.50^{a}	72.7 ± 23.66^{b}	104.5 ± 27.63			
Mean±SD	145.5 ± 33.37	81.5 ± 14.66	160.8 ± 44.22	76.4 ± 27.88				

Note: SD: Standard Deviation, F: Fermentation, P: Various types of tea leaves, F×P: Interaction between fermentation (F) and tea types (P), ^{abc} Superscript with different letters indicates significant difference at $p<0.05$, ns: Non-significant

Green tea, black tea, oolong tea, and white tea undergo different processing methods, including oxidation or fermentation, which affect in vitro digestibility. As shown in Table 2, In Vitro Dry Matter Digestibility (IVDMD) was highest in oolong tea; however, it decreased by 3.68% after fermentation. This decline may be attributed to the presence of tannins in tea, which bind to proteins and inhibit digestibility, negatively impacting rumen microbes. The presence of tannins in the rumen can reduce digestibility by limiting microbial degradation of protein and impairing protein solubilization. A higher protein content generally enhances microbial degradation efficiency. Similar to IVDMD, the highest In Vitro Organic Matter Degradability (IVOMD) was observed in fresh oolong tea (Table 2). However, IVOMD also followed the same pattern as IVDMD, decreasing after fermentation. Table 2 shows that white tea had the lowest values for both IVDMD and IVOMD. This may be due to the effects of tannins and saponins in tea, which have been associated with reduced protozoa counts (Jayanegara et al. 2020). Previous studies have reported that plants rich in tannins and saponins as feed additives lower IVDMD and IVOMD, particularly at inclusion levels of 300 mg/g in Napier grass and 700 mg/g in concentrate, or 700 mg/g in Napier grass and 300 mg/g in concentrate. Furthermore, Junior et al. (2017) showed that the addition of tannins can improve ruminal fermentation efficiency. Apart from their beneficial effects for ruminants, tannins may also generate positive effects on production performance and gut health of poultry when administered at low to moderate levels (Hidayat et al. 2021).

Fermentation (F) and tea types (P) had no significant effects on ammonia concentration or total Volatile Fatty Acids (VFA) (Table 2). Protein sources that enter the rumen are broken down by proteolytic bacteria into ammonia (NH_3), which is subsequently utilized by rumen microbes for microbial protein synthesis and serves as a nitrogen source for ruminant productivity (Hackmann and Firkins 2015). However, excess of rumen ammonia concentration is primarily excreted into urine (Schuba et al. 2017). Ammonia concentrations in green tea, black tea, oolong tea, and white tea ranged from 24.48 to 34.00 mM. According to McDonald et al. (2002), the optimal NH_3 concentration for supporting microbial growth in the rumen is between 6 up to 12 mM. In the present study, NH_3 values exceeded this normal range, but no adverse effects on microbial survival in the rumen liquid were observed. Ammonia is a byproduct of protein metabolism in the rumen, and its concentration is correlated with the extent of protein degradation. Fresh oolong tea produced the highest ammonia concentration, whereas fermented black tea produced the lowest. A higher protein content enhances microbial activity, leading to increased protein degradation. In addition to protein solubility, other factors influencing ammonia production include the proportion of available carbohydrates. Strach is a readily fermentable carbohydrate that provides energy for rumen microbes, enabling them to synthesize microbial protein more efficiently (Mulakala et al. 2022).

Volatile Fatty Acids (VFAs) are the final products of carbohydrate fermentation and serve as the primary energy source for ruminants. As shown in Table 2, the total VFA concentration ranged from 55.8 mM to 104.9 mM, while the normal range reported by McDonald et al. (2002) is 70 mM to 150 mM. This indicates that the total VFA concentration in this study was below the normal range. VFA production reflects the fermentability of feed in the rumen. Factors influencing VFA production include microbial population dynamics during fermentation and the content of readily degradable carbohydrates in feed (Lukitawesa et al. 2020). An increase in VFA concentration is associated with enhanced digestion and higher ammonia concentrations in the rumen (Nasrollahi et al. 2019). Acetic acid is the final product of fiber fermentation, while the proportion of VFA composition typically consists of 70% acetic acid, 19% propionic acid, and 9% butyric acid. These findings are consistent with the report of Wang et al. (2022), which states that VFA composition in the rumen fluid ranges between 60-70% acetic acid, 15-20% propionic

acid, and 10-15% butyric acid. The ratio of acetic acid to propionic acid (C2:C3) serves as an indicator of energy utilization efficiency and fermentation quality. A higher C2:C3 ratio suggests that rumen fermentation favors acetic acid production, whereas a lower C2:C3 ratio indicates a shift towards propionic acid production. The increase in the C2:C3 ratio in the current study may be attributed to the fibrous nature of the feed, which promotes formation of acetic acid (He et al. 2024). A high C2:C3 ratio indicated lower feed efficiency and energy utilization. The highest microbial protein content was observed in black tea, while the lowest was found in green tea. High microbial protein reflects optimal rumen microbial fermentation activity, supporting the efficiency of protein synthesis from feed ingredients. Different types of tea exhibit varying contributions to microbial protein synthesis, possibly due to variations in bioactive compounds such as tannins and polyphenols, which influence rumen microbial activity. Meanwhile, the number of protozoa is influenced by feed types, age, the animal species. Protozoa are anaerobic organisms, therefore, elevated oxygen levels and excessively high rumen pH can lead to their demise. The range of rumen pH typically falls between 5.55 to 7.5, depending on the basal components of the provided feed, and protozoa will not survive at pH levels below 5.4.

Regarding the in vitro rumen fermentation profiles, the fermentation process from all different types of tea resulted in normal pH (6.8-7.2). This result indicated that none of the observed treatments adversely affected fermentation activity in the rumen (Santoso et al. 2020). Total gas production is primarily a result of substrate fermentation by rumen microbes. Fresh green tea exhibited the highest in vitro gas production among the treatments (74 mL), while fresh white tea produced the lowest (38.4 mL) (Table 2). This suggests that green tea has higher nutritive potential compared to black tea, oolong tea, and white tea. Similarly, previous studies by Kondo et al. (2014) reported that gas production from green tea silage was higher than that from black tea silage. Although fermentation did not significantly affect gas production, reductions were observed in green tea, black tea, and oolong tea. Gas production is a byproduct of microbial fermentation in the rumen. Table 2 presents in vitro gas production from different types of tea, both before and after fermentation. Gas production tended to be higher in fresh (non-fermented) tea than in fermented tea. This reduction may be attributed to the loss of fermentable substrates for ruminal gas production during the fermentation process. In this study, gas production was higher in green tea compared to other tea types, which is consistent with findings by Kondo et al. (2004, 2006). Green tea is generally recognized for its higher polyphenol content than other types of tea, particularly catechins like EGCG, which are known for their antioxidant properties (Kim et al. 2014; Tang et al. 2019). Several studies have reported that tannin suppress the activity of ruminal bacteria (Besharati et al. 2022) and reduce ruminal in vitro gas production (Jayanegara et al. 2015). Furthermore, the reduction in gas production after fermentation may be due to oxidation during tea processing, tannin content, and antimicrobial activity. Oxidation occurs most extensively in black tea (full-fermented), followed by oolong tea (partially oxidized or semi-fermented), while green tea undergoes no fermentation during its production. Apart from oxidation, the lower gas production observed in white tea may be due to its higher tannins content compared to the other tea types, which may have allowed for increased gas production. Based on several studies, white tea contains the highest tannin level (26,68%), followed by green tea (21.11%), black tea (3.44%), and oolong tea (8.66%) (Orak et al. 2013; Khasnabis et al. 2015). The higher concentration of tannins in white tea may have contributed to reduced microbial activity in the rumen, thereby limiting fermentation and suppressing gas production. However, the results did not indicate any detrimental effects of green tea, black tea, and oolong tea on gas production. This suggests that tannin content in white tea was too low to significantly suppress rumen bacterial activity. In addition, the proportion of fermentation products depends on feed composition, particularly Crude Protein (CP) content. However, most CP is degraded into amino acids and ammonia, which contributes to less gas production. Fresh green tea may contain more degradable protein than black tea, oolong tea, and white tea, as indicated by its lower CP content. In addition, gas production in the in vitro rumen incubation system primarily results from nutrient degradation and fermentation, particularly of carbohydrates (Jayanegara et al. 2019).

Ruminal methane production was evaluated by correlating gas production parameters with the chemical composition of various tea types. In this study, different types of tea affected enteric methane NH_4 emission, with oolong tea exhibiting a reduction in methanogenesis after fermentation. The reduction may be attributed to the inhibitory activity of flavonoids, polyphenols, catechins, tannins, and saponins, which interfere with methanogenesis, particularly by suppressing methanogenic Archaea and protozoa (Teng et al. 2024). Jayanegara et al. (2020) also reported that NH_4 production decreases with increasing dietary tannins and polyphenols, which inhibit methanogen populations and reduce protozoa counts. Similarly, Kolling et al. (2018), suggested that catechins in green tea acts as a hydrogen sink under anaerobic rumen conditions, competing with methanogens and ultimately reducing NH_4 production. Table 2 shows that different types of tea can reduce NH_4 emissions, categorized as follows: oolong tea, white tea, black tea, and green tea. In the present experiment, we observed that oolong tea may have reduced NH_4 emissions due to antimicrobial activity or by modifying the abundance of methanogens and other microorganisms involved in NH_4 production. The active compounds in tea leaves may influence ruminal protein fermentation either by reducing the number of bacteria associated with protein degradation or by affecting ruminal ammonia production. In this study, the results differed from those of a previous study by Kolling et al. (2018), which reported that green tea reduced NH_4 production. Green tea can reduce methane production in ruminants, particularly in the rumen, by inhibiting protozoa and methanogenic archaea, thus altering the rumen fermentation pattern. Green tea extracts contain compounds like catechins, alkaloids, and saponins, which have antiprotozoal and antimicrobial effects, potentially reducing

the population of methane-producing microorganisms (Kolling et al. 2018). However, in this study, green tea increased NH_4 production. Moreover, Guyader et al. (2017) found that different levels of tea saponins (0.1 g/L and 0.5 g/L) reduced CH_4 5.8% and 29%, respectively. The results of the current study showed a lower methane reduction from green tea, black tea, oolong tea, and white tea compared with the findings of previous studies (Guyader et al. 2017). Several studies have also suggested that the initial methane-suppressing effect of saponins is transient (Jayanegara et al. 2014; Chaudhary et al. 2018; Ramos-Morales et al. 2019). Then, a new balance is established between hydrogen producers other than protozoa and methanogens when protozoa are reduced or eliminated by saponins.

Further research is needed to investigate the effect of oolong tea on methanogen reduction and to clarify the mechanism by which methane production is inhibited. In the current study, the dynamics of ruminal gas production kinetics between non-fermented and fermented tea after 48 h in vitro were significantly different among tea types. The lowest gas production from the immediately soluble fraction (a) was observed in fresh green tea (-2.8 mL), with potential total gas production (a+b) of 158.8 mL. Moreover, the lowest gas fraction (b) was observed in fresh black tea (72.2 mL), likely due to its high tannin content, which reduces the gas production rate constant for the tea insoluble fraction. The lower gas production of the soluble and insoluble substrate fractions seems likely attributable to the high tannin content in the green tea. However, the tannin content of the observed tea was not quantified in this study.

In conclusion, this study demonstrates that tea leaves, depending on their type and fermentation status, exhibit varying capacities to influence ruminal fermentation characteristics. The different tea types significantly affected in vitro dry matter and organic matter degradability, ruminal pH, volatile fatty acid composition, microbial protein synthesis, and methane production. Fermentation, while maintaining the nutritional integrity of tea leaves, exerted a more pronounced effect on fermentation pH than on digestibility parameters. Importantly, the inclusion of fermented oolong tea resulted in the most notable reduction in methane emission (approximately 16.82%), whereas fermented green tea led to an increase, emphasizing the divergent responses among tea types to fermentation. These findings highlight the potential of tea leaves for contributing both to improved nutritional value and environmental sustainability. The lowest methane production was in fermented oolong tea (0.89%). Fermented oolong tea as a feed additive in ruminant diets may reduce enteric methane production, potentially improving feed efficiency. However, the inconsistent effect of fermentation on methane mitigation calls for further research to elucidate the bioactive mechanisms involved and optimize the application of tea leaves in ruminant diets for reducing greenhouse gas emissions throughout the in vivo testing research.

ACKNOWLEDGEMENTS

The authors are grateful to the *Riset Kolaborasi Indonesia* (RKI) Projects for the financial support through the RKI Projects research grant, year 2023-2024.

REFERENCES

Adejuwon KP, Osundahunsi OF, Akinola SA, Oluwamukomi MO, Mwanza M. 2021. Effect of fermentation on nutritional quality, growth and hematological parameters of rats fed sorghum-soybean-orange flesh sweet potato complementary diet. Food Sci Nutr 9 (2): 639-650. DOI: 10.1002/fsn3.2013.

Anyiam PN, Nwuke CP, Uhuo EN, Ije UE, Salvador EM, Mahumbi BM, Boyiako BH. 2023. Effect of fermentation time on nutritional, antinutritional factors and in-vitro protein digestibility of macrotermes nigeriensis-cassava mahewu. Meas Food 11: 100096. DOI: 10.1016/j.meafoo.2023.100096.

AOAC [Association of Official Analytical Chemists]. 2005. Official Methods of Analysis (18th ed). Association of Official Analytical Chemists, Gaithersburg, MD, USA.

Arndt C, Hristov AN, Price WJ et al. 2022. Full adoption of the most effective strategies to mitigate methane emissions by ruminants can help meet the 1.5°C target by 2030 but not 2050. Proc Natl Acad Sci USA 119 (20): e2111294119. DOI: 10.1073/pnas.2111294119.

Beauchemin KA, Ungerfeld EM, Abdalla AL et al. 2022. Invited review: Current enteric methane mitigation options. J Dairy Sci 105 (12): 9297-9326. DOI: 10.3168/jds.2022-22091.

Besharati M, Maggiolino A, Palangi V, Kaya A, Jabbar M, Eseceli H, De Palo P, Lorenzo JM. 2022. Tannin in ruminant nutrition: Review. Molecules 27 (23): 8273. DOI: 10.3390/molecules27238273.

Cardoso-Gutierrez E, Aranda-Aguirre E, Robles-Jimenez LE, Castelán-Ortega OA, Chay-Canul AJ, Foggi G, Angeles-Hernandez JC, Vargas-Bello-Pérez E, González-Ronquillo M. 2021. Effect of tannins from tropical plants on methane production from ruminants: A systematic review. Vet Anim Sci 14: 100214. DOI: 10.1016/j.vas.2021.100214.

Chaudhary SK, Rokade JJ, Aderao GN, Singh A, Gopi M, Mishra A, Raje K. 2018. Saponin in poultry and monogastric animals: A review. Intl J Curr Microbiol Appl Sci 7 (7): 3218-3225. DOI: 10.20546/ijcmas.2018.707.375.

Chen C-N, Liang C-M, Lai J-R, Tsai Y-J, Tsay J-S, Lin J-K. 2003. Capillary electrophoretic determination of theanine, caffeine, and catechins in fresh tea leaves and oolong tea and their effects on rat neurosphere adhesion and migration. J Agric Food Chem 51 (25): 7495-7503. DOI: 10.1021/jf034634b.

Cohort. 2022. CoSTAT Version 6.45 Copyright 1998-2022 Cohort Software 798. Lighthouse Ave, Monterey, California.

Dias TR, Tomás G, Teixeira NF, Alves MG, Oliveira PF, Silva BM. 2013. White tea (*Camellia Sinensis* (L.)): Antioxidant properties and beneficial health effects. Intl J Food Sci Nutr Diet 2 (2): 19-26. DOI: 10.19070/2326-3350-130005.

Dijkstra J, Bannink A, Congio GFS, Ellis JL, Eugène M, Garcia F, Niu M, Vibart RE, Yáñez-Ruiz DR, Kebreab E. 2025. Feed additives for methane mitigation: Modeling the impact of feed additives on enteric methane emission of ruminants-Approaches and recommendations. J Dairy Sci 108 (1): 356-374. DOI: 10.3168/jds.2024-25049.

Galyean ML. 1980. Laboratory Procedures in Animals Nutrition Research. Department of Animal and Food Sciences. Texas Tech University, Lubbock.

Guyader J, Eugène M, Doreau M, Morgavi DP, Gérard C, Martin C. 2017. Tea saponin reduced methanogenesis in vitro but increased methane yield in lactating dairy cows. J Dairy Sci 100 (3): 1845-1855. DOI: 10.3168/jds.2016-11644.

Hackmann TJ, Firkins JL. 2015. Maximizing efficiency of rumen microbial protein production. Front Microbiol 6: 465. DOI: 10.3389/fmicb.2015.00465.

He C, Zhou J, Li Y, Zhang D, Ntezimana B, Zhu J, Wang X, Xu W, Wen X, Chen Y, Yu Z, Wang Y, Ni D. 2023. The aroma characteristics of oolong tea are jointly determined by processing mode and tea cultivars. Food Chem X 18: 100730. DOI: 10.1016/j.fochx.2023.100730.

He S, Zhang R, Wang R, Wu D, Dai S, Wang Z, Chen T, Mao H, Li Q. 2024. Responses of nutrient utilization, rumen fermentation and

microorganisms to different roughage of dairy buffaloes. BMC Microbiol 24: 188. DOI: 10.1186/s12866-024-03342-0.

Hidayat C, Irawan A, Jayanegara A, Sholikin MM, Prihambodo TR, Yanza YR, Wina E, Sadarman S, Krisnan R, Isbandi I. 2021. Effect of dietary tannins on the performance, lymphoid organ weight, and amino acid ileal digestibility of broiler chickens: A meta-analysis. Vet World 14 (6): 1405-1411. DOI: 10.14202/vetworld.2021.1405-1411.

Honan M, Feng X, Tricarico JM, Kebreab E. 2021. Feed additives as a strategic approach to reduce enteric methane production in cattle: Modes of action, effectiveness and safety. Anim Prod Sci 62 (14): 1303-1317. DOI: 10.1071/an20295.

Hosoda K, Wang M-F, Liao M-L, Chuang C-K, Iha M, Clevidence B, Yamamoto S. 2003. Antihyperglycemic effect of oolong tea in type 2 diabetes. Diabetes Care 26: 1714-1718. DOI: 10.2337/diacare.26.6.1714.

Huang Y, Qiu C, Wang Y, Zhang W, He L. 2022. Effect of tea polyphenols on the fermentation quality, protein preservation, antioxidant capacity and bacterial community of stylo silage. Front Microbiol 13: 993750. DOI: 10.3389/fmicb.2022.993750.

Jayanegara A, Makkar HPS, Becker K. 2015. Addition of purified tannin sources and polyethylene glycol treatment on methane emission and rumen fermentation in vitro. Media Peternakan 38 (1): 57-63. DOI: 10.5398/medpet.2015.38.1.57.

Jayanegara A, Wina E, Takahashi J. 2014. Meta-analysis on methane mitigating properties of saponin-rich sources in the rumen: Influence of addition levels and plant sources. Asian-Australas J Anim Sci 27 (10): 1426-1435. DOI: 10.5713/ajas.2014.14086.

Jayanegara A, Yaman A, Khotijah L. 2019. Reduction of proteolysis of high protein silage from *Moringa* and *Indigofera* leaves by addition of tannin extract. Vet World 12 (2): 211-217. DOI: 10.14202/vetworld.2019.211-217.

Jayanegara A, Yogianto Y, Wina E, Sudarman A, Kondo M, Obitsu T, Kreuzer M. 2020. Combination effects of plant extracts rich in tannins and saponins as feed additives for mitigating in vitro ruminal methane and ammonia formation. Animals 10: 1531. DOI: 10.3390/ani10091531.

Junior FP, Cassiano ECO, Martins MF, Romero LA, Zapata DCV, Pinedo LA, Marino CT, Rodrigues PHM. 2017. Effect of tannins-rich extract from *Acacia mearnsii* or monensin as feed additives on ruminal fermentation efficiency in cattle. Livest Sci 203: 21-29. DOI: 10.1016/j.livsci.2017.06.009.

Kebreab E, Bannink A, Pressman EM, Walker N, Karagiannis A, van Gastelen S, Dijkstra J. 2023. A meta-analysis of effects of 3-nitrooxypropanol on methane production, yield, and intensity in dairy cattle. J Dairy Sci 106 (2): 927-936. DOI: 10.3168/jds.2022-22211.

Khasnabis J, Rai C, Roy A. 2015. Determination of tannin content by titrimetric method from different types of tea. J Chem Pharm Res 7 (6): 238-241.

Kim H-S, Quon MJ, Kim J-A. 2014. New insights into the mechanisms of polyphenols beyond antioxidant properties; lessons from the green tea polyphenol, epigallocatechin 3-gallate. Redox Biol 2 (1): 187-195. DOI: 10.1016/j.redox.2013.12.022.

Kolling GJ, Stivanin SCB, Gabbi AM, Machado FS, Ferreira AL, Campos MM, Tomich TR, Cunha CS, Dill SW, Pereira LGR, Fischer V. 2018. Performance and methane emissions in dairy cows fed oregano and green tea extracts as feed additives. J Dairy Sci 101 (5): 4221-4234. DOI: 10.3168/jds.2017-13841.

Kondo M, Kita K, Yokota H-O. 2004. Effects of tea leaf waste of green tea, oolong tea, and black tea addition on sudangrass silage quality and in vitro gas production. J Sci Food Agric 84 (7): 721-727. DOI: 10.1002/jsfa.1718.

Kondo M, Kita K, Yokota H-O. 2006. Evaluation of fermentation characteristics and nutritive value of green tea waste ensiled with byproducts mixture for ruminants. Asian-Australas J Anim Sci 19 (4): 533-540. DOI: 10.5713/ajas.2006.533.

Kondo M, Hirano Y, Kita K, Jayanegara A, Yokota H-O. 2014. Fermentation characteristics, tannin contents and in vitro ruminal degradation of green tea and black tea by-products ensiled at different temperatures. Asian-Australas J Anim Sci 27 (7): 937-945. DOI: 10.5713/ajas.2013.13387.

Kondo M, Hirano Y, Kita K, Jayanegara A, Yokota H-O. 2018. Nutritive evaluation of spent green and black tea leaf silages by in vitro gas production characteristics, ruminal degradability and post-ruminal digestibility assessed with inhibitory activity of their tannins. Anim Sci J 89 (12): 1656-1662. DOI: 10.1111/asj.13106.

Lin H, Lin S, Awasthi MK, Wang Y, Xu P. 2021. Exploring the bacterial community and fermentation characteristics during silage fermentation of abandoned fresh tea leaves. Chemosphere 283: 131234. DOI: 10.1016/j.chemosphere.2021.131234.

Lin S, Huang H, Zheng J, Lin H, Wang Y, Xu P. 2022. Microbial enrichment evaluation during the fermentation of ensiling pruned branches from tea plants. Intl J Food Microbiol 374: 109742. DOI: 10.1016/j.ijfoodmicro.2022.109742.

Lukitawesa, Patinvoh RJ, Millati R, Sárvári-Horváth I, Taherzadeh MJ. 2020. Factors influencing volatile fatty acids production from food wastes via anaerobic digestion. Bioengineered 11 (1): 39-52. DOI: 10.1080/21655979.2019.1703544.

Luo C, Cai S, Jia L, Tang X, Zhang R, Jia G, Li H, Tang J, Liu G, Wu C. 2015. Study on accurate determination of volatile fatty acids in rumen fluid by capillary gas chromatography. In: 5th International Conference on Information Engineering for Mechanics and Materials. DOI: 10.2991/icimm-15.2015.73.

Makkar HPS, Sharma OP, Dawra RK, Negi SS. 1982. Simple Determination of microbial protein in rumen liquor. J Dairy Sci 65 (11): 2170-2173. DOI: 10.3168/jds.S0022-0302(82)82477-6.

McDonald P, Edwards R, Morgan CA, Greenhalgh JFD. 2002. Animal Nutrition. 6th eds. Pearson India.

Molina-Botero IC, Arroyave-Jaramillo J, Valencia-Salazar S, Barahona-Rosales R, Aguilar-Pérez CF, Burgos AA, Arango J, Ku-Vera JC. 2019. Effects of tannins and saponins contained in foliage of *Gliricidia sepium* and pods of *Enterolobium cyclocarpum* on fermentation, methane emissions and rumen microbial population in crossbred heifers. Anim Feed Sci Technol 251: 1-11. DOI: 10.1016/j.anifeedsci.2019.01.011.

Mulakala BK, Smith KM, Snider MA, Ayers A, Honan MC, Greenwood SL. 2022. Influence of dietary carbohydrate profile on the dairy cow rumen meta-proteome. J Dairy Sci 105 (10): 8485-8496. DOI: 10.3168/jds.2022-21812.

Nasrollahi SM, Zali A, Ghorbani GR, Khani M, Maktabi H, Beauchemin KA. 2019. Effects of increasing diet fermentability on intake, digestion, rumen fermentation, blood metabolites and milk production of heat-stressed dairy cows. Animal 13 (11): 2527-2535. DOI: 10.1017/S1751731119001113.

Ng K-W, Cao Z-J, Chen H-B, Zhao Z-Z, Zhu L, Yi T. 2018. Oolong tea: A critical review of processing methods, chemical composition, health effects, and risk. Crit Rev Food Sci Nutr 58 (17): 2957-2980. DOI: 10.1080/10408398.2017.1347556.

Ogimoto K, Imai S. 1981. Atlas of Rumen Microbiology. Japan Science Society Press, Tokyo.

Olijhoek DW, Løvendahl P, Lassen J, Hellwing ALF, Höglund JK, Weisbjerg MR, Noel SJ, McLean F, Højberg O, Lund P. 2018. Methane production, rumen fermentation, and diet digestibility of Holstein and Jersey dairy cows being divergent in residual feed intake and fed at 2 forage-to-concentrate ratios. J Dairy Sci 101 (11): 9926-9940. DOI: 10.3168/jds.2017-14278.

Orak H, Yagar H, Isbilir S, Demirci A, Gumus T. 2013. Antioxidant and antimicrobial activities of white, green and black tea extracts. Acta Alimentaria 42 (3): 379-389. DOI: 10.1556/AAlim.2013.2222.

Ørskov ER, McDonald I. 1979. The estimation of protein degradability in the rumen from incubation measurements weighted according to the rate of passage. J Agric Sci Cambridge 92 (2): 499-503. DOI: 10.1017/S0021859600063048.

Ouatahar L, Bannink A, Lanigan G, Amon B. 2021. Modelling the effect of feeding management on greenhouse gas and nitrogen emissions in cattle farming systems. Sci Total Environ 776: 145932. DOI: 10.1016/j.scitotenv.2021.145932.

Ramdani D, Jayanegara A, Chaudhry AS. 2022. Biochemical properties of black and green teas and their insoluble residues as natural dietary additives to optimize in vitro rumen degradability and fermentation but reduce methane in sheep. Animals 12 (3): 305. DOI: 10.3390/ani12030305.

Ramos-Morales E, Lyons L, De La Fuente G, Braganca R, Newbold CJ. 2019. Not all saponins have a greater antiprotozoal activity than their related sapogenins. FEMS Microbiol Lett 366 (13): fnz144. DOI: 10.1093/femsle/fnz144.

Rojas-Downing MM, Nejadhashemi AP, Harrigan T, Woznicki SA. 2017. Climate change and livestock: Impacts, adaptation, and mitigation. Clim Risk Manag 16: 145-163. DOI: 10.1016/j.crm.2017.02.001.

Santoso B, Widayati TW, Hariadi BT. 2020. Improvement of fermentation and the in vitro digestibility characteristics of agricultural waste-based complete feed silage with cellulase enzyme treatment. Adv Anim Vet Sci 8 (8): 873-881. DOI: 10.17582/journal.aavs/2020/8.8.873.881.

Schuba J, Sudekum KH, Pfeffer E, Jayanegara A. 2017. Excretion of faecal, urinary urea and urinary non-urea nitrogen by four ruminant species as influenced by dietary nitrogen intake: A meta-analysis. Livest Sci 198: 82-88. DOI: 10.1016/j.livsci.2017.01.017.

Suwignyo B, Munawaroh LL, Budisatria IGS. 2015. Effect of material and fermentation time on quality and digestibility of complete feed, average daily gain of bligon goat and Farmer's income. J Indones Trop Anim Agric 40 (1): 23-30. DOI: 10.14710/jitaa.40.1.23-30.

Tang G-Y, Meng X, Gan R-Y, Zhao C-N, Liu Q, Feng Y-B, Li S, Wei X-L, Atanasov AG, Corke H, Li H-B. 2019. Health functions and related molecular mechanisms of tea components: An update review. Intl J Mol Sci 20 (24): 6196. DOI: 10.3390/ijms20246196.

Teng Z, Liu S, Zhang L, Zhang L, Liu S, Fu T, Zhang N, Gao T. 2024. Tea polyphenols inhibit methanogenesis and improve rumen epithelial transport in dairy cows. Animals 14 (17): 2569. DOI: 10.3390/ani14172569.

Thang CM, Hiep T. 2020. Combined effects of cottonseed oil and tannin from tea by-products (*Camellia sinensis*) supplementation on nutrient digestibility, performance and enteric methane production of fattening cattle. Adv Anim Vet Sci 8 (6): 633-638. DOI: 10.17582/journal.aavs/2020/8.6.633.638.

Theodorou MK, Williams BA, Dhanoa MS, McAllen AB, France J. 1994. A simple gas production method using a pressure transducer to determine the fermentation kinetics of ruminant feeds. Anim Feed Sci Technol 48 (3-4): 185-197. DOI: 10.1016/0377-8401(94)90171-6.

Tshikhudo PP, Ntushelo K, Mudau FN, Salehi B, Sharifi-Rad M, Martins N, Martorell M, Sharifi-Rad J. 2019. Understanding *Camellia sinensis* using Omics technologies along with endophytic bacteria and environmental roles on metabolism: A review. Appl Sci 9 (2): 281. DOI: 10.3390/app9020281.

Wang M, Chen M, Bai J, Zhang J, Su R, Franco M, Ding Z, Zhang X, Zhang Y, Guo X. 2022. Ensiling characteristics, in vitro rumen fermentation profile, methane emission and archaeal and protozoal community of silage prepared with alfalfa, sainfoin and their mixture. Anim Feed Sci Technol 284: 115154. DOI: 10.1016/j.anifeedsci.2021.115154.

Wang Y, Zhou H-Z, Gao Y, Wang N-W, Liu H, Yang F-Y, Ni K-K. 2023. Ensiling vine tea (*Ampelopsis grossedentata*) residue with *Lactobacillus plantarum* inoculant as an animal unconventional fodder. J Integr Agric 22 (4): 1172-1183. DOI: 10.1016/j.jia.2022.10.001.

Xu C, Wang H, Yang F, Zhang J. 2012. Fermentation quality and nutritive value of total mixed ration silage of green tea grounds. Adv Mater Res 347-353: 285-289. DOI: 10.4028/www.scientific.net/AMR.347-353.285.

Yang F, Chen C, Ni D, Yang Y, Tian J, Li Y, Chen S, Ye X, Wang L. 2023. Effects of fermentation on bioactivity and the composition of polyphenols contained in polyphenol-rich foods: A review. Foods 12 (17): 3315. DOI: 10.3390/foods12173315.

Yang F, Wang Y, Zhao S, Wang Y. 2020. *Lactobacillus plantarum* inoculants delay spoilage of high moisture alfalfa silages by regulating bacterial community composition. Front Microbiol 11: 1989. DOI: 10.3389/fmicb.2020.01989.

Zeng C, Lin H, Liu Z, Liu Z. 2020. Metabolomics analysis of *Camellia sinensis* with respect to harvesting time. Food Res Intl 128: 108814. DOI: 10.1016/j.foodres.2019.108814.

Zhang F, Li B, Ban Z, Liang H, Li L, Zhao W, Yan X. 2021. Evaluation of origanum oil, hydrolysable tannins and tea saponin in mitigating ruminant methane: In vitro and in vivo methods. J Anim Physiol Anim Nutr 105 (4): 630-638. DOI: 10.1111/jpn.13501.