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Rumen-protected gambir catechins encapsulated using CaO-PFAD: Their effects on rumen fermentation, nutrient digestibility, and methane production *in vitro*

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Abstract: The objective of this study was to evaluate the impact of rumen-protected gambir catechins encapsulated using calcium oxide (CaO) and palm fatty acid distillate (PFAD) on rumen fermentation profiles, microbial activity, nutrient digestibility, and methane production in vitro. A randomised block design was used, consisting of four treatments and five replicates. The treatments were assigned based on different levels of gambir catechin-CaO-PFAD encapsulate supplementation, namely 0% (A), 5% (B), 10% (C), and 15% (D) in the diet. The results indicated that the treatments significantly affected NH_3 concentration, partial VFA profiles, methane production, microbial protein synthesis, degradation, and nutrient digestibility ($P < 0.05$), but no significant treatment effects were detected for total VFA, pH, or microbial biomass ($P > 0.05$). Treatment C produced the highest concentrations of propionate, microbial protein synthesis, degradation, and nutrient digestibility, while reducing methane production and the acetate to propionate ratio. The Cao-PFAD-encapsulated gambir catechin supplementation improved rumen fermentation efficiency, as indicated by increased propionate concentration, microbial protein synthesis, nutrient digestibility, and reduced methane production. Thus, gambir catechin-CaO-PFAD encapsulates could be used as a functional feed supplement to improve nutrient utilisation and mitigate enteric methane emissions in ruminant livestock.

Keywords: enteric methane mitigation; functional supplement; microbial protein; SDG 13; volatile fatty acids

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There is a continuing increase in demand for livestock-based food products with the growth of the human population and increasing public awareness of the importance of healthy, high-quality food. Modern consumers not only demand sufficient quantities of meat and dairy products but also they seek livestock products with higher levels of functional compounds particularly CLA, PUFA, omega-3, and omega-6 fatty acids (Frona et al. 2019).

On the other hand, the ruminant livestock sector faces major challenges in the form of low feed utilisation efficiency and high enteric methane emissions produced during the rumen fermentation process. Beyond its contribution to global warming, enteric methane emissions typically account for 5–7% of gross energy intake in typical production systems, with the global standard baseline commonly evaluated at 6.5% for cattle (Chiariotti 2023). In addition, methane released from ruminant animals is said to contribute a notable part of global greenhouse gas emissions (Astuti et al. 2024).

Various nutritional strategies have been developed to improve rumen fermentation efficiency while reducing methane production, one of which involves the use of plant bioactive compounds and lipid supplementation. Tannin-containing plants have gained attention because, at moderate levels, tannins help regulate ruminal protein degradation, suppress the activity of methanogenic microorganisms, and improve nitrogen utilisation efficiency (Poli et al. 2025).

In addition, tannins may interact with rumen microorganism enzymes and feed cell wall components to modulate fermentation in the rumen to help maintain stable fermentation conditions (Wei et al. 2024). Gambir, also known as *Uncaria gambir*, is a tropical biological resource with high potential in terms of containing condensed tannins and antioxidant and antimicrobial properties. *In vitro* rumen fermentation results showed that gambir catechins contribute to nutrient digestibility while simultaneously decreasing methane production, confirming findings from earlier studies (Pazla et al. 2025a).

Despite all this, the direct inclusion of gambir catechins in ruminant feed is yet to be more widely tested as the biological activity of catechins is diminished before reaching the post-rumen digestive tract, as the catechins degrade relatively fast in the rumen. Hence, there is a need for a technology which can help minimise the extra-ruminal degradation of these bioactive materials. Often, active ingredients are encapsulated to protect them from both degradation by enzymes and instability in the rumen. As coating materials used in this study, calcium oxide (CaO) and palm fatty acid distillate (PFAD) were used as coating materials for gambir catechins. PFAD is a by-product of the palm oil industry; it is used in the composition of long-chain fatty acids and coated in the protective matrix-forming process, while CaO participates in the saponification process. CaO is expected to reduce the rate of ruminal degradation of gambir catechins, followed by the activity of PFAD, which will limit the degradation of some bioactive compounds in the rumen and, consequently, allow them to enter the post-rumen phase.

In addition to serving as a coating material, the fatty acids derived from PFAD may modulate rumen fermentation through the diversion of fermentation hydrogen, thereby helping to reduce methane production and improve feed energy efficiency. Lipid supplementation is known to reduce methane production through the inhibition of methanogenic microbial activity and the reduction of free hydrogen in the rumen (Matra et al. 2025). Previous research reported that gambir catechin-PFAD encapsulates can increase rumen fermentation products, microbial activity, and nutrient digestibility while reducing methane production (Pazla et al. 2025b). However, most previous studies still used these encapsulates as feed additives at low inclusion levels. Information regarding the use of rumen-protected gambir catechins encapsulated using CaO-PFAD as a feed supplement at higher levels remains very limited.

This study aims to investigate the effect of rumen-protected gambir catechins encapsulated using CaO-PFAD functional feed on *in vitro* rumen fermentation characteristics, microbial biomass, microbial protein synthesis, protozoa population, *in vitro* digestibility of nutrients and methane production under *in vitro* rumen incubation conditions. The results obtained from this study are anticipated to serve as the scientific foundation for the development of a functional sustainable feed that can increase the efficiency of livestock production along with the reduction of GHG emissions from the livestock sector in order to mitigate the climate change.

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MATERIAL AND METHODS

Ethical approval. This study was conducted using an *in vitro* approach without directly involving animals in the experimental treatments. However, rumen fluid was collected from donor animals raised in accordance with standard management practices, and the procedures were carried out in accordance with institutional ethical guidelines, with the appropriate ethical approval or exemption obtained prior to sampling.

Experimental material. In this study gambir (*Uncaria gambir*) extract, CaO, and PFAD were used as treatment materials. The basal diet consisted of forages (Indigofera and elephant grass) and concentrate ingredients (palm kernel cake, maize, rice bran, soybean meal, and a mineral mixture) as presented in Tables 1 and 2. The analytical tools used in this study were an analytical balance (AS220 R2, Poland), digital scale (Fujitsu FSR-B620, Indonesia), shaker incubator (Eppendor™ Innova™ 40R, Sweden), and oven (Memmert UN 30, Germany). A centrifuge (Gemmy PLC-05 Top Table, Taiwan) was also used, along with standard laboratory equipment, for proximate analysis and *in vitro* rumen fermentation.

Experimental design. This *in vitro* fermentation experiment was arranged in a randomised block design consisting of four dietary treatments and five blocks. The blocking factor was the source of rumen fluid, represented by rumen fluid collected from different donor goats. It served as one block, and all four treatments were incubated using the rumen fluid from each donor goat. The treatments

Table 1. Nutrient contents (%)

Nutrient content	Feed ingredient						
	indigofera	odot grass	encapsulate	corn	palm kernel meal	bran	soybean meal
Dry matter	87.3	92.3	99.2	85.5	89.7	87.5	85.9
Organic matter	89.9	92.4	64.4	96.2	96.6	89.4	94.4
Crude protein	28.8	11.3	6.64	11.2	20.7	11.0	41.3
Ash	10.0	7.57	35.5	3.78	3.34	10.5	5.6
Crude fibre	18.3	27.3	2.68	10.2	15.9	9.23	16.8
NFE	35.8	50.8	50.1	68.3	54.8	62.8	33.4
TDN	71.0	60.2	59.0	78.7	75.6	74.8	83.8
NDF	29.9	57.6	60.7	58.6	77.8	48.1	44.6
ADF	18.8	37.6	41.7	34.6	39.7	23.0	27.3
Cellulose	13.3	26.7	35.0	19.7	25.5	11.0	25.6
Hemicellulose	7.11	19.9	18.9	23.9	38.0	25.0	17.2
Lignin	3.54	6.54	4.80	7.50	9.42	3.78	5.95

ADF = acid detergent fibre; NDF = neutral detergent fibre; NFE = nitrogen-free extract; TDN = total digestible nutrients

Table 2. The ration formulation

Feed ingredient	Treatment (%)			
	A (0%)	B (5%)	C (10%)	D (15%)
Indigofera	10	10	10	10
Odot grass	50	50	50	50
Encapsulate	0	5	10	15
Palm kernel meal	5	4	4	3
Corn	19	15	11	10
Bran	12	11	8	4
Soybean meal	3	4	6	7
Mineral	1	1	1	1
Total	100	100	100	100

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were assigned based on different supplementation levels of encapsulated gambir catechin-CaO-PFAD in the diet, namely A = 0%, B = 5%, C = 10%, and D = 15% of the ration. Treatments were randomly assigned to fermentation bottles within each block. Thus, each treatment had five experimental units. Data on the chemical composition of the feed are shown in Table 3.

Preparation of encapsulated gambir catechin-CaO-PFAD. The main source of catechin, gambir (*Uncaria gambir* Roxb.), was taken from Talang Maur, Lima Puluh Kota Regency, West Sumatra, Indonesia. Indonesian gambir is broadly known for its top quality, and it remains one of the most important gambir-producing commodities worldwide. Before using it, the gambir material was first dried at 45 °C, then it was ground, and later passed through a 60-mesh sieve. Afterwards, it was kept in airtight containers so that oxidation would be slowed down. As for the lipids, palm fatty acid distillate (PFAD) became the main lipid component inside the encapsulation matrix, and it was obtained from a palm oil refinery located in North Sumatra.

For the encapsulation material, the preparation generally followed the method reported by Pazla et al. (2025b), with a saponification step carried out using PFAD and calcium oxide (CaO). Here, CaO acted as a calcium soap-forming agent, allowing a protective encapsulation matrix to be created with no synthetic chemicals involved. The PFAD-CaO pairing was kept because earlier work showed high encapsulation efficiency (86–91%) plus a capacity to preserve the catechin stability, so the matrix ended up comparatively stable against ruminal degradation.

The procedure began by taking 60 g of PFAD and heating it at 60 °C until it melted completely. After that, 20 g of CaO was poured into the melted PFAD and the mixture was stirred until it appeared as one uniform blend. Then 24 g of gambir was added to the PFAD-CaO mixture and stirring continued until the particles appeared consistent in texture. The proportion of PFAD, CaO, and gambir extract in the encapsulation mixture was 60 : 20 : 24 (w/w/w), equivalent to 57.69%, 19.23% and 23.08% of the final mixture, respectively. The gambir extract contained 71.32% catechin; therefore, the calculated final catechin concentration in the encapsulate was 16.46% (w/w). Finally, the product was dried in an oven at 105 °C, then it was ground into a powder.

***In vitro* fermentation procedure.** Using the Tilley and Terry (1963) protocol, *in vitro* fermentation was conducted but with some modifications. Basically, 2.5 g of substrate was placed in a 250 ml Erlenmeyer flask, 200 ml McDougall's buffer and 50 ml rumen fluid were added. Rumen fluid was collected from five healthy male goats of average body weight (about 35 kg) at a local abattoir in 5–10 min after slaughter. The rumen fluid was filtered using gauze and placed in a thermos capable of maintaining a temperature of 39 °C under anaerobic conditions. The thermos was flushed with

Table 3. Nutrient content of the ration

Nutrient content	Value of content (%)			
	A (0%)	B (5%)	C (10%)	D (15%)
Dry matter	88.7	89.3	89.9	90.5
Organic matter	91.8	90.3	88.9	87.5
Crude protein	14.3	14.2	14.6	14.6
Ash	7.11	8.60	9.91	11.1
Crude fibre	22.3	21.8	21.3	20.8
Nitrogen free extract	52.3	50.9	48.8	47.6
TDN	67.4	66.6	65.8	65.1
NDF	53.9	53.8	53.9	54.1
ADF	32.4	32.6	32.8	33.4
Cellulose	21.1	21.7	22.4	23.3
Hemicellulose	20.1	19.5	18.7	18.1
Lignin	5.97	5.78	5.61	5.53

ADF = acid detergent fibre; NDF = neutral detergent fibre; TDN = total digestible nutrients

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CO_2 gas before use, tightly sealed, and subsequently the thermos containing the rumen fluid was taken to the laboratory for *in vitro* feed evaluation.

The basic ration that was given to the donor goats was based on Napier grass and rice bran at a 70:30 ratio. They were offered feed twice a day regularly. At sampling time, the rumen pH of the sample was around 6.8 to 6.9 and all the donor goats had an identical diet for at least 14 days before slaughter. The outputs of each flask were placed in series using a connecting tube, a gas-tight balloon, and a gas collection system. They were fermented under anaerobic conditions in a shaking water bath at 39 °C for a total period of 48 hours.

Gas samples were collected at 3, 6, 9, 24, and 48 h of incubation from the connecting tube using a gas-tight syringe. The pH value of the fermentation medium was measured directly at the end after incubation. The fermented contents were then centrifuged and the supernatant was separated from the residue. The culture fluid obtained after the first incubation was analysed for ammonia (NH_3), volatile fatty acid (VFA) profile, microbial biomass, and number of protozoa.

The leftovers after the initial incubation were then subjected to further digestion in a pepsin-HCl solution (0.2% pepsin) at 39 °C for 48 h to further simulate digestion in the intestine. The residue after the fermentation was then filtered, dried, and tested for nutrient disappearance.

Analytical measurement. Gas was collected during incubation to obtain samples for methane analysis. Methane production was determined from gas samples collected after 24 h of incubation and analysed using gas chromatography according to Fievez et al. (2005). At the end of incubation, the pH of the fermentation medium was determined using a digital pH meter. Ammonia (NH_3) concentration was subsequently analysed using Conway's microdiffusion method (Conway and O'Malley 1942), while partial VFA concentrations were determined using gas chromatography (GC) following the steam distillation of the rumen fluid samples. Nutrient disappearance, including dry matter, organic matter, and crude protein, was calculated from the residue weight and chemical composition according to AOAC procedures (AOAC International 2019). The measurement of microbial protein synthesis was carried out according to the method of Lowry et al. (1951), and the protozoal population was counted microscopically (Ogimoto and Imai 1981).

Statistical analysis. Before analysis, all data were checked for normality and homogeneity of variance. Normality of the residuals was evaluated using the Shapiro–Wilk test, while homogeneity of variance was assessed using Levene's test. The data were analysed using analysis of variance (ANOVA) according to a randomised block design. The statistical model used was:

$$Y_{ij} = \mu + T_i + B_j + \varepsilon_{ij} \quad (1)$$

where:

Y_{ij} – the observed value;

μ – the overall mean;

T_i – the fixed effect of the i^{th} treatment;

B_j – is the effect of the j^{th} block represented by the source of the rumen fluid from different donor goats;

ε_{ij} – the experimental error.

When a significant treatment effect was detected, treatment means were compared using Duncan's multiple range test at $P < 0.05$. All statistical analyses were performed using SPSS v25.0.

RESULTS

Rumen fermentation characteristics. The administration of gambir catechin-CaO-PFAD encapsulates led to significant differences in NH_3 concentration ($P < 0.05$), while total VFA concentration and rumen pH basically remained the same, with no meaningful changes ($P > 0.05$) (Table 4). Although treatment C showed the numerically highest total VFA concentration, this difference was not statistically significant; the lowest NH_3 concentration of 13.4 mg/100 ml was seen in treatment D. In general, the rumen pH appeared rather constant across treatments, remaining in a narrow band from 6.83 to 6.89.

Partial VFA profile and acetate to propionate ratio. The treatment significantly affected the partial VFA profile ($P < 0.05$). Treatment C produced the highest propionate concentration (34.6) (Table 5), while the acetate concentration tended to decrease with increasing levels of encapsulate supplementation. The acetate to propionate ratio in treatment C was the lowest (1.84) compared to the other treatments. Conversely, treatment A produced the highest acetate to propionate ratio (3.19).

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Table 4. Rumen fermentation characteristics

Parameter	VFA (mM)	NH_3 mg/100 ml	pH
A	105 ± 7.07	16.3^{b} ± 0.81	6.88 ± 0.04
B	108 ± 8.66	16.7^{b} ± 1.49	6.83 ± 0.03
C	114 ± 14.36	18.1^{b} ± 1.11	6.86 ± 0.05
D	103 ± 9.57	13.4^{a} ± 0.28	6.89 ± 0.05
SE	4.96	0.461	0.023
P-value	0.516	0.002	0.372

[a,b]Superscripts indicate significant differences between treatments ($P < 0.05$); Values are presented as mean ± SD

The treatments consisted of different supplementation levels of encapsulated gambir catechin-CaO-PFAD in the diet: A = 0%, B = 5%, C = 10%, and D = 15% of the ration

CaO-PFAD = calcium oxide-palm fatty acid distillate; NH_3 = ammonia; SE = standard error; VFA = volatile fatty acids

Table 5. Partial VFA profile and acetate to propionate ratio

Parameter	VFA partial (mM)						Ratio of C2 & C3
	C2	C3	iC4	nC4	iC5	nC5	
A	68.9^{a} ± 2.41	21.6^{c} ± 1.25	2.16^{b} ± 0.65	7.82^{c} ± 1.32	2.73^{b} ± 0.98	1.68^{b} ± 0.44	3.19^{d} ± 0.09
B	67.3^{a} ± 1.00	27.4^{b} ± 0.93	1.41^{c} ± 0.27	8.47^{bc} ± 1.33	1.70^{c} ± 0.36	1.12^{b} ± 0.12	2.45^{c} ± 0.14
C	63.6^{b} ± 0.84	34.6^{a} ± 1.92	1.87^{bc} ± 0.42	9.75^{b} ± 1.04	2.33^{b} ± 0.56	1.48^{b} ± 0.18	1.84^{a} ± 0.16
D	54.2^{c} ± 0.90	25.7^{b} ± 1.44	2.73^{a} ± 0.44	12.82^{a} ± 1.70	3.78^{a} ± 0.85	3.25^{a} ± 0.26	2.11^{b} ± 0.13
SE	0.642	0.693	0.223	0.673	0.342	0.235	0.108
P-value	0.001	0.001	0.031	0.003	0.032	0.002	0.001

[a–d]The treatment significantly affected the partial VFA profile ($P < 0.05$); Values are presented as mean ± SD

The treatments consisted of different supplementation levels of encapsulated gambir catechin-CaO-PFAD in the diet: A = 0%, B = 5%, C = 10%, and D = 15% of the ration

CaO-PFAD = calcium oxide-palm fatty acid distillate; SE = standard error; VFA = volatile fatty acids

Microbial activity and methane production. Supplementation of gambir catechin-CaO-PFAD encapsulate significantly affected microbial protein synthesis, protozoa numbers, and methane production ($P < 0.05$); however microbial biomass was not significantly affected ($P > 0.05$) (Table 6). Among the treatments, treatment C exhibited the highest rate of microbial protein synthesis

Table 6. Microbial activity and methane production

Parameter	Microbial protein synthesis (mg/100 ml)	Microbial biomass (mg/ml)	Protozoa population (10^5 cells/ml)	Methane production (ppm)
A	108^{c} ± 1.26	1.67 ± 0.05	1.51^{b} ± 0.05	$44\,850^{c}$ ± 6 871
B	117^{b} ± 0.81	1.68 ± 0.02	1.12^{a} ± 0.08	$43\,134^{bc}$ ± 6 657
C	126^{a} ± 1.22	1.70 ± 0.04	1.05^{a} ± 0.10	$39\,328^{a}$ ± 7 022
D	116^{b} ± 1.41	1.67 ± 0.05	1.45^{b} ± 0.10	$41\,250^{ab}$ ± 6 243
SE	0.590	0.020	0.072	762.17
P-value	0.001	0.642	0.001	0.027

[a–c]Superscripts indicate significant differences between treatments ($P < 0.05$); Values are presented as mean ± SD

The treatments consisted of different supplementation levels of encapsulated gambir catechin-CaO-PFAD in the diet: A = 0%, B = 5%, C = 10%, and D = 15% of the ration

CaO-PFAD = calcium oxide-palm fatty acid distillate; SE = standard error

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Table 7. Degradation of dry matter, organic matter, crude protein, and NDF (%)

Parameter	Degradation of dry matter	Degradation of organic matter	Degradation of crude protein	Degradation of NDF
A	$58.1^{b} \pm 1.78$	$62.3^{ab} \pm 1.76$	$61.0^{b} \pm 1.87$	$62.9^{b} \pm 1.78$
B	$58.4^{b} \pm 1.96$	$62.3^{ab} \pm 2.67$	$60.5^{ab} \pm 2.99$	$62.6^{ab} \pm 1.14$
C	$58.5^{b} \pm 0.55$	$64.0^{b} \pm 0.52$	$58.0^{a} \pm 0.73$	$62.0^{ab} \pm 1.07$
D	$54.4^{a} \pm 2.56$	$59.7^{a} \pm 1.45$	$57.6^{a} \pm 0.68$	$59.5^{a} \pm 1.52$
SE	0.883	0.887	0.783	0.590
P-value	0.008	0.026	0.048	0.015

[a,b]Superscripts indicate significant differences between treatments ($P < 0.05$); Values are presented as mean ± SD

The treatments consisted of different supplementation levels of encapsulated gambir catechin-CaO-PFAD in the diet: A = 0%, B = 5%, C = 10%, and D = 15% of the ration

CaO-PFAD = calcium oxide-palm fatty acid distillate; NDF = neutral detergent fibre; SE = standard error

Table 8. Digestibility of dry matter, organic matter, and crude protein (%)

Parameter	Dry matter digestibility	Organic matter digestibility	Crude protein digestibility
A	$63.9^{b} \pm 0.76$	$66.8^{b} \pm 1.95$	$65.3^{b} \pm 0.44$
B	$65.3^{b} \pm 0.30$	$67.4^{b} \pm 1.65$	$66.3^{b} \pm 1.22$
C	$66.7^{a} \pm 0.56$	$70.3^{a} \pm 1.14$	$68.2^{a} \pm 1.10$
D	$64.5^{b} \pm 0.29$	$67.0^{b} \pm 1.10$	$66.0^{b} \pm 0.79$
SE	0.248	0.734	0.433
P-value	0.001	0.045	0.016

[a,b]Superscripts indicate significant differences between treatments ($P < 0.05$); Values are presented as mean ± SD

The treatments consisted of different supplementation levels of encapsulated gambir catechin-CaO-PFAD in the diet: A = 0%, B = 5%, C = 10%, and D = 15% of the ration

CaO-PFAD = calcium oxide-palm fatty acid distillate; SE = standard error

(126 mg/100 ml) along with the lowest methane production (39 328 ppm). Lower protozoa populations were observed in treatments B and C compared to treatments A and D.

Nutrient degradation. The treatments had a significant effect on the degradation of dry matter, crude protein, organic matter, and NDF ($P < 0.05$). Treatment C resulted in the highest organic matter degradation (64.0%), while treatment D produced the lowest degradation values for most parameters. Crude protein degradation in treatments C and D was lower than in treatment A (Table 7).

Nutrient digestibility. Nutrient digestibility was significantly influenced by the addition of gambir catechin-CaO-PFAD encapsulate ($P < 0.05$). Among all treatments, treatment C showed the highest digestibility for dry matter (66.7%), organic matter (70.3%), and crude protein (68.2%). In contrast, treatment A showed the lowest digestibility values for most parameters (Table 8).

DISCUSSION

The absence of a significant effect on the total VFA concentration indicates that CaO-PFAD-encapsulated gambir catechin supplementation did not increase the overall extent of ruminal fermentation. However, the significant changes in partial VFA profiles, particularly the increase in propionate concentration and the reduction in the acetate to propionate ratio, suggest that the treatment modified the direction of fermentation toward a more energetically favourable pattern. Therefore, the beneficial response observed in this study should be interpreted mainly as a shift in the fermentation profile rather than an increase in total VFA production.

Across all treatments, rumen pH was not negatively affected by the treatments and remained within the range required for microbial activity (6.83–6.89). This pH range remains optimal for

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the growth of cellulolytic bacteria and nutrient fermentation within the rumen (Zhang et al. 2026). The administration of gambir catechin-CaO-PFAD encapsulate did not affect total VFA, indicating that the supplementation did not inhibit the overall fermentation activity. However, the decrease in NH_3 concentration in treatment D indicates that protein in the rumen is better protected from excessive degradation. Tannins from gambir catechins are known to form complexes with proteins, thereby reducing protein deamination in the rumen (Antonius et al. 2025).

The stable rumen fermentation conditions observed in this study suggest that CaO-PFAD-encapsulated gambir catechin supplementation did not cause any excessive disruption of ruminal fermentation. Although the catechin release kinetics and ruminal stability were not directly evaluated, it can be hypothesised that the CaO-PFAD matrix may have reduced the immediate exposure of gambir catechins to the rumen environment. The saponification process between CaO and PFAD could form a lipid-calcium matrix that may partially limit a rapid interaction between catechins and rumen microorganisms. Similar protective concepts have been reported for encapsulation systems that improve the stability of bioactive compounds in the ruminant feed application (Almassri et al. 2024). Calcium- and lipid-based nutrient protection strategies have been recognised for maintaining the rumen fermentation stability while improving the nutrient utilisation efficiency (Jenkins and Harvatine 2014). Therefore, the proposed protective and controlled-release effects should be interpreted as a possible mechanism rather than as a demonstrated response in the present study. Further studies evaluating catechin release kinetics, ruminal stability, and post-ruminal availability are needed to confirm this mechanism.

The increase in propionate concentration in treatment C indicates that the administration of gambir catechin-CaO-PFAD encapsulate can direct the rumen fermentation toward the propionate formation pathway. Elevated propionate levels are typically linked to a more efficient hydrogen use, which in turn limits the availability of hydrogen for methanogenesis (Ungerfeld 2020). The decrease in the acetate to propionate ratio in treatment C further supports the indication of a shift in fermentation patterns toward the more energetically efficient fermentation. Additionally, fatty acids from PFAD are also suspected to play a role in modulating the rumen fermentation through partial inhibition of the activity of acetate- and methane-producing microbes (Reddy et al. 2023).

In this study, the VFA pattern shifts suggest that the gambir catechin-CaO-PFAD complexes act not only as a shield for bioactive compounds, but also as a modulator of rumen fermentation. PFAD is high in long-chain fatty acids, and it is thought to help redirect the fermentation hydrogen toward propionate; furthermore, the encapsulation process allows catechins to be released gradually. Rumen microorganisms may have their activity suppressed because of the slow release of these materials, even though the overall fermentation process does not appear to be clearly inhibited. It is suggested that the net outcome is more productive rumen fermentation, where propionate production increases and the acetate to propionate ratio remains lower. Energy that would otherwise be lost as methane gas is reduced when this ratio is kept low. It has been reported that the lipid supplementation of ruminant diets promotes propionate formation while also lowering free hydrogen levels in the rumen (Martin et al. 2010). Additionally, those tannin compounds that are released gradually from the encapsulate can further tune the behaviour of fermentative microbes, which can then steer the fermentation more effectively toward propionate (Aboagye and Beauchemin 2019).

The increased microbial protein synthesis observed in treatment C suggests that the 10% level of CaO-PFAD-encapsulated gambir catechin may have improved the efficiency of microbial nitrogen utilisation. The high microbial protein synthesis reflects a more synchronised availability of fermentable energy and nitrogen for microbial growth (Sommai et al. 2025). Although the total VFA concentration was not significantly affected, the increase in propionate concentration and the lower acetate ratio indicate a shift in fermentation toward a more energy-efficient pathway, which may support the microbial protein synthesis. In addition, the moderate release of tannin-derived compounds from catechin may have reduced excessive protein degradation and ammonia accumulation, thereby improving the nitrogen capture by rumen microbes. The lower protozoa population observed in treatment C may also have contributed to this response, because rumen protozoa can prey on bacteria and reduce the microbial protein flow. Since rumen

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protozoa and methanogens share a symbiotic relationship, a decrease in the protozoa population may also contribute to a reduction in methane production (Matra et al. 2025). However, the microbial biomass was not significantly affected; therefore, the increase in microbial protein synthesis should be interpreted as an improvement in microbial protein production efficiency rather than an increase in total microbial biomass.

The decrease in methane production in the treatment containing the encapsulate indicates that the CaO-PFAD encapsulation system can maintain the biological activity of gambir catechins more effectively. Without encapsulation protection, catechins are likely to degrade more rapidly in the rumen, thereby limiting the anti-methanogenic effect. Conversely, the use of CaO-PFAD as a coating matrix allows for a slower and more stable release of bioactive compounds. Additionally, the presence of fatty acids from PFAD may be toxic to some methanogenic microbes and rumen protozoa, thereby contributing to reduced methane production and improved fermentation energy utilisation efficiency. Beauchemin et al. (2020) reported that lipid supplementation can reduce the population of methane-producing microbes through mechanisms of biohydrogenation inhibition and reduced hydrogen availability. Compared to unprotected tannins, rumen-protected tannins are considered more effective in preserving the anti-methanogenic activity (Jayanegara et al. 2015).

The encapsulated supplement at the 10% level (treatment C) showed the highest organic matter degradation, suggesting that this level may support substrate utilisation without markedly suppressing overall fermentation. However, crude protein degradation tended to decrease with increasing levels of the encapsulated material, when this response should be interpreted cautiously. On the one hand, the formation of tannin-protein complexes may reduce excessive ruminal protein degradation and potentially increase the supply of rumen-undegraded protein to the post-ruminal digestive tract (Loregian et al. 2023).

On the other hand, reduced protein degradation may also indicate a partial inhibition of proteolytic microbial activity, especially at higher inclusion levels. This possibility is supported by the lower nutrient degradation observed in treatment D, suggesting that an excessive inclusion may begin to limit the microbial degradation activity. Therefore, the reduction in crude protein degradation should not be interpreted solely as a beneficial effect, but rather as a response that depends on the balance between protein protection and possible inhibition of rumen microbial activity.

The increased degradation of organic matter in treatment C indicates that the encapsulation technology does not generally inhibit the fermentation activity but it rather regulates the rate of nutrient degradation within the rumen. The CaO-PFAD coating may be able to control the release of catechins, thereby preventing an excessive interaction between tannins and nutrients. At moderate levels, this condition can actually improve the efficiency of fermentation substrate utilisation by rumen microbes. Conversely, at very high application levels, the protective effects and bioactive activity of catechins start to create resistance to nutrient breakdown; consequently, there is a lower loss of dry matter and NDF in treatment D. Patra and Saxena (2011) noted that tannins at moderate levels can enhance the protein utilisation efficiency, while high concentrations can lower fibre degradation and reduce rumen microbial activity. In the same way, the protein-protective role of the lipid-calcium combination was said to increase the post-rumen protein flow without meaningfully disturbing fermentation (Palmquist and Jenkins 2017).

The higher digestibility of dry matter, organic matter, and crude protein under treatment C suggests that the gambir catechin-CaO-PFAD encapsulated material, when used at this specific level (10%), can improve nutrient utilisation. Among the treatments evaluated, this inclusion level provided the greatest benefit. In general, this encapsulation approach seems to protect certain bioactive compounds from rumen breakdown without actually slowing the fermentation process. Treatment D, though, showed a fairly clear tipping point. When the encapsulated dose was tested at the highest inclusion level, it seemed to stall rumen microbial activity, essentially slowing down nutrient degradation. That matches other findings where tannins at higher levels can suppress microbial activity and, as a result, lower feed digestibility (Phesatcha et al. 2025).

Also, the better nutrient digestibility in treatment C indicates that gambir catechins, together with CaO and PFAD, can form a more effective nutrient protection setup. CaO, besides participating in the encapsulation matrix creation, may also work

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as a calcium supply, which supports the rumen microbial activity. Meanwhile, PFAD, as a source of fatty acids, provides additional energy and helps reduce the fermentation energy loss in the form of methane. Thus, the encapsulation technology using CaO-PFAD not only protects gambir catechins from ruminal degradation but also it contributes to improved fermentation efficiency and overall nutrient utilisation. Wu et al. (2024) reported that the supplementation of protected lipids can improve energy efficiency and nutrient digestibility in ruminant livestock.

CONCLUSION

The administration of gambir catechin encapsulated with CaO-PFAD demonstrated a potential to improve the efficiency of *in vitro* ruminal fermentation, nutrient degradation, microbial protein synthesis, and digestibility, while concurrently reducing the methane production. Under these experimental conditions, the 10% inclusion level emerged as the most promising treatment, yielding the highest propionate concentration and digestibility alongside the lowest acetate to propionate ratio and methane output. Collectively, these findings suggest that the CaO-PFAD encapsulation technology warrants further evaluation *in vivo* as a feed additive for enhancing ruminant performance and mitigating methane emissions.

Conflict of interest

The authors declare no conflict of interest.

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