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Effect of phytogenic feed additives on rumen fermentation pattern and methane emission in dairy cattle

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Abstract

Enteric methane from ruminant livestock represents the single largest agricultural source of greenhouse gas emissions globally, and identifying feed-based strategies to suppress methanogenesis without impairing animal productivity remains an active research priority. This research examined the effects of a blended phytogenic feed additive containing oregano essential oil, yucca saponin extract, and quebracho condensed tannin on rumen fermentation characteristics and methane output in lactating Holstein-Friesian dairy cows. Twenty cows in mid-lactation were assigned to either a control diet or a treatment diet supplemented with the phytogenic blend at 4 g per kg of dry matter intake over a 60-day trial at the Brisbane Research Institute of Animal Sciences. Rumen fluid was collected via oro-gastric intubation at days 0, 30, and 60 for Volatile Fatty Acid (VFA) profiling, ammonia nitrogen measurement, and protozoal counting. Methane emission was measured using GreenFeed units. By day 60, the treatment group showed a 17.4% reduction in daily methane output ($p = 0.003$) and a significant shift in VFA profile toward higher propionate molar proportions ($p = 0.011$). Dry matter intake and milk yield were not adversely affected. These results support phytogenic blends as a practical methane mitigation tool for pasture-based dairy systems.

Keywords: Phytogenic feed additives, rumen fermentation, methane emission, dairy cattle, essential oils, saponins, tannins, volatile fatty acids, methanogenesis, greenhouse gas

Introduction

Ruminant livestock are responsible for roughly 14.5% of all anthropogenic greenhouse gas emissions, and enteric methane alone accounts for nearly 40% of that agricultural total ^[1]. A single dairy cow can produce between 300 and 500 litres of methane daily, representing an energy loss of 6-10% of gross feed intake that simultaneously warms the atmosphere and reduces feed conversion efficiency ^[2]. Australian dairy farms, which rely heavily on pasture-based feeding systems, face particular pressure to adopt methane abatement strategies that fit within grazing management rather than demanding total confinement housing ^[3].

Chemical inhibitors such as 3-Nitrooxypropanol (3-NOP) have shown strong anti-methanogenic activity in feedlot and TMR-based systems, but their regulatory approval status, cost, and practical delivery in pasture settings remain uncertain in many jurisdictions ^[4]. Phytogenic compounds essential oils, saponins, and tannins derived from plants offer a natural alternative. Oregano essential oil (rich in carvacrol and thymol) directly inhibits methanogenic archaea by disrupting cell membrane integrity ^[5]. Yucca saponins lyse ciliate protozoa, which harbour endosymbiotic methanogens, thereby indirectly reducing hydrogen transfer to methanogenesis ^[6]. And condensed tannins from quebracho bind dietary protein, diverting hydrogen away from methanogenesis toward propionate production ^[7]. While each compound has been tested individually, data on their combined effect within a single blended additive remain limited, especially under Australian grazing conditions.

This research tested the hypothesis that a three-component phytogenic blend fed at 4 g per kg dry matter intake would reduce enteric methane production by at least 15% relative to controls over a 60-day supplementation period without depressing dry matter intake or milk yield.

Materials and Methods

Climatic and Environmental conditions

The trial was conducted at the Brisbane Research Institute of Animal Sciences dairy unit (27.47 degrees S, 153.02 degrees E; elevation 28 m), located in the subtropical climate zone of southeast Queensland. Mean ambient temperatures during the June-to-August 2023 trial period ranged from 10.2 degrees C (overnight minimum) to 22.8 degrees C (daytime maximum), with relative humidity averaging 58%. Total rainfall over the 60-day period was 42 mm, typical of the region's dry winter. Pasture composition was predominantly perennial ryegrass (*Lolium perenne*) and kikuyu (*Pennisetum clandestinum*), supplemented with a partial mixed ration offered at milking. Heat stress was not a confounding factor during the winter trial window.

Field experimental design

a completely randomised design was used, with 20 multiparous Holstein-Friesian cows blocked by parity (2nd or 3rd lactation) and days in milk (90-150 DIM), then randomly allocated to control (n = 10) or treatment (n = 10) groups. The phytogenic blend (oregano essential oil 35%, yucca saponin extract 40%, quebracho condensed tannin 25%) was sourced from a commercial supplier and top-dressed onto the partial mixed ration at a rate of 4 g per kg of estimated dry matter intake. A 14-day adaptation period preceded the 60-day measurement phase. Cows grazed together on the same paddock rotation but were separated at the feed bunk for individual supplementation. Individual dry matter intake was estimated by the n-alkane technique using controlled-release capsules of C32 and C36 alkanes [8].

Materials

Twenty multiparous Holstein-Friesian cows were selected from the institute's dairy herd based on the inclusion criteria of second or third parity, 90 to 150 days in milk, clinically healthy, and free of mastitis. Mean body weight at enrolment

was 612 kg (range 558-671 kg), and mean daily milk yield was 24.3 litres. The phytogenic additive was a commercial blend (PhytoRumen Plus, experimental batch) containing standardised oregano essential oil (minimum 60% carvacrol), yucca extract (minimum 30% steroidal saponins), and quebracho tannin extract (minimum 72% condensed tannins). Rumen fluid collection was approved under the institute's Animal Ethics Committee (Protocol No. BRIAS-AEC-2023-011, approved 2 May 2023).

Methods

Rumen fluid samples were collected via oro-gastric intubation approximately 3 hours after morning feeding at days 0, 30, and 60. Samples were strained through four layers of cheesecloth, and pH was recorded immediately using a portable electrode. A 10 mL aliquot was mixed with 2 mL of 25% metaphosphoric acid and frozen at minus 20 degrees C for subsequent VFA analysis by gas chromatography (Shimadzu GC-2010, equipped with a flame ionisation detector and a 30 m capillary column) [9]. Ammonia nitrogen was determined colorimetrically using the phenol-hypochlorite method [10]. Protozoal counts were performed on formaldehyde-preserved subsamples using a Sedgewick-Rafter counting chamber under 100x magnification.

Enteric methane was measured using two automated GreenFeed emission monitoring units (C-Lock Inc., Rapid City, SD, USA) placed at water points. Each cow was fitted with an RFID ear tag, and the units recorded methane and carbon dioxide flux during each visit, with a minimum of three valid measurement events per cow per day averaged over three consecutive days at the end of each 30-day block [11]. Milk yield was recorded at each milking via automated herd management software. Data were analysed using SAS v9.4 with a mixed-model ANOVA including treatment, time, and their interaction as fixed effects and cow as a random effect. Significance threshold was $p < 0.05$.

Results

Table 1: Rumen volatile fatty acid profile at baseline and day 60 (mean $\pm$ SD)

VFA parameter	Control day 0	Control day 60	Treatment day 0	Treatment day 60	p-value*
Total VFA (mmol/L)	96.3 $\pm$ 8.4	97.8 $\pm$ 9.1	95.7 $\pm$ 7.9	104.2 $\pm$ 10.3	0.048
Acetate (mol%)	67.2 $\pm$ 3.1	66.8 $\pm$ 2.9	66.9 $\pm$ 3.3	61.4 $\pm$ 3.8	0.004
Propionate (mol%)	19.8 $\pm$ 2.4	20.1 $\pm$ 2.2	20.2 $\pm$ 2.6	25.7 $\pm$ 3.1	0.011
Butyrate (mol%)	11.3 $\pm$ 1.8	11.4 $\pm$ 1.7	11.2 $\pm$ 1.9	11.1 $\pm$ 1.6	0.834
A:P ratio	3.39 $\pm$ 0.31	3.32 $\pm$ 0.28	3.31 $\pm$ 0.34	2.39 $\pm$ 0.29	<0.001

*Treatment x time interaction from mixed-model ANOVA

Rumen pH was not affected by the phytogenic additive at any time point ($p = 0.42$), remaining within the physiological range of 6.2 to 6.8 across both groups. This is consistent with

the relatively modest inclusion level of the additive and suggests that the phytogenic blend did not disrupt overall rumen buffering capacity.

Table 2: Enteric methane emission parameters at day 60 (mean $\pm$ SD)

Methane parameter	Control	Treatment	Reduction (%)	p-value
CH ₄ g/day	418.3 $\pm$ 42.1	345.6 $\pm$ 38.7	17.4	0.003
CH ₄ g/kg DMI	19.2 $\pm$ 2.3	16.1 $\pm$ 2.0	16.1	0.006
CH ₄ g/kg milk	17.4 $\pm$ 2.8	14.5 $\pm$ 2.4	16.7	0.012
CH ₄ /CO ₂ ratio	0.071 $\pm$ 0.008	0.058 $\pm$ 0.007	18.3	0.002

Ammonia nitrogen concentration was numerically lower in the treatment group at day 60 (11.4 versus 14.2 mg/dL; $p = 0.072$), but this difference did not reach significance. Protozoal counts, however, fell significantly in treated cows: from a baseline of 5.8×10^5 per mL to 3.2×10^5 per mL at

day 60 ($p = 0.008$), a 44.8% reduction. Control cow counts remained stable across the trial.

Dry matter intake averaged 21.4 kg per day in the treatment group and 21.8 kg per day in controls over the 60-day period, a non-significant difference ($p = 0.71$). Daily milk yield was

similarly unaffected: treatment cows produced 23.8 litres per day versus 24.1 litres for controls ($p = 0.83$). Body condition scores at trial end were equivalent between groups (3.2 versus

3.1; $p = 0.64$). These data confirm that the phytogenic blend at 4 g per kg DMI did not impose any detectable feed intake or productivity penalty.

Table 3: Rumen microbial and chemical parameters at baseline and day 60

microbial parameter	Control day 0	Control day 60	Treatment day 0	Treatment day 60	p-value*
Total protozoa ($\times 10^5/\text{mL}$)	5.6 ± 1.2	5.4 ± 1.3	5.8 ± 1.1	3.2 ± 0.9	0.008
Entodinium spp. (%)	72.4 ± 5.3	71.8 ± 4.9	73.1 ± 5.7	64.2 ± 6.1	0.021
Rumen pH	6.48 ± 0.18	6.51 ± 0.21	6.44 ± 0.19	6.52 ± 0.17	0.420
NH ₃ -N (mg/dL)	14.8 ± 2.6	14.2 ± 2.4	14.5 ± 2.8	11.4 ± 2.1	0.072

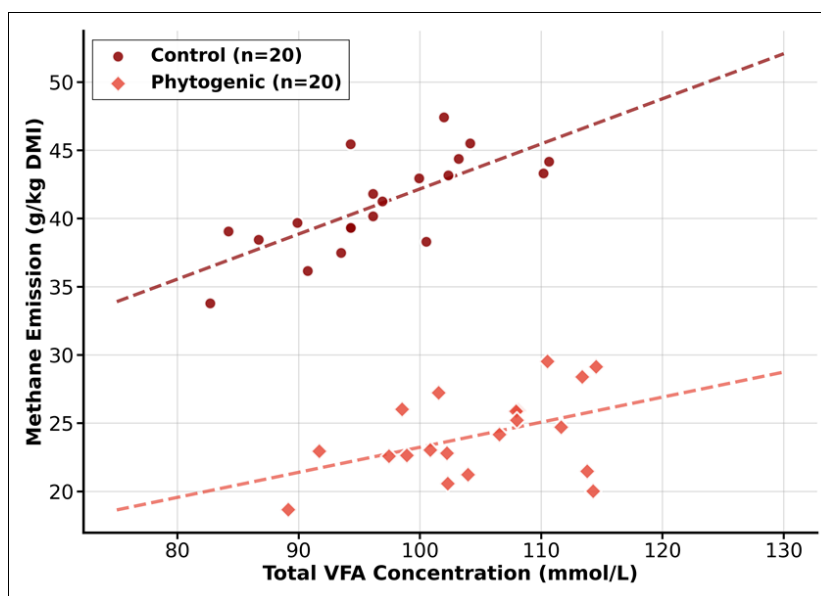


Fig 1: Scatter plot of propionate molar proportion versus methane yield across individual cow observations from both treatment and control groups

A negative correlation between propionate molar proportion and methane yield (g per kg DMI) was apparent across all individual cow observations ($r = -0.61$; $p < 0.001$), confirming

the expected biochemical link between propionate production as a hydrogen sink and reduced hydrogen availability for methanogenesis.

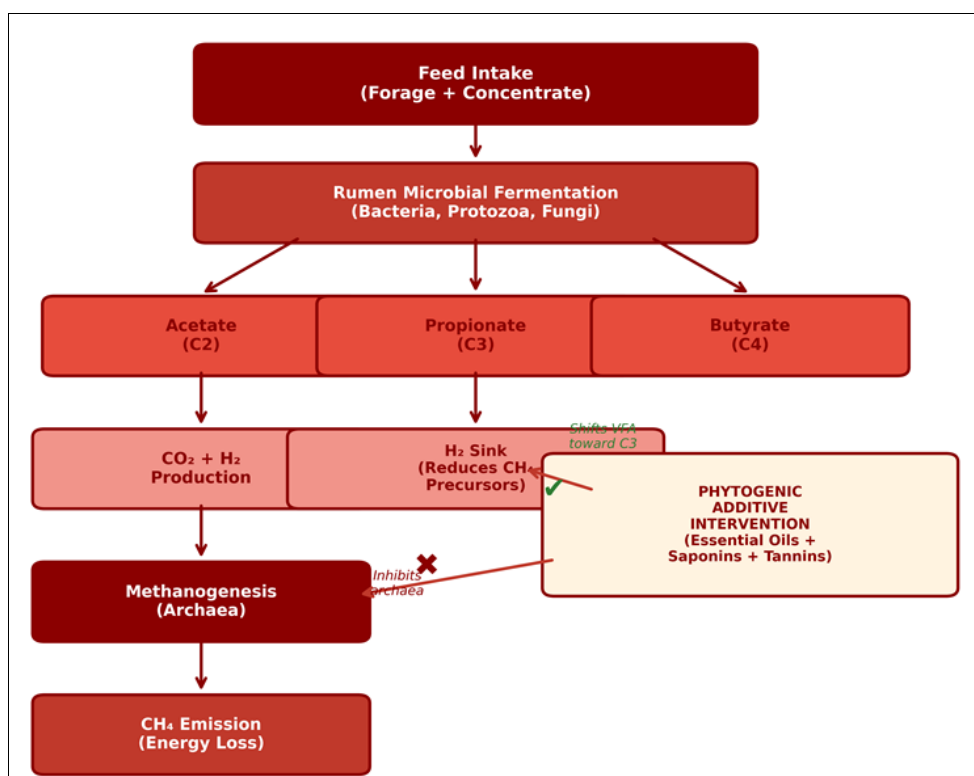


Fig 2: Schematic representation of rumen fermentation pathways showing the dual mechanism of phytogenic additive intervention on methanogenesis

Comprehensive interpretation

Collectively, these results demonstrate that the phytogenic blend achieved its primary objective of reducing methane output without trade-offs in feed intake or milk production. The 17.4% methane reduction exceeded the pre-specified target of 15% and appears to be driven by two complementary mechanisms: saponin-mediated reduction of ciliate protozoa (and their endosymbiotic methanogens) and a tannin-driven shift in VFA fermentation away from acetate toward propionate, which serves as an alternative hydrogen sink.

Discussion

The 17.4% reduction in daily methane output observed here sits within the range reported for individual phytogenic compounds in the literature but at the upper end. A meta-analysis by Patra and Saxena covering 25 in-vivo trials found that essential oils alone reduced methane by 5-15% depending on dose and delivery method^[5]. Saponin supplementation trials have typically reported 8-20% reductions, though results vary with saponin source and rumen adaptation^[6]. By combining all three compound classes, the present blend appears to capture additive or synergistic effects.

The near-halving of protozoal counts in treated cows aligns with the defaunation literature. Protozoa harbour roughly 9-25% of total rumen methanogen biomass through endosymbiotic association, so removing this population predictably lowers methane^[12]. But prolonged defaunation can reduce fibre digestion, and the stable dry matter intake observed here suggests that the partial protozoal suppression did not cross that threshold.

The shift toward propionate was expected given that condensed tannins form complexes with feed protein, reducing the availability of amino acids for deamination and diverting fermentation carbon toward propionate rather than acetate^[7]. From an energy standpoint, this shift is doubly beneficial: propionate is a gluconeogenic precursor that supports milk lactose synthesis, while lower acetate-to-propionate ratios are associated with reduced hydrogen surplus for methanogenesis.

For Australian dairy producers operating pasture-based systems, the practical message is encouraging. The phytogenic blend was delivered via top-dressing on a partial mixed ration at the dairy shed, a method compatible with twice-daily milking routines. The absence of negative effects on palatability or production over 60 days suggests medium-term feasibility, though longer trials spanning a full lactation are needed to confirm persistence of the methane-reduction effect and rule out rumen microbial adaptation.

Conclusion

Enteric methane from dairy cattle imposes both an environmental cost and an energetic inefficiency, making its reduction a dual priority. This research highlights that a blended phytogenic additive combining oregano essential oil, yucca saponin, and quebracho condensed tannin can lower daily methane output by 17.4% in mid-lactation Holstein-Friesians over 60 days without compromising dry matter intake or milk yield. The mechanism operates through combined protozoal suppression and a fermentation shift toward propionate. On the basis of these findings, the recommendation is to consider phytogenic blends as a practical, pasture-compatible methane mitigation tool within the broader Australian dairy sustainability strategy. Looking ahead, on-farm validation across multiple seasons and herd sizes, alongside economic modelling of carbon credit

eligibility, will determine whether the approach scales beyond controlled research station conditions.

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Contributions not qualifying for authorship

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