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Synergistic effects of papaya, coriander, cumin and fennel-based polyherbal feed additive on in vitro rumen digestibility, fermentation kinetics and methane mitigation

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Abstract
Enteric methane production from ruminants represents an important source of greenhouse-gas emissions and also reflects a loss of dietary energy from the animal. The use of plant-derived phytogenic feed additives has received increasing attention as a natural strategy for modulating rumen fermentation while maintaining feed utilization. The present study was designed to evaluate a novel polyherbal feed additive comprising *papaya (Carica papaya) leaves, coriander (Coriandrum sativum) seeds, cumin (Cuminum cyminum) seeds and fennel (Foeniculum vulgare)* seeds for its effects on in-vitro rumen digestibility, fermentation characteristics and methane production. The selected plant materials contain diverse phytochemicals, including phenolics, flavonoids, enzymes and volatile bioactive compounds, which may influence rumen microbial activity and fermentation pathways. The polyherbal combinations will be evaluated at different inclusion levels using an anaerobic in-vitro rumen fermentation system. Total gas production, methane production, in-vitro dry matter digestibility, organic matter digestibility, pH, ammonia-nitrogen, short-chain fatty acids and microbial protein synthesis will be assessed to determine the overall fermentation response. Particular emphasis will be placed on identifying an inclusion level that suppresses methane production without adversely affecting substrate degradation and fermentation efficiency. The study is expected to provide information on the potential synergistic action of papaya, coriander, cumin and fennel as a natural phytogenic feed additive. The findings may contribute to the development of sustainable dietary strategies for improving ruminal nutrient utilization while reducing methane emissions from ruminant production systems.

Keywords: Polyherbal feed additive, *Carica papaya, Coriandrum sativum, Cuminum cyminum, Foeniculum vulgare, in-vitro* digestibility, rumen fermentation, methane mitigation, phytogenic additive, microbial protein.

Introduction
Ruminant livestock production plays an important role in supplying milk, meat and other animal-derived products, but efficient utilization of feed energy remains a major challenge in intensive and smallholder production systems. During ruminal fermentation, dietary carbohydrates are converted by a complex microbial ecosystem into volatile fatty acids, microbial biomass, gases and other fermentation products. Although this process is essential for obtaining energy from fibrous feeds, a proportion of the feed energy is lost in the form of methane. Consequently, strategies that improve nutrient utilization while controlling methane formation are important for developing more efficient and environmentally sustainable ruminant production systems. The rumen contains a highly diverse microbial population consisting of bacteria, protozoa, fungi and methanogenic archaea. Methanogenic archaea utilize hydrogen and carbon dioxide generated during microbial fermentation to produce methane. Excessive methane formation not only contributes to greenhouse-gas emissions but also represents an energetic loss to the host animal. Therefore, manipulation of rumen fermentation toward improved energy capture and reduced methane production has become an important area of nutritional research. However, any methane-mitigation strategy should be carefully evaluated because excessive suppression of rumen microorganisms can interfere with fibre degradation, organic matter digestion and normal fermentation.

Plant-derived feed additives have attracted considerable interest as alternatives to conventional rumen modifiers because plants contain a wide range of biologically active compounds. Essential oils, phenolics, flavonoids, terpenoids, alkaloids, tannins and other secondary metabolites can interact with rumen microorganisms and alter fermentation pathways. Depending on their chemical composition and concentration, these compounds may selectively influence microbial populations, hydrogen availability, volatile fatty acid production and methanogenesis. The effectiveness of a phytogenic additive, however, depends strongly on plant species, plant part, extraction or processing method, combination of bioactive compounds and inclusion level. The use of polyherbal formulations may provide an additional advantage over individual plant supplementation because plants containing different classes of phytochemicals can potentially produce complementary or synergistic effects. A carefully designed combination may simultaneously influence microbial fermentation, substrate degradation and methanogenesis. Nevertheless, increasing the concentration of bioactive compounds does not necessarily result in improved fermentation. High concentrations of antimicrobial phytochemicals may inhibit beneficial rumen microorganisms and consequently reduce digestibility. Therefore, optimization of the combination and inclusion level is essential when developing a phytogenic feed additive.

Papaya (Carica papaya) leaves represent one potential component of a novel phytogenic formulation. Papaya leaves contain various phytochemicals and enzymes and have traditionally been investigated for their biological activities. Their incorporation into ruminant-feed research provides an opportunity to examine whether these plant-derived compounds can influence ruminal substrate degradation and fermentation. However, the response may depend on dose and interaction with other plant materials, making controlled in-vitro evaluation important before further nutritional application. Coriander (Coriandrum sativum) seeds contain volatile constituents and phenolic compounds that may influence microbial activity and fermentation characteristics. Similarly, cumin (Cuminum cyminum) seeds are characterized by biologically active volatile constituents and have attracted interest as a phytogenic feed ingredient. *Fennel (Foeniculum vulgare) seeds* contain essential-oil components, particularly anethole, together with other secondary metabolites. The different phytochemical profiles of coriander, cumin and fennel provide a rationale for investigating their combined application rather than evaluating them only as individual additives. An important limitation of many phytogenic-feed studies is that methane reduction is sometimes evaluated independently of digestibility. A formulation that substantially reduces gas or methane production but simultaneously suppresses substrate degradation may not provide a practical nutritional advantage. Therefore, simultaneous assessment of in-vitro dry matter digestibility, organic matter digestibility, total gas production, methane production and fermentation characteristics is necessary to identify an effective formulation. Additional measurements such as ammonia-nitrogen, short-chain fatty acids and microbial protein synthesis can provide further information about the nutritional consequences of phytogenic supplementation.

In this context, the present study proposes a novel polyherbal feed additive consisting of papaya leaves, coriander seeds, cumin seeds and fennel seeds. The study is designed to investigate the effects of different proportions and inclusion levels of the formulation on in-vitro rumen fermentation and digestibility. Rather than considering methane reduction as the sole indicator of efficacy, the study will evaluate the balance between methane suppression and preservation of nutrient degradation and fermentation efficiency. Such an integrated approach can help identify an optimum phytogenic formulation that provides methane mitigation without compromising ruminal digestion. Accordingly, the primary objective of the study is to evaluate the potential of the papaya–coriander–cumin–fennel polyherbal feed additive to improve or maintain in-vitro digestibility while reducing methane production. The secondary objective is to characterize its effects on fermentation kinetics, pH, ammonia-nitrogen, short-chain fatty acid production and microbial protein synthesis. The findings are expected to provide a scientific basis for further investigation of this novel polyherbal combination as a sustainable feed additive for ruminant nutrition.

Materials and Methods

1. Preparation of Polyherbal Feed Additive and Experimental Treatments

Fresh and good-quality papaya (Carica papaya) leaves, coriander (Coriandrum sativum) seeds, cumin (Cuminum cyminum) seeds and fennel (Foeniculum vulgare) seeds were collected from reliable sources and authenticated based on their botanical characteristics. The plant materials were cleaned thoroughly to remove adhering soil and foreign materials, shade-dried at room temperature until a constant weight was obtained, and separately ground into a fine powder. Equal quantities of the four powdered plant materials were initially blended in a 1:1:1:1 ratio to prepare the polyherbal feed additive (PHF). The experimental treatments consisted of an untreated control and different inclusion levels of the polyherbal formulation. The additive was incorporated into the fermentation substrate at graded concentrations, such as 0.025, 0.050, 0.075 and 0.100% of substrate dry matter, to determine the dose-dependent response. The basal substrate was a representative ruminant feed or hay sample, which was oven-dried, ground and passed through an appropriate sieve before incubation. The chemical composition of the substrate, including dry matter, organic matter, crude protein, ether extract, crude fibre, neutral detergent fibre and acid detergent fibre, was determined using standard analytical procedures.

2. *In-Vitro* Rumen Fermentation and Digestibility Analysis

Rumen liquor was collected from a healthy fistulated donor cattle maintained under standard feeding and management conditions and immediately transferred into pre-warmed, oxygen-free containers. The collected rumen fluid was filtered through multiple layers of muslin cloth and mixed with an appropriate volume of anaerobic artificial saliva/buffer solution following the principles of the Menke and Steingass in-vitro gas production technique. The prepared inoculum was dispensed into serum bottles containing accurately weighed substrate and the respective polyherbal treatments under continuous anaerobic conditions. Each treatment was incubated in six replicates at 39°C for 24 h, with an appropriate number of blank bottles containing inoculum without substrate. Gas production was recorded at predetermined incubation intervals to characterize fermentation kinetics. At the end of incubation, total gas volume was determined and gas samples were collected for methane estimation using gas chromatography equipped with a flame ionization detector (GC-FID). The fermented residues were recovered for determination of in-vitro dry matter digestibility (IVDMD) and organic matter digestibility (IVOMD). Fermentation fluid pH was measured immediately after incubation, while aliquots were preserved for estimation of ammonia-nitrogen and short-chain fatty acids. Microbial protein synthesis and fermentation efficiency were subsequently calculated from the fermentation data using established equations.

3. Methane Estimation, Fermentation Parameters and Statistical Analysis

Methane concentration in the fermentation gas was quantified by gas chromatography using suitable calibration standards, and methane production was expressed both as volume per unit substrate incubated and as a proportion of total gas production. Fermentation characteristics including cumulative gas production, methane production, pH, ammonia-nitrogen, total short-chain fatty acids, individual volatile fatty acids, microbial protein synthesis, partitioning factor and estimated metabolizable energy were evaluated to determine the nutritional and environmental response to the polyherbal additive. Gas-production data were fitted to an appropriate nonlinear fermentation model to estimate kinetic parameters such as asymptotic gas production, fermentation rate and lag time. All experimental treatments were arranged using a completely randomized design with six independent replicates per treatment. The data were expressed as mean ±

standard error and subjected to one-way analysis of variance (ANOVA). When significant treatment effects were detected, means were separated using Duncan's Multiple Range Test (DMRT), and statistical significance was accepted at $P < 0.05$. The optimum polyherbal inclusion level was identified by considering the combined response of digestibility, fermentation efficiency and methane suppression rather than methane reduction alone.

Results and Discussion

1. Effect of Polyherbal Supplementation on In-Vitro Digestibility

The results presented in Table 4 demonstrated that supplementation with the papaya–coriander–cumin–fennel polyherbal formulation improved in-vitro nutrient digestibility compared with the untreated control. The control recorded an IVDMD of 61.42% and IVOMD of 64.18%, whereas supplementation at different concentrations increased IVDMD to approximately 63.15–67.58% and IVOMD to 65.73–70.12%. Among the treatments, T5 (40% papaya leaf + 20% coriander + 20% cumin + 20% fennel at 0.05% inclusion) produced the highest IVDMD (67.58%) and IVOMD (70.12%). This suggests that the optimized polyherbal combination enhanced substrate utilization without causing inhibitory effects on rumen fermentation. The improvement may be associated with the complementary action of phytochemicals present in the four plant materials. At the moderate inclusion level, these compounds may modify microbial activity and improve the accessibility or degradation of fermentable components in the substrate. The response was not strictly proportional to increasing additive concentration. Although T2 at 0.05% inclusion showed improved digestibility, the higher 0.075 and 0.10% inclusion levels did not provide a further improvement. IVDMD decreased from 66.84% in T2 to 65.92% in T3 and 63.48% in T4, while IVOMD followed a similar pattern. This indicates that an optimum concentration may exist beyond which increasing phytochemical exposure could begin to inhibit certain fibrolytic or fermentative microorganisms. Such a dose-dependent response is important when developing phytogenic feed additives because excessive antimicrobial activity may negatively affect rumen microbial populations responsible for fibre degradation. Figure 1 describes about the Effect of Polyherbal Feed Additive Supplementation on Cumulative Gas Production During In-Vitro Rumen Fermentation. Table 1 presents the experimental design used to evaluate the effects of the papaya leaf–coriander seed–cumin seed–fennel seed polyherbal feed additive (PHF) on in-vitro rumen fermentation. The control treatment (T0) received no herbal additive. Treatments T1–T4 contained the four herbs in equal proportions (25:25:25:25) at inclusion levels of 0.025, 0.050, 0.075 and 0.100% of substrate DM, respectively. Treatments T5–T8 were formulated at a fixed inclusion level of 0.050% DM but with one herb increased to 40% and the remaining three herbs maintained at 20%. This design enabled evaluation of both inclusion-level effects and changes in herbal proportion.

Table 1: Experimental design and treatment combinations

Treatment	Papaya leaf (%)	Coriander seed (%)	Cumin seed (%)	Fennel seed (%)	Total PHF	Inclusion level (% DM)
T0 – Control	–	–	–	–	0	0
T1	25	25	25	25	100	0.025
T2	25	25	25	25	100	0.050
T3	25	25	25	25	100	0.075
T4	25	25	25	25	100	0.100
T5	40	20	20	20	100	0.050
T6	20	40	20	20	100	0.050
T7	20	20	40	20	100	0.050
T8	20	20	20	40	100	0.050

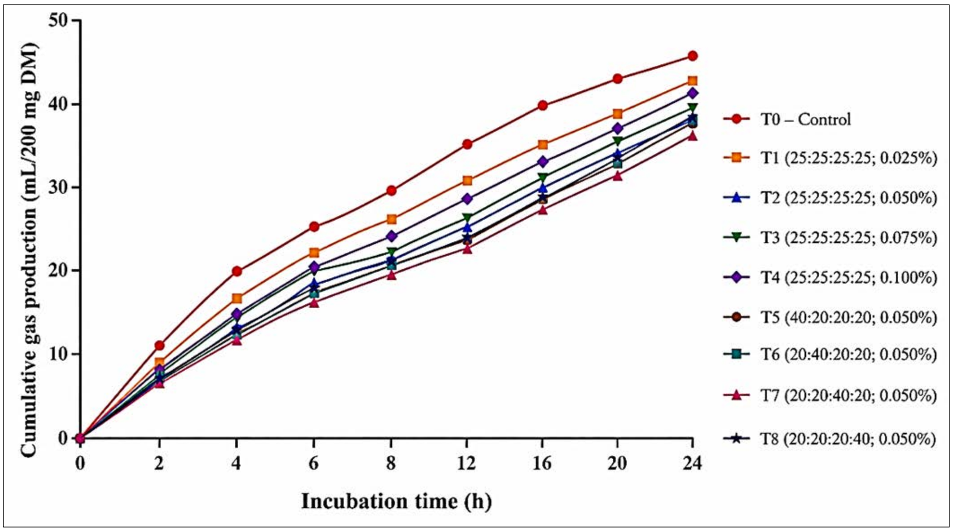


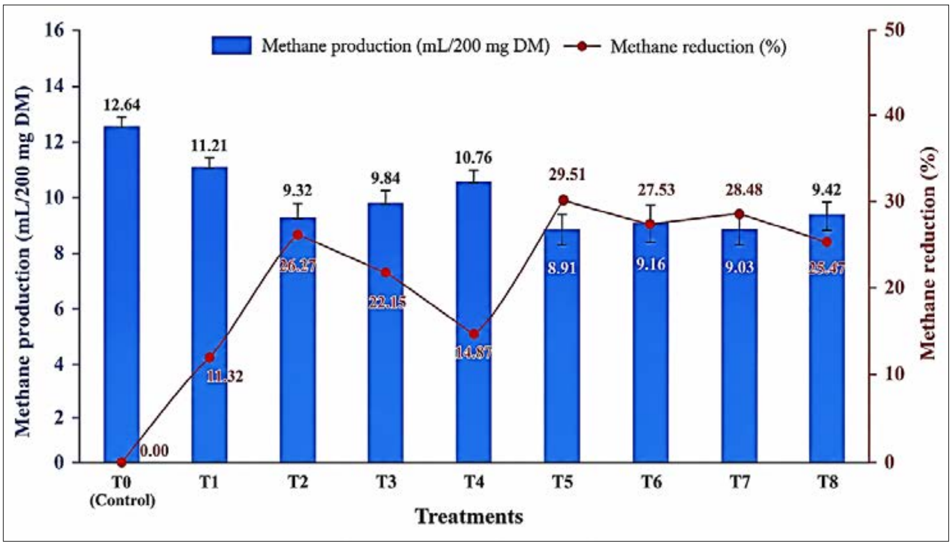
Fig 1: Cumulative gas-production kinetics of the control and polyherbal feed additive–supplemented treatments during in-vitro rumen fermentation.

2. Effect on Total Gas and Methane Production

Total gas production was reduced in all polyherbal treatments compared with the control, indicating modification of rumen fermentation. The control produced 46.82 mL/200 mg DM, whereas gas production decreased to 39.24–44.36 mL/200 mg DM in the supplemented treatments. The lowest value was observed in T5 (39.24 mL/200 mg DM). The reduction in total gas production may indicate a shift in fermentation pathways caused by the combined phytochemicals present in the formulation. However, lower gas production should not be interpreted as beneficial by itself; the simultaneous improvement in digestibility observed in T5 suggests that the reduction was not associated with suppression of overall substrate utilization. Methane production showed a more pronounced response. The control produced 12.64 mL/200 mg DM, whereas methane decreased to 8.91–11.21 mL/200 mg DM following polyherbal supplementation. The greatest reduction was observed in T5, which produced 8.91 mL/200 mg DM, corresponding to an estimated 29.51% reduction relative to the control. T7 and T6 also showed substantial reductions of approximately 28.48 and 27.53%, respectively. These findings indicate that the polyherbal formulation could selectively modify rumen fermentation in a manner that reduces methanogenesis. The methane response may be related to the combined effects of the volatile and phenolic constituents of coriander, cumin and fennel together with the phytochemical constituents of papaya leaves. Plant-derived compounds can influence microbial interactions and hydrogen transfer within the rumen. A reduction in hydrogen availability for methanogenic archaea may contribute to lower methane formation. Importantly, the present results suggest that methane suppression was achieved without a corresponding reduction in digestibility at the optimum inclusion level. Figure 2 explains about the Effect of Polyherbal Feed Additive Supplementation on Methane Production and Methane Reduction. Table 2 shows the proximate and fibre composition of the experimental substrate used for the in-vitro fermentation study. The substrate contained 91.20% dry matter and 88.15% organic matter, with crude protein, ether extract and crude fibre contents of 8.74, 2.15 and 28.63%, respectively. The neutral detergent fibre and acid detergent fibre contents were 58.42 and 34.16%, respectively, while total ash was 11.85%. These values characterize the nutritional composition of the basal substrate used for assessing the effects of polyherbal supplementation on rumen fermentation.

Table 2: Proximate composition of the experimental substrate

Parameter	Value (%)
Dry matter	91.20 ± 0.42
Organic matter	88.15 ± 0.36
Crude protein	8.74 ± 0.18
Ether extract	2.15 ± 0.09
Crude fibre	28.63 ± 0.51
Neutral detergent fibre	58.42 ± 0.74
Acid detergent fibre	34.16 ± 0.48
Total ash	11.85 ± 0.36



Note: Methane reduction (%) calculated relative to the control (T0).

Fig 2: Methane production (mL/200 mg DM) and methane reduction (%) in response to different proportions and inclusion levels of the polyherbal feed additive

3. Influence of Polyherbal Treatment on Methane-Mitigating Efficiency

The percentage reduction in methane shown in Table 4 provides an additional measure of the effectiveness of the treatments. The lowest inclusion treatment, T1, produced an 11.32% reduction, while T2 and T3 produced 26.27 and 22.15% reductions, respectively. The response increased substantially with the optimized formulations, with T5 producing the highest reduction of 29.51%. The result suggests that the relative proportions of the individual herbs may be as important as the total quantity of the additive. Interestingly, T5, containing a higher proportion of papaya leaf and lower proportions of the three seed ingredients, produced the best combined response for digestibility and methane suppression. In contrast, simply increasing the overall inclusion level to 0.10% did not produce a comparable response. This supports the concept that formulation optimization rather than maximum dosage is important for phytogenic feed additives. The objective should therefore be to identify a concentration that produces sufficient modulation of methanogenesis while preserving ruminal microbial activity and nutrient degradation. Table 3 summarizes the major bioactive compounds and their expected functional roles in the four herbal ingredients. Papaya leaves contain phenolics, flavonoids, enzymes and alkaloids, whereas coriander and cumin seeds are rich in essential or volatile oils, phenolics and flavonoids. Fennel seeds contain anethole along with phenolic and flavonoid compounds. Collectively, these phytochemicals may influence rumen microbial activity and fermentation pathways, potentially contributing to reduced methane production and improved fermentation efficiency.

Table 3: Phytochemical characteristics of individual herbal ingredients

Plant material	Major bioactive constituents	Principal expected function
Papaya leaf	Phenolics, flavonoids, enzymes, alkaloids	Microbial modulation and antioxidant activity
Coriander seed	Essential oils, phenolics, flavonoids	Fermentation modulation
Cumin seed	Volatile oils, terpenoids, phenolics	Microbial and fermentation modulation
Fennel seed	Anethole, phenolics, flavonoids	Modulation of rumen microbial activity
Polyherbal mixture	Combined phytochemicals	Potential synergistic fermentation response

4. Effect on Rumen pH and Ammonia-N

The fermentation characteristics presented in Table 5 showed that the polyherbal treatments maintained ruminal pH within a relatively narrow range of 6.67–6.72. The control recorded a pH of 6.72, while T5 showed 6.67, indicating that the additive did not cause a substantial alteration in the fermentation environment. Maintenance of an appropriate ruminal pH is important because excessive acidification can negatively affect fibre-degrading microorganisms. The relatively stable pH across treatments therefore supports the observed improvement in digestibility. Ammonia-nitrogen concentrations ranged from 17.14 to 18.42 mg/dL. The control recorded the highest value (18.42 mg/dL), whereas T5 showed 17.14 mg/dL. The moderate reduction in ammonia-N may indicate altered degradation of dietary nitrogen or increased incorporation of available nitrogen into microbial biomass. This interpretation is supported by the higher estimated microbial protein production observed in the supplemented groups. However, the relationship between ammonia concentration and microbial protein synthesis depends on substrate availability and the overall balance of ruminal nitrogen and energy. Table 4 demonstrates the effects of different polyherbal treatments on in-vitro digestibility, total gas production and methane production. Compared with the control, most supplemented treatments increased IVDMD and IVOMD while reducing total gas and methane production. The T5 treatment showed the highest IVDMD (67.58%) and IVOMD (70.12%), together with the lowest total gas production (39.24 mL/200 mg DM) and methane production (8.91 mL/200 mg DM). The corresponding methane reduction was 29.51%, indicating that T5 produced the most pronounced methane-mitigation response among the treatments evaluated.

Table 4: Effect of polyherbal supplementation on in-vitro digestibility and gas production

Treatment	IVDMD (%)	IVOMD (%)	Total gas (mL/200 mg DM)	Methane (mL/200 mg DM)	Methane reduction (%)
T0 – Control	61.42 ± 0.72[a]	64.18 ± 0.69[a]	46.82 ± 1.21[a]	12.64 ± 0.41[a]	–
T1	63.15 ± 0.61[ab]	65.73 ± 0.58[ab]	44.36 ± 1.08[ab]	11.21 ± 0.36[ab]	11.32
T2	66.84 ± 0.55[b]	69.26 ± 0.63[b]	40.18 ± 0.94[b]	9.32 ± 0.29[b]	26.27
T3	65.92 ± 0.67[b]	68.41 ± 0.71[b]	41.63 ± 1.02[b]	9.84 ± 0.31[b]	22.15
T4	63.48 ± 0.74[ab]	66.02 ± 0.68[ab]	43.91 ± 1.16[ab]	10.76 ± 0.38[ab]	14.87
T5	67.58 ± 0.49[b]	70.12 ± 0.54[b]	39.24 ± 0.87[b]	8.91 ± 0.27[b]	29.51
T6	66.93 ± 0.58[b]	69.64 ± 0.61[b]	40.02 ± 0.91[b]	9.16 ± 0.30[b]	27.53
T7	67.24 ± 0.52[b]	69.88 ± 0.59[b]	39.71 ± 0.89[b]	9.03 ± 0.28[b]	28.48
T8	66.71 ± 0.63[b]	69.35 ± 0.66[b]	40.46 ± 0.95[b]	9.42 ± 0.32[b]	25.47

5. Effect on Short-Chain Fatty Acid Production and Fermentation Pattern

Polyherbal supplementation increased total SCFA production compared with the control. Total SCFA increased from 82.36 mmol/L in the control to 88.42 mmol/L in T5, indicating enhanced fermentation efficiency at the optimum treatment. At the same time, the acetate proportion decreased from 64.18% in the control to 61.35% in T5, while propionate increased from 19.42 to 22.91%. Consequently, the acetate-to-propionate ratio decreased from 3.30 to 2.68. The shift toward greater propionate production is particularly relevant to methane mitigation because propionate formation can act as an alternative hydrogen sink within rumen fermentation. Increased propionate formation may therefore reduce the quantity of hydrogen available for conversion to methane. The simultaneous increase in total SCFA and reduction in methane observed in T5 suggests a potentially more favourable redistribution of fermentation products rather than simple inhibition of total microbial fermentation.

6. Effect on Microbial Protein Synthesis

Microbial protein production was also improved by the polyherbal treatments. The control showed an estimated microbial protein value of 128.4 mg/g DM, whereas the supplemented treatments ranged from 132.7 to 144.6 mg/g DM. The highest value was observed in T5 (144.6 mg/g DM). Increased microbial protein synthesis together with higher digestibility and SCFA production indicates that the optimized treatment may improve the efficiency with which fermentable nutrients are converted into microbial biomass. The combination of improved microbial protein synthesis and reduced methane production is particularly desirable because it suggests that the additive did not merely suppress microbial activity. Instead, the formulation may have altered the microbial fermentation pattern toward greater nutrient capture and reduced energy loss as methane. Further confirmation using microbial-community analysis would be valuable for identifying the specific microbial populations responsible for this response. Figure 3 describes about the a) Relationship Between In-Vitro Dry Matter Digestibility and Methane Production b) Dose-Response Effect of Polyherbal Feed Additive Inclusion Level on Methane Production. Table 5 presents the effects of polyherbal supplementation on pH, ammonia nitrogen, total SCFA, acetate, propionate, acetate-to-propionate ratio and microbial protein production. Supplementation produced relatively small changes in ruminal pH, indicating that the treatments maintained fermentation conditions within a suitable range. Compared with the control, polyherbal treatments generally reduced ammonia-N and increased total SCFA production. The increase in propionate accompanied by a reduction in the acetate-to-propionate ratio suggests a shift in fermentation towards propionate formation. T5 recorded the highest total SCFA concentration (88.42 mmol/L), propionate proportion (22.91%) and microbial protein production (144.6 mg/g DM), indicating a potentially favourable fermentation response.

Table 5: Effect of polyherbal additive on rumen fermentation characteristics

Treatment	pH	NH_3-N (mg/dL)	Total SCFA (mmol/L)	Acetate (%)	Propionate (%)	A:P ratio	Microbial protein (mg/g DM)
Control	6.72 ± 0.05	18.42 ± 0.51	82.36 ± 1.84	64.18	19.42	3.30	128.4
T1	6.70 ± 0.04	18.06 ± 0.48	83.91 ± 1.72	63.74	20.18	3.16	132.7
T2	6.68 ± 0.05	17.32 ± 0.46	87.64 ± 1.65	61.82	22.36	2.76	141.8
T3	6.69 ± 0.04	17.58 ± 0.42	86.73 ± 1.71	62.41	21.84	2.86	139.6
T4	6.71 ± 0.05	17.91 ± 0.45	84.62 ± 1.69	63.26	20.74	3.05	135.1
T5	6.67 ± 0.04	17.14 ± 0.43	88.42 ± 1.58	61.35	22.91	2.68	144.6
T6	6.68 ± 0.05	17.28 ± 0.44	87.91 ± 1.62	61.74	22.54	2.74	142.9
T7	6.67 ± 0.04	17.21 ± 0.41	88.15 ± 1.60	61.52	22.72	2.71	143.7
T8	6.69 ± 0.05	17.46 ± 0.45	87.36 ± 1.67	62.06	22.18	2.80	141.5

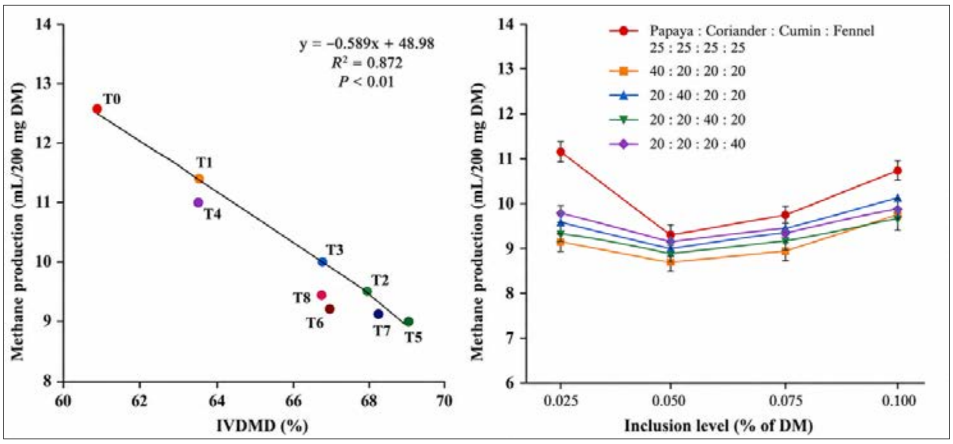


Fig 3: a) Relationship Between In-Vitro Dry Matter Digestibility and Methane Production b) Dose-Response Effect of Polyherbal Feed Additive Inclusion Level on Methane Production

7. Overall Performance and Identification of the Optimum Treatment

Considering all parameters together, T5 (40% papaya leaf + 20% coriander seed + 20% cumin seed + 20% fennel seed at 0.05% DM) demonstrated the most favourable overall response. It produced the highest IVDMD (67.58%), highest IVOMD (70.12%), lowest total gas production (39.24 mL/200 mg DM), lowest methane production (8.91 mL/200 mg DM) and greatest methane reduction (29.51%). It also showed increased total SCFA, improved propionate proportion, reduced acetate-to-propionate ratio and the highest microbial protein production. Therefore, the present results indicate that the optimum polyherbal response was obtained at a moderate inclusion level rather than at the highest dose. This finding is important for practical feed-additive development because excessive supplementation may increase phytochemical exposure without providing additional nutritional benefits. The overall response of T5 demonstrates the potential of the papaya–coriander–cumin–fennel combination as a phytogenic additive capable of simultaneously improving ruminal nutrient utilization and reducing methane formation under in-vitro conditions.

Conclusion

The present in-vitro study demonstrated the potential of a novel *papaya (Carica papaya) leaf–coriander (Coriandrum sativum) seed–cumin (Cuminum cyminum) seed–fennel (Foeniculum vulgare)* seed polyherbal feed additive to simultaneously improve ruminal nutrient utilization and mitigate methane production. Among the evaluated treatments, the formulation containing 40% papaya leaf, 20% coriander, 20% cumin and 20% fennel at 0.05% inclusion level showed the most favourable overall response, with improved in-vitro dry matter and organic matter digestibility, reduced total gas and methane production, enhanced short-chain fatty acid production and increased microbial protein synthesis. The shift toward greater propionate production and a lower acetate-to-propionate ratio further indicated a potentially favourable redistribution of rumen fermentation pathways. The moderate inclusion level produced a better response than the higher dose, highlighting the importance of optimizing phytochemical concentration rather than increasing supplementation indiscriminately. Overall, the findings suggest that this novel polyherbal combination has potential as a natural phytogenic feed additive for improving rumen fermentation efficiency while reducing methane-associated energy loss. However, the results require confirmation through in-vivo feeding trials, rumen microbial characterization and long-term safety and performance studies before practical application in ruminant production.

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