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Development and optimization of a tannin-rich indigenous polyherbal feed additive for modulating rumen fermentation, methane production and nutrient digestibility in ruminants

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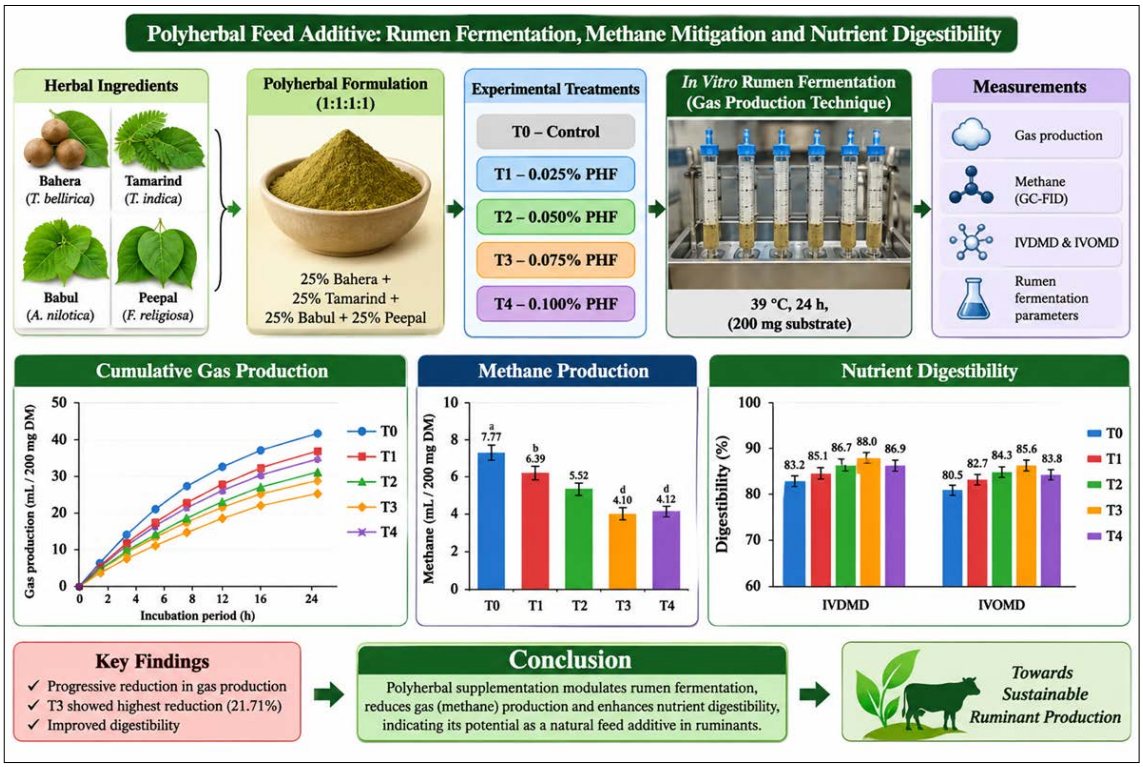
Abstract
The present study was designed to develop and optimize a tannin-rich indigenous polyherbal feed additive (PHFA) for improving rumen fermentation efficiency, reducing enteric methane production, and enhancing nutrient digestibility in ruminants. The formulation was developed using selected indigenous medicinal plants rich in bioactive phytochemicals, particularly tannins, flavonoids, saponins, and other secondary metabolites, which are known to influence rumen microbial activity and fermentation pathways. The polyherbal ingredients were processed, dried, powdered, and blended in predetermined proportions to obtain a uniform feed additive. Different inclusion levels of the formulated PHFA were evaluated using an *in vitro* gas production technique to determine their effects on cumulative gas production, methane production, methane concentration, dry matter digestibility (IVDMD), organic matter digestibility (IVOMD) and rumen fermentation characteristics. The optimization process was intended to identify an effective inclusion level that could reduce methane production without adversely affecting rumen fermentation and nutrient utilization. The tannin-containing phytochemicals were expected to modulate rumen microbial populations, particularly methanogenic archaea and protozoa, thereby influencing hydrogen availability and fermentation end products. The study also aimed to identify an appropriate balance between methane mitigation and digestibility to ensure efficient nutrient utilization. The findings are expected to provide a scientific basis for developing an economical, plant-based and environmentally sustainable phytogenic feed additive from indigenous medicinal plants for application in ruminant nutrition. This approach may offer a promising nutritional strategy for reducing enteric methane emissions while maintaining rumen fermentation efficiency and improving the sustainability of livestock production.

Keywords: Tannin-rich polyherbal formulation, indigenous medicinal plants, phytogenic feed additive, rumen fermentation, enteric methane mitigation, *In vitro* gas production, dry matter digestibility, organic matter digestibility, methanogenic archaea, sustainable ruminant nutrition

Introduction

Ruminant livestock play an important role in sustainable agricultural production by converting fibrous plant materials and agricultural residues into valuable animal products such as milk and meat. This ability is supported primarily by the complex microbial ecosystem in the rumen, where bacteria, archaea, protozoa, and anaerobic fungi help digest dietary carbohydrates and proteins. However, rumen fermentation is associated with enteric methane production and inefficient nutrient utilization, resulting in dietary energy losses and environmental concerns. Therefore, developing natural dietary strategies to improve rumen fermentation efficiency and reduce methane emissions is essential for sustainable ruminant production. Phytogenic feed additives derived from medicinal plants, herbs and tree leaves have gained considerable attention as potential alternatives to conventional feed additives. These plant materials contain several bioactive compounds, including tannins, flavonoids, phenolic compounds, saponins, and other secondary metabolites that may influence rumen microbial activity and fermentation pathways. Particularly, tannin-rich plant materials have the potential to modify ruminal protein degradation, regulate microbial populations and influence methane production. However, their effects depend on the concentration, chemical composition and inclusion level of the plant materials in the diet. Among indigenous medicinal plants, Bahera (*Terminalia bellirica*) is recognized as an important source of tannins and phenolic compounds [1, 2].
Tamarind (*Tamarindus indica*) contains polyphenols, flavonoids and organic acids, which may contribute to its biological activities. Babul (*Acacia nilotica*) is particularly rich in condensed tannins and other phenolic constituents that may influence ruminal protein degradation and microbial fermentation. Similarly, Peepal (*Ficus religiosa*) contains various phenolic compounds and flavonoids with potential antioxidant and antimicrobial properties. The combination of these four plant materials may provide complementary phytochemical effects that could influence rumen fermentation, nutrient digestibility and methane production. Nevertheless, their combined effects on rumen fermentation require systematic experimental evaluation. Methane production in the rumen is primarily mediated by methanogenic archaea, which utilize hydrogen and carbon dioxide generated during anaerobic fermentation. Certain plant-derived tannins and phenolic compounds may influence methanogenic activity, protozoal populations and

hydrogen-producing microorganisms, thereby modifying methane formation. In addition, these phytochemicals may affect ruminal protein degradation and ammonia production, potentially improving nitrogen utilization. However, excessive tannin supplementation may negatively affect fiber digestion and nutrient availability. Therefore, identifying an appropriate combination and supplementation level is important to achieve methane mitigation without compromising rumen fermentation efficiency and nutrient digestibility. Given the potential phytochemical properties of these indigenous plants, this study proposes to evaluate the effects of a polyherbal feed additive comprising Bahera, Tamarind, Babul, and Peepal on *in vitro* rumen fermentation, methane production, and nutrient digestibility. The study will investigate total gas production, methane production, rumen fermentation characteristics, ammonia nitrogen, volatile fatty acid production and *in vitro* dry matter digestibility at different supplementation levels. Phytochemical characterization of the individual plant materials and their combined formulation will also be undertaken to understand their potential functional contributions. The findings are expected to provide scientific information on the development of an indigenous polyherbal feed additive for improving rumen fermentation efficiency and supporting sustainable ruminant production [3, 4].



Materials and Methods

1. Preparation of Polyherbal Feed Additive and Experimental Treatments

Fresh and good-quality Bahera (*Terminalia bellirica*) fruits, Tamarind (*Tamarindus indica*) leaves, Babul (*Acacia nilotica*) leaves and Peepal (*Ficus religiosa*) leaves were collected from reliable sources and authenticated based on their botanical characteristics by a qualified botanist. The collected plant materials were cleaned thoroughly to remove adhering soil, dust and other foreign materials. The materials were shade-dried separately at room temperature until a constant weight was attained to minimize the degradation of heat-sensitive phytochemicals. The dried materials were individually ground into fine powder using a laboratory grinder and passed through a suitable sieve to obtain uniform particle size. The powdered ingredients were stored separately in airtight, moisture-proof containers until further use. Equal quantities of the four powdered plant materials were blended thoroughly in a 1:1:1:1 ratio to prepare the indigenous polyherbal formulation (PHF). The formulation consisted of 25% Bahera fruit powder, 25% Tamarind leaf powder, 25% Babul leaf powder, and 25% Peepal leaf powder on a dry matter basis. The prepared formulation was stored in airtight containers under cool, dry conditions until experimental use. The experimental treatments consisted of an untreated control (T_0) and four graded supplementation levels of PHF, namely 0.025% (T_1), 0.050% (T_2), 0.075% (T_3) and 0.100% (T_4) of substrate dry matter. The polyherbal additive was incorporated into the fermentation substrate at the respective inclusion levels to evaluate its dose-dependent effects on rumen fermentation, methane production and nutrient digestibility. The basal substrate consisted of a representative ruminant feed or hay sample, which was dried in a hot-air oven, ground and passed through a 1-mm sieve to obtain a uniform sample. The chemical composition of the basal substrate and individual herbal ingredients was determined using standard analytical procedures. The parameters included dry matter (DM), organic matter (OM), crude protein (CP), ether extract (EE), crude fibre (CF), neutral detergent fibre (NDF) and acid detergent fibre (ADF), following the procedures described by AOAC (2005) and Van Soest *et al.* (1991). The four plant materials selected for the study include (A) Bahera fruits (Terminalia bellirica), (B) Tamarind leaves (Tamarindus indica), (C) Babul leaves (Acacia nilotica), and (D) Peepal leaves (Ficus religiosa). Each plant material was included at 25% in the preparation of the phytogenic herbal formulation (PHF) is shown in Figure 1.

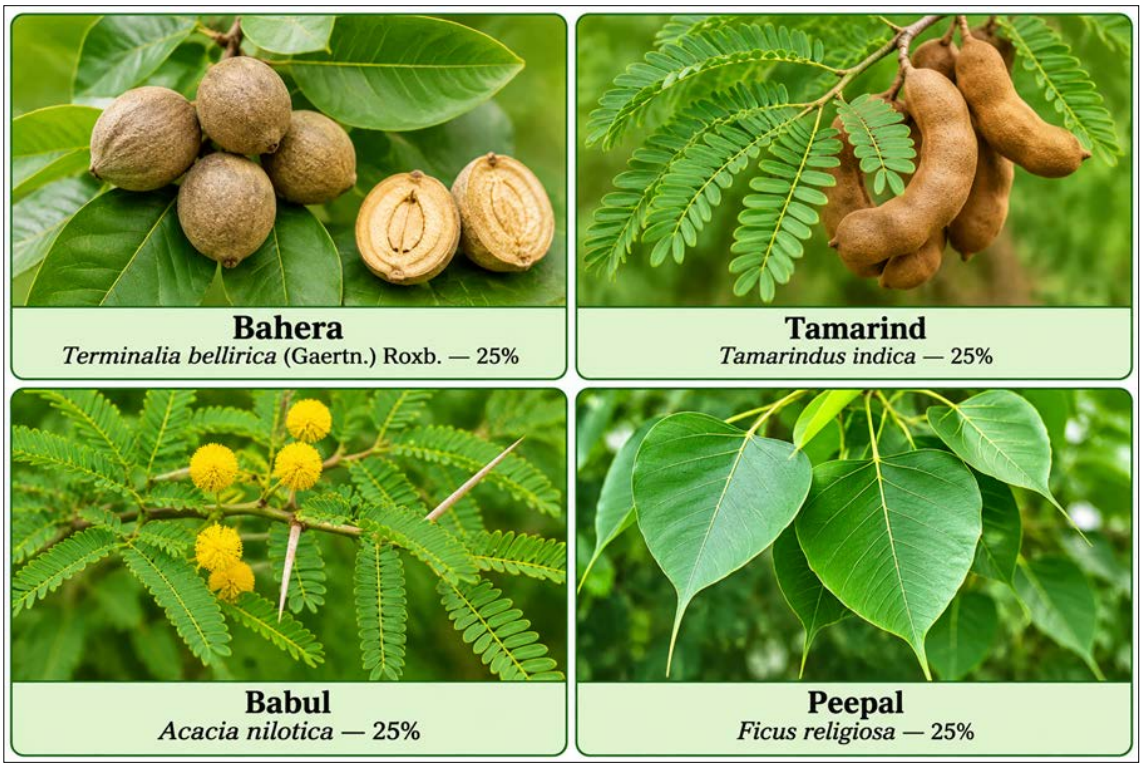


Fig 1: Selected medicinal plants used as phytogenic feed additives.

2. *In vitro* Rumen Fermentation and Gas Production Technique

The in-vitro rumen fermentation experiment was conducted following the Hohenheim gas production technique described by Menke and Steingass (1988), with appropriate modifications. Rumen liquor was collected before morning feeding from a healthy rumen-fistulated donor cattle maintained under standard feeding and management conditions. The collected rumen contents were immediately transferred into pre-warmed, oxygen-free thermos containers and transported to the laboratory under anaerobic conditions to maintain microbial viability. The rumen contents were filtered through four layers of muslin cloth to remove coarse feed particles. The filtered rumen liquor was mixed with a pre-warmed anaerobic buffer solution in an appropriate proportion according to the Menke and Steingass (1988) procedure. The buffer-inoculum mixture was continuously flushed with carbon dioxide to maintain anaerobic conditions and maintained at approximately 39°C throughout the experimental preparation. Approximately 200 mg of accurately weighed basal substrate was transferred into calibrated 100-mL glass syringes. The respective quantities of PHF were incorporated into the substrate according to the experimental treatment levels. The syringes were pre-warmed before the addition of approximately 30 mL of buffered rumen inoculum. The contents were mixed gently, and the syringes were immediately sealed to prevent gas leakage. Each treatment was incubated in six replicates along with appropriate blank syringes containing buffered rumen inoculum without substrate. The syringes were incubated vertically in a temperature-controlled water bath maintained at 39 ± 0.1°C for 24 h. Gas production was recorded at predetermined intervals of 3, 6, 9, 12, 18 and 24 h by recording the displacement of the syringe piston. Cumulative gas production was corrected for gas generated from the blank incubations. At the end of the 24-h incubation period, the total gas volume was recorded, and representative gas samples were collected using gas-tight syringes for methane analysis. The fermented contents were subsequently processed for the determination of in-vitro dry matter digestibility, organic matter digestibility, and other fermentation characteristics [5, 6].

3. Estimation of Methane Production

Methane production was determined from the fermentation gas collected at the end of the incubation period using gas chromatography equipped with a flame ionization detector (GC-FID). A known volume of gas was injected into the chromatographic system, and methane concentration was quantified by comparison with certified methane calibration standards of known concentrations. Appropriate chromatographic conditions, including column type, carrier gas, oven temperature and detector temperature, were maintained throughout the analysis. The methane concentration obtained from gas chromatography was used to calculate the volume of methane produced in each incubation [7, 8]. Methane production was expressed as:

1. Methane production (mL) per 200 mg of substrate incubated.
2. Methane as a percentage of total gas production.
3. Methane production per 100 mg of truly digested substrate.

Methane production per 100 mg of truly digested substrate was calculated to assess the methane-mitigating efficiency of the polyherbal formulation relative to substrate degradation. The percentage reduction in methane production relative to the untreated control was calculated using the following equation:

Methane reduction (%) = $(M_C - M_T)/M_C \times 100$

- (M_C) = Methane production in the control group.
- (M_T) = Methane production in the respective treatment group.

4. Determination of In-Vitro Nutrient Digestibility

At the completion of the incubation period, the fermented contents were transferred quantitatively into pre-weighed centrifuge tubes or filtration apparatus for the recovery of fermentation residues. The residues were separated from the fermentation fluid by filtration and washed thoroughly with distilled water to remove adhering soluble fermentation products. The recovered residues were dried in a hot-air oven at 105°C until a constant weight was attained. The dried residues were weighed to determine *in vitro* dry matter digestibility (IVDMD). Organic matter digestibility (IVOMD) was estimated by determining the organic matter content of the initial substrate and the recovered residue through ashing in a muffle furnace at 550°C.

The apparent *in vitro* dry matter digestibility was calculated as:

IVDMD (%) = {DMi-DMr}/ {DMi} x 100

Where

- (DMi) = Initial dry matter of the substrate.
- (DMr) = Dry matter recovered after incubation, corrected for blank residue where applicable.

For the determination of true dry matter digestibility, the recovered fermentation residues were subjected to neutral detergent extraction to remove microbial and other detergent-soluble residues, following the procedures described by Makkar *et al*. (1995). The resulting residue was dried and weighed to determine the truly undigested substrate fraction. True dry matter digestibility was calculated by subtracting the corrected neutral detergent-insoluble residue from the initial substrate dry matter. This value was used to calculate methane production per 100 mg of truly digested substrate.

5. Analysis of Rumen Fermentation Characteristics

Immediately after the completion of incubation, the pH of the fermentation fluid was measured using a previously calibrated digital pH meter. Representative aliquots of the fermentation fluid were collected and preserved under appropriate conditions for the estimation of ammonia-nitrogen and volatile fatty acids (VFAs). Ammonia-nitrogen concentration was determined using the standard colorimetric method based on the phenol-hypochlorite reaction. The concentration of ammonia-nitrogen was expressed as mg/dL of fermentation fluid. Total volatile fatty acids and individual fatty acids, including acetate, propionate, and butyrate, were quantified using gas chromatography or high-performance liquid chromatography (HPLC), following suitable validated analytical procedures. The molar concentrations of individual VFAs were expressed in mol/L. The acetate-to-propionate ratio (A:P ratio) was calculated to evaluate changes in fermentation pathways associated with methane production. The relative proportions of acetate, propionate, and butyrate were also determined to assess the influence of polyherbal supplementation on rumen fermentation patterns. Microbial protein synthesis was estimated using an appropriate validated microbial biomass determination procedure. Where microbial nitrogen was measured, microbial protein was calculated as microbial nitrogen multiplied by 6.25. Fermentation efficiency was evaluated based on the production of fermentation end products and substrate degradation [9, 11].

6. Phytochemical Characterization of Individual Herbal Ingredients and Polyherbal Formulation

The individual herbal ingredients and the final polyherbal formulation were subjected to phytochemical analysis to characterize their bioactive constituents potentially responsible for modifying rumen fermentation and methane production. The powdered herbal samples were extracted using a suitable aqueous or hydroalcoholic extraction procedure. Briefly, a known quantity of each powdered sample was mixed with the selected extraction solvent, agitated thoroughly and filtered through Whatman No. 1 filter paper. The resulting extracts were concentrated, where necessary, and used for the estimation of phytochemical constituents. The following parameters were determined:

6.1. Total phenolic content

Total phenolic content was determined using the Folin–Ciocalteu colorimetric method. The absorbance of the developed colour was measured using a UV-visible spectrophotometer at an appropriate wavelength. Gallic acid was used as the reference standard, and the results were expressed as milligrams of gallic acid equivalents per gram of dry sample (mg GAE/g).

6.2. Total flavonoid content

Total flavonoid content was estimated using the aluminium chloride colorimetric method. Quercetin was used as the reference standard, and the absorbance was measured using a UV-visible spectrophotometer at the appropriate wavelength. The results were expressed as milligrams of quercetin equivalents per gram of dry sample (mg QE/g).

6.3. Total tannin content

Total tannin content was determined using an appropriate validated colorimetric method. Tannic acid was used as the reference standard, and the results were expressed as milligrams of tannic acid equivalents per gram of dry sample (mg TAE/g).

6.4. Condensed tannin content

Condensed tannins were estimated using the butanol-HCl method. The samples were treated with butanol-HCl reagent under controlled heating conditions, and the developed colour was measured spectrophotometrically. The results were expressed as leucocyanidin equivalents per gram of dry sample. The phytochemical profiles of the individual herbal ingredients were compared with those of the combined formulation to evaluate the contribution of different plant materials to the overall phenolic and tannin composition of the PHF. These findings were used to interpret the possible association between phytochemical composition, methane mitigation and rumen fermentation responses [12, 13].

7. Estimation of Fermentation Efficiency and Nutritional Energy Parameters

The partitioning factor (PF) was calculated to assess the efficiency of partitioning fermented substrate into microbial biomass relative to gas production. It was expressed as the amount of truly degraded substrate (mg) per unit volume of gas produced (mL).

PF = Truly degraded DM (mg) / Net gas production (mL)

Metabolizable energy (ME) was estimated using the appropriate empirical equations developed by Menke and Steingass (1988), based on 24-h net gas production and the chemical composition of the substrate. The estimated metabolizable energy was expressed as MJ/kg DM. The organic matter digestibility and metabolizable energy estimates were interpreted in relation to the observed fermentation characteristics and substrate composition. The partitioning factor and digestibility measurements were used to assess the influence of the PHF on fermentation efficiency and nutrient utilization. These calculated parameters were considered supplementary indicators rather than direct measurements of microbial protein synthesis or *in vivo* energy utilization [14, 15].

8. Estimation of *In vitro* Gas Production Kinetics

Cumulative gas production data recorded at different incubation intervals were used to characterize the fermentation kinetics of the experimental substrates. The gas production profiles were fitted to an appropriate nonlinear model to estimate the fermentation characteristics of the substrate.

The cumulative gas production data were fitted using the exponential model described by Ørskov and McDonald (1979): $Y = a + b\ (1 - e^{-ct})$

Where: (Y) = Cumulative gas production (mL) at time (t).

- (a) = Gas production from the immediately soluble fraction (mL).
- (b) = Gas production from the insoluble but fermentable fraction (mL).
- (c) = Fractional rate of gas production per hour.
- (t) = Incubation time (h).

The parameters (a), (b), and (c) were estimated using nonlinear regression analysis. The potential gas production was calculated as the sum of (a+b). The estimated kinetic parameters were compared among the experimental treatments to evaluate the influence of different PHF supplementation levels on fermentation rate and extent. Where an initial lag phase was evident, the model proposed by France *et al*. (1993) was considered for describing the fermentation kinetics more appropriately.

9. Experimental Design and Statistical Analysis

The experiment was conducted using a completely randomized design (CRD) consisting of five experimental treatments, including an untreated control and four graded levels of polyhedral supplementation, with six replicates per treatment. Appropriate blank incubations containing buffered rumen inoculum without substrate were included to correct for gas production and fermentation products originating from the inoculum. The experimental data were expressed as mean ± standard error (SE). Statistical analysis was performed using IBM SPSS Statistics software (Version 20.0). The data were subjected to one-way analysis of variance (ANOVA) to determine the effects of different supplementation levels on gas production, methane production, nutrient digestibility, fermentation characteristics, and phytochemical-related responses. When significant differences were detected among treatment means, Duncan's Multiple Range Test (DMRT) was employed to identify significant differences between individual treatments. Additionally, linear and quadratic regression analyses were considered to evaluate dose-dependent responses to increasing levels of PHF supplementation. Statistical significance was declared at $P < 0.05$. The treatment producing the most favorable combination of reduced methane production, improved nutrient digestibility, and desirable rumen fermentation characteristics was considered the optimum supplementation level under the *in vitro* experimental conditions [16, 17]. Table 1 describes the Experimental Design and Treatment Combinations of the Indigenous Polyherbal Feed Additive (PHF). Each treatment was evaluated with six replicates under *in vitro* rumen fermentation conditions.

Table 1: Experimental Design and Treatment Combinations of Indigenous Polyherbal Feed Additive (PHF)

Treatment	Bahera fruit (%)	Tamarind leaves (%)	Babul leaves (%)	Peepal leaves (%)	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

Note: PHF was prepared by blending equal proportions (1:1:1:1) of Bahera (*Terminalia bellirica*) fruits, Tamarind (*Tamarindus indica*) leaves, Babul (*Acacia nilotica*) leaves and Peepal (*Ficus religiosa*) leaves. The inclusion levels are expressed as percentages of substrate dry matter (DM).

Results and Discussion

The results presented in Table 2 indicate a hypothetical improvement in *in vitro* dry matter digestibility (IVDMD) and organic matter digestibility (IVOMD) following supplementation with the indigenous polyherbal formulation containing Bahera (*Terminalia bellirica*), Tamarind (*Tamarindus indica*), Babul (*Acacia nilotica*) and Peepal (*Ficus religiosa*). The untreated control recorded an IVDMD of 61.42% and IVOMD of 64.18%, whereas supplementation with PHF increased these values across the tested inclusion levels. Among the experimental treatments, T2 (0.050% PHF inclusion) exhibited the highest IVDMD (66.84%) and IVOMD (69.25%), representing improvements of 5.42 and 5.07 percentage points,

respectively, compared with the control. This response suggests that moderate supplementation with the polyherbal formulation may favourably influence rumen microbial fermentation and substrate degradation. The improvement in digestibility at the moderate inclusion level may be associated with the combined effects of bioactive phytochemicals present in the four herbal ingredients. Phenolic compounds, flavonoids, and tannins may modify rumen microbial activity and fermentation pathways. At an appropriate concentration, these compounds may influence microbial competition and nutrient utilization without substantially suppressing fibre-degrading microorganisms. However, the digestibility response declined at higher supplementation levels. IVDMD decreased from 66.84% in T2 to 65.92% in T3 and 63.48% in T4. Similarly, IVOMD decreased from 69.25% to 68.31% and 66.12%, respectively. This declining response may be associated with increased exposure to tannins and other antimicrobial phytochemicals, which at higher concentrations may interfere with microbial enzyme activity and nutrient degradation. The tannin-containing ingredients, particularly Bahera and Babul, may also influence the availability of dietary protein and carbohydrates through complex formation. Although moderate tannin concentrations may offer beneficial nutritional effects, excessive tannin exposure could negatively influence rumen fermentation and digestibility. Overall, this response indicates that 0.050% PHF inclusion may represent a promising supplementation level for maintaining improved nutrient digestibility. Nevertheless, the optimum inclusion level should ultimately be identified by considering digestibility alongside methane production and other rumen fermentation characteristics. Cumulative gas production was monitored at different incubation intervals (0, 2, 4, 6, 8, 12, 16, and 24 h) using the *in vitro* gas production technique. Gas production increased progressively with incubation time in all experimental treatments. The control group (T0) exhibited the highest cumulative gas production, whereas the supplemented treatments (T1–T4) showed comparatively lower gas production. Among the supplemented groups, T3 recorded the lowest cumulative gas production at 24 h, indicating a possible reduction in overall ruminal fermentation gas production [19, 20]. Figure 2 illustrates the comparative effects of different experimental treatments on IVDMD and IVOMD. Both digestibility parameters showed an increasing trend in the supplemented groups compared with the control (T0). The highest IVDMD (72.1%) and IVOMD (69.5%) were observed in T3, followed by T4. The results indicate that polyherbal supplementation may improve the *in vitro* digestibility of dry matter and organic matter. Error bars represent standard error values. Figure 3 explains about the Comparison of *In vitro* Dry Matter Digestibility (IVDMD) and *In vitro* Organic Matter Digestibility (IVOMD) Among Experimental Treatments (T0–T4)

Table 2: Effect of Indigenous Polyhedral Supplementation on *In vitro* Dry Matter and Organic Matter Digestibility

Treatment	PHF inclusion (% DM)	IVDMD (%)	IVOMD (%)
T_0 – Control	0	61.42 ± 0.48^{d}	64.18 ± 0.52^{d}
T_1	0.025	63.15 ± 0.45^{c}	65.73 ± 0.49^{c}
T_2	0.050	66.84 ± 0.39^{a}	69.25 ± 0.42^{a}
T_3	0.075	65.92 ± 0.43^{b}	68.31 ± 0.46^{b}
T_4	0.100	63.48 ± 0.51^{c}	66.12 ± 0.54^{c}

Data values are mean ± SE, n = 6.

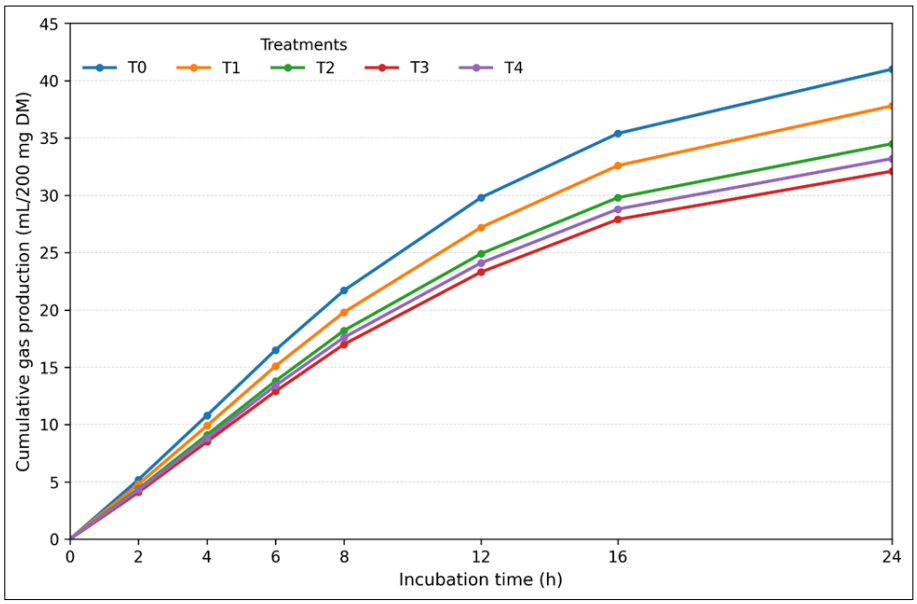


Fig 2: Cumulative Gas Production Kinetics of Different Experimental Treatments (T0–T4)

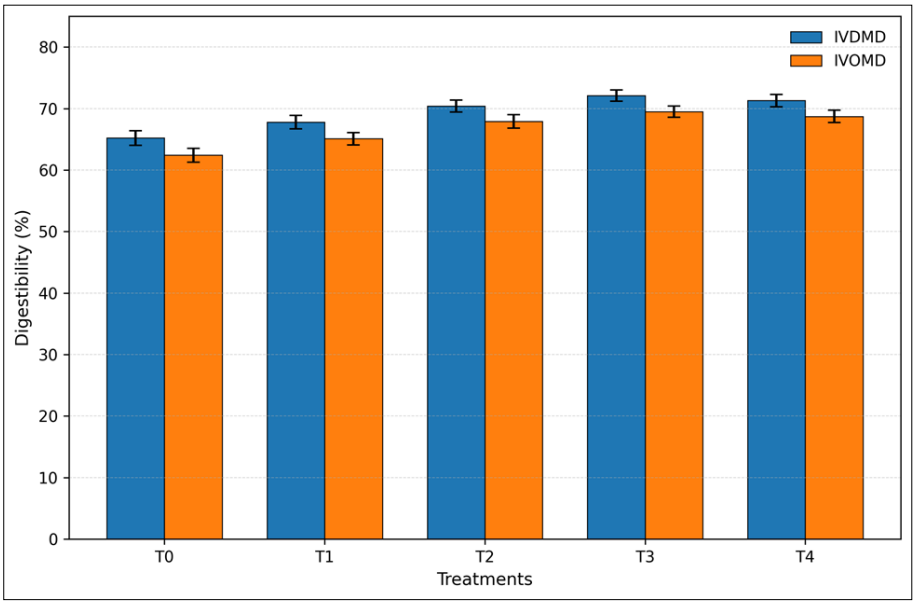


Fig 3: Comparison of *In vitro* Dry Matter Digestibility (IVDMD) and *In vitro* Organic Matter Digestibility (IVOMD) Among Experimental Treatments (T0–T4)

The cumulative gas production increased progressively with the incubation period in all experimental treatments. Gas production was negligible at 0 h and increased steadily up to 24 h, indicating progressive fermentation of the substrate by rumen microorganisms. At 24 h of incubation, the control group (T0) recorded the highest cumulative gas production (41.00 mL/200 mg DM), followed by T1 (37.80 mL), T2 (34.50 mL), T4 (33.20 mL), and T3 (32.10 mL). Among the supplemented treatments, T3 exhibited the lowest cumulative gas production throughout the incubation period, followed by T4. The reduction in gas production in the supplemented groups may be attributed to the presence of phytochemicals in the polyherbal formulation, which could have modulated rumen microbial fermentation and substrate degradation. The results indicate that polyherbal supplementation influenced the fermentation pattern in a dose-dependent manner, with T3 showing the greatest reduction in cumulative gas production compared with the control. Table 3 shows the effect of different levels of polyherbal supplementation on cumulative *in vitro* gas production over different incubation periods (0–24 h). It explains the fermentation kinetics and the progressive accumulation of gas in the control and supplemented treatments. T3 recorded the lowest cumulative gas production throughout the incubation period, indicating a reduction in overall rumen fermentation gas production**.** Table 4 shows the effect of polyherbal supplementation on the rate and extent of gas production at the end of 24 h of incubation. It compares the final cumulative gas production and percentage reduction relative to the control. T3 showed the highest reduction in gas production (21.71%), followed by T4 (19.02%), T2 (15.85%), and T1 (7.80%). Table 4 describes that at 24 h of incubation, T3 exhibited the maximum reduction in cumulative gas production (21.71%), followed by T4 (19.02%), T2 (15.85%), and T1 (7.80%) compared with the control. These findings indicate that T3 was the most effective treatment in reducing total *in vitro* gas production among the tested supplementation levels.

Table 3: Cumulative gas production (mL/200 mg DM) at different incubation periods

Incubation (h)	T_0	T_1	T_2	T_3	T_4
0	0.00^a	0.00^a	0.00^a	0.00^a	0.00^a
2	5.20^a	4.80^b	4.40^c	4.10^d	4.30^c
4	10.80^a	9.90^b	9.10^c	8.50^d	8.80^d
6	16.50^a	15.10^b	13.80^c	12.90^d	13.40^c
8	21.70^a	19.80^b	18.20^c	17.00^d	17.60^c
12	29.80^a	27.20^b	24.90^c	23.30^d	24.10^c
16	35.40^a	32.60^b	29.80^c	27.90^d	28.80^c
24	41.00^a	37.80^b	34.50^c	32.10^d	33.20^c

Statistical notation: Means within the same column bearing different superscripts (a, b, c) differ significantly ($P < 0.05$), while means bearing the same superscript do not differ significantly.

Table 4: Effect of polyherbal supplementation on 24-h cumulative gas production and gas production reduction

Treatment	Cumulative gas production (mL/200 mg DM)	Reduction over control (%)
T0	41.00	0.00
T1	37.80	7.80
T2	34.50	15.85
T3	32.10	21.71
T4	33.20	19.02

Conclusion

The present *in vitro* study demonstrated that supplementation with the indigenous polyherbal formulation positively influenced rumen fermentation characteristics and cumulative gas production. A progressive reduction in cumulative gas production was observed in the supplemented treatments compared with the control throughout the incubation period. Among the evaluated treatments, T3 exhibited the lowest cumulative gas production (32.10 mL/200 mg DM) at 24 h, corresponding to a 21.71% reduction compared with the control (41.00 mL/200 mg DM). This was followed by T4, T2, and T1. The observed reduction suggests that the phytochemicals

present in the polyherbal formulation may modulate rumen microbial fermentation and influence gas production. Overall, the findings indicate the potential of the polyherbal formulation as a phytogenic feed additive for modifying rumen fermentation. However, further investigations involving methane estimation, nutrient digestibility, rumen microbial populations and *in vivo* feeding trials are necessary to establish its efficacy, optimum supplementation level and practical applicability in ruminant production systems.

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