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EFFECT OF HERBAL MIXTURE SUPPLEMENTATION IN TOTAL MIXED RATIONS ON NUTRIENT DIGESTIBILITY, METHANE MITIGATION AND MILK PRODUCTION IN INDIGENOUS DAIRY CATTLE

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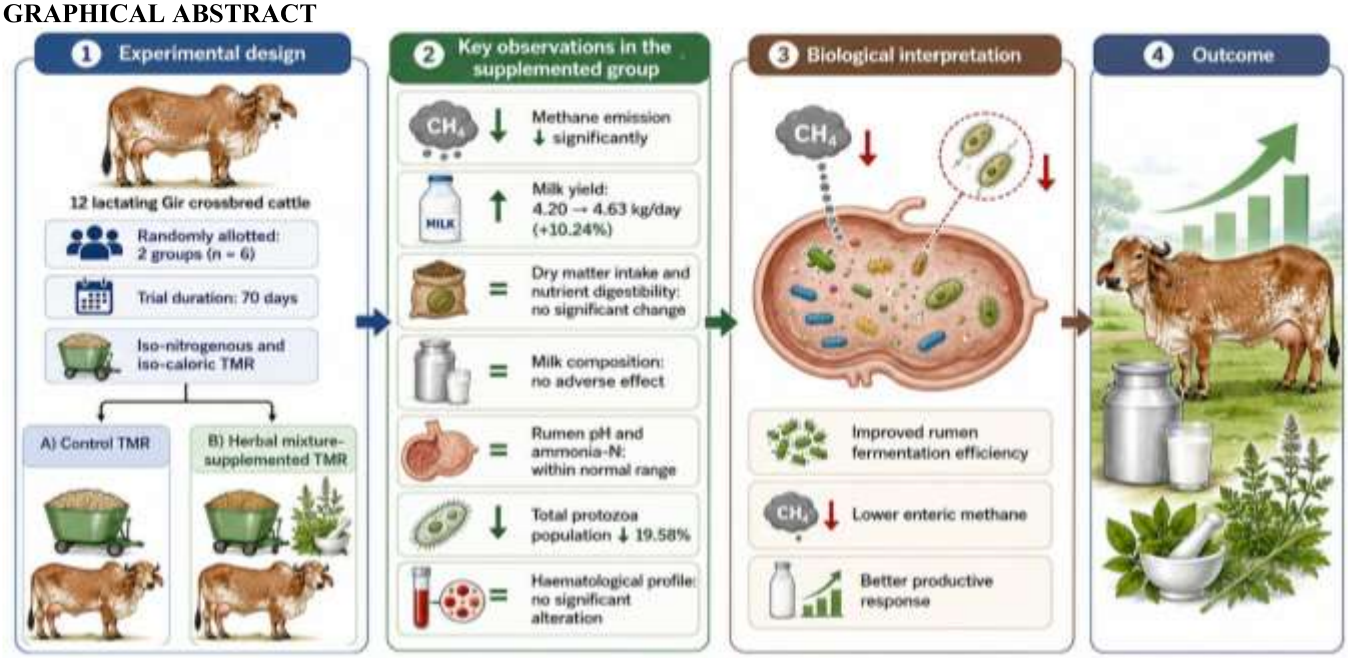
ABSTRACT
A biological trial was conducted to evaluate the effect of a potential herbal mixture combination supplemented in total mixed rations on nutrient digestibility, enteric methane emission, milk production, milk composition, rumen fermentation characteristics, and haematological profile in indigenous dairy cattle. Twelve lactating Gir crossbred cattle were randomly allotted to two groups of six animals each and fed either a control total mixed ration or an herbal mixture-supplemented total mixed ration for 70 days. The rations were iso-nitrogenous and iso-caloric. Dry matter intake and nutrient digestibility were not significantly affected by herbal mixture supplementation. Methane emissions were significantly reduced in the supplemented group, both in g/cow/day and when expressed relative to body weight, metabolic body weight, dry matter intake, and digestible dry matter intake. Average daily milk yield increased from 4.20 to 4.63 kg/day, corresponding to an improvement of approximately 10.24%, while milk composition was not adversely affected. Rumen pH and ammonia nitrogen remained within the normal range, while total protozoa population was reduced by 19.58% in the herbal mixture-supplemented group. Haematological parameters were not significantly altered. The results indicate that supplementation with the selected herbal mixture in total mixed rations can reduce methane emissions and improve milk yield in indigenous dairy cattle without compromising nutrient digestibility, milk quality, or animal health indicators.

KEYWORDS: herbal mixture, methane mitigation, total mixed ration, nutrient digestibility, milk yield, indigenous dairy cattle, rumen fermentation

GRAPHICAL ABSTRACT



1. INTRODUCTION

Methane emission from ruminants is a major environmental concern and an important source of dietary energy loss in dairy production. In cattle, enteric methane is primarily generated by methanogenic archaea during anaerobic fermentation of feed in the rumen. The extent of methane production depends on several factors, including diet composition, feed intake, nutrient digestibility, rumen fermentation characteristics and microbial activity. Excessive methane production not only contributes to greenhouse gas emissions but also represents a loss of feed energy that could otherwise be utilized for animal growth and milk production. Therefore, dietary approaches that enhance nutrient utilization while reducing enteric methane emissions are essential for improving the efficiency and sustainability of dairy farming [1-4]. Total mixed ration (TMR) feeding is an effective nutritional management strategy in dairy cattle because it provides roughages, concentrates, minerals, and other feed ingredients in a uniform mixture. A properly formulated TMR can promote consistent feed intake, improve nutrient availability and support stable rumen fermentation and milk production. However, further manipulation of rumen fermentation through suitable feed additives may improve feed-use efficiency and minimize energy losses through methane production. In this context, herbal feed additives have received increasing attention as natural and potentially sustainable alternatives to conventional synthetic rumen modifiers [5-8].

Plants contain a wide range of biologically active compounds, including tannins, saponins, flavonoids, alkaloids, terpenoids and essential oils, which can influence rumen microbial activity and fermentation pathways. These phytochemicals may reduce methane formation by suppressing methanogenic archaea and protozoal populations, modifying hydrogen availability and shifting rumen fermentation towards pathways that favour greater energy recovery. Depending on their composition and concentration, herbal additives may also enhance nutrient digestibility and improve rumen fermentation efficiency. The use of a combination of herbs may provide complementary or synergistic effects because different phytochemicals can act on rumen microorganisms through multiple mechanisms. Indigenous dairy cattle constitute an important component of smallholder dairy production in India and are well adapted to local climatic and feeding conditions. Nevertheless, information regarding the effects of herbal mixtures incorporated into TMR on nutrient digestibility, enteric methane emission, rumen fermentation, and productive performance in indigenous cattle remains limited [9-12]. Previous investigations have largely examined individual herbs or in vitro fermentation systems, while comparatively fewer studies have evaluated herbal combinations under practical feeding conditions in lactating indigenous cattle. A systematic evaluation of herbal mixtures is therefore necessary to determine whether their inclusion in TMR can simultaneously improve nutrient utilization and reduce methane losses without adversely affecting animal performance. Considering the need for sustainable nutritional strategies in dairy production, the incorporation of a selected herbal mixture into TMR may offer a promising approach for improving rumen efficiency and productive performance while mitigating enteric methane emissions [13-15]. Therefore, the present study was undertaken to evaluate the effect of herbal mixture supplementation in total mixed rations on nutrient digestibility, enteric methane mitigation, rumen fermentation characteristics, and milk production and composition in indigenous dairy cattle.

2. MATERIALS AND METHODS

2.1 Experimental site and animals

The biological trial was conducted at the Instructional Livestock Farm Complex, Madras Veterinary College, Tamil Nadu Veterinary and Animal Sciences University, Madhavaram Milk Colony, Chennai-600 051. Twelve lactating Gir crossbred cattle with an average body weight of 284 ± 17.45 kg were randomly divided into two groups consisting of six animals each. The animals were maintained in a well-ventilated concrete-floor shed with ad libitum access to clean drinking water and were managed under standard farm conditions [16-18].

2.2 Experimental design and feeding

The trial was conducted for 10 weeks (70 days). Animals in the control group were fed a control total mixed ration, while animals in the treatment group received the herbal mixture-supplemented total mixed ration. The selected herbal mixture combination was chosen based on prior in vitro screening and microbial quantification studies, in which the 60 mg combination at 0.05% level showed a reduction in methanogenic archaea and was selected for validation in the biological trial [19-21]. The ingredient composition of the rations is presented in Table 1.

Table 1. Ingredient composition of control and herbal mixture-supplemented total mixed rations (% dry matter basis).

Ingredient	Control (T1)	Treatment (T2)
CO4 hay	60.000	60.000
Maize	24.000	24.000
Soyabean meal	7.000	7.000
De-oiled rice bran	6.000	5.850
Mineral mixture	2.000	2.000
Salt	1.000	1.000
Herbal mixture	-	0.150
Total	100.000	100.000

TDN*	60.04	60.01
CP*	12.08	12.07

**Calculated values. Source data*

2.3 Chemical analysis and digestibility trial

Representative samples of feed offered and refusals were collected daily and analysed for moisture and proximate composition following standard AOAC (2007) procedures. The samples were ground through a 1-mm sieve and stored in airtight containers until analysis. A digestibility trial was conducted from the 45th to 50th day of the experimental period using a 6-day total faecal collection method. Daily faecal output was recorded, and representative faecal samples were collected, preserved, and analysed to determine nutrient digestibility. Digestible crude protein and total digestible nutrients were calculated from the respective digestible nutrient fractions. Dry matter intake, digestible dry matter intake and nutrient intake per unit metabolic body weight were calculated for both experimental groups [22-25]. Figure 1 describes the Materials and Methods Used for the Evaluation of Herbal Mixture Supplementation in Lactating Gir Crossbred Cattle

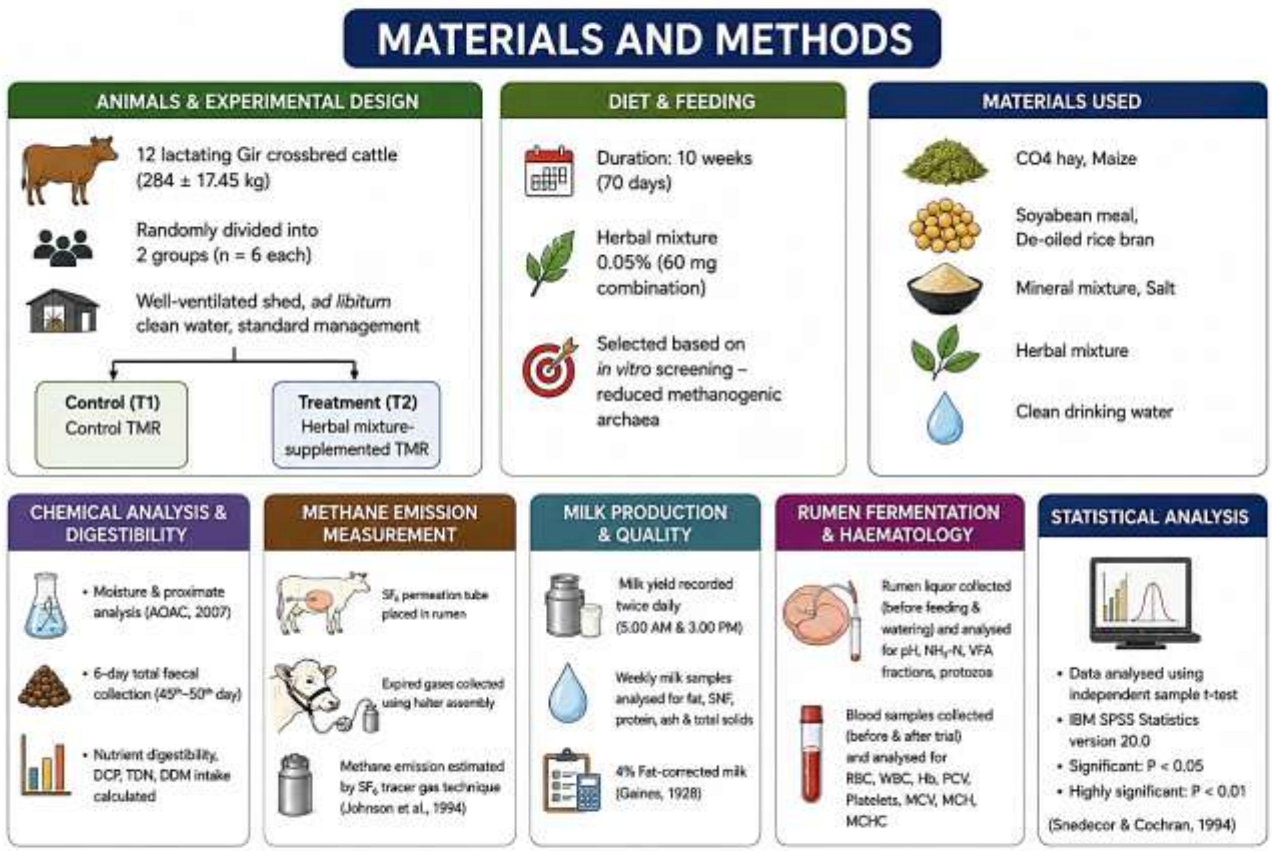


Figure 1. Materials and Methods Used for the Evaluation of Herbal Mixture Supplementation in Lactating Gir Crossbred Cattle

2.4 Methane emission measurement

Methane emission was estimated using the sulfur hexafluoride (SF6) tracer gas technique described by Johnson et al. (1994). Brass permeation tubes containing SF6 with predetermined release rates were placed in the rumen before the collection period. Expired gases were collected using evacuated canisters attached to a halter assembly. Methane emission was expressed as g/cow/day and also relative to body weight, metabolic body weight, dry matter intake and digestible dry matter intake. Background correction and procedural considerations followed the SF6 method as described in earlier studies (Williams et al., 2011).

2.5 Milk production and milk quality

Milk yield was recorded twice daily at 5.00 AM and 3.00 PM. Weekly milk samples were analysed for fat, solids-not-fat, protein, ash and total solids. Milk fat was determined by Gerber's method, protein by the Kjeldahl method using a conversion factor of 6.38, and total solids were calculated from fat and solids-not-fat. Four per cent fat-corrected milk was calculated using the formula of Gaines (1928).

2.6 Rumen fermentation and haematology

Rumen liquor was collected before feeding and watering using a stomach tube and strained through muslin cloth. Volatile fatty acids were estimated after deproteinization using metaphosphoric acid. Rumen pH, ammonia nitrogen, volatile fatty acid fractions, and total protozoa population were recorded. Whole blood samples were collected before and after the trial in EDTA vacutainers and evaluated for RBC, WBC, haemoglobin, packed cell volume, platelets, MCV, MCH and MCHC.

2.7 Statistical analysis

The data collected from the lactation trial were analyzed using an independent-samples t-test with IBM SPSS Statistics version 20.0, following the statistical procedures of Snedecor and Cochran (1994). Differences were considered significant at $P<0.05$ and highly significant at $P<0.01$.

3. RESULTS AND DISCUSSION

3.1 Chemical composition of total mixed rations

The chemical composition of the control and herbal mixture-supplemented rations was comparable, indicating that both rations were iso-nitrogenous and iso-caloric. Organic matter, crude protein, ether extract, crude fibre, nitrogen-free extract and fibre fractions did not show meaningful differences between the two rations (Table 2). This confirms that any observed response in methane emission or milk production was associated with herbal mixture supplementation rather than major differences in basal nutrient supply [22-25].

Table 2. Chemical composition of total mixed rations fed to indigenous dairy cattle (% dry matter basis; mean ± SE).

Nutrient	Control	Treatment
Organic matter	89.58 ± 0.34	89.25 ± 0.49
Crude protein	12.47 ± 0.62	12.26 ± 0.73
Ether extract	2.35 ± 0.04	2.27 ± 0.14
Crude fibre	19.86 ± 0.79	20.43 ± 0.63
Total ash	10.42 ± 0.83	10.75 ± 0.53
Nitrogen-free extract	54.90 ± 0.45	54.29 ± 0.79
Neutral detergent fibre	53.82 ± 0.53	55.82 ± 0.71
Acid detergent fibre	26.47 ± 0.78	28.53 ± 0.52
Cellulose	21.58 ± 0.83	22.76 ± 0.31
Acid detergent lignin	4.74 ± 0.37	4.92 ± 0.46
Hemicellulose	27.35 ± 0.46	27.29 ± 0.38

No significant difference was reported between control and treatment rations.

3.2 Dry matter intake and nutrient digestibility

The average dry matter intake was 8.32 kg/day in the control group and 8.70 kg/day in the herbal mixture-supplemented group, with no significant difference. Dry matter intake expressed as percentage body weight and g/kg W0.75/day also remained statistically similar between groups (Table 3). This suggests that palatability of the total mixed ration was not adversely affected by herbal mixture supplementation [26-28]. Similar non-significant effects of herbal mixture or complete diet supplementation on intake have been reported by Jain et al. (2011) and Kumar et al. (2010).

Table 3. Dry matter intake and digestible dry matter intake in indigenous dairy cattle fed control and herbal mixture-supplemented rations (mean ± SE).

Parameter	Control	Treatment
Average body weight (kg)	301.67 ± 3.39	305.33 ± 5.13
Metabolic body weight	72.38 ± 2.48	73.04 ± 1.89
Average DMI (kg/day)	8.32 ± 0.26	8.70 ± 0.45
Average DMI (% body weight)	2.76 ± 0.06	2.85 ± 0.09
Average DMI (g/kg W0.75/day)	114.94 ± 3.87	119.11 ± 4.72
DDMI (kg/day)	5.07 ± 0.24	5.48 ± 0.37
Average DDMI (g/kg W0.75/day)	*70.04 ± 0.87*	*75.03 ± 0.62*

DMI: dry matter intake; DDMI: digestible dry matter intake.

The digestibility coefficients of dry matter, organic matter, crude protein, ether extract, crude fibre, nitrogen free extract, neutral detergent fibre, acid detergent fibre, hemicellulose and cellulose were not significantly different between groups (Table 4). Slight numerical increases were observed for most digestibility parameters in the treatment group. These findings indicate that tannins and other plant secondary metabolites present in the herbal mixture did not exert negative effects on nutrient utilization. This agrees with Rejil et al. (2008), Patra and Saxena (2011), Thakur et al. (2006) and Wanapat et al. (2013), who reported that herbal or plant secondary metabolite supplementation did not necessarily impair nutrient digestibility when used at appropriate levels.

Table 4. Nutrient digestibility coefficients in indigenous dairy cattle fed control and herbal mixture-supplemented rations (%; mean ± SE).

Nutrient	Control	Treatment
Dry matter	61.68 ± 0.61	62.39 ± 0.97
Organic matter	63.66 ± 1.73	65.53 ± 1.48
Crude protein	66.78 ± 2.76	67.49 ± 2.44
Ether extract	72.17 ± 2.48	74.15 ± 3.43
Crude fibre	58.80 ± 2.51	59.35 ± 2.92
Nitrogen free extract	67.83 ± 2.17	68.51 ± 2.74
Neutral detergent fibre	56.29 ± 2.10	58.72 ± 1.95
Acid detergent fibre	47.45 ± 1.37	48.06 ± 1.93
Hemicellulose	63.51 ± 1.70	64.26 ± 2.38
Cellulose	53.61 ± 2.24	55.79 ± 2.13

Values were reported as statistically non-significant between groups.

Digestible crude protein and total digestible nutrient values were similar between the groups (Table 5). Although DCP percentage was numerically lower in the treatment group, DCP intake and TDN intake were slightly higher due to the marginally higher dry matter intake [29-31]. TDN intake in both groups met ICAR (2013) recommendations for a 300 kg cow producing approximately 5 litres of milk.

Table 5. Nutritive value of control and herbal mixture-supplemented total mixed rations (mean ± SE).

Nutrient/parameter	Control	Treatment
DCP (%)	8.50 ± 0.28	8.34 ± 0.37
DCP intake (g/day)	707.18 ± 21.69	726.31 ± 33.91
DCP intake (g/kg W0.75/day)	9.79 ± 0.40	9.96 ± 0.52
TDN (%)	60.04 ± 0.85	60.01 ± 0.56
TDN intake (kg/day)	4.99 ± 0.06	5.22 ± 0.07
TDN intake (g/kg W0.75/day)	69.10 ± 1.48	71.52 ± 1.33

DCP: digestible crude protein; TDN: total digestible nutrients.

3.3 Methane emission

Methane emission was significantly reduced in the herbal mixture-supplemented group (Table 6). Daily methane emission decreased from 125.31 to 123.30 g/cow/day. When expressed relative to body weight, metabolic body weight, dry matter intake, and digestible dry matter intake, methane emission was significantly lower in the supplemented group [32-33]. Methane emission per kg dry matter intake decreased from 15.06 to 14.17 g/kg DMI/day, and methane emission per kg digestible dry matter intake decreased from 24.72 to 22.50 g/kg DDMI/day.

Table 6. Methane emission in indigenous dairy cattle fed control and herbal mixture-supplemented rations (mean ± SE).

Parameter	Control	Treatment
Methane emission (g/cow/day)*	125.31b ± 1.11	123.30a ± 0.91
Methane emission (g/kg b.wt/day)**	0.42b ± 0.003	0.40a ± 0.003
Methane emission (g/kg W0.75/day)**	1.73b ± 0.04	1.69a ± 0.03
Methane emission (g/kg DMI/day)**	15.06b ± 0.13	14.17a ± 0.10
Methane emission (g/kg DDMI/day)**	24.72b ± 0.22	22.50a ± 0.16

*Means bearing different superscripts within a row differ significantly. *P<0.05; **P<0.01.*

The reduction in methane emission may be attributed to the combined effects of tannins, saponins and other plant secondary metabolites in the herbal mixture. These compounds can suppress protozoa and protozoa-associated methanogens and may shift fermentation toward propionate, thereby reducing hydrogen availability for methanogenesis. The present findings are consistent with Jain et al. (2011), who observed reduced methane emission in dairy cows supplemented with herbal mixture, and with Singh et al. (2017), who reported modulation of methanogenesis by plant secondary metabolite blends. Dietary tannins have also been associated with reduced methane formation in ruminants (Jayanegara et al., 2012).

3.4 Milk production, milk quality and economics

Average daily milk yield increased significantly from 4.20 kg/day in the control group to 4.63 kg/day in the herbal mixture-supplemented group, corresponding to an increase of approximately 10.24% (Table 7). Feed conversion efficiency also improved significantly, and the cost of feed per kg of milk was reduced from Rs. 35.97 to Rs. 34.10, a reduction of Rs. 1.87

per kg of milk. Similar improvements in milk production following herbal supplementation were reported by Thakur et al. (2006), Mahanta et al. (2003), Tanwar et al. (2008) and Bhatt et al. (2009).

Table 7. Milk production, milk composition, feed efficiency and economics in indigenous dairy cattle (mean ± SE).

Parameter	Control	Treatment
Milk yield (kg/day)**	4.20 ± 0.05	4.63 ± 0.04
Milk fat (%) NS	4.68 ± 0.06	4.81 ± 0.07
SNF (%)*	9.69 ± 0.05	9.43 ± 0.08
Protein (%)*	3.83 ± 0.03	3.95 ± 0.02
Average milk fat yield (g/day)**	201.74 ± 3.34	207.92 ± 2.56
4% FCM (kg) NS	5.19 ± 0.11	5.44 ± 0.12
DMI (kg)/kg milk**	2.01 ± 0.04	1.89 ± 0.02
DMI (kg)/kg 4% FCM NS	1.78 ± 0.05	1.69 ± 0.04
Feed conversion efficiency**	0.50 ± 0.01	0.53 ± 0.01
Cost of feed/kg milk (Rs.)**	35.97 ± 0.71	34.10 ± 0.44

*NS: non-significant; *P<0.05; **P<0.01.*

Milk fat percentage and 4% fat-corrected milk did not differ significantly between groups. Solids-not-fat and protein percentage showed significant differences, but no adverse effect on overall milk quality was evident [34-36]. The lack of major changes in milk composition agrees with previous reports where herbal or feed additive supplementation did not markedly alter milk fat or total solids (Singhal et al., 2006; Mohanta et al., 2009; Tomar et al., 1996).

3.5 Rumen fermentation characteristics

Rumen pH values were 6.70 and 6.73 in the control and treatment groups, respectively, and remained within the normal physiological range. Ammonia nitrogen and total volatile fatty acid concentrations were not significantly affected by herbal mixture supplementation (Table 8). Although total VFA, acetate, butyrate, iso-valeric acid, valeric acid, and the acetate: propionate ratio were numerically lower in the treatment group, propionate concentration was numerically higher. This pattern suggests a shift in fermentation toward lower hydrogen production and improved hydrogen utilization [36-40].

Table 8. Rumen fermentation characteristics in indigenous dairy cattle fed control and herbal mixture-supplemented rations (mean ± SE).

Parameter	Control	Treatment
pH NS	6.70 ± 0.02	6.73 ± 0.01
Ammonia nitrogen (mg/dl) NS	8.37 ± 0.13	8.02 ± 0.26
TVFA (mM/l) NS	68.17 ± 2.03	60.44 ± 1.21
Acetate (mM/l) NS	48.50 ± 1.73	46.53 ± 1.52
Propionate (mM/l) NS	17.52 ± 0.30	18.40 ± 0.19
Butyrate (mM/l) NS	5.68 ± 0.30	5.49 ± 0.30
Iso valeric acid (mM/l) NS	1.34 ± 0.04	1.27 ± 0.01
Valeric acid (mM/l) NS	0.53 ± 0.02	0.52 ± 0.01
Acetate: propionate ratio NS	2.77 ± 0.10	2.51 ± 0.18
Total protozoa (x 10^5/ml)**	5.26b ± 0.14	4.23a ± 0.08

*NS: non-significant; **P<0.01. Means bearing different superscripts within a row differ significantly.*

The total protozoan population was significantly reduced by 19.58% in the herbal mixture-supplemented group. This reduction may be associated with the antiprotozoal effect of tannins and saponins. Lower protozoal populations can reduce protozoal-associated methanogens and contribute to lower methane emission. Similar relationships among plant secondary metabolites, protozoal reduction, and methane mitigation have been reported by Bhatta et al. (2015), Wanapat et al. (2013), Lila et al. (2003), and Malik and Singhal (2008).

3.6 Haematological profile

Haematological parameters including RBC, WBC, haemoglobin, packed cell volume, platelet count, MCV, MCH and MCHC did not differ significantly between groups either before or after the trial (Table 9). These findings indicate that herbal mixture supplementation did not produce adverse effects on general haematological health indicators in indigenous dairy cattle. Similar observations were reported by Manju et al. (2020) and El-Alamy et al. (2001).

Table 9. Haematological profile of indigenous dairy cattle before and after the feeding trial (mean ± SE).

Parameter	Before control	Before treatment	After control	After treatment
RBC (x 10^6/µl) NS	5.91 ± 0.03	5.94 ± 0.04	5.86 ± 0.02	5.93 ± 0.01
WBC (x 10^3/µl) NS	8.43 ± 0.12	8.60 ± 0.09	8.87 ± 0.21	9.04 ± 0.18
Hb (g/dl) NS	7.63 ± 0.09	7.76 ± 0.11	7.37 ± 0.08	7.82 ± 0.05

PCV (%) NS	26.08 ± 0.48	25.84 ± 0.59	26.22 ± 0.37	27.31 ± 0.45
Platelets (x 10^3/µl) NS	359.00 ± 4.95	363.00 ± 5.14	365.00 ± 4.25	371.00 ± 2.43
MCV (fl) NS	48.62 ± 0.85	47.22 ± 0.59	51.04 ± 1.61	52.83 ± 1.95
MCH (pg) NS	13.71 ± 0.47	13.94 ± 0.25	14.34 ± 0.21	14.27 ± 0.15
MCHC (%) NS	26.74 ± 0.32	27.37 ± 0.46	27.62 ± 0.29	28.01 ± 0.44

NS: non-significant.

4. CONCLUSION

Supplementation of the selected herbal mixture combination in total mixed rations demonstrated promising effects on the productive performance and environmental sustainability of indigenous dairy cattle. The herbal mixture effectively reduced enteric methane emission without adversely affecting dry matter intake, nutrient digestibility, nutritive value, milk composition, or haematological parameters, indicating that the supplementation was well tolerated and did not compromise the nutritional status or health of the animals. The supplemented cattle exhibited an improvement in milk production and feed conversion efficiency, suggesting better utilization of dietary nutrients for productive purposes. In addition, the reduction in feed cost per kilogram of milk indicates a potential economic advantage of incorporating the herbal mixture into dairy cattle rations. The supplementation also resulted in a reduction in the total protozoa population, which may have contributed to changes in rumen microbial activity and the observed reduction in enteric methane production. Overall, the findings indicate that the selected herbal mixture can serve as a promising natural feed additive for improving dairy production while simultaneously mitigating methane emissions. Its beneficial effects on milk yield, feed efficiency, feed cost, rumen microbial population, and methane reduction highlight its potential as a practical and sustainable nutritional intervention for indigenous dairy cattle. Further long-term studies involving larger animal populations and different supplementation levels are recommended to validate its consistency, determine the optimum inclusion level, and assess its economic and environmental benefits under field conditions.

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Ethical approval

The animal trial was conducted under standard farm management conditions at the Livestock Research Farm, Madhavaram Milk Colony, Chennai – 600 051. Rumen liquor used for the study was collected from a slaughterhouse in Chennai. Since no live experimental animal intervention was involved for rumen liquor collection, Institutional Animal Ethics Committee approval may be stated as “not applicable,” subject to journal/institutional requirements.

Conflict of interest

The authors declare no conflict of interest.

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