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Artemisia plant oil effectively reduces enteric methane emissions in Sahiwal calves

ANKIT KUMAR[1], NISHU DEVI[1], PARUL CHAUDHARY[2], PRASANTH M NAIR[1], SANJIT MAITI[3] and GOUTAM MONDAL[1]✉

ICAR-National Dairy Research Institute (NDRI) Karnal, Haryana 132 001 India

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ABSTRACT

Livestock sector significantly contributed to atmospheric greenhouse gas (GHG) leading to climate change. Several plants and their products can reduce the enteric methane emission from ruminant livestock. In the present study, the potential of *Artemisia annua* essential oil (EO) to reduce methane production in ruminants was investigated. *In vitro* trials with 0, 50, 100, 150, 200 and 250 ppm of essential oil supplementation in a 60:40 (roughage to concentrate) ratio revealed significant methane reduction at 50 ppm (20.90%) and 100 ppm (27.31%) with improved IVDMD and IVOMD (P<0.005). Acetate production decreased significantly at 50 ppm supplementation while propionate decreased beyond 100 ppm. Subsequently, fifteen male Sahiwal calves were grouped into group (Control, T1 and T2) for 70 day feeding trial. Control animals were fed only basal diet while EO at 50 and 100 ppm level was added to T1 and T2 groups, respectively. Results revealed no significant differences in body weight, daily gain, DMI, FCR, digestibility or nitrogen balance between the groups (>0.05). Enteric methane emission (g/d) decreased significantly in T1 by 13.36% and in T2 by 10.83% compared to the control. Methane emissions (g/kg DMI) also decreased by 18.52% in T1 and 14.18% in T2 as compared to control. *Artemisia annua* essential oil at 50 and 100 ppm mitigated enteric methane while maintaining normal nutrient utilization and growth. Therefore, *Artemisia annua* EO may be an option to reduce enteric emission and sustainable dairy production.

Keywords: Average daily gain, Body weight change, Essential oil, Enteric methane, Livestock, Sahiwal calves

India possesses about 17% of the world's total livestock population and this livestock contributed 58.06% of agricultural and 8% of total GHG emissions in 2019 (MoEFCC GoI, 2021). In ruminants, anaerobic fermentation is a vital digestive process (Cañete Jr, 2025) and inherently yields carbon dioxide (CO_2) and hydrogen (H_2), which are converted into CH_4 by specialized methanogenic archaeo bacteria (Ungerfeld, 2020, Öztürk and Gur, 2021). Livestock methane, a powerful greenhouse gas, intensifies climate change and global warming, subsequently leading to higher global temperatures that reduce crop growth and yields (Lu *et al.* 2025). Plant secondary metabolites effectively reduce enteric methane production in livestock (Dhanasekaran *et al.* 2020). Saponins reduce enteric methane emission in ruminants by inhibiting methanogen in the rumen (Darabighane *et al.* 2021). Reducing methane production involves culling non-productive animals, increasing the concentrate-to-forage ratio in feed and using feed additives reduce livestock methane emissions (Tseten *et al.* 2022). Incorporating natural feed additives such as essential oils and plant extracts into ruminant diets reduce enteric methane (Lambo *et al.* 2024). Essential oils, rich in lipophilic compounds, exert antimicrobial effects by disrupting microbial cell and mitochondrial membrane (Maurya *et al.* 2021). *Artemisia* plants mainly grow in arid and cold regions as wild herbs (Manucharyan, 2025). Certain *Artemisia* species have high concentrations of volatile terpenes in their leaves and flowers (Reddy *et al.* 2023, Cardoso-Gutierrez *et al.* 2021). This study aim to explore role of *Artemisia* essential oil in reducing enteric methane emissions and *in vitro* trials to find the optimal dose for the reduction and assess its effects on digestibility and nutrient utilization.

MATERIALS AND METHODS

Institutional animal ethical committee approval: The animal experiment was approved by the Institutional Animal Ethical Committee (IAEC) under file number 50-IAEC-23-05 and committee's guidelines.

Location of experiment: The study was conducted in Livestock Research Centre, ICAR- NDRI Karnal, India located at 29° 42″ 20 sec N and 76° 58″ 52.5 sec E at altitude of 834 feet (228 m) above sea level from March to August

Present address:[1]Animal Nutrition Division, ICAR- National Dairy Research Institute, Karnal, Haryana-132001.[2]Department of Microbiology, School of Agriculture, Graphic Era Hill University, Dehradun, Uttarakhand, India. [3]Dairy Extension Division, ICAR-National Dairy Research Institute, Karnal, Haryana-132001. ✉Corresponding author email: gmondal1075@gmail.com (Orcid id: 0000-0003-1680-8920) Tel. No: +91 184 2259059

2022. The experiment was conducted in two phases' viz., *in vitro* and *in vivo.*

In vitro experiment: Rumen fluid was collected in early morning through a lubricated sterile tube connected by vacuum pump. Rumen fluid was filtered through muslin cloth and pooled samples were used as an inoculum for *in vitro* gas production (Menke and Steingass, 1988). For *in vitro* experiment, substrate was total mixed ration (TMR, 60R:40C) with different concentrations of *Artemisia annua* EO (essential oil) (0, 50, 100, 150, 200 and 250 ppm). TMR chemical composition is presented in Supplementary Table 1. Separately weighted substrates (200 mg) were added to the bottom side of respective glass syringes. The syringes were incubated in water bath at 39°C. Separately, rumen buffer solution (275ml), micro-mineral solution (0.10 ml), macro mineral solution (0.10 ml), resazurin solution (1.45 ml) and distilled water were mixed to prepared mixture solution (547 ml). This solution was prewarmed at 39°C with CO_2 fizzed and rumen liquor was added. After mixing, solution (30 ml) was placed in syringes using an auto dispenser, gently mixed with outlet closed and incubated for 24 h at 39°C. After every 30 min, syringes were shaken.

Analysis of total gas production: The mixture was incubated for 24 h and total gas production was calculated by subtracting it from the final value.

Estimation of methane production: After feed incubation, a suitable aliquot was removed from incubation syringe's tip and its methane content was determined by gas chromatograph (Nucon 5700, India) equipped with a stainless-steel column packed with Porapak-N and fitted with a flame ionization detector (FID). Injector, column and detector temperatures were 40, 50 and 100°C. Air, fuel gas (IOLAR grade hydrogen) and carrier gas (nitrogen) moved through column at 30, 300 and 30 ml/min, respectively. Through injection port, sample (2 ml) was injected into GC with syringe containing 5 mL **(**Leitanthem *et al.* 2022). The standard gas used for CH_4 analysis (Spantech House, Surrey, England) consisted of 50% mixture of CH_4 and CO_2.

Assessment of in vitro digestibility and fermentation parameters: Syringe samples from replicates were centrifuged after 24h incubation and they were spun at 3,000 rpm for 10 min. After centrifugation, pellets were re-extracted with the neutral detergent solution, filtered through crucibles and then deposits were dried in an oven to assess the true *in vitro* digestibility of dry matter (IVDMD). The residue was burned at 550°C-650°C in a muffle furnace to estimate in vitro organic matter digestibility (IVOMD). IVOMD was used to determine partial fraction and microbial biomass production (Blummel *et al.* 1997).

*Estimation of ammonia nitrogen***:** After 24h incubation, fermentation was stopped by chilling at 4°C, followed by collection of gas for CH_4 estimation and transfer of syringe's contents into centrifuge tubes. The tubes were centrifuged for 10 min at 3000 rpm and supernatant acidified with an equal volume of 0.5 M HCl and was stored at -20°C. The acidified supernatant (5 mL) was mixed with NaOH (1N, 10 mL) for ammonia-N quantification (Komolong *et al.* 2001). Produced NH_3 was collected in a solution of boric acid (20% w/v) with mixed indicator and titrated against known strength of sulphuric acid.

Estimation of volatile fatty acids (VFA): The sample was centrifuged at 3000 rpm for 10 min and the supernatant (4 ml) was mixed with m-phosphoric acid (25%, 2 ml), left at 4°C overnight and then kept at -20°C for VFA analysis. Individual VFA in samples were identified using a gas chromatograph (Nucon 5700, New Delhi) equipped with flame ionization detector and stainless-steel column. The injector port, column and detector temperatures were 210,180 and 230°C respectively and the carrier gas flowed at a rate of 40 ml/min (Bannink *et al.* 2006).

In-vivo experiment: Fifteen healthy Sahiwal male calves (9-10 months of age) were randomly selected from Livestock Research Centre, ICAR-NDRI Karnal and divided into three treatment groups according to body weight and age. All animal calves were fed as follows: Control animals were fed green maize, concentrate mixture (60:40) maize concentrate mixture for entire trial period based on their dry matter intake (DMI) according to ICAR requirements (2013); T1 and T2 animals were fed a similar ration to control with additional 50 and 100 ppm of *Artemisia* essential oil mixed with concentrate mixture uniformly. Drinking water was offered *ad libitum*.

Metabolism trial: Metabolism trials were conducted during the experimental period for 7-day collection to determine the digestibility of nutrients and nitrogen balance at 60 days of the experiment. Animals were weighed before and after trial consecutively every 2 days.

Sampling, processing and storage during metabolism trial: The samples of feed, fodder and residues were recorded daily for DM estimation during metabolism trial. These samples were pooled at the end of collection period and ground to pass through 1 mm sieve and then stored in air tight bags. Feces voided during 24 h were collected daily for seven days and weighed at 9:00 am daily. After thorough mixing, an aliquot of 1% of the total sample on a weight basis was kept for DM estimation at 80ºC in a hot air oven. Dried fecal samples of each day were pooled and ground to pass through 1 mm sieve then analyzed for proximate principles (OM, CP, Ash and EE) and cell wall constituents (AOAC, 2005, Van Soest, 1994). For N estimation, an aliquot (1/500) of total feces voided was collected daily for seven days and stored in plastic containers containing 20 ml of 10% H_2SO_4 solution. Total urine voided from each animal was collected in big plastic containers placed at two ends of the metabolic cages. In each container, 100 ml of 10% H_2SO_4 was daily added. Total urine output for 24 h was measured daily at 9:30 h and an aliquot (1/500 of total output) was taken for nitrogen estimation. The aliquots were stored in plastic containers, containing 2 ml of 25% H_2SO_4 for total nitrogen estimation.

Calculation of apparent nutrient digestibility: The nutrient (DM, OM, CP, EE, NFC, NDF and ADF) digestibility coefficients were calculated from the nutrient

intake and nutrient outgo in feces during the metabolism trial as below:

$$\text{Digestibility} = \frac{(\text{Nutrient intake} - \text{Nutrient outgo in feces}) \times 100}{(\text{Nutrient intake})}$$

Estimation of enteric methane emission: Methane emissions from the animals was measured in five successive, 24-h collection periods toward end of the experiment after digestibility trial using SF_6 tracer gas technique (Johnson and Johnson, 1995). Pre calibrated permeation tube containing SF6 (approximately 0.60 g) was placed into experimental animals' rumen and tubes' allocation was randomized among experimental calves. The release rate of SF6 from permeation tubes and their projected expiry time were known before their placement into the rumen. The vacuum canisters were used to collect the expired and eructed gas samples. They were removed after 24 h and pressurized with nitrogen gas before their GC (Nucon 5700, Nucon Engineers, New Delhi, India) analyses of CH_4 and SF_6 concentrations. The CH_4 and SF_6 concentrations were determined from peak area and their different retention times relative to known standards. The methane emission was calculated using the following formula:

$$CH_4\ (g/d) = \frac{SCH_4\text{-}BCH_4 \times MCH_4 \times QSF6 \times 1000}{SF_6\text{-}BSF_6 \times MSF_6}$$

Where, SCH_4 and BCH_4 were methane concentrations in collected samples, SSF_6 and BSF6 represented concentrations of SF_6 in the samples, MCH_4 and MSF_6 are the molecular weight of methane, SF_6 (g) and QSF_6 represents release rate of SF_6 (mg/d). Methane energy was calculated by multiplying methane emission (g/d) with an energy value of 13.34 Kcal/g followed by conversion to MJ/d. The gross energy was calculated by adding the gross energy of individual feed ingredients. DE and ME were calculated from TDN values (NRC, 2001).

$$DE\ (Mcal/kg) = 0.04409 \times TDN\ (\%)$$

$$ME\ (Mcal/kg) = 1.01 \times DE\ (Mcal/kg) - 0.45$$

GEI, DEI and MEI were calculated by multiplying DMI with the GE, DE and ME values of the rations. Methane energy loss as % of GEI, DEI and MEI was calculated from the above data.

Statistical Analysis: Under *in vitro* study, each trial was repeated three times to ensure reliability and consistency of the results. The results obtained during this study were analyzed using software package SPSS version 16.0, 2010. One-way ANOVA with Tukey's b post-hoc test employed to differentiate between dose rates. For *in vivo* studies, two-way ANOVA with repeated measures for bodyweight changes and DMI while one-way ANOVA was used to analyze digestibility and intake of nutrients. A general linear model was used where dependent variable was observation and fixed factor was the dose.

RESULTS AND DISCUSSION

Effect of essential oil on in vitro fermentation: Total gas production increased up to 100 ppm and beyond this, decreased while methane production (mL/g DM substrate) decreased ($p<0.05$) significantly throughout and the lowest value was observed at 250 ppm. Dry matter and organic matter digestibility were similar at 0, 50 and 100 ppm supplementation while beyond it decreased significantly ($p<0.05$). Partitioning factor (PF) increased significantly with essential oil supplementation ($p<0.05$).

Microbial biomass production (MBP), also decreased significantly ($p<0.05$) with increase in EO supplementation present in Table 1.

In vitro dry matter digestibility (IVDMD), *in vitro* organic matter digestibility (IVOMD), Partitioning factor (PF) and microbial biomass production (MBP) are presented in Table 1.

Ammonia nitrogen reduced significantly ($p<0.05$), however total VFA and butyrate didn't show any significant changes ($p>0.05$) upon EO supplementation, although, a decreasing trend of acetate and propionate beyond 100 ppm of supplementation was observed (Table 2).

Essential oil reduced the methane emission by inhibiting ciliated protozoan population and lowering acetate level that limits hydrogen available for methanogens (Permata *et al.* 2024). Lower dose of essential oil might have influenced enteric fermentation that significantly increase total gas production. Higher concentrations decrease total

Table 1. Effect of supplementation of *Artemisia annua* essential oil on *in vitro* digestibility, total gas, methane production, PF and MBP on 60R:40C ration

Levels (ppm)	Total Gas (ml/g DM)	CH4 (mL/g DM)	IVDMD (%)	IVOMD (%)	PF	MBP (mg)
0	$170.70^{de}\pm0.27$	$29.16^{d}\pm0.63$	$59.35^{d}\pm1.18$	$61.59^{d}\pm1.30$	$3.13^{a}\pm0.01$	$31.88^{b}\pm0.02$
50	$171.90^{e}\pm0.22$	$22.51^{c}\pm0.70$	$57.35^{c}\pm0.19$	$61.08^{cd}\pm0.72$	$3.35^{bc}\pm0.02$	$31.53^{b}\pm0.62$
100	$172.50^{e}\pm0.78$	$21.43^{bc}\pm0.48$	$58.36^{cd}\pm0.33$	$60.04^{cd}\pm1.70$	$3.38^{c}\pm0.02$	$30.52^{ab}\pm0.03$
150	$166.51^{c}\pm0.80$	$21.43^{bc}\pm0.90$	$56.85^{bc}\pm0.01$	$58.15^{bc}\pm0.21$	$3.39^{c}\pm0.03$	$29.92^{ab}\pm0.26$
200	$163.70^{b}\pm0.29$	$19.38^{ab}\pm0.62$	$55.03^{b}\pm0.02$	$56.79^{b}\pm0.65$	$3.37^{bc}\pm0.01$	$29.27^{a}\pm1.06$
250	$159.15^{a}\pm0.52$	$19.27^{a}\pm0.45$	$51.30^{a}\pm0.04$	$52.78^{a}\pm0.26$	$3.36^{bc}\pm0.02$	$28.71^{a}\pm0.81$

^{abcde} Means with different superscript differ significantly ($P<0.05$)

Table 2. Effect of supplementation of *Artemisia annua* essential oil on ammonia nitrogen, acetate propionate, butyrate and total VFA on 60R:40C ratio

Levels(ppm)	NH3-N (mg/dl)	Acetate (mM/100gDM)	Propionate (mM/100gDM)	Butyrate (mM/100gDM)	Total VFA
0	6.47^{b}±0.81	33.40±1.44	8.90±0.50	4.33±0.29	46.63±1.20
50	6.07^{ab}±0.25	30.42±0.70	9.26±0.19	4.57±0.98	45.67±1.79
100	6.06^{ab}±0.32	30.40±0.66	9.25±0.26	4.56±0.62	44.67±0.10
150	6.05^{ab}±0.58	30.41±1.48	9.20±0.30	4.87±0.29	45.63±2.07
200	5.81^{a}±0.41	29.01±1.66	9.17±0.49	4.45±0.52	45.49±2.65
250	5.91^{a}±0.34	28.58±3.27	9.16±0.37	4.61±0.33	45.78±3.85
P value	0.001	0.36	0.43	0.23	0.46

^{ab} Means with different superscript differ significantly (P<0.05)

gas production by reducing substrate digestibility and feed fermentation (Ike *et al.* 2024). In similar line, Nunes *et al.* 2023 reported that *Pittosporum undulatum* EO (120 L/g DM) supplementation in Shaiwal cow caused reduction in total gas production (52%) and methane emission (47%). Sheida *et al.* (2024) studied supplementation of *Inulae rhizomata et radices* (elecampane rhizomes and roots) at 3.0 g/kg and *Artemisiae absinthil* herba at 10.0 g/kg DM in dairy cattle's reduced enteric methane emission and total gas production. Higher EO concentration decreased dry and organic matter digestibility and resulted in reduction of DM digestibility or decline in starch-eating protozoa population (Brice *et al.* 2022).

The partitioning factor (PF) is an important parameter and ranged from 3.13 to 3.39. The partitioning factors obtained in results fell into the theoretical value (2.75 to 4.41). Roughages with high PF had a higher intake. Kurt, (2025) reported that supplementation of dairy calves with carob pod diet cause increase in partitioning factors which improves feed intake and growth performance of animals. Microbial protein (MP) is a superior, highly digestible protein source and is crucial for enhancing ruminant growth, milk yield and reproductive performance. EO supplementation and rumen bypass protein are effective strategies for reducing *in vitro* crude protein (CP) degradation, and cause measurable decrease in ammonia nitrogen (N) concentration (Alabi *et al.* 2023). EO at 50 and 100 ppm significantly increased propionate, the main volatile fatty acid (VFA) used for gluconeogenesis in ruminants, thereby suggesting their capacity to boost energy availability for calves. EO effectively remodel rumen microbiome structure, and significantly increased relative abundance of propionate-producing bacteria. Molho-Ortiz *et al.* 2022 had also reported that combination of disodium fumarate and thyme EO significantly boosted propionate while reduced acetate, butyrate and acetate-to-propionate ratio. In contrast to these findings, Bokharaeian *et al*. (2023) reported at 600 ppm of clove oil supplementation in buffalo wheat straw feed insignificantly altered total VFAs and acetate/propionate ratio.

Effect of essential oil on in vivo studies body weight change, average daily gain, DMI and feed conversion ratio (FCR): Body weight was insignificantly altered (p>0.05) among groups upon EO supplementation. Average daily gain (g), DMI (kg/d), DMI (kg/100 kg BW) and feed conversion ratio (DMI/ADG) were also similar amongst the groups as presented in Table 3.

Similarily, Dry matter (DM), organic matter (OM), crude protein (CP), ether extract (EE), neutral detergent fiber (NDF) and acid detergent fiber (ADF) did not altered among the groups (p>0.05) upon supplementation (Table 4).

DMI (kg/ d), DMI (kg/100kg BW), CPI (kg/d), N intake, N voided in feces, N voided in urine, absorbed N, N retention and N balance significantly not altered (p>0.05) among groups. Total digestible nutrient intake (TDNI (kg/d) was similar among the groups, however, the trend of decreasing order in T2 (100 ppm) group is presented in Table 5.

Supplementation of 50 ppm dose (T1) decreased

Table 3. Effect of *Artemisia annua* essential oil on body weight change, average daily gain, DMI and feed conversion ratio (FCR)

Fortnight	Control (C)	T_1 (50 ppm)	T_2 (100ppm)	P value
BW changes	112.39±10.11	115.92 ± 7.2	113.65 ± 11.63	0.96
ADG (g)	0.48±0.03	0.50±0.02	0.49±0.03	0.38
DMI (kg/d)	3.14±0.11	3.26±0.18	2.98±0.16	0.18
DMI (kg/100 kg BW)	2.66±0.19	2.64±0.20	2.63±0.12	0.45
FCR	6.64±0.75	6.69±0.42	6.50±0.53	0.49

Body weight changes (BW changes), average daily gain (ADG, g), dry matter intake (DMI, kg/d), dry matter intake per 100 kg body weight (DMI/100 kg BW) and feed conversion ratio (FCR).

Table 4. Digestibility coefficients (%) of various nutrients in treatment groups

Parameter (%)	Control (C)	T1(50 ppm)	T2(100 ppm)	P Value
DM	61.26±0.93	62.75±0.33	60.73±0.94	0.226
OM	63.74±0.92	65.32±0.22	62.98±0.94	0.145
CP	67.27±0.97	67.04±0.64	66.39±0.84	0.291
EE	75.13±1.94	76.54±1.22	76.47±0.80	0.733
NDF	48.19±1.05	48.60±1.47	47.12±0.61	0.431
ADF	43.15±1.34	44.26±0.78	42.33±1.31	0.529

Dry matter (DM), organic matter (OM), crude protein (CP), ether extract (EE), neutral detergent fiber (NDF) and acid detergent fiber (ADF).

Table 5. Nitrogen Balances in different treatment groups supplemented with *Artemisia annua* essential oil

Parameter	Control (C)	T1(50 ppm)	T2 (100 ppm)	P value
DMI (kg/ d)	3.58±0.18	3.63±0.10	3.65±0.17	0.228
DMI (kg/100kg BW)	2.85±0.13	2.82±0.23	2.79±0.15	0.548
CPI (kg/d)	0.50±0.02	0.57±0.01	0.49±0.03	0.549
Total N intake(g/d)	79.61±3.16	79.83±1.67	78.91±4.31	0.46
N Voided in feces(g/d)	24.04±1.04	24.70±0.69	23.56±1.92	0.832
N excreted in urine (g/d)	35.85±2.47	36.71±0.98	35.89±1.14	0.47
Total N outgo (g /d)	59.89±3.29	60.41±1.20	58.45±2.89	0.43
Absorbed N (g/d)	55.57±2.63	55.33±1.30	55.35±2.50	0.17
N Balance /Total N retention (g/d)	19.72±1.73	19.42±0.67	19.46±1.44	0.771
N retention (% N Intake)	24.22±1.77	24.5±0.68	23.56±0.69	0.492
N absorbed (% N Intake)	69.27±0.97	69.30±0.64	70.14±0.84	0.29
TDNI (kg/d)	2.10±0.12	2.23±0.06	1.91±0.08	0.091

Dry matter intake (DMI), crude protein intake (CPI), nitrogen (N) and total digestible nutrient intake (TDNI).

methane emissions by 13.36% and 100 ppm dose (T2) by 10.83%. The methane expressed as g/kg DMI, g/kg DDMI, g/kg CPI and MJ/d were lowered in treated groups (T_1 and T_2) than control group. There was no significant difference in methane production when expressed as g/kg NDFI, among the various groups. Similarily, gross energy intake (GEI), digestible energy intake (DEI) and metabolizable energy intake (MEI) also did not alter in treated as well as in control groups as presented in Table 6.

Methane emission decreased in treated groups results indicate both doses were effective in reducing enteric methane emissions, under *in vivo* conditions. Ruminant Dry Matter Intake (DMI) is fundamentally governed by intertwined factors of feed palatability and intrinsic

Table 6. Enteric methane emission in male Sahiwal calves fed with control diet and treatment diet (*Artemisia annua* essential oil)

Parameter	Control	T1 (50 ppm)	T2 (100 ppm)	P value
CH_4, g/d	108.34^{b}±2.98	93.86^{a}±0.26	95.60^{a}±1.57	0.002
CH_4, g/kg DMI	28.90^{b}±0.87	23.55^{a}±0.33	24.89^{a}±0.35	0.001
CH_4, g/kg DDMI	46.62^{b}±1.47	37.29^{a}±0.18	38.55^{a}±0.28	0.002
CH_4, g/kg CPI	210.15^{ab}±4.22	173.52^{a}±0.87	178.07^{a}±15.60	0.002
CH_4, g/kgNDFI	58.46±2.42	56.58±2.24	60.89±4.05	0.090
GEI, MJ/d	64.68±1.30	67.55±0.59	65.87±1.79	0.364
DEI, MJ/d	42.96±0.86	44.87±0.39	43.75±1.19	0.365
MEI, MJ/d	36.28±0.73	37.89±0.33	36.94±1.00	0.364
CH_4,MJ/d	6.05^{b}±0.17	5.49^{a}±0.09	5.64^{a}±0.24	0.064

Dry matter intake (DMI), digestible dry matter intake (DDMI), crude protein intake (CPI), neutral detergent fiber intake (NDFI), gross energy intake (GEI), digestible energy intake (DEI) and metabolizable energy intake (MEI).ab Means with different superscript differ significantly ($P<0.05$)

nutrient characteristics, such as cell wall components and secondary metabolites (Faryabi *et al.* 2023). Diepersloot *et al*. (2025), reported supplementing high-milk yielding dairy cattle with essential oils, monensin or a combination for 10 weeks did not significantly alter (p>0.05) dry matter intake (DMI). EO enhance body weight gain by promoting the elongation of intestinal papillae, thereby improving animal feed digestibility **(**Zhang *et al*. 2021). In contrast to present findings, dietary supplementation with 1% *Halimeda opuntia* powder elicited no significant difference (P>0.05) in the body weight (BW) or average daily gain (ADG) of growing lambs (Abu El-Kassim *et al.* 2021). Castro Filho *et al*. (2021) observed that dietary inclusion of essential oil in cattle elicited no statistically significant effect on the apparent digestibility or concentration of major nutritional components such as dry matter (DM)**,** organic matter (OM)**,** crude protein (CP)**,** ether extract (EE)**,** neutral detergent fiber (NDF) and acid detergent fiber (ADF) remained unchanged. Linseed oil (2–4% of diet) had no effect on feed intake or digestion in lactating cows (Cristobal-Carballo *et al.* 2021). High-level malic acid and heat treated protein rich diet enhanced calf nitrogen balance by successfully increasing protein bypass and subsequent intestinal amino acid availability (Thakur *et al.* 2025). Diet supplemented with vegetable oil in Sahiwal cow had no significant effect (p>0.05) on N-balance, intake or digestibility in treated groups (Andrade *et al.* 2025). Supplementing diet with essential oils reduced all metrics of CH_4 emissions by potentially serving as alternative hydrogen sinks to CO_2 (Xu *et al.* 2022).

The study highlighted the effect of *Artemisia annua* essential oil supplementation at 50 ppm and 100 ppm level on nutrient utilization or growth performance of Sahiwal calves**.** At both levels, nutrient utilization and intake was similar with control group. There was 18.52 % and 14.18 % reduction of enteric methane emission in calves, respectively. These findings underscored the potential of *A. annua* essential oil as a viable and sustainable strategy to mitigate greenhouse gas emissions from livestock. Optimizing Artemisia annua dosage and assessing its economic feasibility in practical feeding are crucial to promote its adoption among livestock farmers seeking to reduce their environmental footprint.

Future research should explore the long-term effects and safety of *A. annua* essential oil on animal health and productivity.

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