



Influence of Dietary Supplementation of Peppermint (*Mentha piperita*) and Lemongrass (*Cymbopogon citratus*) Essential Oils on Health Biomarkers in Crossbred Calves

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ABSTRACT

This study evaluated the effects of dietary supplementation with Peppermint (*Mentha piperita*) essential oil (MPEO) and Lemongrass (*Cymbopogon citratus*) essential oil (CCEO) on the hematological profile, serum biochemical parameters, and blood antioxidant status in crossbred calves. Eighteen crossbred calves (Sahiwal × Holstein Friesian), aged between 15-90 days, were allocated into three groups: T0 (control), T1 (MPEO at 0.2% of calf starter), and T2 (CCEO at 0.2% of calf starter). The study was conducted over 90 days, with blood samples collected at monthly intervals: 0, 30th, 60th and 90th day for study of haemato-biochemical parameters while at 45th and 90th day for antioxidant parameters. Hematological parameters showed a significant ($P < 0.05$) increase in total erythrocyte count (TEC) and packed cell volume (PCV) in the T2 group, while eosinophil counts were significantly ($P < 0.05$) reduced in both T1 and T2 groups of calves. No significant treatment effects were observed on total leukocyte count (TLC), differential leukocyte counts, or platelet counts. Biochemical analysis revealed significant reductions in serum urea ($P < 0.01$) levels and alanine aminotransferase ($P < 0.05$) in T1 and T2 group of calves. Lipid profile results revealed significant ($P < 0.05$) reductions in triglycerides, total cholesterol ($P < 0.01$), LDL, and VLDL in *Cymbopogon citratus* essential oil group and an increase in HDL cholesterol in both *Mentha piperita* essential oil (MPEO) and *Cymbopogon citratus* essential oil groups. As regards antioxidant parameters, lipid peroxidase (LPO) was significantly ($P < 0.05$) reduced, and superoxide dismutase (SOD) activity was significantly ($P < 0.01$) increased in the *Cymbopogon citratus* essential oil group of calves. The results suggest that *Cymbopogon citratus* essential oil supplementation significantly improved hematological parameters, lipid metabolism, and antioxidant status, with potential benefits for calf health and performance.

KEYWORDS: Antioxidant status, Crossbred calves, Essential oils, Hemato-biochemical parameters

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INTRODUCTION

The growing global concern over antibiotic resistance has emerged as a critical public health threat. In response, regulatory authorities have imposed restriction on the use of antibiotics in livestock production systems (Coimbra et al., 2022). As a result, there is growing scientific interest in identifying natural alternatives to antibiotics, such as essential oils (EOs), prebiotics, and other phytobiotics (Nehme et al., 2021). Essential oils are plant-derived compounds, exhibit antimicrobial, antioxidant, and anti-inflammatory properties, are promising

candidates for enhancing livestock health without contributing to the development of antimicrobial resistance (Wells, 2024).

Mentha piperita (peppermint) has been widely utilized in traditional herbal medicine due to its broad range of therapeutic properties. It is particularly noted for its immunomodulatory effects and its potential to help prevent secondary infections. The essential oil of *Mentha piperita* (MPEO), which is abundant in menthol and menthone, has been reported to support digestive health and positively influence various blood biochemical parameters (Brahmi et al., 2017).

Cymbopogon citratus, commonly known as lemongrass, is widely recognized for its medicinal value, primarily attributed to its rich array of health-promoting phytochemicals. These compounds contribute to improved gut health and enhanced nutrient absorption. Phenolic compounds in *Cymbopogon citratus* EO play a significant role in boosting antioxidant capacity by acting as effective reducing agents capable of neutralizing pro-oxidants, free radicals, and metal ions (Ali et al., 2021).

In light of the recognized health-promoting properties of *Mentha piperita* and *Cymbopogon citratus* essential oils, this study was conducted to evaluate the effects of dietary supplementation with *Mentha piperita* essential oil (MPEO) and *Cymbopogon citratus* essential oil (CCEO) on hematological profile, serum biochemical parameters, and blood antioxidant status in crossbred calves.

MATERIALS AND METHODS

The present study was carried out at the Instructional Dairy Farm, Nagla, College of Veterinary and Animal Sciences, G.B. Pant University of Agriculture and Technology (GBPUAT), Pantnagar, Uttarakhand, India.

The experiment was approved by the Institutional Animal Ethics Committee (IAEC) of G.B. Pant University of Agriculture and Technology, established in accordance with Article 13 of the CPCSEA regulations set forth by the Government of India (Registration No. 330/GO/Re/SL/01/CPCSEA, dated 03/01/2001).

Eighteen crossbred (Sahiwal × Holstein Friesian) calves, aged between two weeks and three months, were selected and randomly allocated into three treatment groups based on uniformity in age and body weight, with six calves in each group. The trial was conducted over a period of 90 days.

Prior to the initiation of the study, the animal shed was cleaned, disinfected and all calves were kept in well-ventilated pens to ensure optimal hygiene and comfort. All animals were fed a basal diet comprising milk and calf starter, adjusted according to individual age and body weight, while green fodder was provided ad libitum. Essential oils, obtained from Empirical Aromatics LLP, Gautam Budh Nagar, Uttar Pradesh were supplemented in the treatment groups with calf starter diet.

The treatment groups included: T0, which received only the basal diet (control); T1, which

received the basal diet supplemented with *Mentha piperita* essential oil (MPEO) at 0.2% of the calf starter; and T2, which received the basal diet supplemented with *Cymbopogon citratus* essential oil (CCEO) at 0.2% of the calf starter.

Blood samples were collected at four time points during the experimental period: on day 0 (at the beginning of the study), and subsequently at monthly intervals on 30th, 60th and 90th day for haemato-biochemical studies and on 45th and 90th day for study of antioxidant parameters.

Hematological parameters viz. hemoglobin (Hb) was measured using Sahli's method. Total erythrocyte count (TEC) and total leukocyte count (TLC) were assessed using the hemocytometer technique (Jain, 1986). Packed cell volume (PCV) was determined by the capillary tube method with a microhematocrit centrifuge. Differential leukocyte counts were performed manually through microscopic examination of stained blood smears. Mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC) were calculated using standard formulas as described by Coles (1986). Platelet counts were also determined using the hemocytometer method.

Serum biochemical parameters viz. glucose, triglycerides, urea, total protein, albumin, globulin, and liver enzymes i.e. aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were analysed using commercial diagnostic kits procured from ERBA Mannheim, in a UV-VIS spectrophotometer (Tietz, 1998).

Statistical analysis

The experimental data were statistically analyzed using one-way analysis of variance (ANOVA) with SPSS software version 21, following the methodology described by Snedecor and Cochran (1994). Where significant differences were observed, Duncan's multiple range test was employed for post hoc comparisons. Statistical significance was considered at the levels of $p < 0.05$ and $p < 0.01$.

RESULTS AND DISCUSSION

Feed intake and growth performance of calves

Supplementation with MPEO and CCEO at 0.2% did not significantly affect feed intake or dry matter intake (DMI) in crossbred calves (Table 1). These findings are consistent with Bhat et al. (2019), who

reported no significant effect of *Mentha piperita* on DMI in sheep. Similarly, Soares et al. (2023) and Del Valle et al. (2024) found no impact of CCEO supplementation on DMI or nutrient intake in *Bos indicus* Nellore steers and sheep, respectively. However, Liu et al. (2020) reported increased DMI in calves with essential oil supplementation.

Moreover, both MPEO and CCEO supplementation at 0.2% significantly increased body weight in calves compared to the control. This aligns with findings by Al-Azzawi and Rasheed (2021), who observed increased body weight in lambs supplemented with MPEO, and Okoruwa and

Edoror (2021), who found similar effects with CCEO in goats. Tiwari et al. (2018) in broilers and Fahad and Al-Wazeer (2021) in lambs also reported positive effects of CCEO on body weight. Additionally, Orzuna-Orzuna et al. (2022) and Devi et al. (2018) documented weight increase in EO-supplemented calves and poultry respectively. Singh et al. (2018) also suggested improved rumen fermentation and feed efficiency in buffalo with lemongrass EO supplementation which supported the present findings. These suggest improved body weight may be attributed to enhanced feed efficiency and nutrient absorption.

Table 1. Effect of supplementation of *Mentha piperita*, *Cymbopogon citratus* EOs on feed intake and growth performance parameters of CB calves

Parameters	T0	T1	T2
FI (kg/d)	6.56±0.04	6.60±0.05	6.52±0.05
DMI (kg/d)	2.33±0.01	2.34±0.01	2.30±0.01
DMI (kg/100kg BW)	2.04±0.04	2.06±0.07	2.02±0.02
DMI (kg/BW ^{0.75})	0.065±0.001	0.066±0.001	0.064±0.001
Body weight (kg)*	53.63±2.13 ^b	55.92±2.41 ^a	56.61±2.56 ^a

Means bearing different superscripts vary significantly (*P<0.05)

Effect on hematology of calves

Table 2 depicted the results regarding hemoglobin concentration which indicated no significant treatment effect, although hemoglobin levels were observed to be higher, the difference was not statistically significant (P>0.05). These findings contrast with those of Witkowska et al. (2019) and Al-Janabi et al. (2023), who reported an increase in hemoglobin levels with supplementation of MPEO and CCEO, respectively. However, a significant (P<0.01) day effect was observed, with hemoglobin concentration showing a consistent increase from 0 to 90 day of the trial. This upward trend in hemoglobin levels may be attributed to the natural progression of the calves' growth. As calves age, their digestive systems mature, improving the absorption of essential nutrients like iron, which plays a critical role in hemoglobin production. In the early stages, calves primarily rely on colostrum and milk, which contain limited iron. As there is transition to solid food and their gut microbiome develops, iron absorption improves, leading to a steady rise in hemoglobin levels.

The study's findings demonstrated that there was significant (P<0.05) treatment effect on total erythrocyte count (TEC) with higher values in CCEO supplemented (T2) group. This result aligns with Al-Kassie (2010), who observed no significant change in erythrocyte count with peppermint extract supplementation, and with Al-Janabi et al. (2023), who reported a similar significant increase (Pd^{0.05}) with lemongrass supplementation in lambs. The observed increase in erythrocyte count in the CCEO group may be attributed to improved nutrient absorption and utilization, as suggested by Ademuyiwa and Grace (2015). CCEO is believed to enhance erythropoiesis by improving nutrient bioavailability, thus supporting the rise in total erythrocyte count. Essential oils from *Cymbopogon citratus* are rich in polyphenolic compounds, known for their antioxidant properties, which can reduce oxidative stress an important factor in erythrocyte production. Antioxidants lower lipid peroxide levels in erythrocyte membranes, enhancing membrane integrity and reducing susceptibility to hemolysis.

The treatment and day effect were non-significant for total leukocyte count (TLC), although the T2 group exhibited a higher TLC value, the difference was not statistically significant. These findings are consistent with Al-Kassie (2010), who also reported a non-significant impact of MPEO supplementation on leukocyte count. However, contrasting results have been documented; Al-Janabi et al. (2023) observed a significant reduction in white blood cell count with lemongrass supplementation in lambs, while Arab Ameri et al. (2015) reported a significant increase in TLC following MPEO supplementation. Such variations may be attributed to differences in species, dosage levels, or physiological conditions. The supplementation with MPEO and CCEO appears to support immune resilience without significantly altering leukocyte counts, suggesting a stabilizing effect on immune homeostasis.

This study revealed a significant ($P < 0.05$) increase in packed cell volume (PCV) in the T2 group supplemented with CCEO at 0.2%. These findings align with Alagbe and Oluwafemi (2019), who reported a similar rise in PCV with CCEO supplementation. In contrast, Witkowska et al. (2019) observed increased PCV with MPEO instead. Essential oils like CCEO and MPEO are believed to support immune function by preserving cellular integrity, thereby enhancing nutrient absorption and promoting healthy blood cell formation (Arab Ameri et al., 2015). An elevated PCV indicates a higher proportion of red blood cells, improving oxygen transport and supporting growth, feed efficiency, and overall health in young animals. PCV can decline under stress due to protein denaturation and cell damage (Puvadolpirod and Thaxton, 2000).

The study showed that neutrophil percentage increased in the CCEO-supplemented group, but the difference was not statistically significant. This aligns with Rahman et al. (2021), who reported a similar non-significant effect with peppermint extract supplementation. Lymphocyte percentage also remained unaffected by treatment. These findings are supported by Rahman et al. (2021), Srivastava (2021), and Karageorgou et al. (2023), who observed

no significant changes in lymphocyte counts with supplementation of MPEO, CCEO, or plant bioactive compounds. Monocyte levels were unaffected by supplementation, which is in agreement with Witkowska et al. (2019) and Al-Janabi et al. (2023), who found no significant changes with MPEO or lemongrass supplementation. There was non-significant day effect on neutrophil, lymphocyte and monocyte count.

A significant ($P < 0.05$) reduction in eosinophil percentage was observed in both MPEO and CCEO groups compared to the control, consistent with Al-Janabi et al. (2023), who reported similar findings with lemongrass in lambs. This decrease may result from the anti-inflammatory and immunomodulatory properties of bioactive compounds like menthol and citral, which help reduce eosinophil activity by improving gut health and modulating immune responses (Nehme et al., 2021; Orzuna-Orzuna et al., 2022). The day and treatment*day effect were non-significant for eosinophil count.

Platelet counts remained stable across treatments revealed by non-significant treatment and day effect. This is supported by Patra et al. (2019) and Rahman et al. (2021), who reported no significant impact of menthol-rich compounds or peppermint extract. However, Palhares et al. (2021) noted a decrease in platelet count with essential oil blends in heifers. In healthy calves, where physiological balance is maintained, additional supplementation may have minimal effect on platelet levels.

The study revealed that supplementation with MPEO, and CCEO had no significant impact on MCV, MCH, and MCHC levels in crossbred calves. The day effect was also non-significant for the erythrocytic indices. These findings are consistent with Patra et al. (2019), who reported no significant effects of menthol-rich compound supplementation in growing sheep. Al-Janabi et al. (2023) also reported no significant differences in MCHC across treatment groups ($P \leq 0.05$), suggesting that lemongrass had no measurable effect on these parameters.

Table 2. Effect of supplementation of *Mentha piperita*, *Cymbopogon citratus* EOs on hematological parameters of CB calves

Parameters	T0	T1	T2	SEM	T	D	T*D
Haemoglobin	9.92	10.13	10.16	0.09	0.12	0.00	0.50
TEC*	5.78 ^b	5.88 ^{ab}	5.93 ^a	0.04	0.04	0.38	1.00
TLC	9.64	9.91	10.30	0.21	0.09	0.08	0.83
PCV*	28.59 ^b	28.97 ^{ab}	29.59 ^a	0.26	0.03	0.36	0.93
Neutrophil	29.56	30.44	30.62	0.35	0.08	0.47	0.92
Lymphocyte	61.62	62.31	61.87	0.44	0.54	0.57	1.00
Eosinophil*	3.96 ^a	3.78 ^b	3.73 ^b	0.07	0.03	0.68	0.33
Monocytes	3.34	3.53	3.45	0.14	0.64	0.54	1.00
Platelets	3.67	3.71	3.61	0.09	0.74	0.99	1.00
MCV	49.25	49.33	49.94	0.53	0.60	0.88	1.00
MCH	17.16	17.20	17.24	0.17	0.94	0.31	1.00
MCHC	34.70	34.91	34.44	0.35	0.65	0.10	0.99

Means bearing different superscripts vary significantly (*P<0.05, **P<0.01)

Effect on biochemical parameters

The effect of supplementation of MPEO and CCEO on serum biochemical parameters of crossbred calves have been presented in Table 3. The results indicated that supplementing calves' diets with 0.2% MPEO and CCEO did not lead to significant changes in serum glucose levels throughout the duration of the study. These findings align with those of Coelho et al. (2023) in calves and Patra et al. (2023) in lambs, who also found no significant impact of peppermint on glucose levels. Al-Janabi et al. (2023) reported comparable results, noting non-significant alterations in glucose levels with lemongrass supplementation in small ruminants.

The treatment had no significant effect on serum total protein levels, consistent with findings by Chiofalo et al. (2012) and Dorantes-Iturbide et al. (2022) who also observed similar results in sheep. Similarly, supplementation with MPEO, CCEO and MOS did not significantly influence serum albumin levels, in line with Bhat et al. (2019) and Al-Janabi et al. (2023). Garg et al. (2025) observed similar non-significant effect on calves with herbal supplements. The lack of change may reflect the stable nature of albumin, regulated by liver function

and nutritional status. CCEO supplementation showed a non-significant increase in globulin levels, consistent with Arab Ameri et al. (2015) and Khalifah et al. (2021), who reported similar trends with MPEO and a dose-dependent effect of CCEO. No significant effect was observed on the albumin-to-globulin (A:G) ratio, aligning with Akbari et al. (2016) and Khattab et al. (2017).

The present study revealed that supplementation with CCEO at 0.2% (T2 group) resulted in the lowest serum triglyceride and total cholesterol levels, consistent with Kumar et al. (2011), Alagawany et al. (2021), and Al-Janabi et al. (2023), who reported similar reductions with lemongrass supplementation. No significant effect on lipid profiles was noted with *Mentha piperita* or menthol-rich supplements, as observed by Hosoda et al. (2005) and Patra et al. (2019). A significant (P<0.05) increase in HDL-cholesterol levels was observed in both MPEO and CCEO-supplemented groups, possibly due to the bioactive compounds such as flavonoids and alkaloids influencing lipoprotein metabolism, as noted by Donnelly et al. (2005). Additionally, CCEO at 0.2% significantly reduced LDL and VLDL levels, supporting findings by Alagawany et al. (2021).

The reduction in triglycerides and cholesterol with CCEO may be due to bioactive compounds like citral and geraniol, which inhibit HMG-CoA reductase and lipase activity. Its antioxidant and hormonal effects may further support lipid stability. The increase in HDL and reduction in LDL suggest improved lipid balance, consistent with Kris-Etherton and Yu (1997), and may be partly attributed to PUFAs, known to modulate lipids (Grundy and Denke, 1990).

The results showed a significant ($P < 0.01$) reduction in serum urea levels in both MPEO and CCEO-supplemented groups compared to the control, aligning with findings by Ghanima et al. (2021), El-Essawy et al. (2021), Orzuna-Orzuna et al. (2022), and Dorantes-Iturbide et al. (2022). This reduction may reflect improved nitrogen utilization due to enhanced gut health and protein digestion.

The treatment had no significant effect on AST levels, though a numerical reduction was noted in MPEO (T1) and CCEO (T2) groups, consistent with Rekiel et al. (2007). A significant reduction in ALT levels was observed with both EOs, indicating improved liver function, aligning with Uchida et al. (2017), AL-Azzami and Mohammed (2023), Al-Janabi et al. (2023), and Rahman et al. (2024). This hepatoprotective effect is likely due to antioxidant compounds like citral, menthol, and neral. In contrast, Hosoda et al. (2005) reported no significant changes, suggesting variability based on dosage or animal factors. Overall, the decrease in ALT with EOs supplementation suggests a hepatoprotective effect likely driven by antioxidant-mediated protection of liver cells.

Table 3. Effect of supplementation of *Menthapiperita*, *Cymbopogon citratus* EOs on serum biochemical parameters of CB calves

Parameters	T0	T1	T2	SEM	T	D	T*D
Glucose	64.25	65.96	65.37	1.23	0.61	0.19	0.96
Total protein	7.71	8.16	8.39	0.38	0.45	0.98	0.99
Albumin	4.33	4.58	4.36	0.15	0.43	0.28	0.48
Globulin	4.22	4.36	4.54	0.09	0.06	0.65	0.88
AG ratio	1.03	1.06	0.97	0.04	0.23	0.43	0.59
Urea**	23.39 ^a	21.80 ^b	21.11 ^b	0.45	0.00	0.98	0.95
AST	90.61	87.37	86.85	1.62	0.21	0.44	0.15
ALT*	31.30 ^a	29.89 ^b	29.83 ^b	0.45	0.04	0.56	0.69
Triglyceride*	30.83 ^a	30.61 ^a	29.63 ^b	0.32	0.03	0.91	0.98
Total	82.41 ^a	81.94 ^a	80.69 ^b	0.24	0.00	0.64	0.33
HDL*	41.78 ^b	42.27 ^a	42.44 ^a	0.16	0.01	0.18	0.97
LDL*	33.71 ^a	33.54 ^a	32.70 ^b	0.28	0.04	0.98	0.96
VLDL*	6.16 ^a	6.12 ^a	5.93 ^b	0.06	0.03	0.91	0.98

Means bearing different superscripts vary significantly (* $P < 0.05$, ** $P < 0.01$)

Effect on antioxidant parameters

The present study showed a significant ($P < 0.05$) reduction in lipid peroxidase (LPO) levels with CCEO supplementation at 0.2%, indicating its effectiveness in alleviating oxidative stress in

crossbred calves (Table 4). This aligns with findings by Ojo et al. (2006) and Franz et al. (2010). Strong antioxidant properties of *Cymbopogon citratus* essential oil is due to its high polyphenol and flavonoid content which scavenge free radicals and inhibit lipid peroxidation, thereby lower lipid peroxidase levels,

key marker of oxidative damage. Similar antioxidant effects were noted in studies by Bharti et al. (2013), Su et al. (2018), and Zeeshan et al. (2023), further supporting the present results. The reduction in LPO levels observed in CCEO group is likely due to the radical-scavenging action of the phytochemicals, which effectively neutralize reactive oxygen species (ROS) and enhance oxidative stability.

The study showed a significant ($P < 0.01$) increase in superoxide dismutase (SOD) activity in crossbred calves supplemented with 0.2% CCEO, indicating

enhanced antioxidant defense. This aligns with findings by Su et al. (2018) and Zeeshan et al. (2023), who also reported elevated SOD levels with antioxidant supplementation. The improvement is attributed to the presence of polyphenols and flavonoids in lemongrass oil, which effectively scavenge free radicals and reduce oxidative stress, as noted by Ojo et al. (2006) and Franz et al. (2010). The elevated SOD activity reflects the efficacy of CCEO in strengthening the oxidative defense system and promoting cellular health in calves.

Table 4. Effect of supplementation of *Mentha piperita*, *Cymbopogon citratus* EOs on antioxidant parameters of CB calves

Parameters	T0	T1	T2	SEM	T	D	T*D
LPO*	11.17 ^a	10.58 ^{ab}	9.45 ^b	0.44	0.04	0.19	0.89
SOD**	21.33 ^b	22.35 ^b	27.16 ^a	1.03	0.00	0.11	0.95

Means bearing different superscripts vary significantly (* $P < 0.05$, ** $P < 0.01$)

Cost evaluation

Calf starter, priced at ₹ 30/kg, was supplemented with MPEO (₹ 1350/kg) and CCEO (₹ 1200/kg) in the treatment groups (Table 5). Despite the higher

cost, supplementation led to additional body weight gains of approximately 2.5 kg with MPEO and 3.5 kg with CCEO, along with improved hematological and antioxidant profiles in calves.

Table 5. Effect of supplementation of *Mentha piperita*, *Cymbopogon citratus* EOs on cost of feed of CB calves

Parameters	Amount (kg)/calf	Cost (₹)/calf
Calf starter @ (₹ 30/kg)	55.10	1653.00
Calf starter + MPEO @ (1350 ₹/kg)	55.10 + 0.11	1801.50
Calf starter + CCEO @ (1200 ₹/kg)	55.10 + 0.11	1785.00

CONCLUSION

The supplementation of CCEO at 0.2% improved hematological health and possible anti-inflammatory action. In terms of lipid profile CCEO contributed to elevated HDL levels and reduced triglycerides, total cholesterol, LDL and VLDL reflecting a hypolipidemic effect. Enhanced antioxidant defense was also evident through significantly lower lipid peroxidation (LPO) levels and increased superoxide dismutase (SOD) activity by 0.2% CCEO. Therefore, CCEO supplementation at 0.2%, can improve physiological resilience and metabolic health in calves, supporting its use as effective natural feed additive.

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