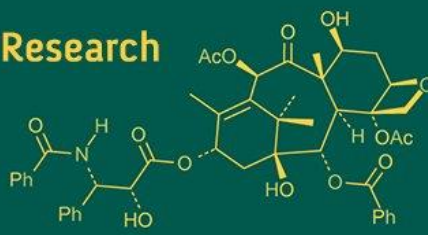
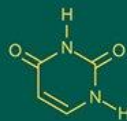
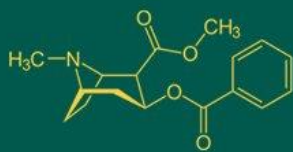


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Gautami Sarma
Department of Livestock Production
Management, College of Veterinary
and Animal Sciences, GBPUA&T,
Pantnagar, Uttarakhand, India

Jyoti Palod
Department of Livestock Production
Management, College of Veterinary
and Animal Sciences, GBPUA&T,
Pantnagar, Uttarakhand, India

Shive Kumar
Department of Livestock Production
Management, College of Veterinary
and Animal Sciences, GBPUA&T,
Pantnagar, Uttarakhand, India

Sanjay Kumar
Department of Livestock Production
Management, College of Veterinary
and Animal Sciences, GBPUA&T,
Pantnagar, Uttarakhand, India

SK Singh
Department of Livestock Production
Management, College of Veterinary
and Animal Sciences, GBPUA&T,
Pantnagar, Uttarakhand, India

RK Sharma
Department of Livestock Production
Management, College of Veterinary
and Animal Sciences, GBPUA&T,
Pantnagar, Uttarakhand, India

Anita Arya
Department of Livestock Products
Technology, College of Veterinary and
Animal Sciences, GBPUA&T,
Pantnagar, Uttarakhand, India

Sumit Gangwar
Department of Livestock Production
Management, College of Veterinary
and Animal Sciences, GBPUA&T,
Pantnagar, Uttarakhand, India

Ankit Sharma
Department of Livestock Production
Management, College of Veterinary
and Animal Sciences, GBPUA&T,
Pantnagar, Uttarakhand, India

Corresponding Author:
Jyoti Palod
Department of Livestock Production
Management, College of Veterinary
and Animal Sciences, GBPUA&T,
Pantnagar, Uttarakhand, India

Natural feed additives: Effects of essential oils and mannan oligosaccharides on growth performance and morphometric traits of crossbred calves

Gautami Sarma, Jyoti Palod, Shive Kumar, Sanjay Kumar, SK Singh, RK Sharma, Anita Arya, Sumit Gangwar and Ankit Sharma

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Abstract

The present study was undertaken to evaluate the effect of dietary supplementation of essential oils of *Mentha piperita* (MPEO), *Cymbopogon citratus* (CCEO) and prebiotic mannan oligosaccharides (MOS) on growth performance and morphometric traits of crossbred calves. A total of 30 crossbred calves (15-90 days old) were selected and randomly allotted to five treatment groups with six animals each. The trial was conducted for a period of 90 days at the Instructional Dairy Farm, College of Veterinary and Animal Sciences, G.B.P.U.A&T Pantnagar, Uttarakhand. The treatments comprised: T₀ (basal feed), T₁ (basal feed + MPEO @ 0.2% of calf starter), T₂ (basal feed + CCEO @ 0.2% of calf starter), T₃ (basal feed + MOS @ 6 g/calf/day), and T₄ (basal feed + MPEO @ 0.1% + CCEO @ 0.1% of calf starter + MOS @ 3 g/calf/day). Calves were maintained in individual pens under uniform housing and feeding conditions, and body measurements along with body weights were recorded at fortnightly intervals. Results indicated that body length, height at withers, and hip height did not differ significantly among groups. However, heart girth was significantly ($p < 0.01$) improved in T₂ and T₄, while paunch girth increased significantly ($p < 0.05$) in T₂ and T₄ compared to the control. Supplementation of essential oils (MPEO, CCEO) and MOS significantly enhanced growth performance in crossbred calves, with the highest body weight, fortnightly and average daily gain observed in the combined supplementation group (T₄). Supplementation of *C. citratus* EO, alone or in combination with *M. piperita* EO and MOS, positively influenced growth and body morphometric traits of crossbred calves, indicating their potential as effective natural growth-promoting feed additives.

Keywords: Body morphometry, crossbred calves, essential oils, growth, mannan oligosaccharides

Introduction

The misuse of antibiotics in livestock production has raised global concerns due to the emergence of antimicrobial resistance, which threatens both human and animal health (Coimbra *et al.*, 2022) [5]. Antibiotics, once widely used as therapeutic agents and growth promoters, are increasingly restricted as resistant bacterial strains can be transmitted through food chains, direct animal contact, or the environment, thereby reducing the effectiveness of antibiotics in human medicine (Van Boeckel *et al.*, 2019) [24]. Consequently, research has shifted toward exploring safe and natural alternatives such as essential oils (EOs), prebiotics, and phytobiotics that can enhance livestock health and productivity without contributing to resistance (Nehme *et al.*, 2021) [12].

Essential oils, derived from aromatic plants, possess antimicrobial, antioxidant, and anti-inflammatory properties, while mannan-oligosaccharides (MOS), as prebiotics, improve gut health, nutrient absorption, and immunity. Several studies have evaluated their effects in ruminants and poultry. Soltan (2009) [17] reported improved feed conversion efficiency in calves supplemented with a blend of essential oils, though no significant impact was observed on body weight. Liu *et al.* (2020) [10] demonstrated enhanced body weight and growth performance in calves fed a combination of essential oils and prebiotics. In small ruminants, Bhat *et al.* (2019) [3] and Al-Azzawi and Rasheed (2021) [2] observed significant improvements in nutrient digestibility, weight gain, and feed efficiency with supplementation of *Mentha piperita*. Likewise, Tiwari *et al.* (2018) [21] and Fahad and Al-Wazeer (2021) [6] reported positive growth responses with *Cymbopogon citratus* supplementation in poultry

and lambs, respectively. Studies on MOS have shown variable results, with some reporting improvements in average daily gain (Lucey *et al.*, 2021; Zeeshan *et al.*, 2023) [11, 25, while others noted only modest or non-significant effects (Uzmay *et al.*, 2011) [23].

Considering the inconsistent outcomes across studies, further research is needed to establish the growth-promoting potential of essential oils and prebiotics in calves. Therefore, the present study was undertaken to evaluate the effect of *Mentha piperita* essential oil (MPEO), *Cymbopogon citratus* essential oil (CCEO) and mannan oligosaccharide (MOS), either alone or in combination, on body measurements and growth performance of crossbred calves.

Materials and Methods

The present study was conducted on 30 crossbred calves aged between 15 and 90 days at the Instructional Dairy Farm (IDF), Nagla, College of Veterinary and Animal Sciences, G.B. Pant University of Agriculture and Technology, Pantnagar, Uttarakhand. All experimental procedures were approved by the Institutional Animal Ethics Committee (IAEC) of the University. The calves were randomly allocated into five treatment groups, each comprising six animals.

The treatment groups were as follows: T₀ (basal feed only), T₁ (basal feed + MPEO @ 0.2% of calf starter), T₂ (basal feed + CCEO @ 0.2% of calf starter), T₃ (basal feed + MOS @ 6 g/calf/day), and T₄ (basal feed + MPEO @ 0.1% + CCEO @ 0.1% of calf starter + MOS @ 3 g/calf/day). The experimental trial was conducted for a period of 90 days.

The calves were maintained under uniform management conditions. They were housed in a well-ventilated calf shed with individual pens for separate feeding, and each animal was identified with numbered tags to maintain accurate records. For exercise and natural exposure, calves were let loose in an open paddock during the first half of the day.

The parameters studied included body morphometric measurements, body weight, and growth performance. Morphometric traits recorded at the beginning and at fortnightly intervals included body length, heart girth, paunch girth, height at withers, and hip height. Measurements were taken using a 300 cm measuring tape while the animal was standing evenly on a firm surface.

Body weight was estimated at fortnightly intervals up to the 90th day using Schaeffer's formula. Growth performance was evaluated by calculating fortnightly body weight gain (difference between consecutive weights) and average daily gain (fortnightly gain/15 days × 1000, g/day).

Statistical analysis

The data were statistically analyzed to determine the mean ± SE. A two-way ANOVA was carried out using the SPSS 21.0 software to assess significant differences among treatment groups and sampling days, as well as to evaluate the effects of dietary interventions on various parameters. Duncan's multiple range test was used for mean separation, and significance was accepted at $p < 0.05$ and $p < 0.01$ (Snedecor and Cochran, 1994) [19].

Results and Discussion

Body measurement parameters

The results presented in Table 1 indicate the effect of dietary supplementation of *Mentha piperita* essential oil (MPEO), *Cymbopogon citratus* essential oil (CCEO), mannan

oligosaccharides (MOS), and their combination on various body measurement parameters of crossbred calves. The body length (BL) of calves showed no significant variation among treatments. Although the T₂ group recorded the highest body length, the differences were statistically non-significant. The heart girth (HG) exhibited a significant ($p < 0.01$) treatment effect. The highest heart girth was recorded in the T₄ group, followed by T₂, T₁ and T₃, while the lowest value was observed in the control group (T₀). This suggests that supplementation, particularly in the combined treatment group (T₄), positively influenced chest development in calves. No significant differences were observed in height at withers (HW) and hip height (HH) among the treatment groups. Paunch girth (PG) was found to be significantly ($p < 0.01$) higher in T₄ group, followed closely by T₂ and T₁. The lowest paunch girth was observed in the control group. Overall, supplementation with essential oils and MOS individually or in combination enhanced key body measurements, particularly heart girth and paunch girth, indicating improved body development and growth performance in crossbred calves. The day effect (D) was highly significant ($p < 0.01$) for all parameters, while treatment × day (T×D) interactions were non-significant.

Table 1: Effect of supplementation of *Mentha piperita*, *Cymbopogon citratus* EOs, MOS and their combination on body measurements of crossbred calves

Parameter	T ₀	T ₁	T ₂	T ₃	T ₄	SEM	T	D	T×D
BL (cm)	79.55	80.27	80.48	79.42	78.93	0.58	0.31	0.00	0.79
HG (cm)**	84.54 ^c	85.78 ^{bc}	86.14 ^b	85.05 ^{bc}	87.81 ^a	0.48	0.00	0.00	0.76
HW (cm)	87.19	89.59	88.68	86.96	88.23	0.78	0.11	0.00	1.00
HH (cm)	91.10	93.70	93.16	91.42	92.16	0.80	0.10	0.00	1.00
PG (cm)**	83.09 ^c	91.38 ^{ab}	93.89 ^a	88.75 ^b	94.58 ^a	1.97	0.01	0.00	0.99

BL: Body length; HG: Heart girth; HW: Height at withers; HH: Hip height; PG: Paunch girth; T₀: Control (Basal diet); T₁: Basal diet + *Mentha piperita* essential oil (MPEO) @ 0.2% of calf starter/calf/day; T₂: Basal diet + *Cymbopogon citratus* essential oil CCEO @ 0.2% of calf starter/calf/day; T₃: Mannan oligosaccharides (MOS) @ 6 g/calf/day; T₄: MP EO @ 0.1% + CCEO @ 0.1% + MOS @ 3 g/calf/day; T: Treatment effect; D: Day effect; T×D: Interaction between treatment and day; Means with different superscripts different significantly (** $p < 0.01$) across the row

The non-significant effects of supplementation on body length, wither height, and hip height are consistent with previous reports by Coelho *et al.* (2023) [4], Heinrichs *et al.* (2003) [9], Silva *et al.* (2012) [16], and Swedzinski *et al.* (2019) [20], who observed no influence of essential oils or MOS on these parameters.

Similarly, the significant increase in heart girth and paunch girth observed in this study agrees with the findings of Santos *et al.* (2015) [15] and Froehlich *et al.* (2017) [7], who reported improved frame growth, including chest and abdominal circumference, in calves fed essential oil compounds. Liu *et al.* (2020) [10] also documented greater ($p < 0.05$) heart girth and paunch girth in calves supplemented with essential oils combined with prebiotics. Similarly, a trend toward increased heart girth with essential oil blends was noted by Coelho *et al.* (2023) [4] during the post-weaning phase.

The discrepancy among studies showing either non-significant or significant responses may be explained by differences in supplementation dosage, composition of essential oils and prebiotics, basal diet quality, and growth

stage of calves. Bioactive components of essential oils such as menthol, citral, and geraniol may stimulate rumen microbial efficiency and nutrient utilization, while MOS enhances gut health and absorption, thereby contributing to increased chest and abdominal development. However, when basal nutrition is already optimal, these effects may not always translate into measurable structural growth. Further investigations are required to define the optimal combinations and dosages for consistent and reproducible improvements.

Growth parameters

During the 90-day experimental trial, supplementation of *Mentha piperita* essential oil (MPEO), *Cymbopogon citratus* essential oil (CCEO), mannan oligosaccharides (MOS), and their combination significantly ($p < 0.01$) influenced the

growth performance of crossbred calves (Table 2). The mean body weight (BW) of calves varied significantly ($p < 0.01$) among treatments, with the highest value observed in the T₄ group (57.87 kg), where calves received a combination of MPEO, CCEO, and MOS. This was followed by T₂ and T₁, while the lowest body weight was recorded in the control group (T₀). Similarly, the fortnightly weight gain (FWG) differed significantly ($p < 0.01$), being highest in T₄ (8.01 kg), followed by T₂, T₃ and T₁. The lowest FWG was recorded in the control group. A comparable trend was observed for average daily weight gain (ADWG), where the T₄ group exhibited the significantly ($p < 0.01$) higher gain followed by T₂ and T₃. The control group (T₀) showed the lowest ADWG. Both treatment and day effects were highly significant ($p < 0.01$) for growth parameters.

Table 2: Effect of supplementation of *Mentha piperita*, *Cymbopogon citratus* EOs, MOS and their combination on body weight and body weight gain of crossbred calves

Parameter	T ₀	T ₁	T ₂	T ₃	T ₄	SEM	T	D	T*D
BW (kg)**	53.63 ^c	55.92 ^{ab}	56.61 ^{ab}	54.58 ^{bc}	57.87 ^a	0.73	0.01	0.00	0.99
FWG (kg)**	6.58 ^c	7.19 ^b	7.76 ^a	7.70 ^a	8.01 ^a	0.17	0.00	0.00	0.08
ADWG (g)**	439.15 ^c	479.49 ^b	517.92 ^a	513.49 ^a	534.27 ^a	10.35	0.00	0.00	0.08

BW: Body weight; FWG: Fortnightly weight gain; ADWG: Average daily weight gain; T₀: Control (Basal diet); T₁: Basal diet + *Mentha piperita* essential oil (MPEO) @ 0.2% of calf starter/calf/day; T₂: Basal diet + *Cymbopogon citratus* essential oil CCEO @ 0.2% of calf starter/calf/day; T₃: Mannan oligosaccharides (MOS) @ 6g/calf/day; T₄: MP EO @ 0.1% + CCEO @ 0.1% + MOS @ 3g/calf/day; T: Treatment effect; D: Day effect; T*D: Interaction between treatment and day; Means with different superscripts different significantly (** $p < 0.01$) across the row

These findings are in agreement with earlier reports where supplementation of MPEO enhanced growth in lambs (Al-Azzawi and Rasheed, 2021) [2] and poultry (Srivastava *et al.*, 2022), while CCEO supplementation improved body weight in goats (Okoruwa and Edoror, 2021) [13] and poultry (Srivastava *et al.*, 2022). Similarly, MOS has been reported to improve growth performance in calves (Quigley *et al.*, 2002; Ghosh and Mehla, 2012) [14, 8], and a synergistic effect of EOs with prebiotics was also observed by Froehlich *et al.* (2017) [7] and Liu *et al.* (2020) [10], which aligns with the present findings.

In contrast, some studies reported non-significant effects of MPEO (Soltan, 2009) [17] and MOS (Heinrichs *et al.*, 2003; Uzmay *et al.*, 2011; Toth *et al.*, 2020) [9, 23, 22] on calf growth performance. Similarly, Abdullah *et al.* (2020) [1] found no effect of lemongrass supplementation on lamb body weight. Such discrepancies could be attributed to differences in dosage, animal species, age, duration of supplementation, or diet composition.

The improved growth performance observed in the present study may be explained by the antimicrobial, digestive, and feed efficiency-enhancing properties of essential oils, which improve gut health and nutrient utilization. The synergistic effect of combining EOs with MOS likely enhanced rumen fermentation, stabilized the gut microflora, and improved nutrient absorption, thereby resulting in greater body weight, fortnightly gain, and ADG compared to the control group.

Conclusion

The study demonstrated that supplementation of *Mentha piperita* and *Cymbopogon citratus* essential oils, individually or in combination with MOS, significantly enhanced heart girth, paunch girth, body weight, fortnightly gain, and ADG of crossbred calves, while other morphometric traits remained unaffected. These results

suggest that such natural supplements might be effectively used as growth-promoting feed additives and could serve as promising alternatives to conventional growth enhancers in calf nutrition.

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