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Effect of essential oils and prebiotic on serum biochemical parameters of crossbred lactating cattle

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Article Info	Abstract
Article history	This study investigated the effects of dietary supplementation with peppermint oil, lemon grass oil and
Received 13 March 2024	mannan oligosaccharides (MOS) on various biochemical parameters in lactating dairy cattle over a period
Revised 29 April 2024	of 90 days. Total 30 animals were randomly distributed into five treatment groups, i.e., control (T0) fed
Accepted 30 April 2024	with basal diet, T1 group supplemented concentrate with 0.1% peppermint oil, T2 group with 0.1%
Published Online 30 June 2024	lemongrass oil, T3 group with mannanoligosaccharide (MOS) @12 g /day /cattle and T4 group was
	 provided a combination of 0.05% of each oil and 6 g MOS. Serum biochemical parameters were studied at
Keywords	0, 30, 60 and 90 days of trial. Key findings indicate that EO supplementation significantly (p <0.05)
Dairy cattle	lowered serum urea levels in T4 group at 30 days, in T2 and T4 groups at 60 days, and in all treatment
Lemongrass oil	groups at 90 days. Moreover, significant ($p < 0.05$) reductions in aspartate transaminase (AST) and alanine
Mannan oligosaccharide	transaminase (ALT) were noted in treatment groups, especially pronounced at 90 days, highlighting a
Peppermint oil	hepatoprotective effect attributed to the bioactive components in EOs and MOS. The other parameters
	like glucose, protein profile and triglycerides did not vary significantly among treatment groups. Overall,
	the results revealed the beneficial role of EO and MOS supplementation in improving liver function and
	protein utilization in ruminants, suggesting a viable dietary strategy to enhance animal health and
	efficiency of nutrient use without negative side effects.

1. Introduction

The misuse of antibiotics in livestock has led to antibiotic resistance, posing global health risks (Coimbra *et al.*, 2022). Consequently, there is growing interest in natural alternatives like essential oils (EOs) and prebiotics to promote livestock health and productivity (Nehme *et al.*, 2021). These phytobiotics synergize with microbial flora, enhance rumen fermentation and overall health status (Kalantar *et al.*, 2017; Alizadeh *et al.*, 2010).

Essential oils (EOs) are natural compounds extracted from aromatic herbs through methods like hydrodistillation, historically pioneered by Arabs. These oils have been extensively studied for their therapeutic properties, including hepatoprotective effects (Bakkali *et al.*, 2008). Studies have reported essential oils to have antioxidant, anti-inflammatory, antimicrobial, anticarcinogenic and antidiabetic properties (Mehrotra, 2021; Deka *et al.*, 2021). Marjani *et al.* (2012) observed a significant reduction in alanine transaminase, alkaline phosphatase and gamma glutamyl transferase on supplementation of peppermint oil at various doses. Peppermint essential oil (PEO) has gained attention in ruminant feeding due to its potential to improve rumen fermentation, reduce methane production, and exhibit antimicrobial properties, thereby enhancing feed efficiency and gut

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Copyright © 2024Ukaaz Publications. All rights reserved. Email: ukaaz@yahoo.com; Website: www.ukaazpublications.com health (Patra *et al.*, 2019). Lemongrass essential oil, derived from the aromatic grass plant *Cymbopogon citratus*, boasts a myriad of therapeutic properties. α -citral and β -citral are the main components, while, citronella, β -myrcene, limonene and geraniol are also present in lemongrass essential oil (LGEO) but in relatively lower concentrations (Schaneberg and Khan, 2002). Ghanima *et al.* (2021) demonstrated that lemongrass essential oil notably decreased levels of uric acid, creatinine, urea as well as ALT and AST levels of serum.

Prebiotics like mannan-oligosaccharides (MOS) sourced from *S. cerevisiae* cell wall promote the growth of beneficial gut microbes, enhance animal performance, stabilize rumen pH, and reduce toxin production (Chaucheyras and Durand, 2010 ; Hady *et al.*, 2012). Considering their potential to enhance livestock health, the current research aimed to assess the impact of supplementation of peppermint oil, lemongrass oil, and MOS on serum biochemical parameters of crossbred lactating cattle.

2. Materials and Methods

The site of the current study was IDF, College of Veterinary and Animal Sciences, GBPUAT, Pantnagar, UK, India. The highest temperature in the region can be reached to 44°C in the summer, and the lowest temperature of 1°C can occur in the winter.

2.1 Experimental animals

Thirty lactating animals up to fourth lactation from the crossbred cattle herd of IDF, Pantnagar, were chosen and equally distributed lactation-wise in different treatment groups. The experiment was performed for a period of 3 months. A combination of green and dry

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fodder with concentrate as per their body weight and milk production was fed to experimental animals. Concentrate feed was provided at the time of milking and supplementation with peppermint oil, lemon grass oil and MOS was done according to different treatment groups.

2.2 Grouping of experimental animals

The experimental cattle were distributed into Control (T0, without any supplement), Treatment 1 (T1, supplemented with peppermint oil @ 0.1% of concentrate diet), Treatment 2 (T2, supplemented with lemon grass oil @ 0.1% of the concentrate diet), Treatment 3 (T3, supplemented with mannan oligosaccharide (MOS) @ 12 g /day /cattle), and Treatment 4 (T4, combination of essential oils and prebiotic was provided, *i.e.*, peppermint oil @ 0.05% of concentrate, lemon grass @ 0.05% of concentrate and MOS @ 6 g/cattle/day). Each group consisted of 6 animals.

2.3 Collection of blood sample and storage

The blood samples were collected at 0, 30^{th} , 60^{th} and 90^{th} day of the experimental study for serum biochemical analysis. The site for blood collection was prepared in a sterile manner by trimming the hair and cleaning the area with sterile gauze and spirit. A 5 ml blood sample was aseptically drawn from the jugular vein using a disposable syringe equipped with a 16-gauge needle, early at 8 am. The blood was immediately placed into a 5 ml vacutainer tube with a clot activator. Subsequently, the samples were quickly transported to the lab in a container chilled with ice packs. The blood samples were then stored at 4°C till the separation of serum from them. Then centrifugation of the samples was done at 3000 rpm for a duration of 10 min. The collected serum sample was then stored at – 20°C until analyses of biochemical parameters.

2.4 Parameters studied

Key serum biochemical parameters, including glucose, triglyceride, urea, total protein, albumin, globulin, and liver enzymes, *i.e.*, alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were assessed. Diagnostic kits of ERBA Mannheim company and UV-VIS spectrophotometer were utilized for these analyses.

2.5 Statistical analysis

The data obtained from study was analyzed statistically by oneway ANOVA (Snedecor and Cochran, 1994). The means were compared using Duncan's post hoc analysis, with a significance estimated at 5% and 1% level.

3. Results

The mean concentrations of glucose and triglycerides remained within the normal range at 0, 30, 60, and 90 days across all treatment groups, with no significant differences observed among them. The overall glucose and triglyceride concentrations showed non-significant differences among different treatments. However, at 30 days, the mean urea concentration was significantly (p < 0.05) lower in animals of T4 and highest in animals of T0 group. Urea concentrations in treatment groups T1, T2, and T3 were comparable to the other groups at 30 days. At 60 days, urea concentrations in animals of T2 and T4 groups were significantly (p < 0.05) lower than control, while concentrations in animals of T1 and T3 groups were similar to other groups. By the 90-days of the trial, urea concentrations in animals of T1, T2, T3, and T4 were significantly (p < 0.05) lower in comparison to animals of group T0. The overall urea concentration was also significantly (p<0.05) lower for animals of T1, T2, T3, and T4 groups than control group (Table 1).

Table	1:	Average	serum	glucose,	triglycerides	and	urea of	different	treatment	groups
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Days	TO	T1	T2	Т3	T4			
Glucose (mg/dl)								
0	59.16 ± 4.11	60.16 ± 3.55	62.50 ± 3.88	62.33 ± 4.26	62.16 ± 4.80			
30	63.16 ± 2.70	63.33 ± 2.98	62.00 ± 4.02	61.16 ± 5.21	66.33 ± 2.85			
60	62.66 ± 4.12	63.00 ± 2.76	65.50 ± 2.21	63.16 ± 4.03	63.83 ± 2.99			
90	60.00 ± 3.25	66.66 ± 2.77	64.83 ± 2.83	65.66 ± 2.37	62.33 ± 3.67			
Overall	61.25 ± 1.71	63.29 ± 1.49	63.70 ± 1.58	63.08 ± 1.94	63.66 ± 1.74			
Triglycerides (mg/dl)								
0	19.58 ± 0.29	18.52 ± 0.63	19.07 ± 0.53	18.83 ± 0.48	19.61 ± 1.79			
30	19.38 ± 0.59	18.67 ± 0.50	17.85 ± 0.49	18.21 ± 0.71	18.14 ± 0.63			
60	16.31 ± 1.01	16.38 ± 0.44	17.41 ± 0.71	17.45 ± 0.79	16.38 ± 0.71			
90	17.66 ± 0.69	17.05 ± 0.51	17.02 ± 0.68	16.27 ± 0.26	16.37 ± 0.40			
Overall	18.23 ± 0.42	17.66 ± 0.31	17.84 ± 0.32	17.69 ± 0.34	17.63 ± 0.56			
Urea (mg/dl)								
0	23.24 ± 1.54	20.17 ± 1.30	23.68 ± 2.14	22.14 ± 1.81	22.80 ± 1.65			
30	24.58 ± 0.76^{b}	21.04 ± 0.87^{ab}	22.29 ± 1.62^{ab}	21.25 ± 1.64^{ab}	18.95 ± 0.67^{a}			
60	24.80 ± 0.99^{b}	22.81 ± 1.12^{ab}	19.84 ± 1.09^{a}	21.62 ± 1.82^{ab}	19.64 ± 1.13^{a}			
90	26.11 ± 0.89^{b}	21.11 ± 1.43^{a}	20.74 ± 1.20^{a}	21.48 ± 1.78^{a}	20.18 ± 1.26^{a}			
Overall	24.68 ± 0.55^{b}	21.28 ± 0.59^{a}	21.63 ± 0.79^{a}	21.62 ± 0.82^{a}	20.39 ± 0.65^{a}			

Values bearing different superscripts within a row differ significantly (p < 0.05).

The average values of protein profile have been presented in Table 2. The mean values of total protein, albumin, globulin and A/G ratio were observed to be in the normal range at all collection days. The protein

profile was found to vary non-significantly across all the treatment groups at 0, 30, 60 and 90 days of treatment. The overall protein profile was non-significantly different for all the treatment groups.

Table 2: Average serum protein profile of different treatment groups

Days	ТО	T1	T2	Т3	T4				
Total protein (g/dl)									
0	8.18 ± 0.86	8.13 ± 0.91	8.40 ± 0.54	8.02 ± 1.08	8.91 ± 0.96				
30	7.96 ± 1.10	8.40 ± 1.02	8.44 ± 0.63	8.51 ± 1.11	8.61 ± 0.98				
60	7.97 ± 1.14	7.85 ± 0.80	8.55 ± 0.79	8.36 ± 1.17	8.16 ± 0.74				
90	7.96 ± 1.04	8.25 ± 0.74	7.65 ± 0.52	8.07 ± 0.87	8.49 ± 0.55				
overall	8.13 ± 0.46	8.38 ± 0.46	8.04 ± 0.37	8.04 ± 0.51	8.67 ± 0.44				
Albumin (g/dl)									
0	4.17 ± 0.20	4.15 ± 0.20	4.30 ± 0.12	4.36 ± 0.27	4.08 ± 0.28				
30	4.26 ± 0.34	4.45 ± 0.20	4.13 ± 0.44	4.83 ± 0.28	4.64 ± 0.33				
60	4.38 ± 0.15	4.16 ± 0.28	4.13 ± 0.18	4.61 ± 0.17	4.71 ± 0.25				
90	4.38 ± 0.22	4.26 ± 0.11	4.55 ± 0.24	4.36 ± 0.22	4.63 ± 0.24				
Overall	4.51 ± 0.14	4.54 ± 0.16	4.48 ± 0.16	4.79 ± 0.14	4.68 ± 0.16				
		Glo	bulin (g/dl)						
0	4.08 ± 0.16	4.23 ± 0.20	4.46 ± 0.16	4.43 ± 0.19	4.28 ± 0.14				
30	4.26 ± 0.13	4.18 ± 0.09	4.50 ± 0.16	4.40 ± 0.24	4.17 ± 0.12				
60	4.13 ± 0.22	4.10 ± 0.16	4.31 ± 0.24	3.99 ± 0.14	4.43 ± 0.28				
90	3.82 ± 0.10	4.16 ± 0.28	4.06 ± 0.28	4.33 ± 0.16	4.33 ± 0.18				
Overall	4.07 ± 0.08	4.17 ± 0.09	4.33 ± 0.10	4.29 ± 0.09	4.30 ± 0.09				
A/G ratio									
0	0.99 ± 0.06	0.95 ± 0.08	0.93 ± 0.07	0.95 ± 0.09	0.95 ± 0.06				
30	0.99 ± 0.06	1.06 ± 0.05	0.90 ± 0.06	1.10 ± 0.04	1.11 ± 0.08				
60	1.15 ± 0.09	1.15 ± 0.10	1.11 ± 0.04	1.24 ± 0.05	1.13 ± 0.12				
90	1.33 ± 0.07	1.22 ± 0.08	1.25 ± 0.14	1.20 ± 0.05	1.19 ± 0.10				
Overall	1.12 ± 0.04	1.10 ± 0.04	1.05 ± 0.05	1.12 ± 0.03	1.10 ± 0.04				

Values bearing different superscripts within a row differ significantly (p<0.05).

Table 3: Average aspartate amino transferase and alanine aminotransferase enzymes of different treatment groups

Days	TO	T1	T2	Т3	T4			
AST (U/L)								
0	85.18 ± 4.29	89.01 ± 5.52	90.48 ± 5.48	89.60 ± 3.15	84.88 ± 4.00			
30	95.49 ± 1.02^{b}	89.89 ± 1.54^{ab}	89.60 ± 4.37^{ab}	88.42 ± 3.02^{ab}	82.52 ± 1.49^{a}			
60	95.49 ± 2.28^{b}	87.83 ± 5.01^{ab}	83.70 ± 3.81^{a}	88.42 ± 1.82^{ab}	81.64 ± 2.30^{a}			
90	93.72 ± 2.18^{b}	79.58 ± 2.28^{a}	81.05 ± 2.93^{a}	81.35 ± 1.70^{a}	75.16 ± 1.35^{a}			
Overall	92.47 ± 1.54^{b}	86.58 ± 2.04^{a}	86.95 ± 2.16^{a}	86.95 ± 1.35^{a}	81.05 ± 1.39^{a}			
ALT (U/L)								
0	33.60 ± 2.28	32.71 ± 4.10	34.48 ± 2.44	27.70 ± 4.44	33.30 ± 2.30			
30	35.95 ± 0.87^{b}	31.24 ± 0.98^{ab}	32.42 ± 1.26^{ab}	32.42 ± 3.01^{ab}	28.29 ± 0.64^{a}			
60	31.83 ± 2.70^{b}	28.29 ± 2.23^{ab}	23.87 ± 0.75^{a}	27.41 ± 2.73^{ab}	22.99 ± 1.02^{a}			
90	32.42 ± 1.41^{b}	27.11 ± 1.08^{a}	26.52 ± 2.14^{a}	26.52 ± 1.58^{a}	25.93 ± 0.87^{a}			
Overall	33.45 ± 0.96^{b}	29.84 ± 1.23^{a}	29.32 ± 1.22^{a}	28.51 ± 1.52^{a}	27.63 ± 1.01^{a}			

Values bearing different superscripts within a row differ significantly (p<0.05).

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The mean values of AST and ALT levels have been presented in Table 3. At the beginning of the study (0 day), the mean values of AST and ALT were non-significantly different for the groups. However, after 30 days, the mean AST and ALT values differ significantly and were lower (p<0.05) in the T4 group and highest in animals of T0. Meanwhile, levels in the animals of T1, T2, and T3 groups were comparable to other groups. By the 60th day of treatment, AST and ALT concentrations were significantly lower (p<0.05) in the T1, T2, and T3 groups. Finally, by the 90th day, both AST (p<0.01) and ALT (p<0.05) concentrations were found significantly lower in animals of T1, T2, T3, and T4 when compared to T0. Overall AST and ALT values were also significantly (p<0.05) lower for treatments T1, T2, T3, and T4 compared to T0.

4. Discussion

The present findings of non-significant changes in glucose concentration align with previous findings by Orzuna-Orzuna et al. (2022) and in a meta-analysis involving ruminants. Furthermore, it was observed that EO supplementation did not negatively impact protein breakdown, as evidenced by the lack of significant effect on serum levels of albumin, globulin, and total protein (Dorantes-Iturbide et al., 2022). El-Essawy et al. (2021) observed similar findings, reporting that EO supplementation had no significant effect on several biochemical parameters in ruminants, including total protein, albumin and globulin for the protein profile, while total lipids, triglycerides, and cholesterol for the lipid profile. Similarly, Joshi et al. (2023) reported a non-significant effect on serum albumin at 3 months on herbal supplementation in lactating Badri cattle. Additionally, El-Essawy et al. (2021) found that EO supplementation led to a decrease in blood urea nitrogen (BUN) concentration, indicating improved protein digestion and utilization, likely due to EO-mediated inhibition of proteolysis (Cardozo et al., 2006; Fraser et al., 2007) which supported the present study. Furthermore, EO supplementation in beef cattle has been associated with a reduction in serum urea concentration (Orzuna-Orzuna et al., 2022). The reduction in urea levels is attributed to an increase in the relatively larger population of the bacterial family Lachnospiraceae, known to negatively correlate with serum urea levels in cattle (Zhou et al., 2020; Qiuet al., 2022). Similarly, Dorantes-Iturbide et al. (2022) demonstrated that EO supplementation decreased urea concentration and had no significant effect on triglyceride levels at low and moderate doses, while at high doses, triglyceride levels were significantly reduced. MOS has been found to reduce ruminal ammonia concentration, maintain ruminal pH and modulate the microbial population which may be associated with reduced urea levels.

In terms of liver health, EO supplementation demonstrated a hepatoprotective effect, as evidenced by decreased concentrations of ALT and AST (Uchida *et al.*, 2017; AL-Azzami and Mohammed, 2023). This protective effect may be attributed to bioactive compounds present in EOs, such as Neral and citral B found in lemongrass essential oil (Uchida *et al.*, 2017). Citral has been proposed to aid in detoxifying processes and lowering oxidative stress in the liver. Plants are natural sources of antioxidants and can minimize oxidative stress by reducing free radical formation (Kilaru *et al.*, 2023). The menthol in peppermint oil helps lower oxidative stress in the body, which in turn reduces lipid peroxidation and thereby

provides hepatocyte protective effect (Marjani *et al.*,2012). Peppermint oil and lemon grass oil were thus found to regulate liver enzyme functions. Gupta and Kori (2022) observed reduced (p<0.05) levels of ALT and AST through herbal extract supplementation and reported increased liver function.

The liver, the primary organ affected by absorbed chemicals from the intestine, releases enzymes upon cell damage, reflected in elevated liver enzyme levels. Thus, lower levels of AST and ALT suggest better liver function and performance. MOS (mannan oligosaccharides) may protect the liver by reducing harmful gut microbes and metabolites produced. Similarly, MOS supplementation led to a significant reduction in AST and ALT levels, indicating a potential protective effect on liver function (Yalcinkaya *et al.*, 2008; Kairalla, 2022; Youssef *et al.*, 2023). Muhammad *et al.* (2020) found nonsignificant differences in protein profile with MOS treatment. However, Shoukry *et al.*(2023) reported that treated groups receiving various levels of prebiotics (MOS + beta-glucan) exhibited significantly elevated concentrations of total protein and albumin in treatment groups. However, other blood parameters such as globulin, urea, creatinine, AST, and ALT did not show significant differences.

5. Conclusion

It can be concluded that supplementation with peppermint oil, lemongrass oil and mannan oligosaccharides (MOS) in diet positively impacts liver health in ruminants, as indicated by the significant reduction in AST and ALT enzyme levels. Furthermore, EO supplementation improved protein digestion and utilization, evident from lower urea concentrations without adversely affecting glucose, triglycerides, total protein, albumin, or globulin levels. These findings suggest that EOs and MOS can be beneficial for ruminant health, supporting efficient nutrient utilization and liver function without negative impacts on metabolic parameters.

Conflict of interest

The authors declare no conflicts of interest relevant to this article.

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