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Effect of Herbal Feed Additives and Sulfate Supplementation on Hematology, Biochemical and Antioxidant Status of Calves

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ABSTRACT

The present study was undertaken to evaluate the effect of herbal feed additives and sulfate supplementation on the hematology, biochemical, antioxidant status and immune response of growing cattle calves. Twenty-four growing cattle calves (12 male Haryana, 8 male Sahiwal, 4 female Sahiwal calves) of 4 to 16 months of age were distributed into four groups with six animals each in a randomized block design. Control (C) group was fed on basal diet without any feed additive, T1 group was fed on basal diet with herbal feed additive (Fennel seed + Clove oleoresin; CLO; @ 1.0% of DMI), T2 group was supplemented with sulfate @ 0.075% of DMI, and group T3 was fed on basal diet with herbal feed additive (Fennel seed + Clove Oleoresin (CLO); @ 1.0% of DMI) and sulfate @ 0.075% of DMI. The basal diet consists of 40% concentrate and 60% wheat straw. Body weight and dry matter intake were recorded fortnightly. Overall body weight, DMI (kg/day), TDN intake (g/kg $W^{0.75}$), and DCP intake (g/kg $W^{0.75}$) were similar in the treatment as well as control group. Hematological parameters like blood hemoglobin concentration and PCV were not impacted by herbal feed additive and sulfate supplementation. Plasmaglucose, triacylglycerol, and cholesterol concentration were found non-significant (P>0.05), plasma total protein, Plasma albumin, plasma globulin and PUN concentration were also not affected (P>0.05). Alanine amino transferase (ALT), aspartate amino transferase (AST), alkaline phosphatase (ALP), plasma bilirubin, plasma creatinine were found normal in range and not significantly different (P>0.05) between the groups. Total immunoglobulin level and antioxidant activity remain similar in all the groups (P>0.05).It may be concluded that supplementation of herbal feed additive (Fennel seed + CLO; @ 1.0% of DMI) may be used as rumen modifiers without any adverse effect in the calves.

KEYWORDS: Antioxidant, Calves, Herbal feed additives, Immunity, Sulfate

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INTRODUCTION

In recent years research on plant secondary metabolites (tannin, saponin, essential oils, etc.) has gained interest in animal nutrition due to their beneficial effect on rumen fermentation and methane mitigation. Plant secondary metabolites can be a rational approach to modulating the rumen microbiome and modifying its function. There is immense potential to manipulate the fate of metabolic hydrogen in the rumen, away from methane synthesis toward propionic acid formation, by feeding plants containing secondary metabolites to ruminants (Dey et al., 2016). Methanogenes is also decreases the efficiency of feed utilization in the animals, as 2-12% of the gross energy of feed taken by the animals is wasted in the form of methane (Johnson et al., 1993) leading to great economic losses for the livestock sector. So, the inhibition of methanogenesis in the rumen may result in enhancing the feed conversion efficiency by diversion of hydrogen for propionate synthesis or by reduction of carbon dioxide to acetate by reductive acetogenesis. Reduction of genera such as *Methanobrevibacter* and *Acetobacter* are potential targets for the reduction of CH_4, on the other hand, an increase in the abundance of the genera *Methanosphaera* and *Eubacterium* led to reductions in CH_4 emissions in heifers (Cunha et al., 2019). A mixture of rumen modifiers (RM-7; neem seed cake, mahua seed cake, Fennel seed seed, harad, fruit pulp of bahera, fruit pulp of amla and ajwain seed) in 2:2:2:1:1:1:1 proportion at 5, 10, 15 and 20% with 0.06% sodium sulphate of substrate did not affect total gas production but there was significant reduction in

methane production upto 10% level (Lakhani et al. 2019a). Bakshi et al. (2022) also reported that herbal feed additive supplementation reduces methane emission.

In the rumen, sulfate reducing bacteria (SRB) are present which have a higher affinity towards hydrogen moiety than methanogens. Thermodynamically, the reduction of sulfate ("G^o= -152 KJ/mole) is more favorable than the reduction of carbon dioxide ("G^o= -130 KJ/mole) (Oremland and Taylor, 1978). The threshold values of H_2 (mmol/liter) for SRBs and methanogens are 0.0013 and 0.067, respectively. The SRBs reduce sulfate into hydrogen sulfide by using ruminal hydrogen. The hydrogen sulfide produced by these bacteria can be used by most of the other microbes which cannot use sulfate directly. Sulphide is also involved in the synthesis of S-containing amino acids, therefore, inclusion of sulphate in the diet improves the growth of rumen microbes, hence, the performance of the animals.

Essential oils have shown some antioxidant activity, which may reduce the lipid peroxidation of meat, thus enhancing the tenderness of meat (Aminzare et al., 2019). Kumar (2017) demonstrated the increased antioxidant status of buffalo calves supplemented with a mixture of essential oil rich poplar and eucalyptus leaves.Clove oleoresin, a concentrated extract from clove buds, primarily consists of essential oil eugenol. Fennel (*Foeniculum vulgare*) is a plant rich in volatile components, mainly estragole, eucalyptol and estragole, limonene, trans-anethole, á-pinene, fenchone, and fenchol (Moosavi-Zadeh et al., 2023). Recently, Moosavi-Zadeh et al. (2023) fed Holstein dairy cows on a diet supplemented with Fennel seed@ 25 or 50 g/d for 45 d and observed increased feed intake and milk yield and decreased ruminal acetate proportion.The supplementation of amla fruit powder (*Emblica officinalis*) @ 0.75% enhanced the overall performance of broilers (Gaur et al., 2023).Kumar (2022) reported that the hemoglobin (Hb), packed cell volume (PCV), serum protein (albumin, globulin, and A:G ratio), and serum enzyme (ALT and AST) levels did not differ ($p > 0.05$) in experimental buffalo calves, fed with herbal feed additives. Bombik et al.(2012) studied the effect of herbal extracts on some haematological parameters of calves during rearing and found a significantly higher mean haemoglobin concentration in the treatment groups. Therefore, the present study was conducted to analyze the effect of supplementation of herbal feed additive (Fennel seed + CLO; @ 1.0% of DMI) as rumen modifiers on hematology, biochemical, antioxidant status, and immune response of calves.

MATERIALS AND METHODS

Ethics approval

Animal care procedures were approved (approval number IAEC/22/2/18) and conducted under the established standard of the Institutional Animal Ethics Committee (IAEC), constituted as per article number 13 of the Committee for Control and Supervision of Experiments on Animals (CPCSEA) rules laid down by the Government of India.

Selection of experimental animals, dietary treatment, and experimental design

Twenty-four growing calves of 4 to 16 months (12 male Hariana calves, 8 male Sahiwal calves, and 4 female Sahiwal calves) were selected from the herd maintained at Livestock Farm Complex (LFC), DUVASU Mathura (Uttar Pradesh), India. Calves were divided into four groups (six animals each) in a randomized block design. The four groups of calves were given different treatments. Control (C) group was fed on basal diet without any supplementation, T1group was supplemented with herbal feed additive (Fennel seed + Clove Oleoresin (CLO); @ 1.0% of DMI), T2 groups were supplemented with sulfate @ 0.075% of DMI(sulfate as Sodium sulfate, Na_2SO_4 contains 67% sulphate; Purity-98%), and group T3 were supplemented with diet containing herbal feed additive (Fennel seed + CLO; @ 1.0% of DMI) and sulfate @ 0.075% of DMI along with basal diet. The Fennel seed contains tannins and essential oils, and Clove Oleoresin contains essential oils. The basal diet contains 40% concentrate and 60% wheat straw.The chemical composition of the concentrate mixture is given in Tables 1 and 2.The experiment was continued for 120 days where all the calves were managed under similar conditions.

Table1. Composition (% DM basis) of diet fed to calves during the feeding trial

Ingredients			Dietary Group (% DM)			
			C	T1	T2	T3
Wheat straw			60	60	60	60
Concentrate	Ingredients	(in Kg)				
	Maize grain	15	40	40	40	40
	Barley grain	15				
	wheat Bran	33				
	Mustard Cake	35				
	Mineral	2				

Table 2. Chemical composition (% or as mentioned) of diet fed during the experimental period

Attribute (%)	Concentrate	Wheat straw
Dry matter	90.0	92.0
Crude protein	20.2	3.20
Ether extract	6.24	1.31
Total ash	9.25	13.2
Crude fiber	16.1	38.9
Neutral detergent fiber	33.1	82.2
Acid detergent fiber	18.1	49.6
Ca %	1.43	0.38
P %	0.39	0.17

Blood sample collection and analysis

Blood samples were collected before feeding and watering of heifers at 07:00 h in heparinized vacutainer tubes (BD Franklin, USA) at 0, 30, 60,and 120 dayspost feeding. A fraction of blood samples was used for analysis of Hb and HITor PCV value as per Sahli's and Wintrobe tube method, respectively. Remaining blood samples were centrifuged at 3000 rpm for 30 min to separate the plasma from packed erythrocytes. Plasma samples were stored at -20 °C until further analysis of biomarkers of liver and kidney function (AST, ALT,ALP, bilirubin, and creatinine), biomarkers of protein metabolism (total protein, albumin, globulin, andPUN). The plasma concentration of AST, ALT, ALP, bilirubin, creatinine,total protein, albumin, and PUN was determined byan automated biochemical analyzer (BS-120Chemistry Analyzer, Shenzhen Mindray Biochemical Electronics Co.Ltd., China) using Span Diagnostic kits (Span Diagnostic Ltd., Surat, India). Plasmaglobulin concentration was determined by subtracting the albumin content from total protein content. Plasma TIg was measured by zinc turbidity method (Mc Ewan et. al., 1970). Total antioxidant status (TAS) was measured as FRAP assay described by Benzie and Strain (1999).

Statistical analyses

All statistical analyses were performed as per the standard method by using the SPSS computer package (SPSS Version 20.0, SPSS Inc, Chicago, USA). The data obtained were statistically analyzed

by using one-way ANOVA procedures. Tukey's Honestly Significant Difference test was used to evaluate significant differences between means of treatments.For all statistical analyses, probability values less than 0.05 were considered significant.

RESULTS AND DISCUSSION

Total DMI (kg/day), CP intake (g/day), DCP intake (g/day), TDN intake (g/day)werefound similar (P>0.05) in all four, control (C1), T1, T2 andT3 groups. Average BW at starting of the study in (C),T1, T2 and T3 groups were 80.3, 80.3, 81.8, 80.3 kg and at the end of the experiment were 129, 127, 129, 127 kg, respectively. The overall BW (kg) was found to be similar in all the experimental groups.FCR also did not differ significantly (P>0.05) between the groups.

Haematological parameters

The blood haemoglobin concentration and packed cell volume percentage of herbal feed additives and sulfate supplemented calves was not differed significantly (Table 3). Similar to our study, Kumar et al. (2022) studied effects of supplementing phytogenic feed additives composed of a mixture of eucalyptus (*Eucalyptus citriodora)* and poplar (*Populus deltoides*) leaf-meal (EPLM) and found hemoglobin (Hb), packed cell volume (PCV), did not differ ($p > 0.05$) in experimental buffalo calves. Although Bombik et al. (2012) have found significantly higher mean haemoglobin concentration and blood cell count in the experimental calves fed with the herbal extracts. Haematological parameters have no significant effect in the present study, this may be due to the lower doses of herbs and sulfate in the treatment groups.

Table 3. Effect of herbal feed additives and sulfate supplementation on hematocrit value and blood biochemical parameters

Parameters	Control	T1	T2	T3	SEM	P value
Hb (g/dl)	9.13	8.90	9.38	8.71	0.113	0.365
PCV (%)	26.4	26.2	28.8	26.2	0.355	0.799
Glucose(mg/dl)	60.1	59.8	59.5	59.9	0.594	0.988
Triglyceride (mg/dl)	67.3	66.1	68.1	67.2	0.947	0.912
Cholesterol (mg/dl)	198	198	197	198	0.949	0.958
Total protein (g/L)	6.25	6.24	6.27	6.24	0.070	0.999
Albumin (g/L)	3.26	3.28	3.26	3.27	0.049	0.999
Globulin (g/L)	3.10	3.08	3.06	3.09	0.078	0.999

C: Control animals having no feed additives supplementation; T1: Treatment Animals supplemented with herbal feed additives; T2: animals supplemented with sulfate; T3: Animals supplemented with herbal feed additives and sulfate; P>0.05: Non significant

Biomarker of energy, lipid, protein metabolism and liver, kidney function tests

The plasma glucose concentration, plasma triglycerides and plasma cholesterol concentration,plasma total protein, plasma albumin and plasma globulin, plasma aspartate aminotransferase (AST), alanine aminotransferase (ALT), blood urea nitrogen (BUN), creatinine, and bilirubin in control and treatment groups did not differ significantly (Table 3 and Table 4). Similar to present study, Kumar et al. (2022) studied effects of supplementing phytogenic feed additives composed of a mixture of eucalyptus (*Eucalyptus citriodora)* and poplar (*Populusdeltoides*) leaf-meal (EPLM) and found serum protein (albumin, globulin, and A:G ratio), and serum enzyme (ALT and AST) levels did not differ ($p > 0.05$) in experimental buffalo calves. Anantasook et al. (2014) found no change in the level of blood urea nitrogen by feeding rain tree pod meal containing tannins and saponins in growing dairy steers. Kumar et al. (2017) also reported no effect of on blood biochemicals and serum enzymes by feeding tea seed saponins. Lakhani et al. (2019) also reported no effect of on blood biochemicals and serum enzymes by feeding rumen

modifier and sodium sulfate.The present study result is no any significant effect on liver and kidney function test and so both herbal and sulfate have no any adverse effect on these vital organs.

Table 4. Effect of herbal feed additives and sulfate supplementation on antioxidant status, liver and kidney function parameters

Parameters	Control	T1	T2	T3	SEM	P value
ALT (U/L)	22.2	22.3	22.1	22.1	0.155	0.953
AST (U/L)	72.9	72.6	72.8	72.8	0.178	0.966
ALP(U/L)	58.3	58.4	58.5	58.6	0.100	0.815
Bilirubin (mg/dl)	0.92	0.935	0.930	0.937	0.009	0.987
Creatinine (mg/dl)	0.910	0.905	0.909	0.905	0.004	0.974
PUN (mg/dl)	6.89	6.86	6.90	6.86	0.032	0.960

Biomarker of antioxidant status and immune response

Total antioxidant activity (TAA) was similar in the group supplemented with herbal feed additives with respect to control groups (Table 5). SOD and FRAP activity remain similar in all groups. Similar to the present study, Passetti et al. (2021) also reported that Adding EO blends (OEO or XEO) and an Emulsifier to barley-based diets of lambs did not change the serum blood oxidative status.Although study of Kumar (2017) demonstrated the increased antioxidant status of buffalo calves supplemented with a mixture of essential oil rich poplar and eucalyptus leaves with increased concentration of major antioxidant enzymes. Total immunoglobulin concentration in plasma was found higher over time period of experiment but no any significant difference among the groups. Similarly to our study, Lakhani et al. (2019b) reported that Supplementing mixture of neem seed cake, mahua seed cake, Fennel seed, harad, fruit pulp of bahera, fruit pulp of amla and ajwain seed in 2:2:2:1:1:1:1 proportion with sodium sulphate at 0.06% of dry matter intake improved immune status of the growing calves. Kumar et al., (2022) also reported that supplementation of phytogenic composite feed additive (EPLM), consisting of an equal proportion of eucalyptus (*Eucalyptus citriodora*) and poplar (*Populus deltoids*) leaves at both the dose levels (50 g and 150 g/h/d) to calves improved the immune response. The present study result is no any significant effect on marker of antioxidant status and immune response, it may be due to lower doses of herbs and sulfate in the treatment groups.

Table 5. Effect of herbal feed additives and sulfate supplementation on antioxidant status, liver and kidney function parameters

Parameters	Control	T1	T2	T3	SEM	P value
SOD (nmol MTT formazan/mg Hb)	381	364	368	344	13.3	0.809
FRAP (µmol/L)	940	983	934	974	21.73	0.822
Totalplasmaimmunoglobulin (mg/ml)	21.7	23.8	23.9	22.6	0.766	0.711

C: Control; T1: Animals supplemented with herbal feed additives; T2: animals supplemented with sulfate; T3: Animals supplemented with herbal feed additives and sulfate.

Effect on plasma mineral levels

The plasma calcium and phosphorus level were numerically higher over time period of experiment but no any significant difference among the groups (Table 6). Similar to present findings, Yadav et al. (2017) reported that Calcium balance was higher in treatment groups but difference was non-significant but phosphorus level here is significantly varied among the groups. A key limitation of this study is the lack of homogeneity among the cattle calves, particularly in terms of breed, age, and sex background. These variations introduced substantial variability in the calves' responses to the nutritional treatments, which likely contributed to the lack of significant differences in the results.

Table 6. Effect of herbal feed additives and sulfate supplementation on plasma mineral profile

Parameters	Control	T1	T2	T3	SEM	P value
Ca (mg/dl)	9.77	9.94	9.97	10.07	0.110	0.816
P (mg/dl)	5.49	5.63	5.53	5.44	0.070	0.790

C: Control animals; T1: Animals supplemented with herbal feed additives; T2: animals supplemented with sulfate; T3: Animals supplemented with herbal feed additives and sulfate.

CONCLUSIONS

The study examined the impact of herbal feed additives and sulfate on cattle calves' hematology, biochemical, and antioxidant status. Results showed no significant differences in blood hemoglobin concentration, packed cell volume percentage, or liver enzymes.Biomarkers of glucose, lipid, and protein indicated no adverse effect of treatment. The study found that all liver enzymes were within the normal physiological range, indicating no detrimental impact on the health of animals. Hence, it may be concluded that the herbal feed additive (Fennel seed + CLO; @ 1.0% of DMI) selected as rumen modifiers may be supplemented in the ration of the cattle calves without any adverse effect.

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