RESEARCH ARTICLE

Influence of Specified vis-a-vis Commercial Mineral Mixture on Haemato-Biochemical Parameters and Serum Mineral Concentration in Crossbred Calves

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Abstract

A study was conducted to discern the effects of supplementation of areas specific mineral mixture in comparison to commercial mineral mixture on the haemato-biochemical and mineral profile of growing crossbred calves. Feeding trial was conducted for 120 days on 15 one-week-old calves that were randomly assigned to 3 equal groups, each of 5 heads. Group 1 received a basal diet (milk, calf starter, green fodder as per NRC, 2001). Group 2 received a basal diet with commercial mineral mixture (Agrimin) supplementation, and Group 3 received a basal diet with areas specified mineral mixture (containing ionic mineral salts like dicalcium phosphate 99.40% and copper sulphate 0.6%). All calves were fed whole milk, calf starter containing broken wheat, soyabean chura and deoiled rice bran, and green fodder (berseem grass, oat and mustard). Milk was discontinued after 2 months. Blood samples were collected at the 1st, 8th, and 16th weeks of age of calves for haemato-biochermical analysis. Throughout the trial, there were no significant ($p>0.05$) differences in haematological parameters. During 1st week, biochemical parameters did not differ significantly among the groups. At 8th week, group 3 showed a significant ($p<0.05$) decrease in blood glucose, while groups 2 and 3 displayed significantly ($p<0.05$) higher total protein and SGOT levels. At 16th week, group 2 and 3 exhibited significantly ($p<0.05$) higher glucose and albumin levels. Mineral concentrations did not differ significantly ($p>0.05$) at 1st week, while significantly ($p< 0.05$) increased during the 8th and 16th weeks. In general, specified mineral supplementation in young calves resulted in numerically improvements in Hb, PCV, and significant improvement in glucose, total protein, albumin, SGOT, calcium, phosphorus and copper concentration, while remaining within normal physiological range. Hence, specified mineral mixture can be safely incorporated into the calves' ration without adversely affecting their blood biochemistry and mineral profiles.

Key words: Crossbred calves, Haematology, Mineral, Serum biochemicals, Specified mineral.

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Introduction

Optimal livestock growth and development hinge on adequate nutrition, with mineral supplementation being a critical facet of their dietary requisites. Vital for maintaining physiological functions like blood health, immune responses, and overall performance in young animals, essential minerals play an irreplaceable role. Blood mineral analysis offers valuable insights into mineral status (Herdt *et al.,* 2000). Notably, soil mineral mapping in Uttarakhand by Shukla *et al.* (2015) uncovers deficiencies in essential minerals such as copper, manganese and zinc. Among these, phosphorus deficiency is globally prevalent and significantly affects grazing livestock. The pivotal roles of calcium and phosphorus in skeletal and tissue development are underscored, their deficiency affecting various developmental stages (De Clercq and Vriens, 2018). Copper (Cu), an indispensable trace element, influences iron metabolism, immune function, oxidative stress protection, and digestive enzyme activities (Amachawadi *et al*., 2011; Huang *et al*., 2015). This study was focused on crossbred calves to evaluate the effects of mineral supplementation on haemato-biochemical attributes and mineral concentrations, contributing to a more comprehensive understanding of livestock production.

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Materials and Methods

Animals, Grouping and Supplementation

This research was conducted after getting approval from Institutional Animal Ethics Committee on fifteen crossbred

calves of HF breed, one week of age. The calves were selected for the on-farm trial of 120 days at Instructional Dairy Farm, GBPUAT, Pantnagar in Tarai Plane region of Uttarakhand (India). These calves were randomly divided into three equal groups each consisting of 5 calves and were given basal diet consisting of whole milk, calf starter and green fodder as per NRC (2001) during phase 1 and milk was discontinued after 2 months in phase 2 in all three groups. Calf starter for all calves was prepared with broken wheat, deoiled rice bran (DORB), soybean chura / groundnut cake in approximately 1:1:1 ratio, and green fodder consisted of berseem grass, green oats and green mustard in 2:2:1 ratio. Additionally, group 2 was supplemented with commercial mineral mixture (Agrimin), while group 3 was given specified mineral mixture formulated based upon the incidence of deficiency prevailing in the animals and in the soil of Uttarakhand (Shukla *et al*., 2015). The specified mineral mixture formulated had ionic mineral salts like dicalcium phosphate (99.40%) and copper sulphate (0.6%). These mineral mixtures were provided daily at specified dose rate of 3.0-7.0 g/day in milk for initial 2 months and 8.0-15.0 g/day in calf starter for next 60 days.

Laboratory Analysis

Blood samples (10 mL) were taken from the jugular vein of each calf at the age of 1st, 8th and 16th week in different types of sterile vacuumized tubes: sodium fluoride-containing tubes for glucose, EDTA-coated tubes for haematological analysis, and clot activator-containing tubes for serum separation. The uncoagulated blood was stored at 2°C, while the separated plasma and serum were stored at -20°C until analysis. The blood samples were analyzed for various parameters including haemoglobin (Hb), packed cell volume (PCV), total erythrocyte count (TEC), total leukocyte count (TLC), differential leukocyte count (DLC), and erythrocytic indices such as mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), and mean corpuscular haemoglobin concentration (MCHC)(Jain, 1986). Serum samples were analyzed using commercial ERBA diagnostic test kits and an ELISA reader for parameters, *viz.,* total protein, albumin, globulin, A:G ratio, glucose, alanine aminotransferase (ALT), aspartate aminotransferase (AST) enzymes, calcium, phosphorus, and copper. The data assembled was statistically analyzed using one-way analysis of variance and Duncan's *post hoc* tests (Snedecor and Cochran, 1994).

Results and Discussion

Haematological Parameters

Table 1 presents comprehensive data on haematological parameters, revealing that the average values obtained fell within the normal physiological range. Across the 1st, 8th, and 16th weeks of crossbred calf development, no noteworthy differences were observed in haematological value encompassing Hb, PCV, TEC, TLC, DLC, as well as erythrocytic indices. However, a numeric uptick in values of Hb, PCV, TEC, TLC, and MCHC was noted within the specified mineral-supplemented group 3 (Table 1). Comparable results were found in studies by Aliarabi *et al*. (2015) concerning lambs and Ramulu *et al*. (2015) focusing on Murrah buffalo calves undergoing mineral supplementation, while Asadi *et al.* (2022) reported a significant increase in the average values of haematological parameters such as TEC, haemoglobin, PCV, MCH, and MCHC. This underscores the potential impact of mineral supplementation. In contrast, Sobhanirad and Naserian (2012) discovered a notable reduction in RBC, haemoglobin, PCV, and MCHC concentration among Holstein dairy cows with zinc supplementation. These diverse findings emphasize the intricate relationship between mineral supplementation and haematological outcomes.

Biochemical Parameters

Table 2 depicts the data of biochemical parameters in crossbred calves during experimental period. During the first week of age of calves, the values obtained showed no significant ($p>0.05$) differences between all the three groups, and all parameters fell within the normal physiological range (Weiss and Wardrop, 2011). By the 8th week, control group 1 exhibited significantly ($p<0.05$) higher glucose concentration than group 2 and 3, mineral supplemented groups 3 had significantly ($p<0.05$) higher levels of total protein than group 2 and 1. For group 2 and 3, SGOT was significantly($p<0.05$) higher in comparison with group 1. All the parameters remained within the normal physiological range (Weiss and Wardrop, 2011). This aligns well with prior studies of Shah *et al*. (2003) in buffalo, Sahoo *et al.* (2017) in crossbred cows, Sharma *et al.* (2002) in heifers and Ramulu *et al.* (2015) in Murrah buffalo on mineral supplementation. At the 16th week, specified mineral supplemented group 3 showed significantly elevated albumin levels (Nikolaev *et al.,* 2022), followed by group 2, compared to group 1. Other parameters like globulin and A:G ratio remained consistent and within the normal range (Kaneko *et al*., 1997) with no significant changes throughout the experiment. Similar results were observed in studies conducted by Samarin *et al.* (2022) and Sobhanirad and Naserian (2012), involving growing lambs, dairy cows, and finishing lambs.

Serum Mineral Concentration

The serum concentrations of calcium (Ca), phosphorus (P), and copper (Cu) in crossbred calves at various age stages are also presented in Table 2. The recorded mineral levels fell within the normal physiological range (Fan *et al.,* 2019). During the initial week of age, the cumulative average mineral concentrations for Ca, P, and Cu exhibited non-significant differences among the distinct groups. However, as the investigation progressed, notable ($p<0.05$) alterations in mineral concentrations were observed for calcium, phosphorus and copper in the specified mineral-

Table. 1: Haematological characteristics assessed during 1st, 8th and 16th week of age of crossbred calves supplemented with selective and commercial mineral mixture

Parameters	Week	Group-1	Group-2	Group-3	p value
Hb (mg/dL)	1	11.34±1.39	10.08±1.05	11.58±1.66	0.723
	8	11.90±1.32	10.46±1.17	12.34±1.15	0.540
	16	12.72±2.99	11.56±1.33	13.82±1.15	0.480
PCV (%)	1	26.26±1.50	27.70±1.27	27.92±1.73	0.713
	8	31.48±2.11	29.72±1.87	32.02±1.79	0.687
	16	32.80±2.10	30.98±2.16	34.74±1.21	0.397
TEC ($x10^6/mm^3$)	1	06.80±0.29	07.04±0.08	07.04±0.35	0.776
	8	07.92±0.29	08.12±0.13	07.97±0.15	0.783
	16	07.97±0.15	08.26±0.50	09.13±0.11	0.228
TLC ($x\ 10^3/mm^3$)	1	07.97±0.76	08.54±0.28	08.27±0.40	0.753
	8	09.73±0.34	09.81±0.41	09.78±0.37	0.987
	16	11.91±0.37	12.11±0.39	12.82±0.64	0.413
Neutrophils (%)	1	21.94±2.08	22.36±3.08	23.12±1.63	0.937
	8	23.32±1.90	24.10±1.90	23.50±1.17	0.944
	16	23.34±1.05	27.04±1.27	27.06±1.13	0.067
Basophils (%)	1	01.00±0.13	00.74±0.08	01.14±0.29	0.917
	8	01.10±0.40	01.06±0.48	01.14±0.26	0.367
	16	01.10±0.40	01.06±0.48	01.14±0.26	0.990
Eosinophils (%)	1	01.74±0.66	01.64±0.38	01.82±0.23	0.963
	8	01.50±0.44	02.10±0.74	01.94±0.63	0.783
	16	01.42±0.75	01.76±0.74	01.48±0.86	0.949
Monocytes (%)	1	01.94±0.32	02.40±0.50	02.0±0.54	0.759
	8	03.40±0.50	03.88±0.42	03.66±0.22	0.710
	16	04.12±0.29	03.70±0.54	03.92±0.55	0.827
Lymphocytes (%)	1	73.38±2.82	72.66±2.26	72.22±2.28	0.946
	8	70.78±2.10	69.18±1.36	69.76±1.21	0.783
	16	70.02±1.12	66.44±2.31	66.40±1.61	0.282
MCV (fl)	1	38.71±2.03	39.27±1.40	40.19±3.7	0.921
	8	39.57±1.52	36.55±2.01	40.10±1.89	0.363
	16	40.01±0.74	37.47±0.78	37.99±0.94	0.114
MCH (pg)	1	16.98±2.50	14.30±1.49	16.63±2.55	0.671
	8	14.87±1.22	12.81±1.21	15.42±1.32	0.340
	16	15.39±0.85	13.91±1.03	15.09±1.14	0.580
MCHC (g/dL)	1	43.06±4.79	36.35±3.20	40.65±4.78	0.558
	8	37.35±1.77	35.23±3.01	38.19±1.79	0.647
	16	38.35±1.76	36.99±1.95	39.61±2.52	0.687

None of the parameters differ significantly within a row ($p>0.05$)

Table. 2: Blood biochemical and mineral parameters assessed during 1st, 8th and 16th week of age of crossbred calves supplemented with selective and commercial mineral mixture

Attributes	Week	Group 1	Group 2	Group 3	p value
Glucose (mg/dL)	1	87.94±2.10	87.78±1.80	88.71±2.07	0.941
	8	79.00^{a}±0.76	75.28^{ab}±1.27	73.52^{b}±1.46	0.017
	16	69.46^{b}±1.39	75.52^{a}±0.77	74.04^{a}±1.47	0.013
Total protein (g/dL)	1	05.40±0.63	04.60±0.25	04.90±0.12	0.443
	8	04.50^{b}±0.14	04.70^{b}±0.17	05.10^{a}±0.22	0.045
	16	05.60±0.43	05.20±0.12	05.80±0.18	0.331
Albumin (g/dL)	1	02.65±0.13	2.68±0.15	02.66±0.11	0.992
	8	02.32±0.28	2.28±0.30	02.40±0.32	0.866
	16	02.70^{b}±0.31	3.30^{a}±0.06	03.50^{a}±0.08	0.035
Globulin (g/dL)	1	02.70±0.68	01.90±0.21	02.20±0.18	0.462
	8	02.10±0.31	02.40±0.37	02.60±0.23	0.581
	16	02.90±0.61	01.90±0.23	02.41±0.23	0.208
A:G ratio	1	01.20±0.35	01.41±0.16	01.20±0.14	0.822
	8	01.20±0.43	01.12±0.36	00.99±0.19	0.829
	16	01.10±0.34	01.70±0.14	01.50±0.16	0.237
SGPT (IU/L)	1	10.49±1.30	08.30±1.60	08.80±2.10	0.671
	8	10.53±2.30	13.11±1.70	11.70±3.00	0.762
	16	12.18±1.10	11.49±1.60	14.21±1.80	0.477
SGOT (IU/L)	1	16.48±1.5	18.4±0.55	16.14±1.06	0.324
	8	17.27^{b}±1.5	22.55^{a}±0.48	19.79^{a}±1.10	0.024
	16	29.27±3.5	29.98±1.31	31.61±1.50	0.796
Total bilirubin (mg/dL)	1	00.54±0.37	00.53±0.30	00.53±0.09	0.991
	8	00.72±0.05	00.87±0.04	00.76±0.50	0.290
	16	00.60±0.5	00.18±0.50	00.11±0.5	0.051
Calcium (mg/dL)	1	07.63±0.41	08.01±0.56	07.81±0.52	0.870
	8	08.24^{b}±0.64	10.43^{a}±0.66	10.72^{a}±0.71	0.046
	16	09.70^{b}±0.87	11.84^{a}±0.76	12.76^{a}±0.43	0.007
Phosphorus (mg/dL)	1	03.64±0.11	04.00±0.20	03.50±0.20	0.239
	8	04.90^{b}±0.13	06.60^{a}±0.20	07.09^{a}±0.10	0.001
	16	06.17^{b}±0.32	07.04^{a}±0.10	06.90^{a}±0.14	0.026
Copper (ppm)	1	00.73±0.01	00.73±0.01	00.72±0.01	0.749
	8	00.79^{b}±0.01	01.31^{a}±0.03	01.31^{a}±0.01	0.006
	16	00.84^{b}±0.03	01.16^{a}±0.11	01.12^{a}±0.07	0.031

Mean values bearing different superscript (a, b) within a row differ significantly (p≤0.05)

supplemented group 3 when compared to group 1, which concurred with the findings of earlier workers on calcium (Ray *et al.,* 2016; Anam *et al*., 2021), phosphorus (Agrawalla *et al*., 2017) and copper (Niaz *et al*., 2017) status with mineral supplementation in cattle and goats. Specifically, by the 8th week of age, the mineral-supplemented group 3 displayed a substantial augmentation in Ca, P, and Cu levels in comparison to group 1, underscoring the vital role of mineral mixture supplementation in heightening mineral absorption and metabolism during this critical growth phase. Furthermore, the enduring impact of mineral supplementation was evident in the 16th week of age, where group 3 and group 2 exhibited a statistically significant ($p<0.05$) increase in Ca (Mohapatra *et al.,* 2012; Butani *et al.,* 2016), P (Singh *et al*., 2010), and Cu (Sahoo *et al.,* 2017) concentrations in comparison to group 1. These findings underscore the prolonged efficacy of mineral mixture supplementation in fostering mineral assimilation and utilization in crossbred calves as they progressed through subsequent developmental stages. Overall, our study highlights the significance of mineral mixture supplementation in enhancing mineral concentrations in crossbred calves during crucial growth stages, providing valuable insights for livestock management and nutrition practices.

Conclusion

Our research demonstrates the positive effects of providing specified mineral mixture to crossbred calves in comparison to commercial mineral mixture and non-mineral supplemented groups. Significant ($p<0.05$) improvement in biochemical parameters like total protein during 8th week, albumin during 16th week and glucose along with serum mineral concentration like of Ca, P and Cu during 8th and 16th weeks of age in specified mineral supplemented group indicates potential to improve health and immune function in calves. However, supplementing specified mineral had no significant ($p>0.05$) effects on haematology and biochemical parameters like globulin, A:G ratio, SGPT and total bilirubin of crossbred calves.

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