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Chelated Mineral Supplementation in Lactating Sahiwal Cows: Effects on Performance, Biochemical Attributes, Immune and Antioxidant Status

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ABSTRACT

The aim of this study was to determine the effect of chelated mineral mixtures supplementation on the performance, nutrients utilization, biochemical attributes, and immune response and antioxidant status in lactating cows. Eighteen lactating Sahiwal cows were randomly allocated into three groups (n=6) in a randomized block design. Cows fed on basal diet without chelated mineral mixture supplement was kept as control (MM_0), whereas; cows in other groups were supplemented with inorganic mineral mixture-1 (inorganic-MM_1) and 2 (Chelated-MM_2), respectively, with dose rate of 30 g/cow/day. Basal diet offered as total mixed ration (TMR) to experimental groups contained concentrate mixture, berseem fodder and wheat straw in the proportion of 40:30:30. Body weight (BW) and dry matter intake (DMI) was recorded fortnightly while blood parameters were measured at monthly intervals. Towards the end of the experimental period, a digestion trial of 7 days collection period was conducted. DMI, BW gain and apparent nutrient digestibility were similar among all the groups. Apparrent digestibility of crude protein (CP) was significantly ($P<0.05$) higher in the cows of both the mineral mixture supplemented groups. There was no effect of treatment on haematological and biochemical attributes. Plasma calcium (Ca) concentration and total antioxidant status (TAS) were significantly higher ($P<0.05$) while level of plasma phosphorous (P) and total immunoglobulin (TIg) were lower ($P<0.05$) in both mineral mixture supplemented cows. Treatment did not exert any effect on milk yield and milk composition. Findings of the present study revealed that the dietary supplementation of chelated mineral mixture improves utilization of Ca and protein without any adverse effect on other attributes.

Key words: Biochemical attributes, Lactating cows, Mineral mixtures, Performance, Digestibility

INTRODUCTION

Minerals are structural components of body and play significant role in activities of enzyme, hormone, as constituents of body fluids and tissues, and as regulators of cell replication and differentiation. Mineral deficiencies, imbalances and toxicity of certain mineral elements may cause reproductive disorders (Sharma *et al.,* 2007). Considering their roles in metabolism it is necessary to prioritize mineral nutrititon in order to optimize production in dairy cattle (Bhindari *et al.,* 2013). Animals obtain minerals through the consumption of natural feeds, fodders and by the supplementation of inorganic salts. Mineral deficiencies and imbalances have long been found responsible for low production among cattle and buffaloes fed on crop residues in tropical agro-climatic condition.

Minerals are supplied to the livestock through mineral mixture in the inorganic form. One of the major disadvantages of using such supplements is that the minerals from such sources are not fully absorbed due to antagonism and anti-nutritional factors present in the diet. In addition, higher levels of inorganic salt based mineral mixture resulted in increased excretion, which may cause environmental pollution. Therefore, in order to meet the increasing demand of bio-available elements and to reduce the contamination of surface water and soil, the concept of chelated mineral came up (Spears, 1989). In animal feed, the primary chelated minerals

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used are the trace elements such as iron, manganese, cobalt, copper, and zinc (Hilal *et al.,* 2016). These organic minerals resemble some of the biological molecules. Thus, they may be more useful in the body than their inorganic counterparts. Chelated minerals are administered mainly during the times of high nutritional demand, such as pregnancy, lactation, weaning, reproductive stress, rapid growth, environmental stress (such as temperature and humidity), or disease challenged conditions (Rabiee *et al.,* 2020).

Even though the basic information regarding the significance of chelated mineral supplementation has been shown in cows (Nayeri *et al.,* 2014), but confirmation of effect of chelated mineral supplementation on production performance of indigenous cows is still lacking. Considering these facts, the present study was designed to investigate the effect of chelated mineral supplementation on the performance of lactating Sahiwal cows.

MATERIALS AND METHODS

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

A total 18 lactating Sahiwal cows were selected from the cattle herd maintained at Livestock Farm Complex (LFC), DUVASU, Mathura, UP, India and randomly assigned into three dietary treatments on body weight (302.33±5.88 kg) and milk yield (6.34±0.46 kg/day) basis. Control group cows were fed on basal diet without any mineral mixture supplementation (MM_0), whereas; group inorganic-MM_1 was supplemented with inorganic mineral mixture-1 at 30 g/animal/day and group Chelated-MM_2 was supplemented with chelated mineral mixture-2 at30 g/animal/day for a period of 90 days. Mineral mixture 1 and 2 were supplemented in experiment were supplied by M/s Himalaya Drug Co. Pvt Ltd, Bangalore, Karnataka, India (composition not mentioned due to IPR issue). The nutrients requirement of experimental cows were met by feeding TMR that consisted of concentrate: berseem fodder: wheat straw in the proportion of 40:30:30 following NRC (2001) guidelines. Experimental cows were housed in a well-ventilated shed having the proper arrangement for individual feeding and watering without having access to the other animal's diet. TMR was prepared daily by hand mixing and was offered at 0900 h. The animals were provided with fresh and clean drinking water free of choice twice daily at 0800 h and 1700 h. Deworming of the experimental animals were done before the start of the experiment by oral administration of Fentas bolus (Intas Pharmaceuticals Pvt. Ltd., India) at the dose level of 10 mg/kg body weight.

A digestion trial with 6 days adaptation period followed by a 7 days collection period was conducted at the end of 90 days of study. Faeces voided during 24 h were recorded for 7 days, and daily representative samples were taken for determination of chemical composition, nitrogen, and mineral balance.

The experimental animals were monitored daily for DMI and fortnightly for body weight change. The representative samples of TMR offered, ort left and faeces voided were dried in a hot air oven at 60°C till a constant weight was attained and ground in a Wiley mill to pass a 1 mm sieve. Processed samples were pooled animal wise and stored at a dry place for the further analysis of dry matter (DM) (Method 973.18c), crude protein (CP) (Method 4.2.08), ether extract (EE) (Method 920.85), and total ash (Method 923.03) contents were analyzed as per methodology of AOAC (2005). The contents of neutral detergent fibre (NDFom) and acid detergent fibre (ADFom) were measured by the methodology of Van Soest *et al.* (1991). Ingredients and chemical composition of the basal diet fed during the experimental period is depicted in Table 1.

Peripheral blood samples were collected before feeding and watering of heifers at 0700 h in heparinised Vacuitainer tubes (BD Franklin, USA) by venipuncture of anterior vena cava at 0, 30, 60 and 90 d. Collected blood samples were analyzed for haematological and blood biochemical attributes. A fraction of whole blood samples were used for assay of haemoglobin (Hb) concentration, packed cell volume (PCV) or haematocrit (HIT) and blood pH. Remaining blood samples were centrifuged at 3000 rpm for 30 minutes to separate the plasma. Plasma samples were stored at -20°C until further analysis of biomarkers of liver and kidney function (aspartate amino transferase (AST), alanine amino transferase (ALT), alakaline phosphatase (ALP) and creatinine), protein metabolism (total protein, albumin, globulin, and plasma urea nitrogen (PUN), and Ca and P concentrations. Hb and HIT or PCV content in blood samples were analyzed as per Sahli's and Wintrobe tube method, respectively. The concentration of AST, ALT, PUN, total protein, albumin, cholesterol, creatinine, Ca and P were determined by an automated biochemical analyzer (BS-120 chemistry analyzer, Mindray, China) using commercial kits (Span Diagnostic Ltd., Surat, India). 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).Data of nutrient intake, production and digestibility trial were tested using the analysis of variance by fitting the following model:

$$Yijk = \mu + Ti + + eijk$$

Where *Yijk* = Response variable; μ = Overall mean

Table 1. Ingredients and chemical composition of TMR fed during the experimental period

Attribute	Group		
	MM_0	Inorganic-MM_1	Chelated-MM_2
Ingredient composition (% DM)			
Berseem fodder	30	30	30
Wheat straw	30	30	30
Concentrate	40	40	40
Ingredients (%DM)			
Barley grain	35	35	35
Wheat bran	20	20	20
Gram chuni	10	10	10
Mustard cake	35	35	35
Mineral mixture	0	30 gm	30 gm
Chemical composition (%)			
DM	68.03	68.03	68.03
	on %DM basis		
OM	90.07	90.07	90.07
EE	2.95	2.95	2.95
CP	14.65	14.65	14.65
Ash	9.72	9.72	9.72
CF	24.92	24.92	24.92
NFE	47.55	47.55	47.55
NDF_{OM}	49.36	49.36	49.36
ADF_{OM}	28.64	28.64	28.64
Ca	1.23	1.23	1.23
P	0.61	0.61	0.61
Mineral mixture*	-	Mineral mixture- 1 (30gm)	Mineral mixture- 2 (30gm)

*Composition not disclosed due to IPR issue; MM_0: control group fed basal diet without mineral mixture; Inorganic-MM_1: 30 g inorganic mineral mixture 1; Chelated-MM_2: 30 g chelated mineral mixture 2.

common to all observations; Ti = Effect of the I th treatment diet; (mineral mixture 1 or 2); åij = Residual error. Rest of the data of *in vivo* experiment was analyzed using the MIXED model with repeated measures of SPSS (IBM SPSS Statistics V 20.0 USA) as per following model:

$$Yijk = \mu + Ti + Dj + (T \times D)ij + eijk$$

Where *Yijk* = Response variable; ì = Overall mean of the population; *Ti* = Mean effect of the *i th* treatments (mineral mixture 1 or 2); *Dj* = Mean effect of day of sampling; *(T×D)ij* = Effect of the interaction between treatment and day of sampling; *eijk* = Residual error assumed to be independent and normally distributed. The model included dietary treatments as fixed effect, whereas period and animals were random effects. The animals were the experimental unit. Results were reported as means with SEM. Differences between the treatments mean were considered significant at P<0.05. Homogenous subsets were separated by Tukey's test.

RESULTS AND DISCUSSION

Supplementation of chelated mineral mixture did not affect daily DMI (kg/day or % BW) and body weight during the feeding trail (Table 2). This could be linked to no effect of organic mineral supplementation on the palatability and feed intake (Garg *et al.,* 2008). However, there are a few reports that indicate decreased feed intake in organic mineral supplemented chicks (Rao *et al.,* 2013) and rams (Gowda *et al.,* 2014). Digestibility of most of the studied nutrients except CP was not impacted by supplementation of mineral mixture. nutrient previuos study indicated improved digestibility and DMI r in -lambs (Mallaki *et al.,* 2015) and crossbred cows (Sahoo *et al.,* 2017; Chaudhary *et al.,* 2017) fed with the diet supplemented with mineral mixture. Digestibility of protein is improved because trace elements like Cu, cobalt (Co), manganese (Mn) and Zn facilitate the growth of cellulolytic rumen microbes resulting in better fermentation and utilization of organic matter especially cellulose, hemicellulose and crude protein.

Blood Hb concentration and PCV of lactating Sahiwal cows supplemented with mineral mixtures was significantly improved (P<0.05). However, there is no significant change (P>0.05) observed in between the mineral supplemented groups. In the present study, blood pH varied within normal range and was similar among all groups. These results are in accordance to results

Table 2. Effect of mineral mixtures supplementation on growth performance, nutrient intake and -digestibility

Attributes	Groups			SEM	Significance
	MM_0	Inorganic-MM_1	Chelated-MM_2		
Initial BW (kg)	304.00	302.83	300.17	5.881	0.96
Final BW (kg)	306.83	309.17	308.50	7.023	0.99
Weight gain(kg)	2.83	6.33	8.33	2.727	0.73
DMI (kg/day)	8.81	8.95	9.08	0.115	0.63
DMI (% of BW)	2.83	2.93	3.00	0.026	0.24
Apparent nutrient digestibility (%)					
DM	61.60	62.88	63.62	0.665	0.45
OM	65.89	67.56	68.47	0.533	0.13
CP	68.73^{b}	71.34^{ab}	72.61^{a}	0.499	0.01
EE	77.19	78.13	78.93	0.452	0.29
NFE	73.33	74.76	75.00	0.494	0.33
NDF	56.53	59.28	59.63	0.800	0.22
ADF	55.51	56.55	57.01	0.858	0.76

P>0.05: non-significant.

Table 3. Effect of mineral mixtures supplementation on blood biochemical parameters in lactating Sahiwal cows

Attributes	Groups			SEM	Significance		
	MM_0	Inorganic-MM_1	Chelated-MM_2		Treatment (T)	Period (P)	T×P
Hemoglobin (g/dl)	8.56	8.55	8.87	0.099	0.190	0.241	0.042
PCV (%)	28.54[b]	28.77[b]	30.19[a]	0.361	0.040	0.680	0.129
Blood pH	7.39	7.41	7.40	0.010	0.641	0.371	0.570
Glucose (mg/dl)	49.38	52.50	50.41	1.150	0.713	0.998	0.998
NEFA (îmol/l)	104.85	104.54	105.35	1.423	0.884	0.991	0.986
Total protein (g/dl)	6.82	6.74	6.85	0.059	0.824	0.525	0.891
Albumin (g/dl)	3.30	3.25	3.17	0.034	0.110	0.645	0.753
Globulin (g/dl)	3.52	3.50	3.68	0.071	0.367	0.723	0.854
PUN (mg/dl)	23.48	22.16	22.56	0.311	0.230	0.975	0.770
ALT (IU/L)	22.40	22.76	22.91	0.960	0.837	0.902	0.801
AST (IU/L)	74.16	72.69	74.46	1.769	0.948	0.834	0.826
Creatinine (mg/dl)	1.44	1.24	1.34	0.044	0.380	0.778	0.896

Means within a row with different superscript letters differ significantly (P< 0.05). T represents the main effect of treatment; P represents the main effect of days of feeding; T×P represents the interaction between main effects of treatment and days.

of Mondal *et al.* (2009) who also observed that supplementation of organic trace minerals at different dose levels linearly increased Hb level. Plasma glucose and non-esterified fatty acid (NEFA) concentration in control and mineral mixture supplemented groups were similar. Similar finding were reported on inorganic and organic (chelated) trace minerals supplementation in dairy cows (Choudhary *et al.,* 2017). Plasma concentration of glucose and NEFA are used as biomarkers of energy and lipid metabolism. NEFA is the product of tissue fatty acid catabolism, and systemic concentrations increase in proportion to the degree of fat mobilization.

Plasma total protein, albumin and globulin concentration were not significantly different in mineral mixtures supplemented groups (Table 3). Plasma urea concentration is a marker of protein intake and digestion, degradation of protein sources, and energy availability in the rumen (Roseler *et al.,* 1993). In the present study, the plasma urea nitrogen varied within normal range and was similar in control and mineral mixtures supplemented groups. Similar finding with

Table 4. Effect of mineral mixtures supplementation on minerals, antioxidant status and immune response parameters in lactating Sahiwal cows

Attributes	Groups			SEM	Significance		
	MM_0	Inorganic-MM_1	Chelated-MM_2		Treatment (T)	Period (P)	T×P
Calcium (mg/dl)	8.76[b]	8.84[ab]	9.24[a]	0.079	0.006	0.759	0.172
Phosphorus (mg/dl)	5.49	5.44	5.62	0.111	0.995	0.614	0.912
FRAP (îmol/L)	1037[c]	10963[b]	1139[a]	22	0.046	0.956	0.829
Total Immunoglobulin (mg/ml)	29.03	29.54	28.95	0.305	0.911	0.995	0.936

Means within a row with different superscript letters differ significantly (P<0.05).

respect to plasma total protein, plasma albumin and globulin and urea were also observed by Satapathy *et al.* (2016) on area specific mineral mixture supplementation.

ALT and AST are used as biomarker of liver function test whereas; plasma creatinine is used as biomarker of kidney function test. In present study, plasma ALT, AST and creatinine concentration of all control and treatment groups were found similar and all value were in normal physiological range indicating that there was no adverse effect of mineral mixtures supplementation on the above parameter. The present findings are in agreement with Satapathy *et al.* (2016).

Plasma Ca concentration was significantly improved ($P<0.05$) in mineral mixtures supplemented groups as compared to control group (Table 4). It was found that plasma Ca concentration was highest ($P<0.05$) in Chelated-MM_2 group as compared to other groups. Plasma P concentration was not significantly different in mineral mixtures supplemented groups. It was also observed that, there was an increasing trend of above minerals concentration as supplementation period progressed. Ashmead and Samford (2004) recorded that amino acid chelates were more bioavailable than inorganic minerals. Most of the positive effects of organic minerals on the performance of the animals appear mainly because of their better bioavailability as compared to their inorganic sources, whereas, inorganic forms of trace minerals make the intestinal pH more alkaline leading to precipitation due to formation of inorganic chelates in the gut (Du *et al.,* 1996) which might have lowered its availability further in the animal system. FRAP assay was used as biomarkers of TAS whereas, plasma TIg level was used as biomarker of immune response. In present finding there was higher FRAP activity in mineral supplemented groups. Higher antioxidant enzyme activities has also been reported on supplementing Zn proteinate to lambs (Nagalakshmi *et al.,* 2009), and organic mineral combination (Fe, Cu, Zn and Mn) (Rao *et al.,* 2013) than the inorganic mineral supplemented groups. There was no affect of mineral supplementation on immunoglobulin concentration in present study. However, supplementation of Cu, Mn, and Zn as chelated sources enhanced immune response of early lactation dairy cows compared with cows supplemented with inorganic sources (Nemec *et al.,* 2012).

Production performance of lactating cows was judged on the basis of quantity as well as quality of milk produced. In the present study, though the group fed with mineral mixtures showed more milk yield, but remained statistically non-significant ($P>0.05$) as compared to the control group (Table 5). The present findings are in agreement with the earlier reports on lactating animals (Hatfield *et al.,* 2001) that reported no increase in milk yield from feeding organic trace mineral complexes. However, contrary to the present findings, some workers have reported consistent and significant improvement of lactation performance in dairy animals following chelated mineral supplementation (Hackbart *et al.,* 2010). Milk fat, protein, solid not fat, lactose and total solids were not significantly different

Table 5. Effect of mineral mixtures supplementation on milk yield and milk composition

Attributes	Group			SEM	Significance		
	MM_0	Inorganic-MM_1	Chelated-MM_2		Treatment (T)	Period (P)	T×P
Milk yield (kg/d)	6.14	6.57	6.71	0.174	0.207	1.000	0.999
Milk fat (%)	4.45	4.55	4.65	0.071	0.233	0.7269	0.687
Milk protein (%)	3.51	3.55	3.51	0.020	0.967	0.937	0.961
Milk SNF (%)	8.99	9.02	9.07	0.034	0.389	0.919	0.926
Milk lactose (%)	5.31	5.25	5.30	0.024	0.930	0.729	0.758
Milk TS (%)	13.44	13.58	13.72	0.064	0.065	0.630	0.557

among the groups. The present finding also corroborated with the earlier findings (*Nemec et al.,* 2012; Sahoo *et al.,* 2017), which reported no significant improvement in milk yield or composition in lactating ruminants.

CONCLUSIONS

It was concluded that dietary supplementation of chelated mineral mixture improved protein digestibility, Ca availability and antioxidant status in Sahiwal cows. There was no adverse effect on nutrient utilization, weight gain and hemato-biochemical parameters.

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