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Supplementation of Critical Minerals
Devasena et al.

A Field Perspective on Supplementation of Specific Critical Minerals in Crossbred Cattle with Reproductive Disorders

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ABSTRACT

A field experiment was conducted in two adopted villages of Chittoor district of Andhra Pradesh to study the effect of supplementation of deficient minerals in crossbred cattle with reproductive inefficiency. Crossbred cattle (40) with different reproductive problems were selected from two adopted villages. The Area Specific Mineral Mixture (ASMM) prepared by the department of Animal Nutrition, SVVU, Tirupati, having specific deficient minerals (Ca, P, Cu and Zn) was supplemented (40g) to selected animals for 90 days. Blood samples were collected from animals before and after supplementation of ASMM and analysed for different minerals (Ca, P, Cu and Zn) and hormones (estrogen and progesterone). Plasma Ca content of repeat breeding cows (8.03 Vs 9.45 mg %) and anoestrous heifers (7.68 Vs 8.86 mg %) indicated significant ($P < 0.05$) improvement. The plasma P (4.10 Vs 5.49 mg %) and Cu (0.52 Vs 0.88 ppm) content in anoestrous heifers significantly ($P < 0.05$) elevated. While Zn content (ppm) in plasma improved significantly ($P < 0.05$) in anoestrous cows, repeat breeder cows as well as anoestrous heifers. The plasma estrogen content of repeat breeding cows (12.68 Vs 16.42 pg/ml) and anoestrous heifers (13.38 Vs 17.19 pg/ml) as well as progesterone content of repeat breeding cows (1.68 Vs 2.31 ng/ml) and anoestrous heifers (0.88 Vs 1.73 ng/ml) elevated ($P < 0.05$) in crossbred animals. Among the animals under study, 61.5% of anoestrous cows, 62.5% of repeat breeder cows and 63.6% of anoestrous heifers responded to supplementation. It can be concluded that, supplementation of specific deficient minerals in the form of ASMM resulted in improved mineral status and hormonal profile of animals thereby improving the reproductive efficiency (62.5%) in crossbred cattle.

KEYWORDS: Area specific mineral mixture, Crossbred cattle, Hormones, Plasma minerals.

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INTRODUCTION

Mineral imbalances are often observed in large number of Indian livestock because, they are mainly maintained on crop residues based rations or grazing without access to mineral supplement (Mc Dowell et al., 1993; Sahooo et al., 2017). Complication of mineral deficiency and metabolic diseases in all categories of dairy livestock have been reported by many scientists due to lower content and low bioavailability of some essential mineral in different feedstuffs. More than 90 percent of mineral deficiencies exist at subclinical level in livestock (Underwood and Suttle, 1999). Normal production and reproductive behavior of animals is associated with hormonal and nutritional status of the animal. Dietary mineral deficiency results in failure of the mineral homeostasis mechanism affecting the productive and reproductive potential of the animal. Some minerals act directly on the gonads, while others act through hypophyseal-pituitary-gonadal axis (Prasad et al., 2007). Deficiency or imbalance of single or multiple minerals results in enzymatic dysfunction and hormonal imbalance associated with fertility of animals (Maurice, 2003), resulting in poor productive performance (Sahooo et al., 2017). In field condition repeat breeding is a major problem in dairy animals (Rohit et al., 2017), results in economic loss (Kavani et al., 2005). Garg and Bhandari. (2005) reported low animal productivity and impaired reproductive behavior due to mineral deficiency and can be combated by supplementation of specific deficient minerals in the ration (Pal et al., 2020).

Present experiment was conducted to study the effect of supplementation of specific deficient minerals in the form of area specific mineral mixture (ASMM) in crossbred cattle showing reproductive disorders under field conditions in Chittoor district of Andhra Pradesh State.

MATERIALS AND METHODS

A field study was conducted in Chittoor district of Andhra Pradesh State, which is situated between 12-37" to 14-8" of Northern latitude and 78-33" to 79-55" of Eastern longitude. Maximum temperature ranges from 36º to 38ºC and in eastern parts it touches 46ºC. Minimum temperature in western parts varies from 12º to 14ºC and in eastern parts it is 16º to 18ºC with average rainfall of 918.1mm. Two villages (viz. village-1 –Pudipatla and village-2 -N. V. Palle) with similar animal husbandry practices were selected from two divisions in Chittoor district for conducting the study (during December to March). A preliminary study was conducted in these villages regarding feeding regimen, mineral status of the feedstuffs and serum mineral profile of animals in order to identify the prevailing mineral deficiencies. Then mineral mixture was prepared with specific minerals and supplemented to the selected crossbred cattle with deficiencies.

Feeding regimen

The crossbred cattle were maintained under semi-intensive feeding system with limited grazing resources, supplemented with paddy straw (*Oryza sativa*), groundnut straw (*Arachis hypogaea*) and sugarcane (*Saccharum officinarum*) tops either alone or in combination. Rice bran (*Oryza sativa*) and groundnut (*Arachis hypogaea* L) cake were common concentrate feeds offered. However, supplementation of concentrates and mineral mixture could be hardly found in case of unproductive animals.

Feeds and Fodders

Samples of green fodder, dry roughages, and individual concentrate ingredients actually fed to the cross bred cattle were collected from all the respondents in the study area. The representative samples of feeds/fodder collected from different farmers, were dried at 80^0C for 24 hours in forced draft oven and subsequently ground to 1mm sieve and were stored in moisture free plastic bags for further analysis.

The feed and fodder samples (0.5g) were digested in microwave sample digester (CEM Mars X-press) using 15 ml nitric acid. Digested samples were diluted with double glass distilled water and filtered through Whatman filter paper no. 1. Macro minerals, Calcium, Sodium, Potassium and magnesium as well as micro minerals Copper, Zinc, Manganese, and Iron were estimated using atomic absorption spectrophotometer (Perkin Elmer, Avanta-PM-A-6287). In case of Ca and Mg, the samples were diluted with 0.1% lanthanum chloride before estimation. Phosphorus and proximate principles were analyzed by AOAC (1995) procedures.

Animals

Twenty crossbred cattle (JBX and HFX) with different reproductive disorders, based on the history (with parity of 3-5, milk production of 6-8 L/day in previous lactation and without any incidence of metabolic disorders) and rectal palpation were selected from each village. The crossbred cows that were not showing any signs of estrous since last six months after calving, did not exhibit estrus, had no palpable corpus luteum or follicle of 10 mm diameter on ovarian surface and < 1 ng progesterone level in plasma were termed as anoestrous cows. Cows that were not conceived after three inseminations were considered as repeat breeders. Heifers which did not come to estrus even once after attaining the age of two years or more/did not obtain the specified body weight at >2 years of age were considered as anestrous heifers. The animals having clear watery secretion, with no anatomical defect in reproductive organs, confirmed through normal calving history and per-rectal examination. These crossbred cattle were having deficiency of one or more minerals as exhibited from the level of minerals in their plasma of these animals analysed prior to the start of study.

Feeding regimen

Farmers in this region were having sparse grazing resources hence semi-intensive feeding system is being adopted. The animals after grazing were supplemented with paddy straw, groundnut straw and sugarcane tops either one or in combination. Rice bran and groundnut cake were the common concentrate feeds offered. However supplementation of concentrates could be hardly found in case of unproductive animals. There was no practice of mineral supplementation.

Mineral mixture with specific minerals

The specific mineral mixture containing di-calcium phosphate 22g, calcium carbonate 6.84g, zinc sulphate 0.72g, copper sulphate 0.44g and common salt 10.g, was prepared based on the deficiencies identified in the feed samples and plasma of animals. The mineral mixture was supplemented at the rate of 40g / animal / day along with concentrates (rice bran and GNC mixture), for a period of 90 days. Selection of supplemental minerals was based on incidence of deficiency prevailing in the animals under investigation (Devasena, 2008).

Collection of blood samples

Blood samples from each animal were collected, before supplementation and at 15 day interval after supplementation of ASMM during 90 days. Blood samples collected by puncturing the jugular vein in heparinized vials aseptically, centrifuged at 3000 rpm for 20 minutes and the plasma was stored at -20^{0}C for subsequent analysis.

Mineral analysis

Plasma samples (2 ml) were digested in microwave sample digester (CEM Mars X press) using 15 ml nitric acid. Digested samples were diluted with double glass distilled water and filtered through Whatman filter paper no-1. Ca, Cu, Zn, Mn, and Fe were estimated using atomic absorption spectrophotometer (Perkin Elmer, Avanta- PM-A-6287). Phosphorus was analysed as per the method of Fiske and Subba Rao (1925).

Hormonal analysis

Estradiol 17 - â and Progesterone content in plasma samples were estimated by using kits. Radio immune assay kits were procured from "Immuno Tech", France (antibody coated tubes along with tracer, calibrators and other required material were provided for estimation of the hormones). Plasma samples were processed as per the prescribed procedure specified in the kits. Processed samples were counted in a multi well Gamma Counter (Cobra II, Packard Gamma Counter, USA) at National Institute of Animal Nutrition and Physiology (NIANP), Bangalore.

Table 1. Macro and micro mineral profile of feedstuffs offered to animals

Feed Stuff	Macro minerals (%)					Micro minerals (mg/Kg)			
	Ca	P	Na	K	Mg	Cu	Zn	Mn	Fe
Critical Value[1]	<0.3	<0.25	<0.06	<0.8	<0.2	<8.0	<30.0	<40.0	<50.0
Paddy straw	0.25±0.06	0.20±0.02	0.09±0.00	0.96±0.02	0.43±0.05	7.1±0.60	25.2±2.10	150±8.6	414±6.3
Groundnut straw	0.89±0.02	0.31±0.01	0.09±0.00	0.89±0.05	0.94±0.03	5.2±0.40	16.0±0.50	68.0±0.80	537±5.3
Sugar Cane tops	0.24±0.02	0.09±0.03	0.09±0.00	1.07±0.04	0.44±0.02	2.2±0.30	19.2±2.6	47.8±2.4	118±5.1
Local grass	0.27±0.02	0.28±0.02	0.16±0.00	0.98±0.03	0.71±0.01	6.1±0.1	43.6±1.3	43.4±0.9	355±2.1
Groundnut Cake	0.30±0.03	0.71±0.01	0.12±0.00	0.83±0.03	0.55±0.02	14.8±0.40	44.9±6.70	51.4±5.8	236±8.5
Rice bran	0.24±0.04	1.23±0.06	0.10±0.00	1.25±0.06	0.56±0.02	4.22±0.30	24.1±8.3	75.8±6.9	263±38.9

[1] McDowell et al., (1993).

Reproductive performance

The crossbred cattle under experiment were examined throughout the study period (90 days of supplementation) as well as three months after insemination for changes in body condition and reproductive status. The observations on general body condition, exhibition of estrous symptoms and date of insemination and pregnancy confirmation by per rectal palpation were recorded individually.

Statistical analysis

The data were analysed by paired't' test, as per the standard statistical procedures (Snedecor and Cochran 1980).

RESULTS AND DISCUSSION

Preliminary study

A preliminary study was conducted in the elected villages regarding feeding regimen, mineral status of the feedstuffs and serum mineral profile of animals to identify the deficiencies. Then mineral mixture was prepared with specific minerals and supplemented to the selected crossbred cattle with deficiencies.

Feeds and Fodders

Macro minerals Ca and P concentration in feedstuffs fed in the area under study indicated deficiency (Table 1) except the groundnut cake for calcium and rice bran for phosphorus. Whereas Na, K and Mg concentrations in the feeds were found to be more than the critical levels as indicated. Among the micro minerals, Cu concentration was below the critical level (<8.0 ppm) in both roughages and concentrates. Whereas Zn was below the critical level (<30.0 ppm) in roughages, but found to be higher in concentrate feeds except ground nut cake. The Mn concentration was more than the critical level (<40.0 ppm) in all feeds except in rice bran. It was observed that Fe concentration was exceptionally high (236 to 537 ppm), in all feedstuffs compared to its critical level (<50.0ppm). The results are in accordance with the reports of Tiwary et al. (2007). Most of the feed ingredients available for feeding livestock are deficient in one or other mineral (Gupta et al., 2017).

A survey work in various states, conducted by NDDB indicated that Zn, Cu, S, Mn, and Co were deficient in the ration of dairy livestock (Bhanderi et al., 2006).

Table 2. Plasma mineral profile of crossbred cattle

	Macro Minerals (mg/100 ml)					Micro Minerals (ppm)			
	Ca	P	Na	K	Mg	Cu	Zn	Mn	Fe
Critical Value[1]	<8.0[1]	<4.5[1]	-	-	<1.0[1]	<0.65[1]	<0.8[1]	<0.02[1]	<0.1[1]
Mean	8.25±	4.68±	162±	23.6±	1.99±	0.59±	0.68±	0.12±	1.59±
	0.49	0.52	46	6.3	0.39	0.04	0.04	0.04	0.36
% animals showing deficiency	57	53	-	-	-	69	62	39	-

[1]McDowell, (1985)

The results presented in table 2 indicated that, crossbred cattle in the area under study were showing deficiency regarding Ca (57%), P (53%), Cu (69%) and Zn (62%). Whereas the K, Na, Mg, Mn and Fe profiles of serum were well above the critical levels.

Supplementation study

Based on the mineral concentration in feeds and fodders and serum profile of the crossbred cattle reared by the farmers in the selected area the deficiencies were identified. Accordingly mineral mixture was prepared, which was supplemented to the crossbred cattle with reproductive disorders selected for a period of 90 days. During this period the animals were continuously under close examination both physically and serologically and the data related was recorded.

General condition of animals

The improvement in general health and reproductive efficiency of animals under study during the supplementation period (90 days) was regularly monitored and recorded at every fortnight. Among the animals supplemented with area specific mineral

mixture, 45% showed improvement in feed intake within 30 days. Improvement in general health condition, glossy skin, improved body score were observed after 45days and appearance of estrous symptoms started after 45 to 60 days of supplementation in 55-60% of animals. Phosphorus is associated with energy metabolism and trace elements like Cu and Zn act as co-factors, activates enzymes and influence biochemical functions, resulting in improved appetite and digestibility, thereby improving the general body condition. The present results confirm the reports of Prasad et al. (2007) regarding improvement in general health condition of the cows after area specific mineral mixture supplementation.

Table 3. Plasma mineral profile of animals with reproductive disorders supplemented area specific mineral mixture

Condition of animal	Macro Minerals (%)				Micro Minerals (ppm)			
	Ca		P		Cu		Zn	
Critical value	<8.0		<4.5		<0.65		<0.8	
	Initial	Final	Initial	Final	Initial	Final	Initial	Final
Anoestrous cows	8.63	8.98	5.03	5.86	0.66	0.78	0.74^{b}	1.06^{a}
(n =13) *	±0.26	±0.42	±0.32	±0.20	±0.04	±0.18	±0.19	±0.21
Repeat breeding cows	8.03	9.45^{a}	4.88	5.23	0.73	0.83	0.78^{b}	0.91^{a}
(n =16)*	±0.36	±0.28	±0.23	±0.28	±0.11	±0.26	±0.11	±0.20
Anoestrous heifers	7.68^{b}	8.86^{a}	4.10^{b}	5.49^{a}	0.52^{b}	0.88^{a}	0.61^{b}	0.82^{a}
(n=11) *	±0.19	±0.26	±0.33	±0.36	±0.22	±0.31	±0.23	±0.19

*Means bearing different superscripts in a row differs significantly ($P < 0.05$)

Macro mineral status

Supplementation of mineral mixture improved ($P< 0.05$) plasma Ca concentration (mg of repeat breeding cows (8.03 Vs 9.45 mg %) and anoestrous heifers (7.68 Vs 8.86 mg %). Plasma P (mg %) showed significant ($P<0.05$) increase after supplementation in anoestrous heifers (4.10 Vs 5.49 mg %), but this improvement was non significant in anoestrous and repeat breeding cows (Table 3). Reduced fertility and reduced or delayed conceptions are the prime signs of phosphorus deficiency and this can be overcome with proper phosphorus supplementation whereas, moderate deficiency may lead to repeat breeding condition and poor conception rate (Rohit et al., 2017). Altered Ca and P ratio has a blocking action on the pituitary and consequently on gonadal function affecting reproductive efficiency (Maurice, 2003). Supplementation of ASMM increased plasma Ca and P levels, which are involved in maintaining normal metabolic process and normal reproductive physiology (Mc Dowell et al., 1993), in turn resulted in improvement of reproductive performance as evidenced in the present study. Similar trend was observed by Samanta et al. (2005), Devasena et al., 2010; Pal et al., 2020.

Micro mineral status

The plasma Cu was influenced by mineral supplementation as indicated by elevation ($P<0.05$) in Cu content of plasma (0.52 Vs 0.88 ppm) in the anoestrous heifers group under study. Similar to the present investigation, Devasena et al. (2010) and Samanta et al. (2005) also reported improvement in plasma trace mineral levels in anoestrous animals after their supplementation. Cu has a significant role in maintaining the optimum fertility as Cu behaves in a regular way to be used as an indicator for FSH, LH and estrogen activity (Desai et al., 1982). Copper deficiency might have effect on reproduction probably through an interaction between Cu and estrogen (Hidiroglou, 1979). Role of Cu in ovarian steroidogenesis through Cu - super oxide dismutase

enzyme activity was reported by Olson et al. (1999), as evidenced by improved reproductive performance in the present study.

While Zn content (ppm) in plasma improved significantly ($P< 0.05$) in all the three categories viz. anoestrous cows, repeat breeder cows as well as anoestrous heifers. Samanta et al. (2005) also reported improvement in plasma Zn levels in anoestrous animals after supplementation. A reduction of Zn level might interfere with prostaglandins receptor medicated phase and cause quaintly the luteolytic process which in turn causes some of the reproductive pathology (Carlson et al., 1982). Deficiency of Zn affects all phases of reproductive processes from estrous to parturition and lactation (Mc Dowell et al., 1993). Optimum serum level of Zn is essential to maintain the activity of FSH and LH (Apagar, 1985), prostaglandins bind Zn and facilitate its transport and enzymatic action (super oxide dismutase) involving reproductive functions was reported by Olsen et al. (1999) as indicated by the present results.

Table 4. Plasma hormonal profile of animals with different reproductive disorders supplemented with area specific mineral mixture

Condition of animal	Estradiol -17 β (pg/ml)		Progesterone (ng/ml)	
	Initial	Final	Initial	Final
Anoestrous cows(n = 13)	15.32±1.2	17.68±2.6	1.03±0.24	1.73±0.25
Repeat breeding cows (n =16)	12.68^{b}±2.1	16.42^{a}±2.8	1.68^{b}±0.18	2.31^{a}±0.20
Anoestrous heifers (n = 11) *	13.38^{b}±1.9	17.19^{a}±1.3	0.88^{b}±0.13	1.73^{a}±0.18

*Means bearing different superscripts in a row differs significantly ($P < 0.05$)

Hormones

Plasma hormonal profile of crossbred cattle with different reproductive disorders, supplemented specific deficient minerals has been presented in table 4. There was an increase ($P<0.05$) in plasma estradiol (pg/ml) content in repeat breeding cows (12.68 Vs 16.42 pg/ml) and anoestrous heifers (13.38 Vs 17.19 pg/ml). Whereas progesterone content (ng/ml) of progesterone content of repeat breeding cows (1.68 Vs 2.31 ng/ml) and anoestrous heifers (0.88 Vs 1.73 ng/ml) indicated significant ($P < 0.05$) elevation in plasma of the crossbred animals under study. Present observations were similar to the reported values of Prasad et al. (1989) in repeat breeding and anoestrous cows, observed during different stages of estrous cycle. While Sampath et al. (2006) reported slightly higher values of estradiol and progesterone in anoestrous cows, repeat breeding cows and anoestrous heifers, as compared to the present study. The trend observed in the present study indicated that, because of the improvement in estrous and conception rate, the profile of both the hormones increased after supplementation, as compared to prior to supplementation.

Table 5. Reproductive performance of Animals with different reproductive disorders supplemented area specific mineral mixture

Reproductive disorder	Total (n=40)	%
Anoestrous cows	8/13	61.5
Repeat breeder cows	10/16	62.5
Anoestrous heifers	7/11	63.6
Total responded	25/40	62.5
Not responded	15/40	37.5

n = number of animals

Reproductive performance

The anoestrous cows (61.5%), of repeat breeding cows (62.5%) and anoestrous heifers (63.6%) improved in terms of their reproductive performance and on an average 62.5% animals under study responded to supplementation of specific deficient minerals (Table 5). The results are in accordance with the reported results of Sahoo et al. (2017) who reported 64% conception rate with sign of first heat occurred earlier (16.3 days), post partum estrus time reduction (15.8 days), service period reduced (15.8 days) due to supplementation of mineral mixture in crossbred cattle. Mineral mixture supplementation resulted in improved reproductive efficiency in terms of shorter first post-partum estrus and higher conception rate (Gupta et al., 2017; Jadoun, et al., 2023 and Pal et al., 2020). Signs of phosphorus deficiency and this can be overcome with proper phosphorus supplementation whereas, moderate deficiency may lead to repeat breeding condition and poor conception rate. Feeding of mineral mixture could improve their reproductive cyclicity with mark display of estrus symptoms (Devasena et al., 2010 and Rohit et al., 2017).

CONCLUSIONS

It can be concluded that, supplementation of specific deficient minerals in the form of area specific mineral mixture can improve reproductive efficiency in crossbred cattle and there by economic condition of the farmers. Long term studies are required to work out the cost benefit ratio in terms of improved reproduction and subsequent improvement in milk production.

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