RESEARCH ARTICLE

Effect of Chelated Mineral Mixture on Blood Biochemistry, Hormone and Mineral Status in Repeat Breeder Buffaloes in Tribal Areas of Dahod District in Gujarat, India

PM Joshi[1], DC Patel[2]*, PD Patel[3]

Abstract

An on-farm trial for 90 days was conducted at four tribal villages to assess the effect of chelated mineral mixture (CMM) supplementation on blood biochemistry as well as hormonal and mineral status, nutrient intake and reproductive performance of the repeat breeder buffaloes (n = 24). The animals selected were randomly divided into two groups of 12 animals each. The group T1 was control group (farmer's feeding schedule), and T2 treatment group (T1 + CMM @ 50 g/animal/day). Blood collection was done at 0, 45 and 90 days of the experiment from both the groups for assessment of blood biochemical, hormonal and mineral status of the animals. Average DM, DCP and TDN intake in repeat breeder buffaloes calculated as per information collected from farmers were statistically similar among both the groups and were as per ICAR requirements of animals. Results revealed significant ($p < 0.05$) improvement in hemoglobin, serum glucose, total protein, total cholesterol, progesterone, and macro (Ca, P)–micro (Zn, Fe, Cu, Co, Mn) mineral status of chelated mineral supplemented group as compared to control animals. The number of days taken for a successful conception in repeat breeder buffaloes as well as the cost of feeding was also reduced upon supplementation of CMM in T2 as compared to T1 group. The control group recorded a 22.12% higher cost of rearing than the treatment group during the experimental period. The findings revealed the beneficial role of cheated minerals supplementation in improving health, nutritional and reproductive status of repeat breeding buffaloes.

Keywords: Buffalo, Mineral supplementation, Repeat breeder, Macro-micro minerals, Reproductive status, Cost-benefit ratio.

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Introduction

The dairy buffalo economy lies in the proper and optimal reproductive rhythm of each female (Dhaliwal, 2005). Minerals play a significant role in fertility by maintaining membrane integrity and are involved in hormone production and maintenance of a strong immune system. Minerals have a direct or indirect relationship with the productive and reproductive health of animals. Deficiencies and imbalance of minerals during the periparturient period are solely associated with disorders like retention of fetal membranes, dystocia, abortion, weak calf syndrome and vulval discharge (Gupta *et al.*, 2005). Adding the mineral mixture in the ration of crossbred dairy cows for a period of 60 days recovered the animal from hypoproteinemia, hypoalbuminemia, hypocholesterolemia more efficiently than that of non-supplemented animals (Oliveira *et al.*, 2014). Several research workers have reported the beneficial effects of supplementation of deficient minerals either in ionic/inorganic form through area specific and chelated mineral mixture on reproductive performance of anestrus and repeat breeder dairy animals. However, very few such studies have been conducted in tribal regions of Gujarat state (Chaudhary and Patel, 2019). Keeping this point in mind, the present study was undertaken to demonstrate the effect of feeding chelated mineral mixture to repeat breeder buffaloes in tribal areas of Dahod district of Gujarat, India.

[1-3]Department of Animal Nutrition Research, College of Veterinary Science & AH, Anand Agricultural University, Anand, Gujarat, India

Corresponding Author: DC Patel, Department of Animal Nutrition Research, College of Veterinary Science & AH, Anand Agricultural University, Anand, Gujarat, India, e-mail: dcp1111@aau.in

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Materials and Methods

An on-farm trial of 90 days duration was carried out in tribal areas of Dahod district (20°30′ to 23°30′ North latitude and 73°15′ to 74°30′ East longitude) in Gujarat state. On the basis of survey, 24 buffaloes with the history of repeat breeding (not conceiving following three or more quality inseminations) were selected from four tribal villages. The animals were randomly divided into two groups of 12 animals each. During the experimental period animals in control group (T1) were maintained as per the traditional feeding practices of the farmer (straw based diet with locally available concentrate) without any nutritional supplementation, whereas the animals of treatment group (T2) were fed with T1+ chelated mineral mixture (CMM) @ 50 g per day per animal formulated based on deficiency of minerals prevailed in animals of the region.

Selected buffaloes were fed home-made concentrate mixtures, including mixed *grain bharda*, maize *bharda*, gram *chuni,* and were offered green as well as dry fodder throughout the experimental period. Clean, fresh, and wholesome tap water was made available to all the experimental animals in the morning and afternoon. Observations regarding the amount and types of feeds and fodder offered to animals, daily feed intakes, etc., were recorded with a fair degree of precision on a questionnaire developed for the purpose. Composite samples of concentrates, green and dry fodder were collected for analysis of proximate and mineral constituents (AOAC, 2005). The intake of dry matter (DM) digestible crude protein (DCP) and total digestible nutrients (TDN) of experimental buffaloes calculated from the records of intake of feeds and fodder, using digestibility coefficients/nutritive values given by Ranjhan (1998) was compared with respective requirement for maintenance and milk production given by ICAR (2013). The macro and micro mineral supplied were compared with the NRC (2001) standard.

Blood samples were collected from the jugular vein of individual buffaloes of both the groups in 4 ml sterile vacuumized tubes containing sodium fluoride (for plasma) and another 9 mL tubes containing clot activator (for serum) at 0, 45 and 90 days of the experiment. Samples were transported to the laboratory in an ice box and plasma/serum was separated by centrifugation at 3000 rpm for 15 min and stored at –20°C until analysis. Hemoglobin was determined in fresh blood using Sahli's method. The serum samples were analyzed for total protein (TP), total cholesterol (TC), glucose, calcium (Ca) and phosphorus (P) using kits of coral clinical systems, Goa, India, on Biochemistry analyzer (Model 3000 Evolution). The serum micro-minerals like cobalt, zinc, manganese, copper, and iron were estimated on wet digested samples by atomic absorption spectrophotometer (Ranjhan, 1998). The observations on reproductive performance were also recorded. The cost-benefit ratio of feeding chelated mineral mixture was also calculated based on costs of inputs and days saved in making the animals pregnant. The data on nutrient intake and blood profile were analyzed statistically using ANOVA and 't' test (Snedecor and Cochran, 1994).

Results and discussion

DCP and TDN Intake

The calculated intake of TDM, DCP, and TDN in T1 and T2 groups of repeat breeder buffaloes (Table 1) did not differ statistically and compared well to ICAR (2013) feeding standards. The data indicated that experimental buffaloes of both groups received an adequate quantity of protein and energy to satisfy their requirement. TDN intake, in fact, was 25–33% more, while DCP intake was nearly 11% less particularly in the T2 group than the ICAR standard in buffaloes under study (Table 1).

Blood Profile

The average concentrations of hemoglobin, as well as biochemical and hormonal constituents studied at 0, 45th and 90th day of the experiment in repeat breeding buffaloes, are shown in Table 2. The mean hemoglobin level in the treatment group increased significantly (p <0.05) within 45 days of supplementation of the chelated mineral mixture with subsequent non-significant rise till 90 days of supplementation in T2 group, but no such change over periods was found in control T1 group of repeat breeder buffaloes. These findings corroborated with those of Behera *et al.* (2012) in crossbred heifers, and Mohapatra *et al.* (2012) and Satapathy *et al*. (2016) in crossbred cows.

The average serum glucose and total protein levels in repeat breeder buffaloes were significantly (p <0.05) increased due to mineral mixture supplementation in T2 group over T1 group (Table 2). The period effect was found to be significant (p <0.05) in the treatment group for both the traits. Similar findings were reported by Mohapatra *et al.* (2012), Satapathy *et al*. (2016) and Sahoo *et al*. (2017) in crossbred cows, and Kumar *et al*. (2012) and Butani *et al*. (2016) in repeat breeder buffaloes on account of mineral mixture supplementation. Behera *et al*. (2012) however, did not observe an elevation in blood glucose and serum protein levels of repeat breeder crossbred heifers on account of mineral mixture supplementation for 60 days.

Table 1: Daily nutrient intake in repeat breeder buffaloes

Particular	*Repeat breeder buffaloes*	
	T1 (Control)	*T2 (CMM)*
TDM intake (kg/d)	9.17 ± 0.33	8.69 ± 0.28
DCP intake (g/d)	299.67 ± 17.38	280.17 ± 16.37
Requirement (g/d)	317.75 ± 26.30	320.38 ± 27.28
Intake as % of requirement	96.06 ± 3.14	89.27 ± 2.93
TDN Intake (kg/d)	4.85 ± 0.18	4.59 ± 0.16
Requirement (kg/d)	3.68 ± 0.19	3.69 ± 0.20
Intake as % of requirement	133.01 ± 2.77	125.65 ± 2.79

Table 2: Mean (± SE) hemoglobin, serum biochemical and hormonal status of repeat breeder buffaloes during the period of experimental mineral supplementation

Attribute	*Group*	*Days of sampling*			*Overall*
		0	*45*	*90*	
Hemoglobin (g/dL)	T1	9.15 ± 0.29	9.49 ± 0.29	9.65 ± 0.39	$9.43^{a} \pm 0.32$
	T2	$10.04^{A} \pm 0.27$	$12.24^{B} \pm 0.24$	$12.76^{B} \pm 0.16$	$11.68^{b} \pm 0.22$
Glucose (mg/dL)	T1	51.91 ± 1.88	52.41 ± 1.43	53.08 ± 1.20	$52.47^{a} \pm 1.50$
	T2	$55.83^{A} \pm 1.38$	$61.08^{B} \pm 0.77$	$62.83^{B} \pm 0.73$	$59.91^{b} \pm 0.96$
Total protein (g/dL)	T1	6.45 ± 0.15	6.55 ± 0.09	6.36 ± 0.09	$6.45^{a} \pm 0.11$
	T2	$6.46^{A} \pm 0.13$	$7.05^{B} \pm 0.08$	$8.19^{C} \pm 0.07$	$7.23^{b} \pm 0.09$
Cholesterol (mg/dL)	T1	100.08 ± 6.58	101.33 ± 5.81	105.00 ± 4.19	$102.13^{a} \pm 5.53$
	T2	$102.91^{A} \pm 3.11$	$152.58^{B} \pm 1.70$	$147.58^{B} \pm 1.60$	$134.36^{b} \pm 2.14$
Progesterone (ng/mL)	T1	1.23 ± 0.38	1.29 ± 0.27	1.82 ± 0.49	$1.45^{a} \pm 0.38$
	T2	$1.38^{A} \pm 0.25$	$2.41^{A} \pm 0.42$	$4.79^{B} \pm 0.37$	$2.86^{b} \pm 0.34$
Estradiol (pg/mL)	T1	74.63 ± 9.36	94.88 ± 18.53	89.13 ± 14.67	86.21 ± 14.18
	T2	82.00 ± 9.52	71.25 ± 8.92	61.88 ± 8.33	71.71 ± 8.92

^{a,b}Means bearing different superscripts in column differ significantly (p <0.05)
^{A,B,C}Means bearing different superscripts in row differ significantly (p <0.05)

The average serum cholesterol concentration was also increased significantly (p <0.05) in the T2 group as compared to the T1 group (Table 2). Similar trend was found by Mohapatra *et al.* (2012), Behera *et al.* (2012) and Butani *et al.* (2016), while Satapathy *et al.* (2016) in crossbred cows and Khan *et al.* (2015) in Murrah buffaloes reported no significant change in serum cholesterol concentration in any groups after mineral supplementation.

In repeat breeder buffaloes, the average serum progesterone levels in both control and treatment groups increased gradually from day 0 to day 45 and 90 with significant (p <0.05) difference only in the treatment group. The pooled mean of the treatment group was significantly (p <0.05) higher than in the control group (2.86 ± 0.34 vs. 1.45 ± 0.38 ng/mL). Conversely, the serum level of estradiol-17β, however, decreased up to 90 days in the treatment group, while it increased non-significantly in control group, and the pooled values of treatment and control groups did not differ (Table 2). This could be due to the establishment of normal cyclicity and luteal phase of an increasing number of animals at the time of blood sampling. Contrary to present findings, Devasena *et al.* (2010) reported a significant increase in blood estrogen level due to mineral mixture supplementation in repeat breeder cows.

Macro-Micro Minerals

The mean serum levels of macro-micro minerals observed at 0, 45th and 90th day of experimental CMM supplementation in repeat breeding buffaloes are presented in Table 3. The period effect was found to be significant (p <0.05) in the treatment group for macro-minerals, *viz.,* calcium and inorganic phosphorus. The levels of phosphorus, in fact, continued increasing significantly after 45 days till 90 days of supplement, but no such consistent rising trend was noted in serum calcium level. The treatment group had significantly (p <0.05) higher pooled values than the control group for both the minerals. Similar findings were reported by Mohapatra *et al.* (2012), Satapathy *et al.* (2016), Butani *et al.* (2016) and Sahoo *et al.* (2017) in repeat breeder animals supplemented with the mineral mixture.

Further, in treatment group T2 the levels of serum trace minerals, *viz.,* copper, zinc, and manganese progressively (p <0.05) increased up to 90th day with pooled values significantly (p <0.05) higher as compared to control group T1. However, the period effect in the control group was non-significant, and the values remained more or less at a constant level until 90 days. Similar findings were also reported by Devasena *et al.* (2010) and Behera *et al.* (2012) in repeat breeder heifers, Mohapatra *et al.* (2012), Satapathy *et al.* (2016) and Gouda *et al.* (2017) in repeat breeder crossbred cows and Sahoo *et al.* (2017) in repeat breeder buffaloes due to mineral mixture supplementation. Garg *et al.* (2007) reported that crucial microminerals like Cu and Zn could be supplemented in the form of chelates for better bioavailability and improved productivity and reproduction efficiency.

The levels of serum cobalt and iron also progressively and significantly (p <0.05) increased up to the 90th day of chelated mineral mixture supplemented T2 group, but not in control T1 group, and the pooled values were significantly higher in T2 over T1 group (Table 3). These findings were more or less similar to values and trend of Mohapatra *et al.* (2012) in repeat breeding cows.

Reproduction Status and Cost-benefit Ratio

The repeat breeder buffaloes of the control group required 73 days and those of the treatment group 54 days for correcting the condition. Accordingly, the treatment group required 26.02% less number of days compared to the control group. Among repeat breeder buffaloes of control group 41.67% and of treatment group 83.34% animals conceived successfully on account of mineral

Table 3: Serum minerals and hormonal status of repeat breeder buffaloes during the experimental period

Attribute	*Group*	*Days of sampling*			*Overall*
		0	*45*	*90*	
Calcium (mg/dL)	T1	7.55 ± 0.10	7.71 ± 0.08	7.44 ± 0.12	$7.57^{a} \pm 0.10$
	T2	$7.15^{A} \pm 0.14$	$11.20^{B} \pm 0.13$	$10.96^{B} \pm 0.12$	$9.77^{b} \pm 0.13$
Phosphorus (mg/dL)	T1	4.02 ± 0.05	4.12 ± 0.10	4.16 ± 0.08	$4.10^{a} \pm 0.08$
	T2	$4.21^{A} \pm 0.07$	$6.16^{B} \pm 0.07$	$6.93^{C} \pm 0.04$	$5.77^{b} \pm 0.06$
Copper (ppm)	T1	0.59 ± 0.02	0.57 ± 0.02	0.58 ± 0.02	$0.58^{a} \pm 0.02$
	T2	$0.61^{A} \pm 0.02$	$0.98^{B} \pm 0.03$	$1.21^{C} \pm 0.06$	$0.93^{b} \pm 0.04$
Zinc (ppm)	T1	0.80 ± 0.02	0.82 ± 0.02	0.81 ± 0.01	$0.81^{a} \pm 0.02$
	T2	$0.81^{A} \pm 0.01$	$2.10^{B} \pm 0.04$	$2.91^{C} \pm 0.04$	$1.94^{b} \pm 0.03$
Manganese (ppm)	T1	0.25 ± 0.01	0.26 ± 0.02	0.25 ± 0.01	$0.25^{a} \pm 0.01$
	T2	$0.25^{A} \pm 0.01$	$0.30^{A} \pm 0.01$	$0.40^{B} \pm 0.01$	$0.32^{b} \pm 0.01$
Iron (ppm)	T1	0.91 ± 0.03	0.92 ± 0.03	0.87 ± 0.02	$0.90^{a} \pm 0.09$
	T2	$1.02^{A} \pm 0.07$	$1.82^{B} \pm 0.09$	$2.43^{C} \pm 0.05$	$1.76^{b} \pm 0.07$
Cobalt (ppm)	T1	0.40 ± 0.01	0.40 ± 0.01	0.42 ± 0.01	$0.41^{a} \pm 0.01$
	T2	$0.42^{A} \pm 0.01$	$0.65^{B} \pm 0.03$	$1.02^{C} \pm 0.05$	$0.70^{b} \pm 0.05$

^{a,b}Means bearing different superscripts in column differ significantly between groups T and T2 (p <0.05)
^{A,B,C}Means bearing different superscripts in row differ significantly between periods (p <0.05)

mixture supplementation for a period of 90 days. In the case of repeat breeder buffaloes of the control group, the feeding cost worked out was 6661 and in treatment group it was 5187. The control group thus recorded a 22.12% higher cost of rearing than the treatment group during the experimental period. Prasad *et al*. (1991) reported 67% of repeat breeder buffaloes to conceive when Cu, Mn, and Zn were supplemented in ionic form. The respective success rate was 73% and 62% in repeat breeders when the same minerals were supplied in chelate form @ of 100 and 50% of requirement. The success rate of conception on account of supplementation of the ionic mineral mixture has been reported in the repeat breeder buffaloes as 39% by Kumar *et al*. (2012) and in crossbred cows as 47% by Behera *et al*. (2012) and 35% by Mohapatra *et al*. (2012). In a recent study, Chaudhary and Patel (2019) also reported that the repeat breeder buffaloes required 74 and 52 days in ionic and chelated mineral mixture supplemented groups, respectively, to correct the disorder, i.e., ~43% less number of days in chelated group with conception rates of 40 and 70% in respective groups. They also recorded the cost of feeding to be higher by 42% in ionic than the chelated mineral group.

From the study, it could be concluded that supplementation of chelated mineral mixture @ 50 g/animal/day for 90 days improved hemoglobin as well as serum biochemical, mineral and endocrine status of the repeat breeder buffaloes, resulting into the better conception rate and cost-benefit ratio as compared to the unsupplemented control group.

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