RESEARCH ARTICLE

Augmenting Blood Profile and Reproduction in Buffaloes of Tribal Areas of Vadodara District (Gujarat) through Appropriate Mineral Mixture Supplementation

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Abstract

An on-farm trial for 90 days was conducted using 20 anestrus and 20 repeat breeder buffaloes to evaluate the effect of supplementing appropriate mineral mixtures (AMMs) on their nutrient intake, blood profile, estrus occurrence, and conception. Both anestrus and repeat breeder buffaloes were randomly allotted to 2 treatments, *i.e.*, T1 (Ionic mineral mixture) and T2 (T1 + 25% extra zinc in the chelate form). The average intake of DCP, TDN, and copper in anestrus and repeat breeder buffaloes calculated as per information collected from farmers were almost the same among treatments and as per requirements of animals. The average intake of calcium and phosphorus was higher by 65% and 45% than the requirements in anestrus and repeat breeder buffaloes. However, Zn intake was lower by about 35% of the requirement in all treatments. Blood glucose, serum total protein, and serum albumin levels increased marginally on supplementation of T1 and T2 mineral mixtures for 90 days, particularly in anestrus buffaloes. The serum triglycerides and total cholesterol increased, but non-significantly ($p > 0.05$), particularly in T1 groups of both anestrus and repeat breeder animals, whereas levels of BUN and creatinine increased significantly ($p < 0.05$) in both the groups of repeat breeders and T1 group of anestrus buffaloes. Activities of serum alkaline phosphatase decreased non-significantly, whereas those of alanine and aspartate aminotransferases remained mostly unaltered. In contrast, the serum Ca, P, Mg, Na, K, and Zn concentration increased significantly ($p<0.05$; $p<0.01$) following 90 days supplementation of T1 and T2 minerals in both anestrus and repeat breeders. The number of days taken for return to the heat of anestrus and repeat breeder buffaloes as well as the cost of feeding was reduced upon supplementation of T2 as compared to T1 mineral mixture. The study showed the beneficial role of mineral supplementation in improving the health and reproductive status of infertile buffaloes.

Keywords: Anestrus, Buffalo, Mineral supplementation, Repeat breeder, Return to heat.

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Introduction

The success of the dairy buffalo economy lies in the proper and optimal reproductive rhythm of each buffalo within the normal physiological range (Dhaliwal, 2005). Anestrus and repeat breeding are two major reproductive disorders in buffaloes. Deficiency of certain minerals in dairy animals under field conditions has been reported by several researchers. The deficiencies can be corrected by an appropriate mineral supplementation through specifically tailored, high quality, economically feasible mineral supplements that contain only required minerals for correction of disorders. Several authors have reported the beneficial effects of supplementation of deficient minerals either in ionic/inorganic form through area specific 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. Keeping this point in mind, the present study was undertaken to demonstrate the effect of feeding appropriate mineral mixtures to anestrus and repeat breeder buffaloes in tribal areas of Vadodara district of Gujarat state.

Materials and methods

Selection of Buffaloes

Forty buffaloes, with history of postpartum anestrus (n = 20) and repeat breeding (n = 20) were selected for the on-farm trial of 90 days duration at 2 tribal villages, *viz.*, Khandivav and Tejavav of Pavijetpur taluka of Vadodara district. The anestrus and repeat breeding buffaloes were supplemented with mineral mixtures, *i.e.*, T1 (Ionic mineral mixture) and T2 (T1 + 25% extra Zinc in the chelate form) by dividing them into two groups of 10 each. Two appropriate mineral mixtures (AMMs) were prepared and supplemented to each buffalo daily @ 30 g until the buffaloes responded by returning to estrus or maximum up to 90 days. The AMMs were formulated based upon the incidence of deficiency prevailing in the animals under investigation. Ionic mineral salts like dicalcium

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phosphate, magnesium oxide, sulfate salts of Na, Cu, Mn, Fe, Co and K iodide were used in the formulation of the ionic mineral mixture (T1). While the deficient micro mineral zinc (Zn) was added extra @ 25 % in the chelate form to the ionic mineral mixture (T2).

Feeding Practices and the Plane of Nutrition

Selected buffaloes were fed home-made concentrate mixtures, including mixed *grain bharda*. In addition to concentrates, buffaloes were offered green maize and green bajra fodder, straws of maize and paddy during the study period. The conventional practice of feeding concentrates in two equal portions at the time of milking in the morning and evening was followed. The roughage was offered separately twice or thrice in a day. Clean, fresh and wholesome tap water was made available to all the experimental animals in the morning and afternoon. Observations regarding the quantity and type of feeds and fodder offered daily were recorded with a fair degree of precision on a questionnaire developed for the purpose. The amount of digestible crude protein (DCP) and total digestible nutrients (TDN) available to dairy buffaloes were calculated, based on input data, using digestibility coefficients/ nutritive values given by Sen *et al.* (1978) and Anonymous (2005). The intake of DCP, TDN, and minerals by experimental buffaloes was compared with respective requirements for maintenance and milk production given by ICAR (1998) and NRC (2001) standards, respectively.

Laboratory Analysis

Blood samples were collected from the jugular vein of individual buffaloes in 4 ml sterile vaccumized tubes containing sodium fluoride (for plasma) and another 9 ml sterile vaccumized tubes containing clot activator (for serum) at 0 and 90 days of feeding AMMs. Plasma and serum separated were stored at –20°C until analyzed. Serum samples were analyzed for total protein (TP), albumin, globulin, blood urea nitrogen (BUN), glucose, creatinine, cholesterol, triglycerides, sodium (Na), potassium (K), calcium (Ca), magnesium (Mg), phosphorus (P), zinc (Zn), alkaline phosphatase (AKP), alanine aminotransferase (ALT) and aspartate aminotransferase (AST), using semiautomatic analyzer for clinical chemistry test (Model 3000 EVOLUION) of Coral Clinical Systems. The commercial diagnostic kits for analyses were procured from Crest Biosystems, Ltd., Goa, India. The observations on reproductive performance were also recorded. The data on nutrient intake and blood profile were analyzed using a randomized block design and 't' test (Snedecor and Cochran, 1994).

Results and discussion

DCP and TDN intake

The chemical composition of feeds and fodders were within the normal range. The calculated intake of DCP and TDN in T1 and T2 groups of anestrus and repeat breeder buffaloes (Table 1) did not differ statistically. The average

Table 1: Daily nutrient intake in anestrus and repeat breeder buffaloes (% of requirement) at the start of experiment

		Anestrus buffaloes		*Repeat breeder buffaloes*	
	Nutrient intake	*T1*	*T2*	*T1*	*T2*
DCP	Intake (g/d)	585.81 ± 8.07	582.35 ± 7.14	588.04 ± 15.59	590.32 ± 8.78
	Requirement (g/d)	584.26 ± 7.65	579.42 ± 7.07	584.23 ± 14.99	586.10 ± 8.88
	Intake as % of requirement NS	100.26 ± 0.12	100.50 ± 0.09	100.64 ± 0.13	100.73 ± 0.10
TDN	Intake (kg/d)	6.40 ± 0.03	6.37 ± 0.03	6.46 ± 0.06	6.46 ± 0.0.5
	Requirement (kg/d)	5.69 ± 0.06	5.65 ± 0.05	5.68 ± 0.11	5.70 ± 0.06
	Intake as % of requirement NS	112.66 ± 0.84	112.84 ± 0.76	113.92 ± 1.59	113.57 ± 1.07
Ca	Intake (g/d)	65.88 ± 1.56	67.18 ± 1.39	63.78 ±1.62	67.16 ± 1.91
	Requirement (g/d)	40.05 ± 0.82	40.22 ± 0.65	39.29 ± 1.26	41.32 ± 0.98
	Intake as % of requirement NS	165.35 ± 5.90	167.16 ± 3.18	162.90 ± 3.25	162.75 ± 3.63
P	Intake (g/d)	35.36 ± 0.45	36.46 ± 0.64	34.53 ± 1.42	36.89 ± 1.01
	Requirement (g/d)	24.65 ± 0.51	24.76 ± 0.40	24.19 ± 0.78	25.44 ± 0.61
	Intake as % of requirement NS	143.82 ± 2.61	147.28 ± 1.69	142.64 ± 2.83	145.16 ± 2.95
Cu	Intake (mg/d)	103.25 ± 1.13	105.86 ± 1.37	100.48 ± 2.92	106.54 ± 2.67
	Requirement (mg/d)	110.33±1.07	112.27 ± 0.86	110.33 ± 0.75	112.15±0.92
	Intake as % of requirement NS	93.63 ± 1.07	94.33 ± 1.32	91.01 ± 2.31	94.92 ± 1.84
Zn	Intake (mg/d)	577.79 ± 10.63	596.32 ± 12.25	557.28 ± 20.44	593.01 ± 16.55
	Requirement (mg/d)	882.64 ± 8.57	898.16 ± 6.85	882.64 ± 5.96	897.20 ± 7.35
	Intake as % of requirement NS	65.52 ± 1.38	66.40 ± 1.33	63.09 ± 2.12	66.04 ± 1.52

NS = Non-significant differences between treatments and between groups.

daily DCP intake was as per the need, whereas TDN intake was 12–14% higher than that of the requirement (ICAR, 1998) in all groups of buffaloes. The treatment groups did not differ from each other. The data indicated that experimental buffaloes of both the groups received an adequate quantity of protein and energy to satisfy their requirement.

Intake of Minerals

Daily Ca, and P intake was higher by 65% and 45%, respectively, in both the groups of anestrus and repeat breeder buffaloes (Table 1). Intake of Cu was lower by 6–8% in both the groups of animals. However, intake of Zn was lower by 34–35% of the requirements in both anestrus and repeat breeder buffaloes. Further, T2 mineral mixture improved Zn and Cu levels

Table 2: Blood serum profile of anestrus and repeat breeder buffaloes following 90 days of ionic (T1) and chelated (T2) forms of mineral mixture supplementation

		Anestrus buffaloes		*Repeat breeder buffaloes*	
Particular	*Day*	*T1*	*T2*	*T1*	*T2*
Glucose (mg/dL)	0	61.60 ± 1.90	61.20 ± 2.26	63.00 ± 1.36	63.00 ± 1.05
	90	64.10 ± 1.95	63.40 ± 1.86	61.80 ± 1.35	64.60 ± 2.21
Total protein (g/dL)	0	7.18 ± 0.13	7.27 ± 0.17	7.48 ± 0.16	7.39 ± 0.11
	90	7.27 ± 0.29	7.32 ± 0.20	7.34 ± 0.22	7.41 ± 0.19
Albumin (g/dL)	0	2.84 ± 0.27	2.73 ± 0.46	3.14±0.20	3.19 ± 0.13
	90	3.15 ± 0.33	3.08 ± 0.25	3.13 ± 0.38	2.95 ± 0.23
Globulin (g/dL)	0	4.34 ± 0.26	4.54 ± 0.36	4.34 ± 0.09	4.30 ± 0.13
	90	4.12 ± 0.22	4.14 ± 0.14	4.21 ± 0.23	4.46 ± 0.15
Triglycerides (mg/dL)	0	25.90 ± 2.36	25.60 ± 2.28	20.90 ± 2.28[a]	25.30 ± 3.38
	90	29.30 ± 2.49	27.90 ± 2.41	25.60 ± 3.03[b]	29.90 ± 4.12
Cholesterol (mg/dL)	0	80.40 ± 2.11[a]	82.40 ± 2.29	82.30±2.38	84.40 ± 1.83
	90	85.60 ± 2.57[b]	83.10 ± 3.47	86.50±4.76	85.20 ± 4.10
BUN (mg/dL)	0	23.70 ± 3.56	23.90 ± 1.99	19.4±2.02a	20.1 ± 2.08[a]
	90	24.20 ± 0.95	24.60 ± 1.15	22.6±0.79[b]	22.3 ± 0.99[b]
Creatinine (mg/dL)	0	1.80 ± 0.09[a]	1.74 ± 0.08	1.74 ± 0.07[a]	1.75 ± 0.09[a]
	90	1.98 ± 0.08[b]	1.77 ± 0.11	1.85 ± 0.05[b]	1.90 ± 0.09[b]
AKP activity (U/L)	0	240.50 ± 30.87	287.00 ± 48.18	227.70 ± 29.45	282.60 ± 33.63
	90	235.00 ± 28.49	244.50 ± 20.44	218.00 ± 17.03	230.30 ± 18.08
ALT activity (U/L)	0	66.50 ± 3.39	65.70 ± 2.42	71.40 ± 4.32	68.50 ± 5.96
	90	67.00 ± 3.86	66.10 ± 2.12	66.80 ± 4.93	64.40 ± 6.03
AST activity (U/L)	0	178.10 ± 11.62	176.60 ± 8.94	163.30 ± 6.00	166.90 ± 14.16
	90	177.70 ± 13.24	170.50 ± 15.66	158.20 ± 9.39	152.90 ± 9.90
Calcium (mg/dL)	0	8.44 ± 0.15[b]	8.47 ± 0.14[a]	9.77 ± 0.14[a]	9.66 ± 0.16[a]
	90	9.06 ± 0.21[b]	9.15 ± 0.19[b]	10.32 ± 0.11[b]	10.33 ± 0.15[b]
Phosphorus (mg/dL)	0	5.94 ± 0.14[a]	5.92 ± 0.24[a]	5.73 ± 0.13[a]	5.68 ± 0.10a
	90	6.42 ± 0.15[b]	6.39 ± 0.09[b]	6.60 ± 0.32[b]	6.76 ± 0.21c
Magnesium (mg/dL)	0	2.03 ± 0.12[a]	2.06 ± 0.12[a]	1.96 ± 0.13[a]	1.93 ± 0.08a
	90	2.53 ± 0.18[b]	2.51 ± 0.16[b]	2.50 ± 0.14[b]	2.61 ± 0.20[b]
Sodium (mmol/L)	0	121.50 ± 2.35[a]	122.00 ± 2.74[a]	124.30 ± 1.78[a]	123.40 ± 1.69[a]
	90	134.70 ± 2.57[b]	130.70 ± 2.35[b]	130.60 ± 2.15[b]	129.80 ± 2.71[b]
Potassium (mmol/L)	0	3.45 ± 0.17[a]	3.58 ± 0.18[a]	3.89 ± 0.14a	3.94 ± 0.20a
	90	3.92 ± 0.13[b]	3.98 ± 0.16[ab]	4.79 ± 0.33[b]	4.80 ± 0.27[b]
Zinc (ppm)	0	1.36 ± 0.26[a]	1.39 ± 0.33[a]	1.56 ± 0.15[a]	1.41 ± 0.21[a]
	90	2.77 ± 0.30[b]	2.95 ± 0.24[b]	2.53 ± 0.16[b]	2.59 ± 0.32[b]

Means bearing different superscripts ([a,b]) in a column for a particular parameter differ significantly ($p < 0.05$) between days.

non-significantly over T1 formulation, particularly in repeat breeder buffaloes. Results of the present study indicate that the experimental buffaloes had a severe deficiency of Zn, an essential micromineral for reproduction, which might have led to anestrus and repeat breeder conditions in these buffaloes. Supplementing deficient nutrients is thus expected to improve reproductive performance of such infertile animals. 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.

Glucose and Total Protein Profile

The average blood glucose levels on day 0 were almost same in T1 and T2 group of anestrus and repeat breeder buffaloes, and increased insignificantly by day 90 only in anestrus buffaloes. Behera *et al*. (2012) also did not find any difference in blood glucose level of anestrus crossbred heifers supplemented with the mineral mixture. However, Mohapatra *et al.* (2012) recorded significantly higher values of blood glucose on account of supplementation of the mineral mixture. Level of total protein in T1 and T2 groups of anestrus and repeat breeder buffaloes did not differ statistically from each other. Although higher total serum protein levels in anoestrous as well as in repeat breeder animals have been reported earlier with supplementation of area specific mineral mixture (Kumar *et al*., 2012; Mohapatra *et al.,* 2012).

Albumin and Globulin

On supplementation with the mineral mixture for 90 days, serum albumin increased by 10.91 and 12.82%, and globulin decreased by 5.07 and 8.81%, respectively, in T1 and T2 groups of anestrus buffaloes, but no such shift was seen in repeat breeder buffaloes. In fact, the trend in T2 treatment was inversed (Table 2). However, no significant difference was observed between the treatment groups of anestrus or repeat breeder buffaloes. Similar findings were reported by Mohapatra *et al.* (2012) in the ASMM supplemented a group of anestrus and repeat breeder cows than in un-supplemented one.

Triglycerides and Total Cholesterol

The serum triglycerides levels increased in both groups upon supplementation, but the increase was significant (p <0.05) only in repeat breeder buffaloes. Level of serum total cholesterol increased from 80.40 ± 2.11 to 85.60 ± 2.57 and 82.30 ± 2.38 to 86.50±4.76 mg/dL on supplementing T1 treatment for 90 days in anestrus and repeat breeder buffaloes, but differed significantly (p <0.05) only in anestrus animals, which was in agreement with the findings of Mohapatra *et al.* (2012).

BUN and Creatinine

BUN level increased marginally in anestrus animals, and significantly (p <0.05) in repeat breeder buffaloes by 16.49 and 10.94% on supplementing both T1 and T2 treatments, respectively. Behera *et al*. (2012) reported no effect of mineral supplementation on levels of BUN. Serum creatinine increased (p <0.01) by 10.1 and 8.57% in T1 groups of anestrus and repeat breeder buffaloes, respectively, but in T2 groups the increase was marginal.

Serum Enzyme activity

A non-significant decrease in serum AKP activity was found in T1 and T2 groups of anestrus and repeat breeding buffaloes on supplementation of AMMs with a greater difference in only T2 groups. Behera *et al.* (2012) observed significantly increased the activity of AKP following supplementation of Minfa (mineral mixture) in anestrus heifers. Serum ALT and AST activities after 90 days of ionic and chelated mineral supplement remain almost unchanged in anestrus buffaloes, while in repeat breeder buffaloes the activity of both the enzymes decreased non-significantly (Table 2). Behara *et al.* (2012) however, found a significant increase in activity of AST of anestrus heifers on supplementation of Minfa (mineral mixture).

Macro-micro Minerals

Serum levels of Ca, *P* and Mg (mg/dL) increased significantly (p <0.01) following 90 days of ionic and chelated minerals supplementation in both anestrus as well as repeat breeder buffaloes (Table 2). Similar findings were reported by Devasena *et al.* (2009) and Behera *et al*. (2012) in anestrus heifers and Mohapatra *et al.* (2012) in anestrus cows. Whereas, Lall *et al.* (2004) reported a non-significant effect of mineral mixture supplementation on Ca level in anestrus buffalo heifers. Further, serum Na and K levels in both treatment groups of anestrus and repeat breeder buffaloes also increased significantly (p <0.05). These results were in close agreement with the findings of Behera *et al*. (2012) in anestrus heifers. Serum Zn concentration increased highly significantly (p <0.01) following 90 days of ionic and chelated minerals supplementation in both anestrus and repeat breeder buffaloes (Table 2). Due to the presence of Zn in the chelate form in the mineral mixture, the overall mean value was higher than in the ionic mineral mixture group, particularly in anestrus animals. These observations supported earlier findings of Lall *et al.* (2004), Garg *et al.* (2008), Devasena *et al.* (2009) and Behera *et al.* (2012)in anestrus cattle, buffalo or heifers, and of Kumar *et al.* (2012) and Mohapatra *et al.* (2012) in lactating anestrus buffaloes and cows.

Reproduction Status

The numbers of days of mineral mixture supplementation required for correcting the condition of anestrus was 69 and 54 in T1 and T2 groups, respectively, indicating ~28% less number of days in T2 as compared to T1 group. The repeat breeder buffaloes required 74 and 52 days in T1 and T2 groups, respectively. Accordingly, the chelated group required ~43% less number of days compared to the ionic group. In the case of anestrus buffaloes, the success rate of treatment observed was 50% and 70% in T1 and T2 groups, respectively, on supplementation for 90 days. Corresponding values in repeat breeder buffaloes was 40% and 70% in T1 and T2 groups, respectively. Total feeding cost of anestrus buffaloes was Rs. 7042 in T1 and ₹5545 in T2 mineral mixture supplemented groups, which was higher by 27% in T1 supplemented group. In the case of repeat breeder buffaloes, the cost of feeding was Rs. 7701 and Rs. 5429 in T1 and T2 supplemented groups, respectively, which was higher by 42% in T1 supplemented group due to fewer days of mineral mixture supplementation required for correcting the condition of anestrus and repeat breeding. The success rate of conception in the anestrus buffaloes on account of supplementation of the ionic mineral mixture was variable in different studies. Accordingly, 90% (Lall *et al.,* 2004), 60% (Kumar *et al.,* 2011) and 46% (Kumar *et al*., 2012) of anestrus buffaloes were reported to successfully conceive. Prasad *et al*. (1991) reported 73 and 67% of anestrus and repeat breeder buffaloes to conceive when Cu, Mn, and Zn were supplemented in ionic form. The respective success rate was 87 and 63% in anestrus and 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 in the repeat breeder buffaloes on account of supplementation of the ionic mineral mixture has been reported as 39% by Kumar *et al*. (2012) and in crossbred cows as 47% (Behera *et al*., 2012) and 35% (Mohapatra *et al*., 2012).

Conclusion

The present findings re-established the fact that identification of specific mineral deficiencies and supplementation in proper form is essential for improving the nutritional status and reproduction efficiency of infertile buffaloes.

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