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Effect of area specific mineral mixture supplementation on productive and reproductive performance of Murrah buffaloes

M FAIZ[1], D ROY[1✉], A KUMAR[1], R K SINGH[1], N ALI[2], A FAHIM[1], A TRIPATHI[1], G CHANDRA[1], V THAKUR[1], S KAPIL[1] and Y SINGH[2]

Sardar Vallabhbhai Patel University of Agriculture & Technology, Modipuram, Meerut (UP) India-250 110

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ABSTRACT

Eighteen periparturient Murrah buffalo were allocated randomly into 3 groups having 6 animals in each group, on basis of body weight and history of previous lactation to find effect of area specific mineral mixture supplementation on productive and reproductive performance. Animals in T_1 (control) group were fed roughage based basal diet i.e., wheat straw, chaffed green berseem fodder and concentrate mixture at a ratio of 35:40:25. The concentrate mixture was prepared by mixing barley grain, wheat grain, oat grain, wheat bran, gram chunni and mustard oil cake. Treatment (T_2) animals were fed basal diet with supplementation of area specific mineral mixture (ASMM) @50g/d. Treatment (T_3) animals were fed basal diet supplemented with mineral mixture of BIS (Type-1) specification @50g/d. There was no significant difference in dry matter intake (DMI) and body condition score (BCS) among T_1, T_2, and T_3. The average milk yield (kg/d) for T_1, T_2 and T_3 were 6.14, 7.20 and 6.80, respectively. There was a significant increase in the average milk yield of T_2 as compared to T_1 as ASMM also fulfil mineral requirement which are important for efficient milk production. There was a significant increase in average protein % in T_2 as compared to T_1 and T_3. There was a significant decrease in the time to shed complete placenta after parturition in T_2, as compared to T_1. Rate of uterine involution (uterine cleansing as well as the status of the regressing caruncles) was faster in the buffaloes supplemented with ASMM and BIS mineral mixture as compared to the control group. On days 14th, 21st and 28th post-partum, significant ($P < 0.05$) difference in the average uterine horn diameter between the control and treatment groups was recorded. 16.67% buffaloes (1/6) in ASMM fed group showed large sized follicle on day 21st while none of the control and BIS group animals had large sized follicles till day 21st post-partum. On day 28th post- partum, 16.67% buffaloes (1/6) in BIS and 33.33% buffaloes (2/6) in ASMM groups were having large sized follicles on their ovaries while control group still did not show any large sized follicular activity. Early resumption/ rebound of ovarian cyclicity was observed in buffaloes of T_2 and T_3 group than control group. T_2 fed with ASMM had even better response than that of T_3. The digestibility of nutrients was comparable among the treatment groups. It can be concluded that dietary supplementation of area specific mineral mixture improved milk production and reproduction performance without any untoward effect on nutrient utilization in Murrah buffaloes.

Keywords: Area Specific Mineral Mixture, Buffaloes, Murrah, Milk production, Milk composition, Reproduction

India has major genetic resource of bovines with an estimated number of 303.76 million population (Livestock Census, 2024). India is renowned for the best buffalo germplasm in the world (Kumar *et al*. 2019). The total population of buffaloes is around 109.85 million which estimates around 20.45% of total livestock population in India (Livestock Census, 2024). It has a major contribution of the livestock sector in the economy of India, which is integrated with the Indian agriculture (Kumar *et al*. 2019). The buffaloes also contribute about 45.32% of total milk production in India (BAHS, 2024). Murrah an important dairy breed of the country. In India, livestock are mostly allowed free grazing on common land resources of the locality and farmers add only salt to their diets without any mineral mixture. The mineral content of the soil and forages differs according to the region that affects the mineral profile of the animal diets. Garg *et al*. (2008) highlighted that blanket supplementation normally fails to fulfil specific deficiencies. Since there is lack of many macro and micro-nutrients in these pastures it leads to poor productive and reproductive performance. Imbalances and deficiencies of minerals can lead to poor conception rates, repeat breeding, anestrus and decreased milk production (McDowell, 1992). Hence, based on the soil profile of the local area, forages and animal serum mineral profiles, formulation of the ASMM should be done, that avoid any particular mineral deficiencies in animals. Availability of the minerals to the livestock is also influenced by the different

Present address: [1]College of Veterinary and Animal Science [2] College of Agriculture, Sardar Vallabhbhai Patel University of Agriculture & Technology, Modipuram, Meerut (UP) India-250110. ✉Corresponding author email: deb_ashis2k4@rediffmail.com

production system, feeding practices, and environmental conditions. Studies by Mohapatra *et al*. (2012) and Singh *et al*. (2017) showed that the supplementation of ASMM notably improved the milk production, percentage of fat, and reproductive performance in buffaloes under field conditions. By proper supplementation of area specific mineral mixture normal production and reproduction of animals can be ensured (Bhagat, 2017). Based on these considerations, the present study was conducted to evaluate the effect of area-specific mineral mixture supplementation on productive and reproductive performance of Murrah buffaloes.

MATERIALS AND METHODS

Ethical approval: All experimental procedures and animal welfare protocols were evaluated and sanctioned by the Institutional Animal Ethics Committee of Sardar Vallabhbhai Patel University of Agriculture and Technology, Meerut (Approval No. IAEC/SVPUAT/2024/145).

Experimental animals and diets: Eighteen Periparturient Murrah buffaloes were selected from Livestock Research Centre, SVPUAT and were allocated randomly into 3 groups of 6 animals in each. The buffaloes were selected considering their live weight and history of previous lactation.

Feeding of experimental animals: The animals were fed as per Kearl (1982) feeding standards to meet the nutrient requirements. The diets comprised of concentrate mixture, wheat straw and green fodder. The buffaloes of Treatment (T_1) were fed basal diet without mineral mixture supplementation wherein buffaloes of Treatment (T2) were fed basal diet and supplemented with ASMM @50g/d/animal and buffaloes of Treatment (T3) were fed basal diet and mineral mixture of BIS (Type-1) specification @50g/d/animal. Samples of concentrate mixture, green fodder, wheat straw, feed residues and faecal matter were grounded and analyzed for dry matter (DM), organic matter (OM), crude protein (CP), ether extract (EE), crude fiber (CF) and total ash as per standard procedures of AOAC-2005. Fibre fraction of feeds, fodders and faecal samples were analysed by using detergent method of fibre estimation (Van Soest *et al*. 1991).

A five-day digestibility trial was conducted at the end of the experiment. Feed was offered to the animals after weighing, and refusals were collected and weighed. Faeces were collected and weighed, and representative samples were taken for analysis. Dry matter intake was recorded fortnightly by subtracting the residual dry matter from the quantity of dry matter offered. Body condition score (BCS) of experimental buffaloes were measured fortnightly from day 1. Daily milk yield of experimental buffaloes was recorded individually in morning and evening by using digital electronic weighing scale. Milk samples (20 ml) from individual animals were collected on weekly interval after thorough mixing and transferred to properly labeled sample bottle. Milk samples were immediately analyzed for fat, protein, lactose, SNF and pH using milk analyzer.

The delivery time of the calf was recorded and then the animals were monitored for time taken to shed the placenta completely (in minutes) i.e., completion of third stage of labor. Following proper restraining of the animal in the travis, the rectal and ultrasonographic examination was carried out at day 10, 14, 21, 28 and 32 to record days required for complete cleansing of the uterus and days post-partum (dpp) required for complete involution of the uterus (Minitube portable ultrasound, rectal probe, 6.5 MHz) i.e., recording of the uterine diameter with cleansing and complete regression of the caruncles. The post-partum ovarian activity was also observed (day 7th onwards post-partum together with uterine involution study) by recording the days required post-partum for the appearance of the large sized follicles greater than 6 mm size. The ovulation of the experimental animals was assessed regularly using ultrasonography (Minitube, 7.5 MHz, Linear probe) at weekly interval from day 14 to day 21.

Statistical analysis: Data obtained was subjected to analysis–following completely randomized design with the simple analysis of variance technique (Snedecor and Cochran, 1994) using SPSS-2011. Homogenous subsets were separated by using Duncans multiple range test described by Duncan (1955). Differences among treatments were significant when P is less than or equal to 0.05 ($P \leq 0.05$).

RESULTS AND DISCUSSION

The chemical composition of different dietary ingredients was found to be in normal ranges (Table 1).

Dry matter intake, digestibility and BCS: The range of DMI (kg/d) for T_1, T2 and T3 was 12.39 to 15.51, 13.29 to 16.20 and 12.83 to 15.99 (kg/d), respectively (Table 2). The average DMI (kg/d) for T_1, T2 and T3 was 14.08, 15.16 and 14.67, respectively. The mean DMI (% BW) for T_1, T2 and T3 was 2.78, 2.77 and 2.75 %, respectively. No significant difference in DMI was found among the three groups. Through the statistical analysis, it was revealed that there was no significant difference between the digestibility of DM, OM, CP, EE, CF, NFE, NDF and ADF among all the groups, indicating that the mineral mixture did not change overall nutrient digestibility in adult Murrah buffaloes during the research period. The average BCS of T_1, T2 and T3 was recorded 2.83, 3.25 and 3.84, respectively. Khadda et al. (2024) also aligns with our study and observed that nutrient digestibility was not reported as significantly changed by the combined bypass fat + area-specific mineral mixture in the on-farm trial. Gupta et al. (2024) also found no significant difference in nutrient digestibility between supplemented and non-supplemented groups. Sumedhan *et al.* (2024) also observed that there was no significant difference ($P>0.05$) in average intake of DM, OM, CP, NDF and ADF as compared to control animals (crossbred cows). Singh *et al*. (2017) had also reported increased dry matter intake, organic matter digestibility, crude protein digestibility, NDF and ADF digestibility in supplemented groups.

Table 1. Chemical composition (%DM basis) of feeds

Items	Wheat Straw	Berseem fodder	Jowar Fodder	Concentrate	Total Mixed Ration
DM	89.98±0.62	21.85±0.15	26.32± 0.18	90.98 ± 0.22	70.61±0.27
OM	86.50±0.50	88.80±0.60	89.70± 0.05	91.10 ± 0.20	89.16±0.03
Total ash	13.50±0.50	11.20±0.60	10.31± 0.05	8.90 ± 0.20	10.84±0.03
CP	3.53±0.38	19.97 ± 0.29	10.97± 0.28	21.05 ± 0.82	13.82±0.22
EE	0.51±0.06	3.56±0.41	3.52± 0.04	3.79 ± 0.10	2.73±0.13
CF	37.81 ±0.56	26.38±0.99	31.75±0.39	7.43 ± 0.16	23.03±0.02
NFE	44.66 ±0.38	40.90 ± 0.51	43.46±0.66	58.83 ± 1.08	49.58±0.14
NDF	84.83±1.83	63.34±0.95	54.81±0.56	37.50 ± 1.50	58.17±1.09
ADF	52.48±1.10	53.13±1.13	30.81±0.45	15.60± 0.40	34.58±0.27
Hemicellulose	32.35±2.93	10.21±0.19	24.00±0.11	21.90 ± 1.10	23.60±1.36
ADL	3.75±0.25	2.75±0.15	4.35±0.05	1.45 ± 0.05	2.77±0.13
Cellulose	48.73±0.85	50.38±0.98	26.46±0.50	14.15 ± 0.45	31.81±0.15

Table 2. Effect of supplementation of mineral mixture on dry matter intake, BCS and nutrient digestibility (%) in Murrah buffaloes

Attributes	Treatment			SEM	P value
	T_1	T_2	T_3		
Average body weight (kg)	506.72	547.93	534.41	27.72	0.58
Metabolic body weight ($W^{0.75}$)	106.58	113.16	111.01	4.38	0.57
DMI (kg/d)	14.08	15.16	14.67	0.76	0.61
DMI (%BW)	2.78	2.77	2.75	0.02	0.66
DMI (g/kg$W^{0.75}$)	131.50	133.75	132.04	1.84	0.67
BCS	2.83	3.25	2.84	0.28	0.49
Digestibility (%)					
DM	67.31	67.00	68.93	2.34	0.82
OM	69.35	68.77	70.71	2.26	0.83
CP	69.32	73.20	71.20	3.78	0.77
EE	81.15	81.46	85.96	2.95	0.46
CF	64.55	65.81	65.46	2.72	0.94
NFE	73.00	72.90	73.35	2.22	0.98
NDF	58.89	62.50	59.57	3.26	0.71
ADF	53.81	58.29	58.14	3.49	0.61

Milk yield and composition: The average milk yield (kg/d) for T_1, T2 and T3 are 6.14, 7.20 and 6.80 (kg/d/animal), respectively (Table 3). There was a significant increase in the average milk yield of T2 as compared to T_1 while T3 shows more milk yield than T1 and less milk yield than T2. The average milk fat for T_1, T2 and T3 were 7.09, 7.55 and 7.50 %, respectively. Statistical analysis reveals that there was no significant difference of fat % among the groups. Similarly, no significant differences were observed among T_1, T2, and T3 for SNF (%), lactose (%), total solids (%), and pH. However, there was a significant increase in the protein content (%) in T2 group as compared to T_1 and T3 with the average milk protein (%) for the group being were 3.58, 3.71 and 3.59%, respectively. Khadda *et al*. (2016) and Tanwer *et al*. (2019) had also observed similar results of increase in the milk yield of the buffaloes with supplementation of mineral mixture. Similarly, Bharti *et al*. (2025) reported the importance of supplementing mineral

Table 3. Effect of supplementation of mineral mixture on milk yield and milk composition in Murrah buffaloes

Parameter	Treatment			SEM	P Value
	T_1	T_2	T_3		
Milk Yield (kg/d)	6.14[a]	7.20[b]	6.80[ab]	0.27	0.05
6% FCM yield	6.97[a]	8.48[b]	8.03[ab]	0.39	0.04
Composition of milk (%)					
Fat	7.09	7.55	7.50	0.13	0.05
SNF	10.12	10.28	10.17	0.07	0.36
Lactose	4.79	4.83	4.82	0.03	0.60
Protein	3.58a	3.71b	3.59a	0.03	0.01
Total Solids	17.22	17.83	17.67	0.17	0.05
pH	6.50	6.54	6.52	0.02	0.42

Means bearing different superscript in a row differ significantly ($P < 0.05$)

mixture in cows and noted the increased milk yield. The results of Kantwa *et al*. (2021) also aligns with our study as they observed that the group of buffaloes supplemented with chelated minerals showed increased milk production as compared to control group. Ramteke *et al*. (2022) also observed similar results that there was no statistically significant variation of milk composition in the ASMM supplemented group and control group. Similar outcomes were observed by Singh (2019) after supplementation of ASMM in Murrah buffaloes significantly increased.

Third stage of labour and uterine involution: The average values from parturition to complete shedding of placenta for group T_1, T2 and T3 were 412, 359.67 and 376 minutes, respectively (Figure 1). There was a significant (P<0.05) decrease in the time to shed complete placenta after parturition in T2 as compared to T_1 whereas a slight decrease in T3 as compared to T_1. On day 10th, the diameter of uterine horns was similar in both control and treatment groups. However, thereafter, the rate of uterine involution (uterine cleansing as well as the status of the regressing caruncles) was faster in the buffaloes supplemented with ASMM and BIS Type-1 mineral mixture as compared to control group. On days 14, 21 and 28 post-partum, significant (P < 0.05) difference in the average uterine horn diameter was recorded between the control and treatment groups. Similarly, in a previous study, rate of uterine involution was found faster in group fed by-pass fat and area

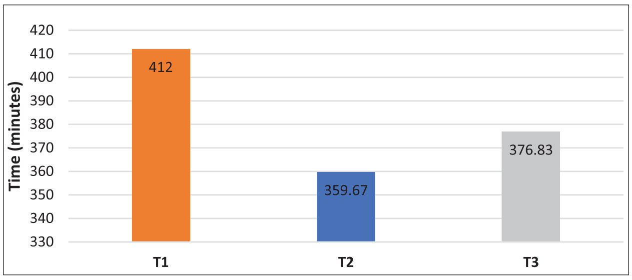

Fig. 1 Effect of supplementation of mineral mixture on third stage of labour (minutes) in Murrah buffaloes

specific mineral mixture than control (Tripathi and Mehta, 2022). As supplementation of macro- and microminerals restores ionic balance and enzyme cofactors essential for prostaglandin and oxytocin-mediated myometrial contractility, promoting stronger, more coordinated uterine contractions that speed lochial clearance and involution (Kalasariya *et al*. 2017).

At day 14 post-partum, more number of buffaloes had detectable fluid accumulation in uterus in T_1 group as compared to the T2 and T3 groups. Similarly Kalasariya *et al*. (2017) also observed the reduction in the duration of the complete expulsion of placenta in the animals administered with the injection of microminerals as compared to the control group. Rao *et al*. (2016) also aligns with our study and reported that the animals supplemented with vitamin

Table 4. Effect of supplementation of mineral mixture on reproductive performance in Murrah buffaloes

Attributes	Days	Treatment			SEM	P Value
		T_1	T_2	T_3		
Uterine horn diameter (cms)	10	7.57	7.12	7.25	0.14	0.09
	14	6.53^a	5.55^b	5.67^b	0.19	<0.01
	21	5.68^a	4.53^b	4.88^b	0.13	<0.01
	28	4.73^a	3.62^b	3.93^b	0.20	<0.01
	32	3.60	3.58	3.62	0.14	0.99
Detectable fluid in the uterus (% of animals)	10	100	100	100	-	-
	14	100	83.33	83.33	-	-
	21	83.33	50	50	-	-
	28	50	16.67	33.33	-	-
	32	16.67	NIL	NIL	-	-
Large sized follicles (≥6.00 mm) (% of animals)	10	NIL	NIL	NIL	-	-
	14	NIL	NIL	NIL	-	-
	21	NIL	16.67	NIL	-	-
	28	NIL	33.33	16.67	-	-
	32	100	50	83.33	-	-
First post-partum ovulation (% of animals)	10	NIL	NIL	NIL	-	-
	14	NIL	NIL	NIL	-	-
	21-28	NIL	16.67	NIL	-	-
	28-32	NIL	33.33	16.67	-	-
	32-35	100	50	83.33	-	-

Means bearing different superscript in a row differ significantly (P < 0.05)

E and selenium shows reduced time to expel placenta. Mohapatra *et al*. (2012) also observed that the reproductive performance of cows improved in the groups supplemented with ASMM as compared to control group.

Post-partum ovarian activity and first post-partum ovulation: On day 21st post- partum, 16.67% buffaloes (1/6) in T2 fed group showed presence of large sized follicle (>6 mm) while none of the T_1 and T3 group animals had large sized follicles (Table 4). On day 28th post- partum, 16.67% buffaloes (1/6) in T3 and 33.33% buffaloes (2/6) in T2 groups were having large sized follicles on their ovaries while T_1 group still did not reveal any large sized follicular activity. However, day 32nd post-partum, remaining 50 % (3/6) of buffaloes in T2 group and remaining 83.3% buffaloes in T3 group showed large sized follicles along with all the animals from T_1 group showed large sized follicular activity on day 32. Similar to present results, Tripathi and Mehta (2022) also found early resumption of post-partum estrus in buffaloes fed bypass fat and area specific mineral mixture than control group revealing early rebound of ovarian activity in the treatment group. Similarly, Shinde *et al*. (2024) have also reported early resumption of estrus in buffaloes supplemented with area-specific mineral mixture than the non-supplemented counterparts. Ullah *et al*. (2010) have reported early resumption of postpartum ovarian activity in the group supplemented with minerals as compared to non-supplemented group. Khan *et al*. (2014) reported reduction in days required to show first post-partum estrus and better conception rates in macro- and micro-mineral supplemented group than the control group. Kumar *et al*. (2024) found that supplementation of a proprietary mineral mixture to postpartum anestrous Murrah buffaloes induced estrus in 33.3% of treated animals and achieved a 50% conception rate, whereas untreated controls neither showed estrus nor the conception.

The present study revealed that supplementation of area specific mineral mixture in periparturient Murrah buffaloes significantly improved milk yield, milk protein content, and reproductive performance (placenta shedding, uterine involution, ovarian activity) without affecting nutrient utilization. Therefore, ASMM is recommended over BIS mineral mixture for improving productive and reproductive efficiency of Murrah buffaloes under field conditions of Western Uttar Pradesh.

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