Indian Journal of Animal Sciences **88** (4): 453–457, April 2018/Article

Effect of multi-nutrient liquid supplement on serum minerals and hormone profile in buffalo heifers

ANKITA[1], A K VERMA[2], PUTAN SINGH[3] and ASIT DAS[4]

ICAR-Indian Veterinary Research Institute, Izatnagar, Uttar Pradesh 243 122 India

Received: 14 June 2017; Accepted:13 December 2017

ABSTRACT

Eighteen Murrah buffalo heifers (average BW of 290±2.5 kg) were randomly distributed into three groups of six each in an experiment based on randomized block design to investigate the effect of partial replacement of concentrate mixture with molasses based multi-nutrient liquid supplements (MMLS) on serum mineral and reproductive hormonal profile. During 240 d of experimental period, all animals were supplied with green forages (2 kg DM/d) and wheat straw *ad libitum*. Animals in group T_1 (control) were fed a concentrate mixture (maize 40, wheat bran 40, soyabean meal 17, mineral mixture 2, and salt 1%) to meet their nutrient requirements. However, 20% of the concentrates were replaced with MMLS and MMLS plus in groups T_2 and T_3, respectively. Blood was collected on d 0, 120 and 240 days of experiment to evaluate serum mineral and reproductive hormonal (progesterone and estradiol) profile. Serum concentrations of Ca, P, Fe and Mn were similar among the groups. Serum concentration of Cu and Zn was higher in groups T_2 and T_3. Concentrations of progesterone and estradiol were similar among the groups, but showed an increasing trend on day 240. It was concluded that partial replacement of concentrate mixture with MMLS and MMLS plus improved Cu and Zn status without any adverse impact on other minerals and reproductive hormone profile.

Key words: Buffalo heifer, Estradiol, Hormones, Minerals, Multi-nutrient liquid supplement, Progesterone

Normal growth, reproduction and productivity in domestic animals depend upon the nutritional status of the animal. Minerals play crucial role in maintaining the functional and structural integrity of the tissues (McDowell 1995, Underwood and Suttle 1999) and in exploiting the actual genetic potential of the animals. Mineral disorders such as deficiencies and imbalances have significant consequences on animal health and production (McDowell 1995). Mineral deficiency is quite common in livestock and the severity of the deficiency depends upon the type of feed, physiological status of the animals and the agro-climatic conditions of the region.The deficiency of macro and micro minerals may lead to various reproductive failures, such as infertility, delayed puberty, delayed ovulation, lower conception rate and poor conception (Hidiroglou 1979, Boland 2003).

To overcome these detrimental effects, mineral supplementations to the livestock are essential (Underwood 1981). Several trace elements act as cofactors and have role in hormone synthesis that may influence biochemical functions associated with reproduction. Minerals such as Cu, Co, Zn, Fe and Mn affect reproduction in ruminants. Copper and zinc are activators of certain enzyme systems that assist in maintaining the activity of hormones in blood (Georgievskii 1982) and are known particularly to have a significant correlation with reproductive hormones such as progesterone and estradiol (Prasad *et al*. 1989). Copper deficiency leads to reduced insulin concentration, which adversely affects oestrus cyclicity, corpus luteum function and progesterone hormone production (Robinson *et al*. 2006). An appropriate supplementation of minerals acts as a stimulus for ovarian rebound and initiation of ovarian activity (Markandaya *et al*. 2002). Molasses based supplements were originally developed as a scarcity feed (Ranjhan *et al*. 1973, Verma *et al*. 1995). Later, it was observed that supplementation of urea molasses mineral block can improve the productivity of livestock fed on poor quality of roughages (Mohini and Gupta 1993). Thus, it would be logical to assume that partial replacement of concentrates with molasses based multi-nutrient liquid supplement (MMLS) would be beneficial. There are several reports which indicate that deficiency/ imbalances of trace elements are major determinants of reproductive performance of heifers (Borghese 2005). We hypothesized that supplementation of trace elements through molasses based multi-nutrient liquid supplement (MMLS) would positively influence the serum mineral and hormonal profile of buffalo heifers. Specific objective of this experiment was to study the effect of super dosing of trace minerals through

Present address: [2]Head and Director-CAFT (vermaak62 @gmail.com), [1]PhD Scholar (yadav08ankita@gmail.com), [3,4]Principal Scientist (putan60@gmail.com, drasitdas @rediffmail.com), Animal Nutrition Division.

MMLS on serum mineral profile and reproductive hormone levels in growing buffalo heifers.

MATERIALS AND METHODS

Experimental site, animals and housing: Eighteen healthy Murrah buffalo heifers of 10 to 16 months old (BW ranging from 230 to 355 kg) were selected from the Cattle and Buffalo farm, LPM section, IVRI, for the present investigation. All the animals were ear tagged and housed in a separate shed having provision of both open and close space.All the buffalo calves were vaccinated and dewormed for both endo- and ecto- parasites as per the schedule before start of the experiment.

Experimental design and dietary treatments: The animals were divided into three groups of six each according to their age and live body weights following randomized block design. All animals were supplied with green forages (2 kg DM/d) and wheat straw *ad lib*. Animals in group T_1 (control) were fed a concentrate mixture (maize 40, wheat bran 40, soyabean meal 17, mineral mixture 2, and salt 1%) to meet their nutrient requirements (ICAR 2013). However, 20% of the concentrates were replaced with MMLS and MMLS plus in groups T_2 and T_3, respectively. The MMLS consisted of urea (10%), molasses (74%), deoiled mahua seed cake (5%), guar meal (5%), mineral mixture (2% in MMLS and 4% in MMLS plus), salt (1%) and water. The animals were maintained on standard diet comprised of concentrate mixture, green fodder (oats) and wheat straw without inclusion of MMLS and MMLS plus, for 20 days before the actual start of 240 days trial. Feed samples were analyzed for dry matter and crude protein following standard procedures (AOAC 2005). Blood samples were collected from the jugular vein of each animal on days 0, 120 and 240 of the experiment and were allowed to clot, serum was harvested by centrifuging the samples at 2,000 rpm for 20 min and were stored at -20°C for further analysis. Serum concentrations of Ca and P were determined using commercial kit (Span Diagnostics, India). Serum concentrations of Fe, Mn, Cu and Zn were analyzed by Atomic Absorption Spectrophotometer (ECIL, Hyderabad). Samples for trace element analysis were processed according to the wet digestion procedure (Kolmer 1951). Serum samples were assayed for progesterone (P4) and estradiol by using a commercial RIA kits (Immunotech, France). The samples were incubated with ^{125}I-labelled progesterone and estradiol, in antibody coated tubes to measure the bound radioactivity. A calibration curve was established and unknown values were determined by interpolation from the curve.

Statistical analysis: Data obtained were subjected to two-way analysis of variance. Treatment means were separated by Duncan's multiple range test (Duncan 1955) and was considered significant at P<0.05. All analysis were performed using statistical package SPSS (20.0)

RESULTS AND DISCUSSION

The chemical and mineral composition of concentrate mixture, green fodder, wheat straw and multi-nutrient liquid supplements (MMLS and MMLS plus) are presented in Table 1.

Data pertaining to serum concentration of minerals are presented in Table 2. Serum concentration of Ca, P, Mn and Fe were similar among the groups. However, the serum concentration of Zn and Cu was higher (P<0.05) in MMLS and MMLS plus supplemented groups. It is evident that supplementation of MMLS and MMLS plus increased the dietary supply of Cu and Zn that was reflected in elevated serum concentration of the elements. Our result corroborated well with that of Mohapatra *et al*. (2012) who reported elevated serum concentration of Cu and Zn in mineral supplemented group as compared to non-supplemented group. In another study (Garg *et al*. 2008), it was observed that supplementation of chelated minerals resulted in increased serum concentration of Cu and Zn. We expected that a super-dosing of minerals would be beneficial to improve the utilization of minerals. However, the results of this experiment indicate that addition of 4% mineral in the MMLS may not have any extra benefit and addition of mineral mixture at 2% in MMLS seems to be adequate for growing heifers. Thus replacing some part of concentrate mixture with MMLS and MMLS plus had no adverse effects on any serum mineral concentration. However, impact of super-dosing of minerals through MMLS needs to be ascertained during more nutrient demanding life processes like lactation.

The serum concentration of progesterone and estradiol are presented in Table 3. The blood level of P_4 >1 ng/ml is considered a good indicator of the beginning of puberty in heifers (Khan *et al*. 2015, Terzano *et al*. 2012). The initial level of progesterone was <1 ng/ml and the estradiol concentration varied from 9.95–12.15 (pg/ml) among all groups on 0 day, indicating all heifers to be in their prepubertal stage. Jain and Pandey (1983) also reported P_4 level <1 ng/ml and estradiol level between 6.8–12.8 pg/ml in prepubertal buffalo heifers during the first 12 month of

Table 1. Chemical and mineral composition of concentrate mixture, green fodder, wheat straw and multi-nutrient liquid supplements (MMLS and MMLS plus)

Ingredient	Concentrate mixture	Green fodder	Wheat straw	MMLS	MMLS plus
Dry matter (%)	88.43	19.97	94.06	70.06	72.83
On dry matter basis					
Crude protein (%)	18.9	9.3	4.1	35.42	34.89
Total ash (%)	6.04	12.76	6.53	13.61	15.46
Ca (%)	0.58	0.41	0.23	1.48	1.94
P (%)	0.53	0.21	0.06	0.39	0.57
Zn (ppm)	66.24	23.34	10.23	64.49	106.98
Fe (ppm)	152.02	324.42	171.89	234.68	327.59
Mn (ppm)	57.24	46.46	28.28	105.86	149.95
Cu (ppm)	16.41	14.12	2.04	40.36	73.81

MMLS, Molasses based multi-nutrient liquid supplements containing 2% mineral mixture; MMLS plus, Molasses based multi-nutrient liquid supplements containing 4% mineral mixture.

Table 2. Effect of replacement of concentrates with multi-nutrient liquid supplements on serum mineral concentration of buffalo heifers

Mineral	Treatment	0 day	120 day	240 day	Treatment mean	T	P	T*P
Ca (mg/dl)	T_1	9.84±0.55	9.45±0.73	9.71±0.57	9.67±0.34	0.66	0.53	0.93
	T_2	9.74±0.17	9.80±0.28	10.26±0.58	9.93±0.22			
	T_3	9.74±0.11	9.88±0.16	10.32±0.39	9.98±0.15			
	Period mean	9.77±0.19	9.71±0.25	10.10±0.29				
P (mg/dl)	T_1	6.26±0.58	6.20±0.08	6.22±0.23	6.23±0.20	0.41	0.47	0.91
	T_2	6.30±0.26	6.47±0.15	6.67±0.19	6.48±0.12			
	T_3	6.26±0.15	6.40±0.08	6.68±0.15	6.45±0.08			
	Period mean	6.27±0.20	6.36±0.07	6.52±0.12				
Zn (ppm)	T_1	1.50±0.08	1.60±0.11	1.52±0.11	1.54^{b}±0.06	0.01	<0.01	0.17
	T_2	1.48±0.10	1.92±0.09	1.87±0.11	1.76^{a}±0.07			
	T_3	1.52±0.09	1.95±0.18	2.03±0.08	1.83^{a}±0.09			
	Period mean	1.50^{B}±0.05	1.79^{A}±0.08	1.84^{A}±0.08				
Fe (ppm)	T_1	3.35±0.17	3.12±0.18	3.33±0.13	3.26±0.09	0.59	0.78	0.87
	T_2	3.27±0.14	3.32±0.26	3.47±0.15	3.35±0.11			
	T_3	3.41±0.16	3.45±0.12	3.40±0.25	3.42±0.10			
	Period mean	3.34±0.09	3.30±0.11	3.40±0.10				
Mn ppm)	T_1	0.79±0.04	0.77±0.06	0.73±0.10	0.76±0.04	0.35	0.95	0.92
	T_2	0.81±0.07	0.82±0.05	0.85±0.08	0.83±0.04			
	T_3	0.81±0.05	0.83±0.06	0.87±0.05	0.83±0.03			
	Period mean	0.80±0.03	0.80±0.03	0.82±0.05				
Cu ppm)	T_1	0.84±0.05	0.85±0.02	0.82±0.03	0.84^{b}±0.02	<0.01	<0.01	<0.01
	T_2	0.81±0.03	0.93±0.02	1.03±0.05	0.92^{a}±0.02			
	T_3	0.83±0.04	0.94±0.03	0.108±0.04	0.95^{a}±0.03			
	Period mean	0.82^{C}±0.02	0.90^{B}±0.02	0.98^{A}±0.03				

Values bearing different superscripts in a row differ significantly (P≤0.05) and (P≤0.01). Values bearing different superscripts in a column differ significantly (P≤0.05) and (P≤0.01). Animals were fed either control, or diet containing molasses based multi-nutrient liquid supplements containing 2 or 4% mineral mixture, in groups T_1, T_2 and T_3, respectively.

Table 3. Effect of multi-nutrient liquid supplements on reproductive hormone profile of buffalo heifers in different groups

	Treatment	0 Day	120 Day	240 Day	Treatment mean	T	P	T*P
Progesterone (ng/ml)	T_1	0.31±0.11 (0.07–0.82)	0.57±0.20 (0.32–1.50)	1.54±0.37 (0.38–2.87)	0.81±0.19	0.194	<0.001	0.680
	T_2	0.23±0.05 (0.10–0.46)	1.26±0.51 (0.35–3.59)	2.32±0.50 (0.52–3.78)	1.27±0.31			
	T_3	0.28±0.04 (0.10–0.40)	1.19±0.25 (0.76–2.40)	2.48±0.70 (0.90–5.53)	1.31±0.32			
	Period mean	0.27^{C}±0.04	1.00^{B}±0.20	2.12^{A}±0.31				
Estradiol (pg/ml)	T_1	9.95±2.44 (0.60–14.56)	19.91±3.53 (12.30–33.22)	24.56±7.72 (12–62.81)	18.14±3.14	0.871	<0.001	0.760
	T_2	9.76±2.21 (4.82–17.66)	21.66±2.45 (16–32.57)	27.10±1.32 (23.65–31.89)	19.50±2.08			
	T_3	12.15±0.58 (10.21–14.03)	16.18±1.68 (10.66–23.11)	26.96±2.06 (21.62–36.23)	18.43±1.74			
	Period mean	10.62^{C}±1.08	19.25^{B}±1.55	26.21^{A}±2.55				

Values bearing different superscripts in a column differ significantly (P≤0.01). Animals were fed either control, or diet containing molasses based multi-nutrient liquid supplements containing 2 or 4% mineral mixture, in groups T_1, T_2 and T_3, respectively.

age. Irrespective of treatment, progesterone and estradiol level increased (P<0.05) with the advancement of age in heifers. The increasing trend of progesterone (>1 ng/ml) and estradiol in supplemented groups indicated that heifers started attaining puberty early (Jain and Pandey 1983). The increase in the level of estradiol is reflected as a transformation of the follicles into the interstitial tissues that augments the potential capacity of ovary to form estrogen during the prepubertal stage (Baker 1972). The estradiol concentration show two peaks i.e one before the day of oestrus while second during days 10–11 of the cycle (Baruselli *et al*. 1997, Manik *et al*. 1998), because of these

Table 4. First heat detection record in buffalo heifers

Treatment	Animals in heat	Age at first heat detection (days)	Percentage of animals in heat
T_1	1	834	
	2	802	
	3	715	66.66
	4	773	
		Average: 781	
T_2	1	683	
	2	530	
	3	621	83.33
	4	746	
	5	711	
		Average: 658.2	
T_3	1	695	
	2	699	
	3	705	83.33
	4	714	
	5	596	
		Average: 681.8	

fluctuations estradiol is not considered as reliable as progesterone level in detecting the onset of puberty in prepubertal animals. Even though the level of progesterone on day 240 of the experiment was 52–61% in MMLS supplemented groups which could be linked to the early attainment of puberty in MMLS fed group. It is, however, acknowledged that interpretation of data on reproductive hormonal profile needs more frequent sampling. It will be thus a matter of interest of future studies that would unveil the probable link of reproduction cycle with improved mineral supply through MMLS supplementation.

The first heat detection record obtained from 240 d study also confirmed early puberty in supplemented groups. Five out of six heifers exhibited heat symptoms in T_2 group at the average age of 658.2 days and in T_3 groups at 681.8 days while only 67% animals came into heat from control group around the average age of 781 days (Table 4). Bashir (2006) reported that the average age of puberty in buffalo heifers is 37 months (1110 days), however, supplementation of most critical nutrients can accelerate the growth rate of calves and help attain puberty at 19–20 months (570–600 days) in heifers (Terzano *et al*. 1997). Thus, in the present study, super dosing of minerals mainly Zn at 64.49 and 106.98 ppm and Cu at 40.36 and 73.81 ppm through MMLS and MMLS plus which are crucial for reproduction (Campbell and Miller 1998) resulted in early onset of puberty in supplemented heifers, which was in agreement with the results of Mohapatra *et al*. (2012). However, it was found that in terms of reproductive performance also super dosing of mineral at 4% did not show any extra benefit as compared to supplementation at 2%, thus showing that animals receiving better quality feed respond less to super dosing of mineral supplementation as compared to nutritionally deprived animals (Chaudhry *et al*. 1991).

On the basis of these results it can be concluded that super dosing of mineral mixture through molasses based multi-nutrient liquid supplements replacing 20% of concentrate mixture had significantly (P<0.05) better serum mineral status of Zn and Cu in growing buffalo heifers. The trend of reproductive hormone profile and heat records also indicated rapid onset of puberty in multi-nutrient liquid supplemented groups.

REFERENCES

AOAC. 2005. *Official Methods of Analysis.* Association of Official Analytical Chemists. 18th ed. USA.

Baker T G. 1972. Oogenesis and ovarian development. *Reproductive Biology*. Excerpt Med. Found, Amsterdam, pp. 398.

Baruselli P S, Visintin J A, Barnabe V H, Barnabe R C, Amaral R and Souza A C.1997. Early pregnancy ultrasonography and embryonic mortality occurrence in buffalo. *Proceedings of Vth World Buffalo Congress,* Italy. pp 776–778.

Bashir M K. 2006. 'Genetic and phenotypic aspects of some performance traits of Nili-Ravi buffaloes in Pakistan'. PhD Thesis, University of Agriculture, Faisalabad, Pakistan.

Boland M P. 2003. Trace minerals in production and reproduction in dairy cows. *Advances in Dairy Technology* **15**: 319–30.

Borghese A. 2005. *Buffalo Production and Research*. Technical Series 67. Food and Agriculture Organization, Rome, Italy.

Campbell M H and Miller J K. 1998. Effect of supplemental dietary vitamin E and Zinc on reproductive performance of dairy cows and heifers fed excess iron. *Journal of Dairy Science* **81**: 2692–99.

Chaudhry M A, Asghar A A and Ahmad M. 1991. Productive and reproductive performance of NiliRavi buffalo heifers as influenced by mineral and concentrates mixture. *Buffalo Journal* **1**: 41–49.

Duncan D B. 1955. Multiple 'F' test. *Biometrics* **1**: 1–42.

Garg M R, Bhanderi B M and Gupta S K. 2008. Effect of supplementing certain chelated minerals and vitamins to overcome infertility in field animal. *Indian Journal of Dairy Science* **61**: 181–84.

Georgievskii V I. 1982. Mineral composition of bodies and tissue of animals. *Mineral Nutrition of Animals,* pp 67–77. London, England.

Hidiroglou M. 1979. Trace element deficiencies and fertility in ruminants: A review. *Journal of Dairy Science* **62**: 1195–206.

ICAR. 2013. Nutrient Requirements for Cattle and Buffalo. Indian Council for Agricultural Research, New Delhi.

Jain G C and Pandey R S. 1983. Changes in the profiles of peripheral plasma progesterone, oestradiol-17β and luteinizing hormone from birth to puberty following conception in buffalo heifers (*Bubalus bubalis*). *Asian Journal of Dairy Research* **2**: 227–33.

Khan H M, Mohanty T K, Bhakat M, Gupta A K, Tyagi A K and Mondal G. 2015. Effect of vitamin E and mineral supplementation on biochemical profile and reproductive performance of buffaloes. *Buffalo Bulletin* **34**: 62–78.

Kolmer J A. 1951. *Approved Laboratory Techniques*. pp 1090–1091. Appleton Century Crafts. New York.

Manik R S, Singla S K, Palta P and Mada M L. 1998. Ovarian follicular dynamics monitored by real-time ultrasonography during oestrous cycle in buffalo (*Bubalus bubalis*). *Asian Australian Journal of Animal Sciences* **11**: 480–85.

Markandaya N M, Bhikane A U and Bharkad G P. 2002. Clinical response to microminerals with vitamin E supplementation in anestrous Deoni cows. *Indian Journal of Animal Reproduction*

23: 78–82.

McDowell L R. 1995. *Nutrition of Grazing Ruminants in Warm Climate*. Academic Press, New York.

Mohapatra P, Swain R K, Mishra S K, Sahoo G and Rout K K. 2012. Effect of supplementation of area specific mineral mixture on reproductive performance of the cows. *Indian Journal of Animal Sciences* **82**: 1558–63.

Mohini M and Gupta B N. 1993. Nutrient utilization in buffaloes fed paddy straw supplemented with UMMB. *Indian Journal of Animal Nutrition* **10**: 217–21.

Prasad C S, Sarma P V, Reddy O A and Chinnaiya C P. 1989. Trace elements and ovarian hormonal levels during different reproductive conditions in crossbred cattle. *Indian Journal of Dairy Science* **42**: 489–92.

Ranjhan S K, Sawhney P C and Jayal M M. 1973. *Application of life saving research in animal feeding*. Farm Information Unit, Directorate of Extension, Ministry of Agriculture, New Delhi, India.

Robinson J J, Ashworth C J, Rooke J A, Mitchell L M and McEvoy T G. 2006. Nutrition and fertility in ruminant livestock. *Animal Feed Science and Technology* **126**: 259–76.

Terzano G M, Galasso A, Barile V L, Pacelli C, Montemurro N and Borghese A. 1997. Effect of feeding system and puberty on blood metabolites trends in buffalo heifers. *Proceedings of Vth World Buffalo Congress, Italy*. pp. 951–56.

Terzano G M, Barile V L and Borghese A. 2012. Overview on reproductive endocrine aspects in buffalo. *Journal of Buffalo Science* **1**: 126–38.

Underwood E J and Suttle N G. 1999. *The Mineral Nutrition of Livestock*. 3rd edn. CABI Publishing, CAB International, UK.

Underwood E J. 1981. *The Mineral Nutrition of Livestock*. pp 189. Commonwealth Agricultural Bureaux, England.

Verma A K, Mehra U R, Dass R S, Varshney V P and Kumar H. 1995. Performance of crossbred heifers during revival period after long term scarcity feeding. *Journal of Applied Animal Research* **8**: 62–70.