Indian Journal of Animal Sciences **88** (4): 463–469, April 2018/Article

Factors affecting milk minerals and constituents in indigenous *vis-à-vis* crossbred cattle and buffaloes

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Received: 13 October 2017; Accepted: 14 December 2017

ABSTRACT

The present investigation was conducted to compare milk minerals and constituents in different bovine species and breeds. The genetic and non-genetic factors affecting milk fat, protein and milk mineral composition in crossbred cattle were investigated. The effect of breeds was highly significant on copper (Cu), zinc (Zn), manganese (Mn), sodium (Na), potassium (K), fat and protein, significant ($p \leq 0.05$) on calcium (Ca) and non-significant on phosphorus (P) and iron (Fe) content. The Ca, Cu, fat and protein were significantly higher in Murrah buffalo followed by Tharparkar cattle and crossbred cattle. However, Mn and Zn were higher in Tharparkar cattle followed by Murrah buffalo and crossbred cattle. Tharparkar milk was also rich in Na and K followed by crossbred cattle and Murrah buffalo. The effect of parity was highly significant only on Mn, whereas, effect of lactation stage (LS) was significant on Fe and Cu in milk of crossbred cattle. The effect of Test Day Milk Yield (TDMY) was significant on Fe and Cu. The product moment correlation was highest (0.414) between Zn and Fe and lowest (-0.347) between Cu and Zn. Among milk minerals, the estimates of heritability for Fe, Ca and Mn were moderate, whereas it was low for other minerals in crossbred cattle.

Key words: Breeds, Buffalo, Cattle, Indigenous, Milk mineral, Murrah, Tharparkar

Milk is primarily composed of water, proteins, amino acids, lactose, vitamins, lipids, fatty acids and minerals. The milk pricing system is mainly based on quantity of milk fat and protein in milk. Milk minerals have a pivotal role in humans and animal body not only in structural, biochemical, catalytic and nutritional functions but also in all spheres. Thus, adequate levels of minerals in the diet are very essential. Minerals such as Ca, P, Na, K, Cl, I, Mg and iron are mainly present in milk. Among all these minerals, Ca and P constitute a larger fraction in cow milk, which is essential for bone growth and proper development of newborns. Content of different milk constituents is affected by several factors which can be either genetic or non-genetic such as lactation period, animal breed, climate, season, dietary composition of animal feed. But scientific literature pertaining to milk mineral composition of different Indian breeds is scanty. In India, there are numerous breeds of indigenous as well as crossbred cattle and buffalo and further all of them have different genetic makeup which may influence the milk composition. The nutritional value of milk of Indian cows is the highest along with many curative properties against diseases. Cow ghee prepared in traditional manner using milk/fat of indigenous cows, works better for human health and posses highest organoleptic properties. Recently, it has been established that majority of indigenous cattle produces A2 milk which are not stimulating immunogenic reaction or gastro-intestinal disorder specially in children unlike A1 milk of exotic or their crosssbred cattle. So, depending upon these facts aim of the present study was to estimate Ca, P, Fe, Cu, Mn, Zn, Na, K, fat and protein contents in milk of Murrah buffaloes, Tharparkar and crossbred cattle. Haug *et al.* (2007) reported that lactation stage of the dairy animals affected mineral composition. Recently, Buitenhuis *et al.* (2015) found high heritability in Danish Holstein (DH) cattle for Ca (0.72), Zn (0.49) and P (0.46), while for Danish Jersey (DJ), high heritabilities were found for Ca (0.63), Zn (0.57) and Mg (0.57). Limited number of studies are available on the effect of genetic and non-genetic factors on milk mineral constituents. The aim of this study was to compare milk mineral and constituents in different bovine breeds and to find out the effect of genetic and non-genetic factors on traits under investigation of crossbred cattle.

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MATERIALS AND METHODS

Experimental animals and farm: The present study was carried out at cattle and buffalo farm of the Indian Veterinary Research Institute, Izatnagar, which falls in the upper Gangetic plain region of India after including104 crossbred cows, 10 Tharparkar cows and 33 Murrah buffaloes. The mean annual temperature is about 21°C, whereas the temperature ranges between 5°C and 40°C. The region receives an annual rainfall approximately 900 to 1200 cm. Animals were maintained under the loose housing system and nutritional requirements of animals were fulfilled through a balanced combination of dry and green fodder with concentrate mixture supplementation. Milk recording was done from the first day of calving till date of drying and calves weaned immediately after birth. Vaccination, deworming and spraying of these animals were done regularly as per recommendation.

Milk samples and chemical analysis: Approximately 40 ml of fresh milk samples were collected from each animal in well cleaned and autoclaved plastic sample bottles. The milk samples were collected twice from each animal, first in month of December and second in month of March. Before taking the sample it was ensured that it should be thoroughly mixed. Milk samples were transported to the laboratory as soon as possible and were processed immediately. Total fat and protein percentage was determined by using Lactoscan. Calcium and phosphorus estimation was done as per the Talapatra *et al.* (1940) in milk samples. The Na and K estimation was done by flame photometer, whereas, trace minerals (Zn, Cu, Fe, Mn) in different milk samples were estimated by atomic absorbance spectrophotometer in their mineral extracts.

Statistical analysis: The Proc GLM procedure of SAS 9.3 was used for estimation of the effects of various non-genetic factors on milk mineral and milk constituent's traits. Fixed effect model used for estimating the effect of breed type was:

$$Y_{ij} = \mu + B_{i} + e_{ij}$$

where Y_{ij} is the j^{th} observation of i^{th} breed, B_i is effect of i^{th} breed type (i=1 to 3); e_{ij} is random error (NID 4 0, σ^2_e). Further, the effect of genetic and non-genetic factors was estimated using least squares mixed models as given below;

$$Y_{ijklmn} = \mu + S_i + M_j + P_k + L_l + T_m + e_{ijklmn}$$

where Y_{ijklmn} is the n^{th} observation (traits) of i^{th} sire recorded in j^{th} month of k^{th} lactation order at l^{th} lactation stage having m^{th} level of test day milk yield, S_i is effect of i^{th} sire (i=1 to n); M_j is effect of j^{th} month of milk collection (j=1 to 2); P_k is effect of k^{th} parity (k=1 to 5); L_l is effect of stage of lactation (l=1 to 3) and e_{ijklmn} is random error (NID 4 0, σ^2_e). For all milk minerals (except Na and K), fat percentage and protein percentage, the above mentioned mixed model was used. However, since Na and K content of milk was estimated only once in month of December, hence effect of month of collection was removed from model for analyzing the Na and K data.

RESULTS AND DISCUSSION

Estimates of different milk constituents: The least squares means of different milk mineral and constituents of different bovine breeds is presented in Table 1. The least-squares means of crossbred cattle for Ca, P, Fe, Cu, Mn, Zn, fat, protein, Na and K were 1441±0.03 mg/l, 1268±0.02 mg/l, 9.680±0.22 mg/l, 1.103±0.04 mg/l, 1.104±0.04 mg/l, 3.77±0.10 mg/l, 4.28±0.08%, 2.81±0.01%, 21.404±0.44 meq/l and 33.442±0.61 meq/l, respectively, whereas, for Murrah buffalo were 1598±0.04 mg/l, 1246±0.04 mg/l, 9.186±0.35 mg/l, 1.478±0.07 mg/l, 2.061±0.04 mg/l, 4.83±0.21 mg/l, 7.69±0.25%, 3.16±0.02%, 16.607±0.58 meq/l and 27.424±0.68 meq/l, respectively, and for Tharparkar cattle were 1502±0.07 mg/ml, 1251±0.10 mg/ml, 8.772±0.79 mg/l, 1.406±0.11 mg/l, 2.128±0.09 mg/l, 5.39±0.30 mg/l, 4.49±0.24%, 3.00±0.03%, 22.8±1.81 meq/l and 36.400±1.52 meq/l, respectively.

Effect of bovine breed type: The effect of breeds was highly significant (P≤0.01) on Cu, Zn, Mn, Na, K, fat and protein, significant (P≤0.05) on Ca and non-significant on P and Fe content. The Ca, Cu, fat and protein were significantly higher in Murrah buffalo followed by Tharparkar cattle and then crossbred cattle. However, Mn and Zn were higher in Tharparkar cattle followed by Murrah buffalo and then crossbred cattle. Tharparkar milk was also rich in Na and K followed by crossbred cattle and then Murrah buffalo. The milk of crossbred cattle was only rich in Fe as compared to Murrah buffaloes and Tharparkar cattle (Table 1). Mariani *et al.* (2002) found that amount of Ca, and P in Holstein-Friesian was significantly lesser (7%) than the Brown Swiss, Reggiana and Modenese breeds. Toffanin *et al.* (2015b) reported that Holstein-Friesian has low Ca and P in milk because Ca and P bound to casein, and milk of HF cow has lower casein content than Brown Swiss cow's milk (De Marchi *et al.* 2008). Whereas, Kayastha *et al.* (2008) reported that the milk of native cattle of Asom has higher fat% (5.34±0.06), SNF (8.54±0.03) and protein (%) content (3.04±0.03) compared to most of the Indian breeds of cattle.

Calcium (*Ca*) *concentration:* In the present study, estimates of Ca varied from 171 to 3210 mg/l, 1004 to 2379 mg/l, 1072 to 1930 mg/l in the milk of crossbred cattle, Murrah buffalo and Tharparkar cattle respectively. Zamberlin *et al.* (2012) revealed the range of Ca varied from 107 to 133 mg/100 g in cow milk. The least squares mean of Ca was significantly (P≤0.05) highest in Murrah buffalo (1598±0.04 mg/l) followed by Tharparkar cattle (1502±0.07 mg/l) and crossbred cattle (1441±0.03 mg/l). However, Toffanin *et al.* (2015b) reported lower (1171 mg/l) mean concentration of Ca in Holstein-Friesian cows, whereas, Rodriguez *et al.* (2001) reported a higher concentration in fresh cow milk (1653±207 mg/l) as well as in sterilized cow milk (1309±62 mg/l).

Phosphorus (*P*) *concentration:* The estimates of P ranged from 535 to 2475 mg/l, 649 to 2108 mg/l, and 408 to 2360 mg/l in milk of crossbred cattle, Murrah buffalo and Tharparkar cattle, respectively. However, Tongato (2015)

Table 1. Least square means and standard errors of milk minerals and constituents for different bovine breeds

Breed	Na (meq/l)	K (meq/l)	Ca (mg/l)	P (mg/l)	Fe (mg/l)	Cu (mg/l)	Mn (mg/l)	Zn (mg/l)	Fat (%)	Protein (%)
Overall	20.422±0.39 (147)	32.292±0.52 (147)	1480±0.03 (294)	1262±0.02 (294)	9.507±0.18 (294)	1.208±0.04 (294)	1.388±0.04 (294)	4.12±0.09 (294)	5.06±0.11 (294)	2.90±0.01 (294)
Crossbred	21.404^{a}±0.44 (104)	33.442^{a}±0.61 (104)	1441^{b}±0.03 (208)	1268±0.02 (208)	9.680±0.22 (208)	1.103^{b}±0.04 (208)	1.104^{b}±0.04 (208)	3.77^{b}±0.10 (208)	4.28^{b}±0.08 (208)	2.81^{c}±0.01 `(208)
Murrah	16.607^{b}±0.58 (33)	27.424^{b}±0.68 (33)	1598^{a}±0.04 (66)	1246±0.04 (66)	9.186±0.35 (66)	1.478^{a}±0.07 (66)	2.061^{a}±0.04 (66)	4.83^{a}±0.21 (66)	7.69^{a}±0.25 (66)	3.16^{a}±0.02 (66)
Tharparkar	22.8^{a}±1.81 (10)	36.400^{a}±1.52 (10)	1502^{ab}±0.07 (20)	1251±0.10 (20)	8.772±0.79 (20)	1.406^{a}±0.11 (20)	2.128^{a}±0.09 (20)	5.39^{a}±0.30 (20)	4.49^{b}±0.24 (20)	3.00^{b}±0.03 (20)

SE, Standard error; TDMY, Test day milk yield; meq/l, Millie equivalent per litre; mg/l, Milligram per litre; kg, Kilogram. The least squares means with at least one same superscripts do not differ significantly. Figure in parentheses are number of observations.

reviewed that P ranged from 825 to 1995 mg/l as evidenced from various workers. The least squares mean of P was not differing significantly among Murrah buffalo (1268±0.02 mg/l) and crossbred cattle (1246±0.04 mg/l) whereas, it was slightly lower in Tharparkar cattle (1251±0.10 mg/l). In another study, comparatively lower (1112 mg/l) mean concentration of P was reviewed (Zamberlin *et al.* 2012). Toffanin *et al.* (2015b) reported mean P concentration of 935 mg/l in Holstein Frisian cow's milk.

Iron (*Fe*) *concentration:* In present study, Fe content in milk varied from 4.640 to 22.400 mg/l, 4.958 to 15.750 mg/l, 5.713 to 21.410 mg/l of crossbred cattle, Murrah buffalo and Tharparkar cattle respectively. However, Zamberlin *et al.* (2012) reviewed higher range of Fe from 30 to 70 µg/100g in cow milk. The mean concentration of Fe was non-significantly higher in crossbred cattle (9.680±0.22 mg/l) followed by Murrah buffalo (9.186±0.34 mg/l) and then Tharparkar cattle (8.772±0.79 mg/l). However, lower mean (0.515±0.178) was reported by Rodriguez *et al.* (2001) in fresh cow's milk from Canary Island.

Copper (*Cu*) *concentration:* The estimates of Cu varied from 0.30 to 4.420 mg/l, 0.787 to 3.380 mg/l and 0.86 to 2.19 mg/l in milk of crossbred cattle, Murrah buffalo and Tharparkar cattle respectively. The least squares mean of Cu was close (1.478±0.07 mg/l and 1.406±0.11 mg/l) in Murrah buffalo and Tharparkar cattle, respectively whereas it was significantly ($P \leq 0.01$) lower in crossbred cattle (1.103±0.04 mg/l). However, estimates were slightly variable from 0.034 to 0.164 mg/l in fresh cow milk (Rodriguez *et al.* 2001). In a study, Zamberlin *et al.* (2012) reviewed higher range of Cu from 2 to 30 µg/100g in the milk of cattle. However, Rodriguez *et al.* (2001) reported lower concentration of 0.076±0.032 mg/l in fresh cow milk and 0.110±0.039 mg/l in sterilized cow milk.

Manganese (Mn) concentration: The range of estimates of Mn varied from 0.007 to 2.102 mg/l, 1.568 to 2.724 mg/l, 1.60 to 2.59 mg/l in milk of crossbred cattle, Murrah buffalo and Tharparkar cattle respectively. The least squares mean of Mn was significantly ($P \leq 0.01$) higher in Tharparkar cattle (2.128±0.09 mg/l) than Murrah buffalo (2.061±0.04 mg/l) and crossbred cattle (1.104±0.04 mg/l). However, Zamberlin *et al*. (2012) reviewed higher variability of Mn from 1.3 to 4.0 µg/100g in cow milk and 5.5 µg/100g in goat milk.

Zinc (*Zn*) *concentration:* The estimates of Zn in present study varied from 0.430 to 8.240 mg/l, 1.640 to 8.770 mg/l, and 2.713 to 8.550 mg/l in milk of crossbred cattle, Murrah buffalo and Tharparkar cattle respectively. The least squares mean of Zn was significantly ($P \leq 0.01$) higher in Tharparkar cattle (5.394±0.30 mg/l) followed by Murrah buffalo (4.825±0.21 mg/l) and then crossbred cattle (3.774±0.10 mg/l). Zamberlin *et al.* (2012) reviewed higher concentration of Zn ranging from 74 to 145 µg/100g in cow milk and 242 µg/100g in goat milk. However, in other studies, estimates were slightly variable (2.30 to 6.60 mg/l) in fresh cow milk (Rodriguez *et al.* 2001). However, Rodriguez *et al.* (2001) reported lower concentration of Zn in fresh cow milk (4.409±0.666 mg/l) and in sterilized cow milk (3.060±0.137 mg/l). But Van hulzen *et al.* (2009) reported a lower mean of 3711 µg/kg in Dutch HF cow milk.

Sodium (*Na*) *concentration:* The estimates of Na varied from 12 to 39 meq/l, 10 to 24 meq/l and 15 to 34 meq/l in milk of crossbred cattle, Murrah buffalo and Tharparkar cattle, respectively.The least squares mean of Na was significantly ($P<0.01$) higher in Tharparkar cattle (22.800±1.81 meq/l) than crossbred cattle (21.404±0.44 meq/l) and Murrah buffalo (16.606±0.58 meq/l). Similar variability from 278 to 870 mg/l was found in fresh cow's milk from Canary Island (Rodriguez *et al.* 2001), whereas Zamberlin *et al.* (2012) reviewed a lower range of Na from 40 to 58 mg/100g in cow milk. Rodriguez *et al.* (2001) reported 534±109 mg/l of Na in fresh cow's milk from Canary Island.

Potassium (*K*) *concentration:* The estimates of K varied from 19 to 53 meq/l, 20 to 37 meq/l and 31 to 44 meq/l in milk of crossbred cattle, Murrah buffalo and Tharparkar cattle respectively. The least squares mean of K was significantly ($P<0.01$) higher in Tharparkar cattle (36.40±1.51 meq/l) followed by crossbred cattle (33.442±0.61 meq/l) and then Murrah buffalo (27.424±0.68 meq/l). However, in a study estimates of K were 1035 to 1874 mg/l in fresh cow milk (Rodriguez *et al.* 2001), whereas Zamberlin *et al.* (2012) reviewed a lower range of K from 144 to 178 mg/100g in cow milk and 135 to 235 mg/100g in goat milk. Rodriguez *et al.* (2001) reported similar (1424±200 mg/l) estimate of K in fresh cow's milk from Canary Island.

Fat percentage: The least squares mean of fat percentage was significantly ($P<0.01$) higher in Murrah buffalo (7.693±0.25%) followed by Tharparkar cattle (4.487±0.24%) and crossbred cattle (4.28±0.68%). However, slightly lower mean of 6.99±0.10, 4.37±0.20 and 4.23±0.18% was reported in Murrah buffalo, Tharparkar and Sahiwal cattle respectively (Sarkar *et al.* 2006). Similarly, Mishra and Joshi (2004) and Sarkar *et al.* (2006) reported fat percentage 4.02±0.02% and 3.91±0.14% in milk of Karan-Fries cattle. Whereas Kayashtha *et al.* (2008) reported higher fat percentage (5.34±0.06%) in the Asom native cattle breed.

Protein percentage: The least squares mean of protein percentage was significantly ($P<0.01$) higher in Murrah buffalo (3.156±0.02%) followed by Tharparkar cattle (3.004±0.03%) and crossbred cattle (2.810±0.01%). A similar finding of protein percentage (3.04±0.03%) in Asom native cattle breed was reported by Kayashtha *et al.* (2008). However, higher mean of protein, i.e. 3.78±0.03%, 3.92±0.05% and 3.60±0.05% was reported in Murrah buffalo, Tharparkar cattle and Sahiwal cattle respectively (Sarkar *et al.* 2006). Similarly, Mishra and Joshi (2004) and Sarkar *et al.* (2006) reported protein percentage of 3.35±0.03% and 3.58±0.04% in milk of Karan-Fries cattle.

Effect of different genetic and non-genetic factors in milk

Table 2. Least square means for different milk minerals and constituents in crossbred cattle for different non-genetic effects

Effect	Level	N	Ca (mg/l)	P (mg/l)	Fe (mg/l)	Cu (mg/l)	Mn (mg/l)	Zn (mg/l)	Fat (%)	Protein (%)	Na (meq/l)*	K (meq/l)*
Month	December	104	1450±0.05	1217^{b}±0.03	8.761^{b}±0.30	1.467^{a}±0.06	0.882^{b}±0.06	3.31^{b}±0.14	4.4±0.10	2.79±0.02	Not estimated	Not estimated
	March	104	1432±0.05	1319^{a}±0.03	10.598^{a}±0.30	0.74^{b}±0.06	1.326^{a}±0.06	4.23^{a}±0.14	4.17±0.10	2.83±0.02	Not estimated	Not estimated
Parity	1	50	1435±0.07	1322±0.05	9.276±0.41	1.106±0.09	0.914^{b}±0.07	3.8±0.20	4.35±0.14	2.8±0.02	20.14±0.71 (25)	32.372±0.96 (25)
	2	52	1398±0.07	1250±0.06	9.334±0.40	1.011±0.11	0.739^{b}±0.08	3.58±0.16	4.17±0.15	2.83±0.04	20.583±0.81 (26)	32.927±1.17 (26)
	3	40	1436±0.07	1269±0.05	10.47±0.63	1.055±0.08	1.346^{a}±0.10	3.91±0.27	4.47±0.19	2.82±0.02	20.33±0.69 (20)	31.700±1.03 (20)
	4	20	1345±0.10	1091±0.08	9.794±0.65	1.203±0.11	1.527^{a}±0.07	3.87±0.39	4.44±0.26	2.83±0.04	20.6±1.32 (10)	31.100±0.86 (10)
	>5	46	1541±0.08	1306±0.05	9.775±0.46	1.204±0.09	1.33^{a}±0.07	3.81±0.17	4.09±0.16	2.79±0.02	20.826±1.23 (23)	32.304±1.29 (23)
LS	5 to 90 days	26	1385±0.08	1193±0.05	8.579^{b}±0.60	1.368^{a}±0.10	0.942±0.13	3.58±0.25	4.66±0.21	2.8±0.02	19.42^{b}±0.771 (23)	32.029±1.03 (23)
	90 to 180 days	76	1403±0.06	1277±0.04	9.349^{ab}±0.39	1.148^{ab}±0.06	1.086±0.07	3.6±0.15	4.28±0.13	2.78±0.02	19.67^{b}±0.57 (28)	31.333±0.77 (28)
	>180 days	106	1482±0.05	1280±0.04	10.187^{a}±0.29	1.006^{b}±0.07	1.157±0.06	3.95±0.14	4.19±0.10	2.83±0.02	22.029^{a}±0.66 (53)	34.047±0.91 (53)
TDMY	upto 8 kg	58	1518±0.07	1305±0.05	10.665^{a}±0.47	0.986^{b}±0.08	1.15±0.08	3.62±0.16	4.26±0.14	2.81±0.02	20.488±0.66 (29)	35.681±1.66 (29)
	>8 to 14 kg	96	1389±0.05	1268±0.04	8.934^{b}±0.27	1.229^{a}±0.07	1.051±0.06	3.75±0.15	4.25±0.11	2.82±0.02	20.704±0.57 (48)	33.228±0.96 (48)
	>14 kg	54	1451±0.06	1230±0.05	9.948^{ab}±0.44	1.006^{b}±0.07	1.149±0.08	3.99±0.20	4.36±0.16	2.79±0.02	19.40±0.85 (27)	31.160±0.83 (27)

The least squares means with same superscripts do not differ significantly. LS, Lactation stage; TDMY, Test day milk yield; mg/l, milligram per litre; kg, kilogram; meq/l, millie equivalent per litre. *Since for Na and K effect of month of collection was not investigated, hence their number of observation for factors included in model are given in parentheses along with least squares means whereas for all other traits number of observation were same as mentioned in column 3.

of crossbred cattle: The least squares analysis of variance (mean squares only) of milk Ca, P, Fe, Cu, Mn, Zn, Na, K, fat and protein in crossbred cattle are presented in Table 2.

Effect of month of sample collection on milk mineral and constituent traits: The concentration of P, Fe, Mn, Zn and protein in crossbred cattle were higher in March month whereas Ca, Cu and Fat were higher in December month (Table 2). The effect of month was not investigated on milk Na and K concentration because these minerals were estimated only once in month of December. Nantapo and Muchenje (2013) reported that milk in spring had significantly lower concentrations of zinc, copper, sodium, magnesium, aluminium, boron, iron and manganese but more abundant phosphorus than milk in winter in Friesian, Jersey and crossbred (Friesian×Jersey) cows. Rodriguez *et al.* (2001) reported that the mean contents of Fe and Cu in cow milk from Canary Island remained approximately constant during the year. They found that Zn in milk was lower during autumn than during winter and spring. Lowest fat% (3.33±0.03) in milk was reported by the Mishra and Joshi (2004) from Karan Fries cows during the rainy season.

Effect of parity on milk mineral and constituent traits: The effect of parity was highly significant only on Mn, whereas for Ca, P, Fe, Cu, Zn, K, Na, fat and protein it was non-significant (Table 2). The Mn content remained low in 1st and 2nd parity after that it increased up to 4th parity and again decreased thereafter in crossbred cows. The concentration of Zn increased with lactation order. Lal and Narayan (1984) reported that milk fat (4.9±0.02%) and SNF (9.1±0.01%) contents attained a peak value in the third lactations and declined thereafter to the minimum (4.8±0.06% fat and 9.0±0.05 SNF) in the 10th parity of Sahiwal cows, whereas, it was observed that in Tharparkar, Red Sindhi and Karan swiss cow parity had no significant effect on fat percentage. Mishra and Joshi (2004) reported that the milk constituents had higher value in 1st parity in Karan-Fries cows. According to Toffanin *et al.* (2015b), there is reduction of minerals, mainly Ca and P, has been detected across parities in HF cows.

Effect of lactation stage on milk mineral and constituent traits: The effect of lactation stage (LS) was significant on Fe and Cu and highly significant on Na, whereas it was non-significant for Ca, P, Mn, Zn, K, fat and protein (Table 2). The Cu content was high in 1st lactation stage, then decreased in 2nd and 3rd lactation stage in crossbred cattle, whereas, Fe and Na reported an opposite trend. Toffanin *et al.* (2015b) reported Ca, P and Mg contents are high at the beginning of lactation, then decrease until 6 to 8 weeks and increase thereafter in HF cows. Although some researcher (Haug *et al.* 2007, Van Hulzen *et al.* 2009, Zamberlin *et al.* 2012) found different results. The potassium content is low in colostrum and it increases gradually in milk (Zamberlin *et al.* 2012). However, some studies found a different trend (Gaucheron 2005, Van Hulzen *et al.* 2009). Sodium concentration was high in colostrum and at the end of lactation in Holstein cows (Kume *et al.* 1998, Gaucheron *et al.* 2005, Zamberlin *et al.* 2012). Sarkar *et al.* (2006) estimated significant effect of lactation stage for protein, SNF, lactose, total solid and casein% increases in the later stages but not fat% in Tharparkar cows.

Effect of TDMY on milk mineral and constituent traits: Effect of TDMY was significant on Fe and Cu, whereas its effect was non-significant on Ca, P, Mn, Zn, K, Na, fat and protein percentage. The content of Fe was low in cows yielding 8 to 14 kg milk per day, whereas, it increased in low and high milk producer cows. But, the content was high in cows yielding 8 to 14 kg milk yield, whereas, it decreased in low and high milk producer cows.

Heritability of milk minerals and constituent traits in crossbred cattle: A low estimate heritability was observed for milk Cu (0.044±0.14), Zn (0.017±0.13), Na (0.096±0.29) and fat (0.097±0.16); however, for milk, Ca (0.126±0.19), Fe (0.218±0.21) and Mn (0.246±0.22) had a moderate estimate of heritability. The heritability of K could not be estimated because of failure of matrix inversion during least squares analysis. The protein percentage had highest heritability (0.392±0.27) because of its narrow range of variability without any selection for protein percentage. The trait having a wider range of variability are expected to have lower heritability which may be a reason for moderate to low heritability for milk minerals. Hence, till a few generation individual selection is not implemented under uniform managemental conditions, it is quite difficult to narrow down the existing wider range of individual minerals in milk so that its heritability could be improved. The milk

Table 3. Product moment correlation among various milk minerals and constituents in crossbred cattle

	P	Fe	Cu	Mn	Zn	Fat	Protein	Na	K
Ca	0.177*	0.108	0.071	0.113	-0.090	-0.080	-0.041	-0.050	-0.057
P		0.156*	-0.083	0.133	0.189*	-0.080	-0.039	0.060	-0.008
Fe			-0.231**	0.301**	0.414**	-0.040	0.028	0.090	0.103
Cu				0.011	-0.347**	-0.010	-0.091	0.020	-0.117
Mn					0.162*	-0.080	0.042	-0.125	-0.304**
Zn						0.020	0.062	0.160	-0.075
Fat							-0.082	0.010	-0.071
Protein								-0.010	0.041
Na									0.189

**Significant at 1% level; *Significant at 5% level fat percentage is a prime targeted trait in selection programme in tropical country like India which consequently reduced its additive genetic variance and heritability. The lowest heritability value for fat (0.11±0.15) had been reported in Hariana cows (Kaushik and Tandon 1979). But highest heritability of fat (0.74%) had been reported in Black and white cows in the Netherlands (Meinert *et al.* 1989). The heritability estimates ranged from 0.13 to 0.29 for fat, protein and SNF in KF (Mishra and Joshi 2004). Soyeurt *et al.* (2008) estimated heritability of 0.47 for P and 0.42 for Ca, while Toffanin *et al.* (2015b) reported lower heritability of 0.12 for P and 0.10 for Ca in HF cows. Buitenhuis *et al.* (2015) found high heritability in Danish Holstein (DH) cattle for Ca (0.72), Zn (0.49), and P (0.46), while for Danish Jersey (DJ), the heritability was high for Ca (0.63), Zn (0.57), and Mg (0.57). Furthermore, intermediate heritability was found for Cu in DH, and for K, Na, P and Se in the Danish Jersey.

Product moment correlation among milk fat, protein and mineral in crossbred cattle: The product moment correlation among milk fat, protein, Ca, P, Fe, Cu, Mn, Zn, Na and K are presented in Table 3. Product moment correlation ranged from -0.090 (Zn) to 0.177 (P) with Ca, -0.083 (Cu) to 0.189 (Zn) with P, -0.231 (Cu) to 0.414 (Zn) with Fe, -0.347 (Zn) to 0.071 (Ca) with Cu, -0.304 (K) to 0.301 (Fe) with Mn, -0.347 (Zn) to 0.414 (Fe) with Zn, -0.287 (TDMY) to 0.189 (K) with Na, -0.304 (Mn) to 0.189 (Na) with K, -0.082 (protein) to 0.020 (Zn) with Fat and -0.091 (Cu) to 0.062 (Zn) with protein. Toffain *et al.* (2015a) reported that correlation of protein with Ca, Mg and P were shown because these minerals are bound with milk protein (Casein micelles). Tongato (2015) reported a moderate pearson correlation of Ca with K (0.55) and Na (0.42), and strong pearson correlation with Mg (0.81) and P (0.77); however, K was moderately correlated with Mg (0.52), P (0.56) and Na (0.35), whereas, P and Na were moderately correlated (0.30) in milk of four cattle breeds (HF, BS, Simmental and Alpine Grey).

The effect of bovine breed type was observed to be highly significant on milk constituent traits. Where, Murrah buffaloes and Tharparkar cattle had significantly better mineral profile over crossbred cattle. This study showed the superiority of indigenous animal over the crossbred in respect to milk mineral profile. It is nation's responsibility to cease cross breeding programmes and protect the purity of desi breeds. The majority of non-genetic factors under investigation (other than month of collection) had no significant effect on milk mineral constituents, which suggested role of genetics as a major source of variation in milk minerals. Among milk minerals, the estimates of heritability for Fe, Ca and Mn were moderate to high whereas for other minerals it was low. However, it needs to be investigated on large population.

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