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ORIGINAL ARTICLE

Quantification of minerals and trace elements in raw caprine milk using flame atomic absorption spectrophotometry and flame photometry

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Abstract This study reports minerals and trace elements quantification in raw caprine milk of Beetal breed, reared in Northern India and their feed, fodder & water using flame atomic absorption spectrophotometry and flame photometry. The mineral and trace elements' concentration in the milk was in the order: K > Ca > Na > Fe > Zn > Cu. The results showed that minerals concentration in caprine milk was lesser than reference values. But trace elements concentration (Fe and Zn) was higher than reference values. Multivariate statistical techniques, viz., Pearsons' correlation, Cluster analysis (CA) and Principal component analysis (PCA) were applied to analyze the interdependences within studied variables in caprine milk. Significantly positive correlations were observed between Fe - Zn, Zn - K, Ca - Na and Ca - pH. The results of correlation matrix were further supported by Cluster analysis and Principal component analysis as primary cluster pairs were found for Ca – pH, Ca – Na and Fe – Zn in the raw milk. No correlation was found between mineral & trace elements content of the milk and feed.

Keywords Minerals · Trace elements · Caprine milk · Atomic absorption spectrophotometry Flame photometry · Principal component analysis (PCA)

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Introduction

Milk is a basic component of human diet which contains protein, minerals and trace elements essential for growth and maintenance of human life. The minerals (Na, K, Ca, I etc.) and trace elements (Fe, Zn, Cu etc.) are in chemical equilibrium with free ions, protein, lipids, carbohydrates, citrate and amino acids and present in higher quantities in milk than any other dairy product (Vegarud et al. 2000). Trace elements and minerals significantly alter the quality and nutritional values of milk. Intrinsic factors such as species of animal, lactation stage, fat content, health status, maternal age and parity determine the concentration of minerals and trace elements and thus quality of milk (Gallenberg and Vodicnik 1989). Extrinsic factors including exposure of the animals to contaminants which in turn depends upon feed of animal and locality of farm also effects the milk quality. Therefore, milk's trace elements and minerals concentrations could be an important "direct indicator" of the hygienic status of the milk and its derived products.

Resilience to extreme climatic conditions and capricious environments makes the goat an important dairy animal and is the main supplier of dairy and meat products for rural people (Silanikove 2000). World goat population with 920.6 million heads contribute 2.1 % to the world milk production (FAO 2010). Goat milk and its products play important role in human nutrition by feeding malnourished people and treating people affected with cow milk protein allergies and gastrointestinal disorders (Walker 1964; Haenlein 2004). Goat milk is superior than bovine milk due to its antallergic, therapeutic, higher digesting and buffering properties compared to bovine milk (Haenlein 2004).

Some studies have been undertaken on nutritional status of goat milk (Table 1) in yester years. But no data is available on the minerals and trace elements content in the milk of Beetal breed lactating goats in Northern India. Keeping this in view,

Table 1 Minerals and trace elements in raw milk of goat as reported in literature

Minerals (mg L^{-1})			Trace elements (mg L^{-1})			References
Ca	Na	K	Fe	Cu	Zn	
1,200	500	1,500	0.40	0.20	4.0	Underwood 1981; Miles et al. 2001
701	454	418	0.480	0.30	3.46	Khan et al. 2006
961	309	543	0.320	0.39	2.46	Khan et al. 2006
1,340	510	1,240	0.70	0.18	3.20	Garcia et al. 2006
1,342	433	409	3.88	0.48	4.68	Guler 2007
1,620	150	1,690	0.36	0.36	5.47	Mestawet et al. 2012
805	–	–	0.56	–	2.13	Slačanac et al. 2011
344.8	118	355.1	9.07	<0.025	5.10	Present study

present study was undertaken to quantify minerals and trace elements in the milk of Beetal breed lactating goats reared in Northern India.

Material and methods

Thirty one raw goat milk samples were collected from thirty one apparently healthy lactating goats of indigenous Beetal breed from central sheep breeding farm, Hisar, India. These goats were housed in a loose barn system with adlibitum water and fodder (*Sesbania bispinosa*). 250 g of concentrate mixture (79 % maize, 21 % groundnut cake and 1 % salt) and *Avena sativa* grains mixed in 3:1 ratio was provided to the animals just before milking. Milking of animals is performed twice a day at an interval of 12 h. Goats of the Beetal breed for the study were selected based upon their comparable milk yield (1.5–2.0 kg) and similar lactation stage (post calving) and age varying 2 to 6 years. Fifty ml of milk sample was collected from each animal in morning milking shift in pre-cleaned sterilized polypropylene tubes, labelled, immediately transported to laboratory on ice packs and stored at −20 °C until analysis.

Fodder samples (*Sesbania bispinosa*) were cut above a height of 4 cm from ground to minimise soil adherence and to represent the portion normally used as fodder by goats. Four samples including leaves and shoot parts (≈1.0 kg) were collected from the agricultural fields of the central sheep breeding farm, Hisar, India in perforated polythene bags and taken to the laboratory. Similarly, four concentrate feed, *Avena sativa* grain samples (≈250 g) were collected from the central sheep breeding farm, Hisar, India, packed in plastic bags and taken to the laboratory for further analysis. Collected feed and fodder samples were dried in drying oven at a temperature of 80 °C until a constant weight of the sample was achieved. Dried samples were ashed in a muffle furnace at 350–400 °C, homogenized, digested (0.2 g) in microwave digester (MarsX, CEM USA) using 5.0 ml diacid (HNO_3 and $HClO_4$ ratio of *9:1*) mixture and then made final desired volume. Four samples of the water fed to the goats were also collected and analyzed for their mineral and trace elements content.

For the mineralization of milk samples, closed microwave assisted wet digestion method was used. Two millilitre of each milk sample taken in Teflon digestion vessels (XP-1500 Plus) was mixed with six millilitre HNO_3 (≈71 %). The vessels were kept open overnight to allow the initial reactions to be subsided. This mixture was digested using microwave digester. The operating programme for the closed microwave digestion system consists of two stages. Each digestion stage was optimized at power of 800 W ramped for 20 min. to a temperature of 170 °C with 8 digestion vessels after which the samples were held at the same temperature for 45 min. When digestion was complete, vessels were cooled for 2–3 h at room temperature for internal pressure to be vented. After this, vessels were

Table 2 Instrumental (FAAS) operational conditions for analysis of selected trace elements in goat milk

	Flame chemistry	Background correction	Wavelength (nm)	Slit width (nm)	Gas flow (L min^{-1})	Lamp current (mA)	Detection limits (mg L^{-1})
Zn	Air-Acetylene-Stoichiometric	D_2- Lamp	213.8	0.2	7.5	8	0.01
Cu	Air-Acetylene-Stoichiometric	D_2- Lamp	324.7	0.7	1.8	6	0.025
Fe	Air-Acetylene-Stoichiometric	D_2- Lamp	248.3	0.2	2.2	12	0.45

Table 3 Mineral and trace element concentrations in the Beetal breed caprine milk (n=31)

	Elemental and Physico-chemical properties of raw milk	Minimum	Maximum	Mean	± S. D
Physico-chemical properties	pH	6.1	6.9	6.6	0.2
	Ec (μS cm^{-1})	5.2	8.2	6.8	0.7
	Density (kg / dm^3)	1.027	1.030	1.028	0.001
Minerals	K (mg L^{-1})	259.5	423.0	355.1	32.1
	Ca (mg L^{-1})	307.5	433.5	344.8	30.8
	Na (mg L^{-1})	87.0	238.5	118.9	30.7
Trace elements	Fe (mg L^{-1})	1.5	28.5	9.1	5.5
	Zn (mg L^{-1})	0.6	8.4	5.1	1.7
	Cu (mg L^{-1})	< 0.025	–	–	–

uncapped and digests were diluted to 25 ml using double distilled water and filtered. Extracted solutions were stored in polypropylene bottles and refrigerated until analysis.

Raw milk pH, electrical conductivity and density were determined immediately after receiving the samples in the laboratory using pH meter (Eutech), EC meter (Elico-CM183) and Pycnometer, respectively. Trace elements (Fe, Cu and Zn) in digested milk, feed, fodder and water samples were quantified in triplicate using Flame Atomic Absorption Spectrometer (GBC, model SensAA) and minerals (Na, K and Ca) using Flame photometer (Elico-CL378). Detailed Instrumental analytical conditions for analyses of selected trace elements are given in Table 2. Analytical quality of the results was assured by subtracting the concentration of elements in blank from that of sample and concentrations were finally expressed in mg L^{-1}.

The bivariate correlation analysis with Pearson's correlation coefficient (r) at two-tailed significance level (p), Principal component analysis and Cluster analysis were applied to the data using SPSS software package (version 16.0).

Results and discussion

Among various body fluids milks' mineral profile can be related to nutritional supply and thus used as an important tool in determining the deficiency or toxicity states of dairy animals including goats (Greppi et al. 1995). Beetal breed raw milk samples analyzed for their mineral and trace element content and the results are encapsulated in Table 3. pH of the caprine milk was slightly acidic (6.1) to neutral (6.9) and with a mean value of 6.6±0.14 (Table 3). Guler (2007) have also reported the pH of milk of goats reared in Eastern Mediterranean as 6.7±0.09. Electrical conductivity of the milk samples ranged between 5.2 and 8.2 μS cm^{-1} (Table 3). Variation in electrical conductivity may be due to change in Na, K and Cl content of milk that can very due to a several factors such as nutrition, age, breed, parity, milking interval and milk composition besides with mastitis (Norberg 2005). Density of the milk samples showed a narrow range of variation from 1.027 to 1.030 kg /dm^3 with a mean value of 1.028 kg /dm^3.

Calcium content of milk samples varied from 307.5 to 433 mgL^{-1}. A perusal of data indicated that calcium content in milk samples were lesser than caprine milk reference value of 1,200 mg L^{-1} suggested by Underwood (1981). A comparison of calcium content in goat milk in the present study with available literature showed that it was lower than that reported by Khan et al. (2006) (701 mg L^{-1}), Guler (2007) (1,342 mg L^{-1}) and higher than that reported by Garcia et al. (2006) (340 mg L^{-1}). The sodium content of milk samples varied from 87 to 238 mg L^{-1} and was lesser than the reference value

Table 4 Mineral and trace element concentrations in feed, fodder and water fed to the Beetal breed goats in the study

Feed /Fodder	No. of samples analyzed (n)		K	Ca	Na	Fe	Zn	Cu
Concentrate feed (mg kg^{-1})	4	Mean	257.6	187.9	268.1	123.7	12.6	3.7
		± SD	1.6	2.6	6.0	4.4	0.9	0.3
Avena sativa grain (mg kg^{-1})	4	Mean	465.7	114.9	32.0	162.1	22.6	3.1
		± SD	7.2	5.6	0.5	5.4	1.1	0.3
Sesbania bispinosa (mg kg^{-1})	4	Mean	766.6	546.9	19.5	59.0	14.5	4.8
		± SD	4.8	3.3	0.7	1.2	0.6	0.5
Water (mg L^{-1})	4	Mean	1.1	19.2	2.3	<0.05	<0.01	<0.05
		± SD	0.1	0.1	0.1			

Table 5 Pearson's correlation among minerals and trace elements content present in caprine milk, feed, fodder and water fed to them

	K_M	Ca_M	Na_M	Fe_M	Zn_M	Density
K_F	0.333	0.198	0.049	0.622	0.454	−0.01
Ca_F	0.106	0.338	0.208	0.502	0.224	−0.008
Na_F	−0.461	0.019	0.139	−0.536	−0.547	−0.002
Fe_F	−0.043	−0.316	−0.205	−0.396	−0.119	0.012
Zn_F	0.316	−0.306	−0.303	0.035	0.283	−0.069
Cu_F	−0.079	0.312	0.232	0.279	0.08	−0.048

[M] = stands for milk

[F] = stands for feed

of 500 mg L^{-1} (Underwood 1981). Sodium content in goat milk was four times lesser than reported by Khan et al. (2006), Garcia et al. (2006) and Mestawet et al. (2012) in different goat species (Table 1). Average potassium concentration (355.1±31.2 mg L^{-1}) of goat milk samples was significantly lesser than the reference value of 1,500 mg L^{-1} (Underwood 1981) and varied from 259 to 423 mg L^{-1} (Table 3). The potassium content of caprine milk was lesser than as reported by others (Table 1). The variations in mineral content of goat milk may be attributed to breed of the animal, its lactation stage (Morand-Fehr et al. 2007) and feed (Haenlein and Anke 2011).

The major elements in the goat milk are higher than cow milk and several times higher than human milk (Zamberlin et al. 2012). Iron is an important trace element, necessary for catalase activity, constituent of haemoglobin and myoglobin (important in cellular respiration). Iron concentration in the milk ranged from 1.5 to 28.5 mg L^{-1} with an average value of 9.1±5.5 mg L^{-1} which is significantly higher than the reference value of 0.40 mg L^{-1} (Miles et al. 2001). Guler (2007) has also reported significantly higher iron content in goat milk. A comparison of the data (Table 1) showed that iron content in caprine milk was comparatively higher in this study. Soil ingestion by grazing animals may significantly influence the iron status in them (Lopez-Alonso 2012). Higher concentrations of iron in milk may deteriorate the quality of dairy products due to its catalytic effect on oxidation of lipids, resulting in development of unpleasant smell (Lant et al. 2006).

Copper is an essential element plays crucial role in oxidation-reduction reactions. Although ruminants commonly encounter copper deficiencies but supraoptimal concentration of this element may be found especially in sheep (Puls 1994). Copper content in all milk samples was below detectable limit (<0.025 mg L^{-1}) (Table 3). The copper content in goat milk reported by various authors is highly variable. 0.18 mg L^{-1} copper concentration in goat milk was reported by Garcia et al. (2006) whereas some other researchers have reported 0.36–0.48 mg L^{-1} of copper content in goat milk (Table 1).

Zn is used as a co-factor in several enzyme systems (Virgil and Melvin 1970) and necessary for growth and development, its deficiency may result growth retardation by reduced enzymatic activity, altered testicles, excessive salivation and anorexia (Haenlein 1980). Zinc concentration in the milk varied from 0.6 to 8.4 mg L^{-1}. The result showed that zinc content in most of the milk samples was higher than the reference value of 4.0 mg L^{-1} (Underwood 1981). Mestawet et al. (2012) have reported 5.47 mg L^{-1} zinc content in the milk of Nubian goats. Whereas, 2.13 mg L^{-1} zinc content in the milk of Croatian goats has been reported by Slačanac et al. (2011). As compared to minerals, milk trace elements zinc and iron showed marked variation in their concentration even though the lactating goats were retained on similar feeding and housing habits. Milan and Korenovska (2010) explained the variation in milk properties due to different retention time and mineral absorption of feed in the digestive system of the animals reared under identical environmental and husbandry conditions. Variation in trace elements content in different milk samples may also be attributed to several other factors affecting mineral requirements by the animal which depends upon to speciation of the element, its interrelationships with other nutrients, mineral intake by the animal, animal adaptation, age

Table 6 Pearson's correlation among minerals and trace elements present in Beetal breed caprine milk

	pH	EC	K	Ca	Na	Fe	Zn	Density
pH	1							
EC	−0.48**	1						
K	0.13	−0.08	1					
Ca	0.36*	−0.23	−0.15	1				
Na	0.14	−0.18	−0.43*	0.88**	1			
Fe	0.08	−0.14	0.19	0.28	0.28	1		
Zn	0.04	−0.02	0.37*	−0.12	−0.17	0.37*	1	
Density	0.31	−0.11	−0.043	−0.01	−0.11	−0.25	0.07	1

*. Correlation is significant at the 0.05 level (2-tailed)

**. Correlation is significant at the 0.01 level (2-tailed)

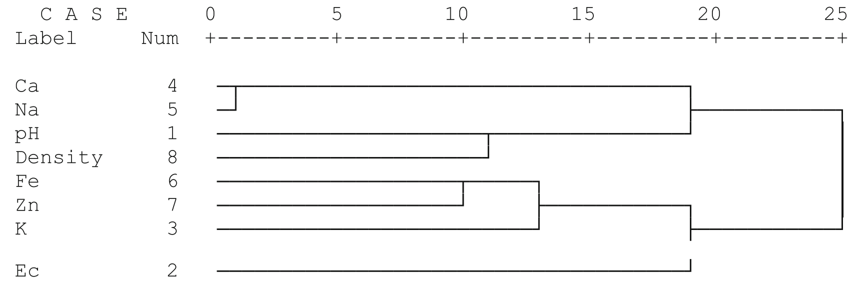

Fig. 1 Dendrogram of the raw milks' minerals and trace elements using Complete Linkage method

and genetic make-up of the animal (Field 1984; McDowell 1985). The mineral and trace elements profiling of the feed, fodder and water fed to the goats was also done and results are given in Table 4. In the present study, no correlation among minerals and trace elements content in milk, feed, fodder and water were observed (Table 5).

Inter elemental correlation, cluster analysis (CA) and principal component analysis (PCA)

Pearson's correlation technique was applied to elemental and physico-chemical properties of the milk to explore the inter-dependence among them and the results of correlation matrix are given in Table 6. The results showed that calcium was positively and significantly correlated with Na (r=0.88*; P<0.05) and pH (r=0.36*; P<0.05). Zinc was positively correlated with K (r=0.37*; P<0.05) and Fe (r=0.37*; P<0.05). Significantly positive correlation between Fe–Zn in milk was also reported by Belewu and Aiyegbusi (2002) and Garcia et al. (2006).

An observational data set of variables selected for the analysis can be grouped together based on similarities by Cluster analysis technique (Coker et al. 2005). In order to find out the natural groupings within the data set, hierarchical cluster analysis of samples using complete linkage method with Pearson correlation interval was performed. At a distance of about 5, in the milk samples were found three primary clusters between Ca – pH, Ca – Na and Fe – Zn (Fig. 1). These primary clusters in the goat milk, strengthen the findings of correlation matrix. Cluster pair between minerals (Ca and Na) and trace elements (Fe and Zn) suggested that they have same origin due to which they form primary cluster pairs.

Principal component analysis (PCA), a multivariate statistical technique allows simplified graphical representations, observation and interpretation of information. PCA was applied to analyse the inter-dependence among studied parameters and for qualitative evaluation of their clustering behaviour and the results are given in Table 7. Three factors having a total variance of 70. 31 % were obtained. Factor-1 contributed 27.96 % to the total variance with a high loading on Ca (r= 0.88), Na (r=0.94) purely supporting Ca – Na primary cluster pair. Factor-2 contributed 21.3 % to the total variance with a high loading on Zn (r=0.76) and Fe (r=0.69) and K (r=0.72), supporting Fe - Zn and Zn - K cluster pairs. Factor −3 with a high loading on pH (r=0.82) and Ca (r=0.26) supported pH - Ca cluster pair and contributed 20.96 % to the total variance.

Similarly the factor loadings for PCA applied to the Slovakian cows' and goats' cheese elemental data set was reported by Milan and Korenovska (2010). Three principal components explaining a total variance of 55.7 % were found loaded with Na and Mg (Factor - 1), Cr and V (Factor - 2) and

Table 7 Factor loading for selected elements in Beetal breed caprine milk

Elemental and Physico-chemical properties of raw milk	Factor 1	Factor 2	Factor 3
pH	0.21	0.12	**0.83**
EC	−0.25	−0.17	−0.66
K	−0.32	**0.72**	0.15
Ca	**0.89**[a]	−0.07	**0.25**
Na	**0.94**	−0.21	0.04
Fe	0.51	**0.69**	−0.13
Zn	−0.12	**0.76**	0.05
Density	−0.28	−0.22	0.18
Total	2.24	1.71	1.68
% of Variance	27.96	21.39	20.97
Cumulative %	27.96	49.35	70.32

[a] = Values of dominant elements/ property in each factor are indicated bold

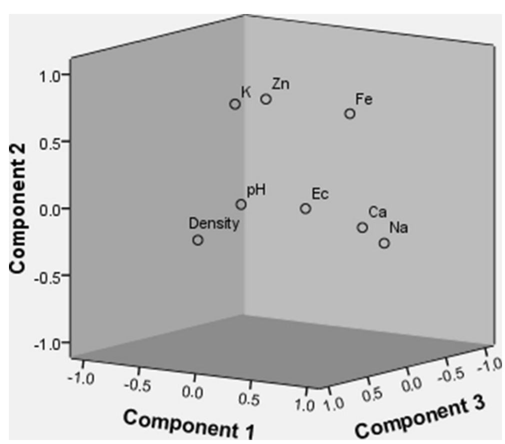

Fig. 2 Component plot of the factor loadings of raw milks' analyzed parameters in three dimensional space

Ca and K (Factor - 3). Five principal components, accounting for 64 % of the total variance indicating associations among minerals, trace elements, proteins and fat in raw cow milk were reported by Larranaga and Blasco (2009). A component plot of loadings with Varimax-Kaiser normalization showed the associations among studied minerals, trace elements and physico-chemical parameters (Fig. 2) of caprine raw milk and allowed in marked visualization of the discriminating efficiency of the principal components.

Conclusions

The results of the present study showed that mean concentration of minerals was several - fold lesser and that of trace elements (Fe and Zn) was higher than reference values in the goat milk in study area. The elemental content of caprine milk varied within the same breed even though the animals were kept on similar feeding and housing habits. These differences in the examined milk properties may be attributed to differences in metabolic background of the animals. Cluster analysis indicated that the minerals (Ca and Na) and trace elements (Fe and Zn) in the milk were having the same origin.

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Conflict of Interest The authors declare that there are no potential conflicts of interest.

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