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Mineral Profiles in Blood and Milk of Sheep

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Abstract: ***The present study was carried out to assess the mineral composition of blood and milk of sheep grazing in similar pasture. The study focused on levels of macrominerals (Ca, P, Mg, Na, K, and Cl) and microminerals (Cu, Co, Fe, Mn and Zn) in blood plasma and milk of ewes, Inter-relationship of minerals in blood plasma and milk and comparing the mineral content in blood and milk of sheep. The findings revealed that the macro and micro minerals were within normal physiological limits, but calcium, phosphorus, magnesium, zinc and potassium levels were lower and sodium and chloride levels were higher in the blood of sheep as compared to milk.***

Keywords: macro-mineral, micro-mineral, blood plasma, milk, sheep

1. Introduction

Indigenous sheep (*Ovis aries*) and goat (*Capra hircus*) are two distinct species in the family Bovidae. They were among the first to be domesticated. Sheep for wool and meat and the goat for milk, meat and fiber. According to FAO (Food and Agriculture Organization of United Nations) stats 2013, the world's total sheep and goat population is 2.17 billion, consisting sheep of 1.17 billion and goat of 1.00 billion. India has a total population of sheep and goat of 237.5 million includes sheep 75.5 million and goat of 162 million. Average wool production of Indian sheep is as low as 0.9 kg against 2.4 kg of the world average. As such, 50 million kg wool is imported per year to meet the wool requirement. Similarly, carcass yield of sheep is also low, i.e. 12 kg as against 17 kg of the world average, although 32 to 36 per cent sheep are slaughtered annually, which is similar to the world average of sheep slaughter (Anonymous, 2006).

In nature all the members of animal and plant kingdom require inorganic elements: minerals, as these are needed for their survival and efficient performance. Twenty two mineral elements are believed to be "essential" for the members of the animal kingdom. These comprise seven major or macromineral viz., Calcium (Ca), Phosphorus (P), Sodium (Na), Potassium (K), Chlorine (Cl), Magnesium (Mg) and Sulphur (S) and fifteen trace or micro minerals. These mineral elements exist in the cells and tissues of the animal in specific concentrations, which is essential for the normal growth, health and productivity of the animal. The three important functions of minerals are (i) Structural components of body, organ and tissues. (ii) Constituents of body fluid and tissues as electrolytes concern with maintenance of osmotic pressure, acid-base balance, membrane permeability and tissue irritability. (iii) Catalysts in enzyme and hormonal system as an integral and specific component of structure of metallic-enzyme or as activators within those systems (Underwood 1981).

Nutritional disorders, including deficiencies, toxicities and imbalance are severely inhibiting grazing livestock in developing countries and have more significant consequences than infectious disease (McDowell, 1985). Infertility has been commonly recorded in copper and manganese deficiencies resulting in depressed or delayed oestrous and poor conception rate in cows (Underwood, 1977). Similarly, zinc has been shown to be important for gonadal growth in rats, lambs and is responsible for infertility in ewes (Underwood, 1981). Role of trace minerals, particularly copper and zinc in maintaining reproductive rhythm is well documented, as they are specific activators of enzymes inthe reproductive system(McDowell *et al*. 1984). Milk is a rich source of Calcium, Phosphorus, Potassium, Chloride and Zinc, milk yield is affected by low dietary levels of Calcium, Phosphorous, Potassium, Sodium and Iron (Underwood, 1981). However, the concentration of these minerals in milk is little affected by dietary intake (Miller, 1979; Underwood, 1981). Milk composition can be related to nutritional supply and milk mineral profile can be an appropriate tool to evaluate trace mineral nutritional status of dairy sheep and goats (Greppi *et al*., 1995). Whole blood or blood serum or plasma is more widely employed in studies of mineral nutrition than any other tissue or fluid of the body because of its invariablereflex in some aspects of its composition, especially the mineral status of animals (Underwood, 1981). Much less information is known about the status of mineral content in blood and milk of sheep. Hence, the present investigation was undertaken to assess the mineral composition of blood and milk of sheep grazing in similar pasture, with the objective to study the levels of macrominerals (Calcium, Phosphorus, Magnesium, Sodium, Potassium and Chloride) and Microminerals (Copper, Cobalt, Iron, Manganese and Zinc) in blood plasma and milk of lactating ewes and their interrelationship.

2. Materials and Methods

2.1. Experimental Animals

Deccani sheep maintained at the farm of College of Veterinary and Animal Sciences, Parbhani were used for the present research work. Lactating Ewes: Seven ewes in lactation, which were on stage of 5^{th} to20^{th} day of lambing.

2.2. Management and Feeding of Animals:

All the selected animals were maintained under free range system and let loose for grazing in the field from morning 8.00 a.m. to 2.00 p.m. The animals had free access to drinking water and not provided with any additional feed and fodder. The animals were dewormed regularly and confirmed to be free from mastitis.

2.3. Collection and Processing of Blood and Milk Samples

Blood and milk samples were collected in the morning hours before the animals were let loose for grazing. From each animal about 10 ml of blood sample was collected by Jugular venipuncture in two sterile glass test tubes, one containing anticoagulant and other without anticoagulant. Milk samples were collected in 50 ml capacity sterile plastic containers with lid. Serum was separated from blood samples within 4-5 hours of collection. Whole blood samples were centrifuged at 3000 rpm for 15 minutes in centrifuge tubes and plasma was collected, serum and plasma were stored at -20^{o}C until further analysis.

Whey preparation:Two ml 1% citric acid (W/V) was added drop by drop to 5 ml of milk in a beaker so that the milk was completely coagulated. The contents were then filtered through qualitative grade filter paper.

2.4. Analytical Techniques

2.4.1. Spectrophotometry

Spectrophotometry (Systronics Semi-Autoanalyser, Model No. 635, Systronics, Mumbai) was used to read the absorbance of colour solution and for estimation of Calcium and phosphorus of blood serum and milk (whey). Serum and milk (whey) calcium were estimated by using the O - CPC method of Gitelman (1967) and Berthelot (1973). The absorbance of final coloured complex is directly proportional to calcium concentration and is read at 570nm and the calibration was done using working standards containing 0.5, 1.0, 1.5 and 2.0 µg of calcium. Serum and milk (whey) Phosphoruswere estimated by using Molybdate method of Tindler (1969), Morin (1973) and Munoz (1981). The absorbance of the Phosphomolybdate complex is directly proportional to the phosphorus concentration and measured at 340 nm and the calibration was done using working standards containing 0.25, 0.5, 0.75 and 1.0 µg of Phosphorus.

2.4.2. Atomic Absorption Spectrophotometry (AAS)

Atomic Absorption Spectrophotometer (AAS, Model No. 4129, ECIL, Hyderabad) was used to estimate Magnesium (Mg), Copper (Cu), Cobalt (Co), Zinc (Zn), Manganese (Mn) and Iron (Fe) in blood plasma and milk as per the procedure described by Hilliyard and Smith (1979). The absorbance of Cu, Co, Zn, Mg, Mn and Fe were measured at 324.8, 240.7, 213.9, 285.2, 279.5 and 248.3 nm. The calibration was done using working standards for Cu, Zn and Mg containing 0.05, 0.1, 0.2, 0.3, 0.4, and 0.5 ppm, for Co containing 0.1, 0.2, 0.3, 0.4 and 0.5 ppm, however, for Fe containing 0.5, 1.0, 2.0, 3.0, 4.0 and 5.0 ppm respectively.

2.4.3. Flame Emission Photometry

Flame Emission Photometer (Model MKII, Systronics, Mumbai) was used for estimation of Na and K in blood serum and milk (whey) samples. Flame photometer had the provision of estimating one element at a time. After igniting the flame the fuel gas and compressor pressure were suitably adjusted so as to give sharp blue flame over the burner. Appropriate filter for the element was set. The sensitivity of the detector was adjusted so that it could give zero reading with distilled water feeding and 100 reading with highest working standard.

2.4.4. Titration

Chloride in blood serum and milk (whey) was estimated by titration method of Schales and Schales as described by Oscer (1965).

2.5. Statistical Analysis

The data obtained were statistically analyzed by applying students paired "t" test (Snedecor and Conkhran, 1967).

3. Results and Discussion

Table 2: Macrominerals and Microminerals in blood of lactating Deccani ewes

Name of the Macrominerals	*Mean ± SE (Range)*	*Name of the Microminerals*	*Mean ± SE (Range)*
Calcium (S) (mg/dl)	9.64 ± 0.30 (7.94 – 10.34)	**Copper (P)** (ppm)	0.77 ± 0.11 (0.25-1.18)
Phosphorus (S) (mg/dl)	4.39 ± 0.31 (3.08 – 5.65)	**Cobalt (P)** (ppm)	0.06 ± 0.00 (0.03-0.09)
Magnesium (P) (mg/dl)	1.37 ± 0.20 (0.525 – 2.65)	**Zinc (P)** (ppm)	1.30 ± 0.14 (1.0-2.1)
Sodium (S) (mEq/l)	141.14 ± 4.11 (125 – 152)	**Manganese (P)** (ppm)	0.71 ± 0.34 (0.12-2.68)
Potassium (S) (mEq/l)	4.02 ± 0.21 (3.0 – 4.8)	**Iron (P)** (ppm)	1.50 ± 0.31 (0.18-2.7)
Chloride (S) (mEq/l)	102.34 ± 2.87 (93.3 – 116.6)		

S - Blood serum **P** - Blood plasma Ratio - Ca: P: Mg = 7.03:3.20:1.00

Table 3: Macrominerals and Microminerals in milk of lactating Deccani ewes

Name of the Macrominerals	*Mean ± SE (Range)*	*Name of the Microminerals*	*Mean ± SE (Range)*
Calcium (W) (mg/dl)	126.16±5.59 (114.29 – 158.72)	**Copper (M)** (ppm)	0.49 ± 0.08 (0.19-0.71)
Phosphorus (W) (mg/dl)	117.11 ± 7.12 (93.44 – 146.96)	**Cobalt (M)** (ppm)	0.09 ± 0.02 (0.02-0.15)
Magnesium (M) (mg/dl)	3.2 ± 0.16 (2.6 – 3.6)	**Zinc (M)** (ppm)	3.24 ± 0.29 (2.34-4.40)
Sodium (W) (mEq/l)	5.94 ± 0.29 (5.0 – 7.1)	**Manganese (M)** (ppm)	0.83 ± 0.17 (0.37-1.84)
Potassium (W) (mEq/l)	11.05 ± 0.95 (6.72 –12.99)	**Iron (M)** (ppm)	1.91 ± 0.40 (0.20-2.70)
Chloride (W) (mEq/l)	68.64 ± 3.3 (51.3 – 81.6)		

W - Whey **M** – Milk

Table 4: Macro and Micro minerals in Blood and Milk of lactating Deccani ewes

Name of the Macrominerals	**BLOOD Mean ± SE**	**MILK Mean ± SE**	**Name of the Microminerals**	**BLOOD Mean ± SE**	**MILK Mean ± SE**
Calcium (S,W) (mg/dl)	9.64 ± 0.30	126.16±5.59**	**Copper (P,M)** (ppm)	0.77 ± 0.11	0.49 ± 0.08
Phosphorus (S,W) (mg/dl)	4.39 ± 0.31	117.11 ± 7.12**	**Cobalt (P,M)** (ppm)	0.06 ± 0.00	0.09 ± 0.02
Magnesium (P,M) (mg/dl)	1.37 ± 0.20	3.2 ± 0.16**	**Zinc (P,M)** (ppm)	1.30 ± 0.14	3.24± 0.29*
Sodium (S,W) (mEq/l)	141.14 ± 4.11	5.94± 0.29**	**Manganese (P,M)** (ppm)	0.71 ± 0.34	0.83± 0.17
Potassium (S, W) (mEq/l)	4.02 ± 0.21	11.05± 0.95**	**Iron (P,M)** (ppm)	1.50 ± 0.31	1.91± 0.40
Chloride (S,W) (mEq/l)	102.34 ± 2.87	68.64± 3.3**			

S - Blood serum, **P** - Blood plasma, **W** - Whey, **M** – Milk. ** Significant ($P< 0.01$), * Significant ($P< 0.05$)

3.1. Macrominerals in Blood of Lactating Ewes

The range and mean values of macrominerals and microminerals in the blood of lactating ewes are shown in Table 2 respectively. Among the macrominerals the mean value of serum calcium in ewes in the present study is comparable to those mentioned by Edmundo *et al* (1982), Kelly (1984), Hooda (1992) and Sudhan *et al* (1996). However, the value is higher than those reported by Tajane *et al* (1990) and Samanta and Samanta (2002), and lower than those reported by Kaneko (1997), Karim (2000) and Sharma (2004). The mean value of serum phosphorus is comparable to those mentioned by Edmundo *et al* (1982), Tajane*et al* (1990) and Samanta and Samanta (2002). However, the value is lower than those reported by Hooda (1992), Kelly (1984), Sudhan*et al* (1996), Sharma (2004) and Kaneko (1997). The mean value of plasma magnesium is lower than those mentioned by Edmundo *et al* (1982), Kelly (1984) Hidroglou *et al* (1987), Hooda (1992), Sudhan *et al* (1996), Kaneko (1997) and Sharma (2004).

A dietary calcium, phosphorus ratio between 1:1 and 2:1 is assumed to be ideal for bone formation andgrowth. The ewes in the present study were in early lactation stage, the blood values of Ca, P and Mg were 9.64 ± 0.30, 4.39 ± 0.31 and 1.37 ± 0.20 mg/dl, respectively Therefore, the ratio of Ca, P and Mg will be 7.00, 3.20 and 1.00. Underwood *et al.* (1981) has stated the blood serum value of Ca, P and Mg as 9.4, 4.6 and 1.7 mg/dl in lactating cows. However, the ratio of Ca, P and Mg comes out to be 5.5, 2.7 and 1.0, respectively which is concomitant with the present findings.

The mean value of serum sodium is comparable to those mentioned by Kelly (1984) and Sharma (2004). However, the value is lower than those reported by Tajane (1990) and Hooda (1992). The mean value of serum potassium is lower than those reported by Kelly (1984), Tajane (1990) and Hooda (1992). The mean value of serum chloride is comparable to those reported by Kelly (1984) and Sharma (2004).

3.2. Microminerals in Blood of Lactating Ewes

Among the microminerals the mean value of plasma copper is comparable to Kelly (1984), Hidroglou (1987), Kaneko (1997), Rodostits (2003) and Sharma (2004). However, the values is lower than those reported by Edmundo *et al* (1982), Karim *et al* (2000), Vijaykumar *et al* (2001), Samanta and Samanta (2002), Randhawa*et al* (2003) and Deger*et al* (2007).The mean value of plasma cobaltis higher than that reported by Rodostits (2003). The mean value of plasma zinc is comparable to those reported by Rodostits (2003), whereas lower than those mentioned by Kumar *et al* (1992), Karim *et al* (2000), Vijay Kumar *et al* (2001) and Sharma (2004) and higher than those reported by Edmundo *et al* (1982), Samanta and Samanta (2002), Abdelrahman *et al* (2003) and Deger *et al* (2007). The mean value of plasma manganeseis higher than those reported by Karim*et al* (2000), Samanta and Samanta (2002) whereas lower than that of Deger*et al* (2007). The mean value of plasma iron in ewes, is comparable to those of Kelly (1984), Kaneko (1997) and Rodostits (2003), whereas higher than that reported by Samanta and Samanta (2002) and lower than those of Edmundo *et al* (1982), Hidroglou *et al* (1987), Randhawa *et al* (2003) and Deger (2007).

Macromineral and micromineral status in ewes varied during oestrous cycle, pregnancy, at lambing, post-lambing lactation days (Bonchev,1985, Bhatt *et al*,1996), season but not oestrous cycle (Ezzo *et al* 1990), pregnancy and lactation (Baranow - baranowski et a 1994), the mineral composition of soil and plants (Saba *et al*, 1995).

3.3 Macrominerals in Milk of Lactating Ewes

The range and mean values of macrominerals and microminerals in the milk of lactating ewes are shown in Table 3 respectively. Among the macrominerals the mean value of milk calcium is comparable to those mentioned by Mehaia (1994), whereas higher than that reported by Arora*et al* (2005) and lower than Underwood *et al* (1981). The mean value of milk phosphorus is lower than those reported by Underwood*et al* (1981) and Zafar *et al* (2006). The mean value of milk magnesium is lower than those reported by Ming (1990), Mehaia (1994) and Zafar (2006). The mean value of milk sodium (5.94 $\pm$ 0.20 mEq/l) is lower than those mentioned by Ming (1990), Mehaia (1994) and Zafar *et al*.(2006). The mean value of milk is lower than those reported by Underwood*et al* (1981), Ming (1990), Mehaia (1994) and Zafar *et al*. (2006). The mean value of milk chloride is higher than that reported by Underwood*et al* (1981).

3.4 Microminerals in milk of lactating ewes

Among the microminerals the mean value of milk copper is comparable to those mentioned by Ming (1990) and Moreno-rojas (1993), whereas higher than that of Underwood *et al* (1981) and Zafar *et al* (2006) and lower than that of Mehaia (1994). The mean value of milk cobalt (0.09 $\pm$ 0.02 ppm) is lower than the values of 0.114 $\pm$ 0.008 in winter and 0.137 $\pm$ 0.009 in summer season in milk of sheep reported by Zafar *et al*. (2006). No other reports are available with which in the present finding could be compared. However, the concentration of copper and cobalt in milk were affected by rearing system (Abdelrehman *et al*. 2003).

The mean value of milk zinc is comparable with that reported by Mehaia (1994), whereas higher than Arora *et al* (2005) and Zafar *et al* (2006) and lower than Underwood *et al* (1981), Ming (1990) and Morenorojas *et al* (1993). The mean value of milk manganese is higher than those mentioned by Underwood *et al* (1981), Ming (1990), Moreno rojas *et al* (1993), Mehaia (1994), Arora*et al* (2005) and Zafar *et al*. (2006).The mean value of milk iron (1.91 $\pm$ 0.40 ppm) is comparable to those reported by Ming (1990) and Mehaia (1994), whereas higher than Underwood *et al* (1981), Moreno-rojas*et al* (1993), Arora*et al* (2005) and Zafar *et al*. (2006).

The mean values and statistical analysis of minerals in the blood and milk of Deccani ewes are shown in Table 3. Among the macrominerals the comparison revealed that blood serum Ca content was significantly ($P < 0.01$) lower than milk (whey) calcium. Similarly, the blood serum phosphorus content was significantly lower ($P < 0.01$) than milk (whey) phosphorus content . The blood plasma magnesium content was also lower than milk magnesiumsignificantly ($P<0.01$). It is interesting to note that ratio of calcium: phosphorus of blood plasma is changed from 2.1:1 to that of in milk as 1.07: 1.0; also and ratio of calcium: phosphorus: magnesium (7.0:3.2:1.0) has become much wider (39.42:36.59:1.0) in milk of ewes as compared to that of blood. Among the electrolytes, the blood serum sodium content was significantly higher than ($P < 0.01$) milk (whey) sodium. The blood serum potassium content was significantly ($P < 0.01$) lower than milk (whey) potassium, whereas the blood serum chloride contentwas significantly higher than ($P < 0.01$) the milk (whey) chloride.

Among the microminerals the plasma copper content was non-significantly higher than that of milk content. The blood plasma Cobalt content was non-significantly lower than milk content. The blood plasma manganese content was non-significantly lower than milk manganese. The blood plasma zinc content was significantly lower ($P < 0.05$) than milk content .The blood plasma iron content was non-significantly lower than that of milk.

Many materials in milk come unchanged from the bloodstream, including minerals, certain hormone and several proteins. Large differences in sugar and salt concentrations are balanced since blood and milk are isotonic to each with the same osmolality or osmotic pressure. A concentration effect is apparent for many constituents including Ca, K, in which the blood level is many times smaller than that found in milk. The reverse is true for other minerals such as Na and Cl where the blood amount is much larger than that found in milk (Larson, 1985).

Thus, in the present work it was found that the mean values of Ca, P, Mg, K and Zn were significantly higher ($P<0.01$except for Zinc, which was $P<0.05$) in the milk as compared to those with blood of ewes and mean values of Co, Mn and Fe in milk were non-significantly higher than blood. This finding could be justified to the concentration effect as stated by Larson (1985). On the other hand, the mean values of sodium and chloride were significantly lower ($P<0.01$) in milk than the value in blood and the Cu content was non-significantly lower, this could be justified to the gradient effect as explained by Larson (1985).

Table 5: Simple correlation coefficients (r) of Macrominerals and Microminerals in blood and milk of lactating Deccani ewes

Minerals in blood	*Minerals in milk*	*Correlation coefficient (r)*
Calcium (S)	Calcium (W)	0.286
Phosphorus (S)	Phosphorus (W)	-0.442
Magnesium (P)	Magnesium (M)	-0.016
Sodium (S)	Sodium (W)	-0.456
Potassium (S)	Potassium (W)	-0.176
Chloride (S)	Chloride (W)	-0.267
Copper (P)	Copper (M)	-0.699*
Cobalt (P)	Cobalt (M)	-0.510
Zinc (P)	Zinc (M)	0.876**
Manganese (P)	Manganese (M)	-0.195
Iron (P)	Iron (M)	-0.117

S - Blood serum, **P -** Blood plasma, **W** - Whey, **M** – Milk ,.
** Significant (P< 0.01), * Significant (P< 0.05)

Simple correlation coefficients of mineral in blood and milk of lactating ewes are presented in Table 5. In ewes milk copper was significantly negatively correlated (r = 0.699, (P<0.05) with blood plasma copper, whereas milk zinc was significantly and positively correlated (r = 0.876, P<0.01) with blood plasma zinc. The non-significant positive correlation was seen for calcium, whereas non-significant negative correlation was seen for Phosphorus, Magnesium, Sodium, Potassium, Chloride, Cobalt, Manganese and Iron.

4. Conclusions

The macrominerals like calcium, phosphorus, sodium and potassium and chloride in the blood and milk of sheep are within the normal physiological limits. The magnesium content of blood and milk of ewes was lower than the reported values which was probably indicative of the lower magnesium content of the pasture on which the animal were grazed. The microminerals like copper, zinc, iron, cobalt and manganese in the blood and milk were within normal physiological limits. Comparing blood and milk mineral composition calcium, phosphorus, magnesium, zinc and potassium were significantly lower and sodium and chloride were significantly higher in the blood as compared to milk. Blood copper content was significantly negatively correlated with that of milk.

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