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Effects of physiological status and seasonal variation on plasma mineral profile of sheep in Kashmir valley

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Evaluating mineral profile of sheep, belonging to different physiological states and in different seasons, is an important indicator of their nutritional and health status. This is important to prevent health disorders which lead to production and reproductive disturbances. A total of 167 blood samples were collected in four different seasons of the year from sheep having varied physiological demands in Shuhama Alusteng area of Kashmir valley. The plasma macro-minerals such as Ca, P_i and Mg were measured using standard kits; while as micro-minerals like Cu, Zn and Fe were estimated using atomic absorption spectrometry. The concentration of Ca was below the critical level during pregnant and lactating periods, and round about the critical level throughout the year. The Mg values were just above the critical concentration in all categories of sheep throughout the year. The concentrations of Cu and Zn were above the critical levels in all categories of sheep especially during the winter season. P_i and Fe levels were adequate in all throughout the year. In addition, a good percentage of samples were deficient in one or the other mineral round the year. The results suggest that sheep in the study area should be supplemented with Ca and Mg round the year; Cu and Zn during spring, summer and autumn seasons. Also, the influence of local agro-geo-climatic conditions plus mineral interactions involving greater sample size must be studied prior to attempting the formulation of area specific mineral supplement(s). Further, the dosage should be recommended as per the physiological need of an animal.

Key words: Sheep, physiological status, seasonal variation, minerals, Kashmir valley.

INTRODUCTION

Livestock sector in Asia forms an important livelihood activity for most of the farmers, supporting agriculture in the form of critical inputs, contributing to the health and nutrition of the household, supplementing incomes, offering employment opportunities, and finally being a dependable “bank on hooves” in times of need (Ben Salem and Smith, 2008). Jammu and Kashmir (India) is a hilly state with total area of 2,22,236 km^2 that sprawls over the western Himalaya and Karakorum mountains between 32.17° N and 36.58° North latitude and 73.26° E and 83.30° East longitude. The state is divided into three agro-climatic zones: Cold arid desert areas of Ladakh, temperate Kashmir valley and the humid sub-tropical region of Jammu. Each has its own specific geo-climatic condition which reflects the diverse profile of livestock species (Wani and Wani 2010).

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Table 1. Total number of plasma (Sheep) samples collected from the study area.

Area	Season	Lambs/Weaners	Dry	Pregnant	Lactating	Rams	Total
Shuhama Alusteng	Winter	13	7	16	15	10	**61**
	Spring	6	7	6	10	7	**36**
	Summer	8	6	Nil	16	6	**36**
	Autumn	6	6	6	10	6	**34**
	Total	**33**	**26**	**28**	**51**	**29**	**167**

Conducive agro-climatic conditions, rich alpine pastures and other natural endowments provide enormous scope and potential for sheep rearing in the state. Free foraging of small ruminants on alpine pastures, sub-alpines and waste lands for 7 to 8 months on semi-migratory mode of rearing and round the year on migratory mode optimize the input costs. In J&K, 75% population is rural with agriculture as the main stay and livestock-rearing as the subsidiary one. As per 18th Livestock Census of 2007, the sheep population in J&K is about 3.69 million (70% crossbred) out of the total 10.99 million livestock strength. The agriculture and allied sectors contribute about 38% to the state GDP of which 11% is contributed by livestock sector (DES, 2007). In Kashmir valley, sheep farming is admittedly a profitable venture as it is capital oriented not labour intensive and forms an integral component of food production and livelihood system of most of the under-privileged communities and agro-pastoral farmers.

Minerals have been recognized as potent nutrients and their deficiency/imbalance exerts a significant effect on health and productivity of livestock (Kincaid, 1999; Aregheore et al., 2007; Gonul et al., 2009). Suboptimal mineral deficiency that affects growth and production is more serious than the manifested mineral deficiency showing clinical signs that can be corrected (Underwood, 1977). Levels of requirements as well as thresholds of deficiency and toxicity vary with age, sex, production level, activity level, species and genetic strain of the animal. Animal age can affect mineral requirement through changes in efficiency of absorption. The pre-ruminant animal absorbs most minerals more efficiently than the older animal (Standing Committee on Agriculture, 1990). Animals most susceptible to trace element deficiencies are young growing animals and animals during their first pregnancy and lactation (Khan et al., 2003). Gender of animal affects susceptibility to mineral disorders probably through differences in growth rate and physiological function (Shallow et al., 1989).

Mineral inadequacies in livestock are often seasonal, resulting from increased demands of pregnancy, lactation or rapid growth coinciding with reduced mineral content or availability in the pasture (Tashi et al., 2005). The mineral content in forages is influenced by several factors such as soil, plant species, and stage of maturity, yield, pasture management and climate (Poland et al., 2001). The physiological response of animals to environmental stress (heat/cold) exerts a profound effect on serum biochemical parameters (Nazifi et al., 1999).

Assessment of mineral status of grazing animals has been considered an important strategy to increase animal productivity, especially in mineral deficient areas (Khan et al., 2003). In Kashmir, sheep are mainly maintained on grazing with little or no mineral supplementation. Hence, the study was undertaken to evaluate the plasma mineral content of sheep in Shuhama Alusteng area so as to devise the supplementation strategy for ensuring optimum production performance and prevention of health disorders.

MATERIALS AND METHODS

The study area in district Ganderbal is located at 34° 12' N 74° 46'E with an average elevation of 1,619 m (5,312 ft) and is characterized by sub-humid temperate climate with mean annual rainfall of 744 mm and mean annual temperature of 13.4°C.

Blood samples

Blood samples from sheep belonging to different physiological states (lambs/weaners aged 3 to 6 months; dry, pregnant, lactating ewes, and rams aged 2 to 5 years; majority of animals belonging to non-descript and Corriedale breed) and in different seasons of the year (spring/summer/autumn/winter as shown in Table 1) were collected by jugular venipuncture in heparinised vials, centrifuged at 3000 rpm for 10 min to harvest the plasma which was then transferred to sterile, acid-washed vials, labelled and stored at -40°C until transport to laboratory where the samples were stored at -20°C for further analysis.

Estimation of plasma minerals

Calcium

Plasma calcium was estimated by O-Cresolphthalein Complexone (OCPC) end point assay (Span Diagnostics Ltd. India).20 µl of plasma samples were taken in labelled test tubes followed by 1000 µl of working Ca reagent. Standard was prepared in triplicate with 20 µl of Ca standard in test tubes mixed with 1000 µl of working Ca reagent. Test tube containing 1000 µl working Ca reagent was used as reagent blank. After mixing the reagents, test tubes were incubated at 37°C or room temperature (15 to 30°C) for 5 min. Analyzer programmed (578 nm) as per assay conditions and absorbance of standards followed by plasma samples was taken against blank.

Calculations:

Plasma Ca (mg/dl) = Absorbance of Test/Absorbance of Standard × 10

Phosphorus

Plasma inorganic phosphorus (Pi) was estimated by UV Molybdate, end point assay (Span Diagnostics Ltd. India).10 µl of plasma samples were taken in labelled test tubes to which 1000 µl of reagent 1 was added and mixed well. Test tubes containing 10 µl of Pi standard and 1000 µl of reagent 1 were taken in triplicate as standard. Reagent blank was prepared by taking 1000 µl of reagent 1 in the test tube. All test tubes containing test samples, standards and blank were mixed properly by shaking and incubated at 37C for 5 min. Analyzer was programmed as per assay parameters (340 nm) and blanked with reagent blank. The absorbance of the standard and plasma samples was taken against blank.

Calculations:

Plasma P_i (mg/dl) = Absorbance of Test/Absorbance of Standard × 5

Magnesium

Plasma Mg was estimated by Calmagite method (Crest Biosystems, India).To labelled test tubes, 0.01 ml of plasma samples were added followed by 0.5 ml of buffer reagent (L_1), and 0.5 ml of colour reagent (L_2). Standard was prepared in triplicate which contained 0.5 ml of L_1 reagent, 0.5 ml of L_2 reagent and 0.01 ml of Mg standard. Test tube containing L_1 and L_2 reagents (0.5 ml each) plus 0.01 ml distilled water was used as reagent blank. The contents in test tubes were mixed well by shaking and incubated at 25°C for 5 min. Absorbance of standard and the samples was recorded against blank at 510 nm.

Calculations:

Mg (mEq/L) = Absorbance of Test/Absorbance of Standard × 2 (Note: 1 mEq/L = 0.5 mmol/L= 2.44 mg/dl)

Digestion of plasma samples

Plasma samples were digested as per the standard procedure (Kolmer et al., 1951). To 3 ml of sample in digestion tubes, an equal volume of concentrated HNO_3 was added and mixed well. The tubes were kept for overnight at room temperature followed by low heat (70 to 80°C) digestion until the volume of the samples reduced to 1 ml. To this, 3 ml of double acid mixture (HNO_3 and $HClO_4$ in 3:1 ratio) was added and low heat digestion continued until the digested samples became watery clear and emitted white fumes. As per need, the addition of 3 ml double acid mixture followed by low heat digestion was repeated couple of times. Heating was continued until the volume of the samples got reduced to ~0.5 ml. Final volume of the filtrate was made 10 ml with triple distilled de-ionized water after warming the solution. During digestion of plasma samples simultaneous digestion of reagent blank was also undertaken and final volume of 10 ml stored to have the blank.

Trace mineral estimation

Atomic Absorption Spectrophotometry (AAS) is considered one of the precise techniques for estimation of trace minerals in biological materials. AAS (Model No ECIL 4141) manufactured by Electronic Corporation of India (ECIL), Hyderabad was used in the present study. It uses a double beam with a wave length range of 190 to 900 nm. Separate hollow UV lamps for each mineral were used. Air/acetylene flame was used as fuel. At least three standards of known concentrations were used for calibration and then the unknown test samples were analyzed. After sample analysis, sufficient distilled water flush was done for at least 10 min. Sample analysis was done by attached computer and concentration of mineral samples was expressed in parts per million (ppm).

Statistical analysis

Data collected during the study were analyzed for mean, standard error and analysis of variance (ANOVA) by using SPSS software (version 16).

RESULTS AND DISCUSSION

For maintenance of normal health and sustained efficient production of livestock, it is necessary to ensure adequate dietary intake of essential nutrients. Intensification of production requires full coverage and appropriate balancing of mineral elements (Hosnedlava et al., 2007). In sheep, nutrients quality and quantity directly affect highly demanding reproductive functions such as expression of estrus, embryo implantation and reduction in spermatogenesis, and indirectly affect overall animal health (Vázquez-Armijo et al., 2011). Additionally, kilograms of offspring weaned per female exposed may be affected by both trace mineral supplementation and source (Ahola et al., 2004). Mineral deficiencies that affect livestock at pasture in most regions of the world include those of macro- and micro- minerals (Khan et al., 2005). Excessive intake of minerals can also have an adverse effect on animal health. There are a number of methods to establish the existence or likely existence of specific mineral deficiency/imbalance for grazing livestock, in which determination of concentrations and proportions of minerals in dietary components along with clinical, pathological and biochemical examination of animals and appropriate tissues and fluids are commonly used for diagnosis of mineral status (McDowell, 1992). Signs of mineral disorders are often non specific and in cases of marginal deficiencies may go unnoticed by the stock owners. The interpretation of such signs is also difficult if more than one mineral is deficient or the deficiency is associated with disorders like increased burdens of gastrointestinal parasites, especially when trace element deficiencies coexist as they increase the susceptibility of animal to diseases (Suttle and Jones, 1989).

The overall plasma mineral concentration in sheep belonging to different physiological states and in different seasons is presented in (Tables 2 and 3).

Effect of physiological status on plasma minerals

In sheep, significantly lower and below the critical limit

Table 2. Effect of physiological status and seasonal variation on plasma minerals in sheep.

Parameter	Physiological Status	Seasons									
		Winter		*Spring*		*Summer*		*Autumn*		Overall	
		n	Mean±S. E	n	Mean±S. E	n	Mean±S. E	n	Mean±S. E	n	Mean±S. E
Calcium (mg/dl)	Lambs/weaners	13	9.29±0.50	6	8.99±0.37	8	9.45±0.35^{B}	6	9.24±0.19^{AB}	33	9.26±0.22^{B}
	Dry	7	9.15±0.22	7	8.90±0.44	6	9.22±0.60^{B}	6	9.16±0.55^{AB}	26	9.10±0.22^{AB}
	Pregnant	16	8.25±0.37	6	8.72±0.39		ND	6	8.74±0.25^{A}	28	8.45±0.23^{A}
	Lactating	15	8.12±0.25^{a}	10	9.14±0.35^{b}	16	7.93±0.33^{aA}	10	9.13±0.32^{bAB}	51	8.46±0.17^{A}
	Rams	10	8.29±0.41^{a}	7	9.86±0.41^{b}	6	10.18±0.39^{bB}	6	10.25±0.45^{bB}	29	9.46±0.26^{B}
	Overall	**61**	8.55±0.18^{a}	36	9.14±0.18^{ab}	36	8.85±0.25^{ab}	34	9.28±0.18^{b}	167	8.89±0.10
Phosphorus (mg/dl)	Lambs/weaners	13	7.11±0.51^{bBC}	6	6.61±0.54^{bC}	8	6.89±0.54^{bB}	6	5.16±0.48^{aAB}	33	6.61±0.29^{B}
	Dry	7	6.27±0.34^{bABC}	7	3.96±0.29^{aA}	6	5.97±0.40^{bAB}	6	5.52±0.50^{bAB}	26	5.41±0.26^{A}
	Pregnant	16	7.33±0.51^{C}	6	5.97±0.57^{BC}		ND	6	6.31±0.59^{B}	28	6.82±0.35^{B}
	Lactating	15	5.52±0.46^{bAB}	10	3.67±0.56^{aA}	16	4.99±0.34^{abA}	10	4.77±0.40^{abAB}	51	4.84±0.23^{A}
	Rams	10	5.29±0.67^{A}	7	4.64±0.40^{AB}	6	5.40±0.68^{AB}	6	4.20±0.88^{A}	29	4.93±0.33^{A}
	Overall	**61**	6.38±0.26^{c}	36	4.79±0.29^{a}	36	5.64±0.26^{bc}	34	5.14±0.26^{ab}	167	5.63±0.14
Magnesium (mg/dl)	Lambs/weaners	13	1.63±0.10^{a}	6	1.48±0.16^{a}	8	1.82±0.16^{a}	6	2.15±0.19^{b}	33	1.74±0.08
	Dry	7	1.76±0.21	7	1.79±0.17	6	1.94±0.23	6	2.20±0.34	26	1.91±0.12
	Pregnant	16	1.77±0.08	6	1.69±0.15		ND	6	1.92± 0.30	28	1.79±0.08
	Lactating	15	1.66±0.08^{ab}	10	1.52±0.10^{a}	16	1.85±0.15^{ab}	10	2.06±0.19^{b}	51	1.77±0.07
	Rams	10	1.75±0.11	7	1.91±0.16	6	2.01±0.40	6	2.37±0.37	29	1.97±0.12
	Overall	**61**	1.71±0.05^{a}	36	1.67±0.07^{a}	36	1.88±0.10^{a}	34	2.13±0.12^{b}	167	1.82±0.04
Copper (ppm)	Lambs/weaners	13	1.39±0.15^{b}	6	0.83±0.16^{a}	8	0.78±0.19^{a}	6	0.75±0.09^{a}	33	1.02±0.09
	Dry	7	1.33±0.26^{b}	7	1.00±0.11^{ab}	6	0.68±0.06^{a}	6	0.76±0.07^{a}	26	0.96±0.09
	Pregnant	16	1.28±0.15	6	0.72±0.13		ND	6	1.03 ± 0.29	28	1.10±0.11
	Lactating	15	1.38±0.18^{b}	10	0.86±0.08^{a}	16	0.68±0.05^{a}	10	0.81±0.06^{a}	51	0.94±0.07
	Rams	10	0.99±0.26	7	0.67±0.04	6	0.96±0.22	6	0.88±0.22	29	0.88±0.11
	Overall	**61**	1.28±0.08^{b}	36	0.82±0.05^{a}	36	0.74±0.06^{a}	34	0.84±0.07^{a}	167	0.98±0.04
Zinc (ppm)	Lambs/weaners	13	0.94±0.14	6	0.81±0.18	8	0.71±0.12	6	0.84±0.14	33	0.84±0.07
	Dry	7	1.26±0.19	7	0.65±0.13	6	0.73±0.10	6	0.93±0.32	26	0.90±0.10
	Pregnant	16	1.22±0.13	6	0.86±0.16		ND	6	0.83±0.17	28	1.06±0.09
	Lactating	15	1.17±0.17^{b}	10	0.71±0.13^{a}	16	0.69±0.07^{a}	10	0.69±0.09^{a}	51	0.83±0.07
	Rams	10	1.42±0.20^{b}	7	0.66±0.07^{a}	6	0.82±0.11^{a}	6	0.69±0.09^{a}	29	0.96±0.10
	Overall	**61**	1.19±0.07^{b}	36	0.73±0.06^{a}	36	0.73±0.05^{a}	34	0.78±0.07^{a}	167	0.91±0.04
Iron (ppm)	Lambs/weaners	13	3.76±0.67^{b}	6	2.02±0.32^{ab}	8	1.53±0.16^{a}	6	2.66±0.44^{ab}	33	2.70±0.32^{AB}
	Dry	7	3.53±1.00	7	2.48±0.71	6	1.76±0.28	6	2.30±0.55	26	2.55±0.36^{A}
	Pregnant	16	3.93±0.36	6	2.53±0.58		ND	6	3.49±0.50	28	3.54±0.28^{B}
	Lactating	15	3.80±0.38^{b}	10	2.27±0.41^{a}	16	1.83±0.25^{a}	10	3.65±0.42^{a}	51	2.85±0.21^{AB}
	Rams	10	4.86±0.75^{b}	7	2.20±0.44^{a}	6	2.30±0.49^{a}	6	3.93±0.39^{ab}	29	3.49±0.37
	Overall	**61**	3.97±0.25^{c}	36	2.30±0.22^{a}	36	1.83±0.15^{a}	34	3.26±0.22^{b}	167	3.00±0.13

Means bearing different uppercase superscripts across the columns for each parameter differ significantly (P<0.05); Means bearing different lower case superscripts across the rows differ significantly (P<0.05); ND=No Data (No sample available).

Table 3. Percent samples (sheep plasma) deficient in minerals.

Mineral	Critical concentration* (mg/dl)	Season	Physiological status					
			Lambs/weaners	*Dry*	*Pregnant*	*Lactating*	*Rams*	Overall
Calcium	9.00	Winter	6/13=46.2%	3/7=42.9%	11/16=68.8%	12/15=80%	6/10=60%	**38/61=62.29%**
		Spring	2/6=33.3%	3/7=42.9%	4/6=66.6%	3/10=30%	1/7=14.3%	**13/36=36.11%**
		Summer	3/8=37.5%	3/6=50.0%	ND	12/16=75%	1/6=16.7%	**19/36=52.77%**
		Autumn	1/6=16.67%	3/6=50.0%	4/6=66.6%	5/10=50%	1/6=16.7%	**14/34=41.18%**
		Overall	**12/33=36.36%**	**12/26=46.15%**	**19/28=67.86%**	**32/51=62.75%**	**9/29=31.03%**	
Phosphorus	4.00	Winter	1/13=7.7%	0/7=0	3/16=18.8%	2/15=13.3%	3/10=30%	**9/61=14.75%**
		Spring	0/6=0	3/6=50%	0/6=0	7/10=70%	2/7=28.6%	**12/36=33.33%**
		Summer	0/8=0	0/6=0	ND	4/16=25%	1/6=16.7%	**5/36=13.89%**
		Autumn	1/6=16.7%	1/6=16.7%	0/6=0	3/10=30%	4/6=66.7%	**9/34=26.47%**
		Overall	**2/33=6.06%**	**4/26=15.38%**	**3/28=10.71%**	**14/51=27.45%**	**10/29=34.48%**	
Magnesium	1.50	Winter	5/13=38.5%	2/7=28.6%	3/16=18.8%	4/15=26.6%	3/10=30%	**17/61=27.87%**
		Spring	4/6=66.7%	1/7=14.3%	2/6=33.3%	4/10=40%	2/7=21.6%	**13/36=36.11%**
		Summer	2/8=25%	1/6=16.7%	ND	2/16=12.5%	1/6=16.7%	**6/36=16.67%**
		Autumn	1/6=16.7%	2/6=33.3%	2/6=33.3%	1/10=10%	1/6=16.7%	**7/34=20.6%**
		Overall	**12/33=36.36%**	**6/26=23.08%**	**7/28=25.0%**	**11/51=21.57%**	**7/29=24.14%**	
Copper	0.60	Winter	1/13=7.7%	1/7=14.3%	0/16=0	3/15=21.6%	5/10=50%	**10/61=16.4%**
		Spring	2/6=33.3%	0/7=0	2/6=33.3%	2/10=20%	2/7=28.6%	**8/36=22.2%**
		Summer	3/8=37.5%	2/6=33.3%	ND	4/16=25%	1/6=16.7%	**10/36=27.8%**
		Autumn	2/6=33.3%	1/6=16.7%	2/6=33.3%	1/10=10%	2/6=33.3	**8/34=23.5%**
		Overall	**8/33=24.24%**	**4/26=15.38%**	**4/28=14.29%**	**10/51=19.61%**	**10/29=34.48%**	
Zinc	0.60	Winter	3/13=23%	1/7=14.3%	1/16=6.3%	1/15=6.7%	0/10=0	**6/61=9.8%**
		Spring	2/7=28.6%	2/7=28.6%	2/6=33.3%	6/10=60%	2/7=28.6%	**14/36=38.9%**
		Summer	3/8=37.5%	1/6=16.6%	ND	4/16=25%	1/6=16.6%	**9/36=25%**
		Autumn	1/6=16.7%	2/6=33.3%	2/6=33.3%	4/10=40%	2/6=33.3%	**11/34=32.4%**
		Overall	**9/33=27.3%**	**6/26=23.1%**	**5/28=17.9%**	**15/51=29.4%**	**5/29=17.2%**	
Iron	1.20	Winter	0/13=0	0/7=0	0/16=0	0/15=0	0/10=0	**0/61=0**
		Spring	1/6=16.7%	1/7=14.3%	1/6=16.7%	2/10=20%	1/7=14.3%	**6/36=16.7%**
		Summer	1/8=12.5%	1/6=16.7%	ND	2/16=12.6%	1/6=16.7%	**5/36=13.9%**
		Autumn	0/6=0	1/6=16.7%	0/6=0	0/10=0	0/6=0	**1/34=2.94%**
		Overall	**2/33=6.1%**	**3/26=11.5%**	**1/28=3.6%**	**4/51=7.8%**	**2/29=6.9%**	

*Radostitis et al. (2000).

Ca concentration was observed in pregnant and lactating ewes as compared to rams, lambs/weaners and dry ewes which could be attributed to dietary imbalances of Ca and P, higher requirements due to pregnancy owing to marked increase in the needs of fetal skeleton for mineralization, increased endogenous Ca loss with the advance of pregnancy, dietary interaction with other minerals (Maynard et al., 1979) and negative Ca balance owing to excessive secretion of Ca through milk (Asif et al., 1996). The outflow of Ca into milk at the onset of lactation may be accompanied by a reduction in the plasma Ca pool (Remberg et al., 1970). The higher concentration of Ca in young growing animals could be due to more efficient absorption of Ca in young than that of older animals (Ricks, 1996). Also, in growing animals, net retention of Ca occurs in the body, while in adults the amount ingested equals that lost if metabolic requirement is met (Church and Pond, 1988). Moreover, absorption efficiency is well known to fall with age which partly relates to the decline in vitamin D stores (Robert, 1989). The percent samples deficient in Ca were in the order of pregnant>lactating>dry>lambs/weaners>rams. The study revealed adequate plasma P_i irrespective of the physiological status and/or season of the year with significantly lower concentration in lactating ewes compared to lambs/weaners. The marked increase in P_i secretion in milk may be the reason (Braithwaite, 1983). Serum phosphate has been seen higher in young animals because the growth hormone increases renal phosphate resorption (Kaneko et al., 1997). The percent samples deficient in P_i were in the order rams>lactating>dry> pregnant>lambs/weaners. The Mg concentration observed was just above the critical level especially in lambs/weaners, pregnant and lactating ewes. These findings might be attributed to hemodilution that occurs during late pregnancy and lactation,

increased physiological demands for Mg in such category of animals, and the more rapid uptake of Mg by young than adult animals (Ahmed et al., 2000). Furthermore, exchange of radio Mg in bone has been recorded 5 to 10 times greater in young than old animals (Breibart et al., 1960) owing to more water content in former than latter (Fontenot et al., 1989). The percent samples deficient in Mg were in the order of lambs/weaners> pregnant>rams >dry>lactating. During gestation, both the mother and fetus are very susceptible to dietary mineral imbalances owing to time of rapid growth and cell differentiation (Ghany-Hefnawy et al., 2007). Lambs born to mineral deficient ewes have lower birth weight, poor energy utilization, insufficient quantity and quality of colostrum, slower suckling reflex, greater risk of hypothermia, and increased risk for diseases such as white muscle disease, enlarged thyroid gland, muscular in co-ordination and bone abnormalities.

The Cu concentration observed was adequate in all categories of sheep though non-significantly higher in pregnant ewes in which it may be attributed to higher progesterone level and/or to the increased fetal demands and utilization of maternal Cu for development of fetal nervous system (Elnageeb and Abdelatif, 2010). Moreover, pregnancy is usually associated with an increase in plasma Cu levels in the form of ceruloplasmin due to increase in oestrogen levels during late pregnancy (Howell et al., 1968). Low Cu content in sheep rations inhibits embryo implantation, causes embryo loss and fetal death (Hidiroglou, 1979). In sheep, postnatal lordosis, detected as muscle weakness and ataxia, is also caused by Cu deficiency during gestation (Ashworth and Antipatis, 2001). Du Plessis et al. (1999) observed a suppressed estrous behaviour in ewes due to induced secondary Cu deficiency that might be attributed to altered production and/or expression of hormones, such as estrogens and luteinizing hormone (LH) and follicle-stimulating hormone (FSH). The percent samples deficient in Cu were in the order of rams>lambs/weaners>lactating >dry>pregnant. Zn concentration besides being adequate in all categories was non-significantly higher in pregnant ewes, and the percent samples deficient in Zn were in the order of lactating>lambs/weaners>dry>pregnant>rams. These observations could be due to increased demands for Zn towards the end of pregnancy (Elnageeb and Abdelatif, 2010), increased rate of Zn accumulation in the fetus, and the higher plasma albumin levels in pregnant animals to which Zn is bound primarily (Davis, 1984). Similar findings have been observed by other authors (Williams, 1977). However, decreased serum Zn level during late gestation as a result of hemodilution has been found in desert ewes (Masters and Fels, 1980). Zn deficiency in males affects spermatogenic process as well as primary and secondary sex organ development, and in females it could affect any phase of the reproductive processes- estrus, gestation or lactation (Smith and Akinbamijo, 2000). Zn also plays a key role in maintaining the integrity of epithelia of the reproductive organs, which is necessary for embryo implantation (Robinson et al., 2006), besides, adequate concentrations of Zn in serum and in diets, are vital for uterine involution and tissue repair post-partum, and particularly the return to estrus.The Fe concentration observed was adequate with significantly higher in pregnant ewes. Relatively high Fe concentration from 3rd to 7th month of pregnancy compared to minimum concentration when lactation commenced has been reported (Tainturier et al., 1984). The percent samples deficient in Fe were in the order of dry>lactating>rams>lambs/weaners> pregnant. Yatoo et al. (2013) reported higher prevalence of copper deficiency followed by zinc, cobalt and iron in sheep of few districts of Kashmir.

Effect of seasonal variation on plasma minerals

The Ca concentration observed was round about the critical limit throughout the year, significantly lower in winter as compared to autumn season, and the percent samples deficient in Ca were in the order of winter> summer>autumn>spring. This might be attributed to higher dietary availability of Ca during dry season than wet season plus the higher absorption efficiency in drier months (Khan, 2003). A good percentage of animals were found Ca deficient throughout the year. The P_i concentration observed though adequate round the year was significantly lower in spring and higher in winter season. The percent samples deficient in P_i were in the order of winter>autumn>spring>summer. The Mg concentration was just above the critical level throughout the year but significantly lower in spring, winter and summer as compared to autumn season. The percent samples deficient in Mg were in the order of spring>winter>autumn>summer. This might be due to maximum Mg excretion through faeces in spring/winter than during autumn, and thus less absorption through the gastrointestinal tract in wet seasons than dry season (Khan, 2003). The Cu levels noticed though adequate were significantly higher in winter as compared to rest of the seasons, and the percent samples deficient in Cu were in the order of summer>autumn>spring>winter. These observations are in agreement with the findings of other researchers (Pastrana et al., 1991). The Zn concentrations (adequate) were higher in winter as compared to rest of the seasons with percent samples deficient being in the order of spring>autumn>summer>winter. Higher plasma Zn concentration in winter than summer has been reported (Khan et al., 2008). Throughout the year, the Fe concentration was more than adequate with significantly higher concentration in winter compared to rest of the seasons, with percent samples deficient being in the order of summer > spring > autumn > winter. Similar observations have been recorded by other authors

(Merkel et al., 1990; Rojas et al., 1993). Significantly higher blood Ca and Mg level has been recorded in summer than winter season, with higher Ca concentration in lambs than adults (Pasha et al., 2009). Lower plasma Ca and Mg concentration has been noticed in winter than summer in lactating and non lactating ewes, and in rams as well but the reverse trend has been observed for Cu, Fe and Zn (Khan et al., 2003).

Conclusion

In this study, a significant influence of physiological status and seasonal variation on plasma mineral concentration of sheep was found, hence the dosage of supplement(s), if any, should be recommended as per the physiological need of an animal under existing climatic conditions. The experimental findings also suggest that sheep of this area should be supplemented with Ca and Mg round the year, Cu and Zn during spring, summer and autumn seasons. This study could well set a platform for much detailed and wider scale investigations in future where in larger sample size and mineral interaction in regional soil-fodder-animal system is evaluated which would further confirm these findings and allow the formulation of Kashmir specific mineral supplement(s).

Conflict of Interests

The author(s) have not declared any conflict of interests.

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Abbreviations

Ca, Calcium; **P_i,** Inorganic Phosphorus; **Mg,** Magnesium; **Cu,** Copper; **Zn,** Zinc; **Fe,** Iron; **AAS,** Atomic Absorption Spectrophotometer.

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