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OS Shah
Department of Clinical Veterinary Medicine Ethics and Jurisprudence, F.V.Sc and A.H, SKUAST K, Jammu and Kashmir, India

U Amin
Department of Veterinary Pathology, F.V.Sc and A.H, SKUAST K, Jammu and Kashmir, India

AR Baba
Department of Veterinary Paristology, IIVER, Rohtak, Haryana

T Hussain
Department of Clinical Veterinary Medicine Ethics and Jurisprudence, F.V.Sc and A.H, SKUAST K, Jammu and Kashmir, India

ZA Dar
Department of Veterinary Microbiology, F.V.Sc and A.H, SKUAST K, Jammu and Kashmir, India

SU Nabi
Department of Clinical Veterinary Medicine Ethics and Jurisprudence, F.V.Sc and A.H, SKUAST K, Jammu and Kashmir, India

HU Malik
Department of Clinical Veterinary Medicine Ethics and Jurisprudence, F.V.Sc and A.H, SKUAST K, Jammu and Kashmir, India

Correspondence
OS Shah
Department of Clinical Veterinary Medicine Ethics and Jurisprudence, F.V.Sc and A.H, SKUAST K, Jammu and Kashmir, India

Studies on seasonal changes in mineral concentrations of cross breed cattle in Kashmir valley

OS Shah, U Amin, AR Baba, T Hussain, ZA Dar, SU Nabi and HU Malik

Abstract
The present study reports the seasonal variations of copper, zinc, magnesium, iron, selenium calcium, phosphorus and copper concentrations in dairy cattle of Kashmir valley. Serum samples were collected in four seasons summer, winter, autumn and spring season. Mineral estimation for different season revealed no significant effect of season on the blood calcium levels. Inorganic phosphate was found to be significantly higher in the summer (7.05 ± 0.61) and significantly lower in the autumn. Blood magnesium levels showed marked seasonal variations with significantly lower level in spring and winter than in the summer (2.43±0.48) and spring (1.75 ± 0.41) while no significant difference was observed in levels between spring (2.33 ± 0.57) and winter (2.52 ± 0.65). Iron levels were seen to be significantly higher in summer (3.95 ± 0.32), while no significant difference was observed in levels between spring (2.33 ± 0.57), autumn (2.52 ± 0.65) and winter (2.88± 0.78). Copper levels was found to be significantly higher in summer(1.43 ±0.42) and significantly lower winter (0.98 ± 0.19), while no significant difference was found in levels of copper between autumn(1.28 ± 0.25) and spring (1.23± 0.21). Present study could not find any seasonal variations on serum Zn levels.

Keywords: Cattle, Kashmir, minerals and seasons

Introduction
The mineral level in animal body depends on large number of factors such as species, breed, age, sex, nutritional and health status, mineral supplementation, seasonal and physiological variations [1]. For maintaining better health and production animals need optimum concentrations of both macro and trace minerals. Recently, the essentiality of major and trace elements in animals for the maintenance of normal metabolic and production levels has been recognized [1]. Factors, including kind and levels of production, age, level and chemical form of elements, breed, mineral intake, interrelationships with other nutrients and animal adaptation play a critical role in mineral requirements of animals [1]. The responses of animals to environmental stress during different seasons have profound effects on some serum biochemical parameters [2]. Sufficient information regarding the effect of seasonal variation on the mineral status of animal in Kashmir valley is lacking. The present study was undertaken to investigate the possible influences of seasonal variations of serum mineral status (Cu, Zn, Mg, Fe, Ca, P and Se) in cattle of Kashmir valley. In present study effort were made to make other variable unchanged from season to season. The study has a potential to serve as the model for seasonal specific mineral supplementation to animals in different seasons of year.

Material and Methods
Design of study
The present study was undertaken to investigate the possible influences of seasonal variations on serum elements Cu, Zn, Mg, Fe, Ca, P and Se. For that reason, only seasonal conditions were evaluated as variables, and efforts were made to maintain all other parameters unchanged. The study was carried out in healthy crossbreed cows aged 5–7 years in similar physiological status and raised under similar managemental conditions. The total of (200) samples were collected from cows in four seasons' winter (50), spring (50), summer (50) and autumn (50).

Blood collection
Blood samples were obtained from the jugular vein of all animals in heparinised vails. The samples were placed in acid-washed polyethylene tubes and centrifuged at 1800g for 15 min at 4 °C. The serum was then frozen at –20 °C until needed for analysis.

Digestion of samples

Plasma samples were digested as per method described by Kolmer *et al.* (1951). To 3 ml of sample in digestion tube an equal volume of concentrated HNO3 was added and mixed well. The tubes were kept overnight at room temperature followed by low heat (70-80 ^{0}C) digestion until the volume of samples reduced to 1 ml. To this 3 ml of double acid mixture (HNO3 and HClO4 at ratio of 3:1) was added and low heat digestion continued until digested samples becomes watery clear and emits a white fume. According to need, the addition of 3 ml double acid mixture followed by low heat digestion was repeated couple of times. Heating was continued to reduce the volume of sample to approximately 0.5 ml. Final volume of filtrate was made up to 15 ml with triple distilled deionized water after making solution lukewarm. During digestion of soil, fodder and plasma samples simultaneous digestion of reagent blanks were also undertaken with final volume of 15 ml to have blank.

Estimation of mineral content

A flame atomic absorption spectrometer equipped with an air–acetylene flame burner and hollow-cathode lamps (Unicam 925, United Kingdom) was used for the determination of Cu, Zn Ca, Fe, Mg, Se (Table 1). while P were measured calorimetrically by spectrophotometer using commercially available kits. Plasma inorganic phosphorus (Pi) was estimated by UV Molybdate, end point assay (Span Diagnostics Ltd. India).

Statistical Analysis

All statistical analyses were performed using SPSS v.10.0 for Windows. Two-way analysis of variance (ANOVA) was performed to compare differences between sampling periods of animals.

Result

The results of present study are shown in (Table 2 and figure 1, 2 and 3). Mineral estimation for different season revealed no significant effect of season was observed on the blood calcium levels. Although some animals showed high level of calcium in winter but there was no significant difference along different seasons in animals. Inorganic phosphate was found to be significantly higher in the summer (7.05 ± 0.61) and significantly lower in the autumn (4.58 ± 0.72). While no significant difference was found in between levels in spring (5.5 ± 0.57) and winter (5.45± 0.62). Blood magnesium levels showed marked seasonal variations with significantly lower level in spring and winter (1.69±0.37) than in the summer (2.43±0.48) and spring (1.75 ± 0.41) while no significant difference was observed in levels between spring (2.33 ± 0.57) and winter (2.52 ± 0.65), similarly no significant difference was observed in levels between summer and winter. Iron levels were seen to be significantly higher in summer (3.95 ± 0.32), while no significant difference was observed in levels between spring (2.33 ± 0.57), autumn (2.52 ± 0.65) and winter (2.88± 0.78). The levels of iron were higher in summer in almost all animals. Copper levels was found to be significantly higher in summer (1.43 ±0.42) and significantly lower winter (0.98 ± 0.19), while no significant difference was found in levels of copper between autumn (1.28 ± 0.25) and spring (1.23± 0.21). Present study could not find any seasonal variations on serum Zn levels.

Discussion

The finding in present study showed no significant effect of season was observed on the blood calcium levels, which is in agreement with findings of [2, 3] in dairy cows, and [4] in humans. However in stallions seasonal effect on blood calcium was reported with lowest level observed in summer [2, 3] in sheep. Similarly 1 reported significantly lower blood calcium in winter as compared to autumn in cattle. There was no significant difference across different seasons in present study as efforts were to collect samples from same animals in different seasons.

The results of serum phosphorus level are in agreement with [3] who reported the seasonal variation in serum inorganic phosphate level, with higher values in September than in March [2]. Reported elevated plasma concentrations of phosphorus in the dry season of the year. The increased inorganic phosphate concentration is probably due to the increased intake and increased absorption in the gastrointestinal tract [4].

The results of present study with respect to magnesium in accordance with findings of [3] and contrarily to findings of [5] in horses and [2] in dairy cattle. Earlier reports [6] have proposed that optimum absorption of magnesium occurs when Na: K ratio of diet is 5:1 and is sigficantly impaired if Na:K is less than 3:1. In spring the rapidly growing lush grass is low in sodium and high in potassium, which significantly decreases the Na: K ratio of rumen fluid, and thus depressing the absorption of magnesium.

Iron levels were seen to be significantly higher in summer which were in accordance with the findings of [4] in horse and [5] who reported higher serum iron level in summer. The iron content were seen to vary according to the variations in the serum copper level, probably because copper-containing proteins hephaestin and ceruloplasmin are required for normal iron transport [6]. This was however contrary to the findings of [3] who reported no seasonal effect on serum iron levels in dairy cattle [7, 8] who observed significant high level of iron during winter.

Copper levels was found to be significantly higher in the summer compared to winter which is in accordance with findings of [6] in dairy catllte and [10] in Standard bred mares. [9] Found lower copper concentrations in August in comparison with concentrations measured in February. Copper deficiency may result from malabsorption, caused by excess of calcium, iron, molybdenum and protein. However our findings are contrary to the findings of [11] who found no seasonal effect on blood copper levels. The contrary observations may be due to differences race, sex, and regional feeding practices and because differences in physiological [3].

We did not find seasonal variations on serum Zn. This result is in partial agreement with that of [12] for cattle [9], but not with [12] for human and [7] for dogs.

Table 1: Standard working conditions of AAS for selected elements

Elements	Wavelength (nm)	Slit setting (nm)	Flame	Normal working range (µg/ml)	Sensitivity at 1% (µg/ml)	Operating current (mA)
Ca	422.7	0.5	Air-C2H2	0.05-5	0.160	10-12
Mg	285.2	0.5	Air- C2H2	0.003-1	0.004	
Cu	324.7	0.5	Air- C2H2	0.04-10	0.040	3.5
Zn	213.9	1	Air- C2H2	0.01-2.0	0.012	5
Fe	248.3	0.2	Air- C2H2	0.05-20	0.090	5
Se	196	1	Air- C2H2	3-250	1.0	10

Table 2: Serum parameter of minerals (Ca, P, Mg, Fe, Cu and Zn) in cattle of Kashmir valley in different seasons of year.

Minerals	Period 1 (winter)	Period 2 (Spring)	Period 3 (Summer)	Period 4 (Autumn)
Ca (mg/dl)	9.83 ± 0.96	9.75±1.09	9.59±0.98	9.97±1.12
P (mg/l)	5.45± 0.62^{a}	5.5 ± 0.57^{a}	7.05 ± 0.61^{b}	4.58 ± 0.72^{c}
Mg (mg/dl)	1.69±0.37^{a}	1.75 ± 0.41^{a}	2.43±0.48^{b}	2.51±0.51^{b}
Fe ppm	2.88± 0.78^{b}	2.33 ± 0.57^{b}	3.95 ± 0.32^{a}	2.52 ± 0.65^{b}
Cu ppm	0.98 ± 0.19^{c}	1.23± 0.21^{b}	1.43 ±0.42^{a}	1.28 ± 0.25^{b}
Zn ppm	1.26 ±0.11	1.21±0.24	1.32±0.22	1.23 ±0.16

Values with different superscripts vary significantly ($p \leq 0.05$)

Fig 1: Ruffled coat due to mineral deficiency.

Fig 2: Anaemic mucous membrane due o iron deficiency.

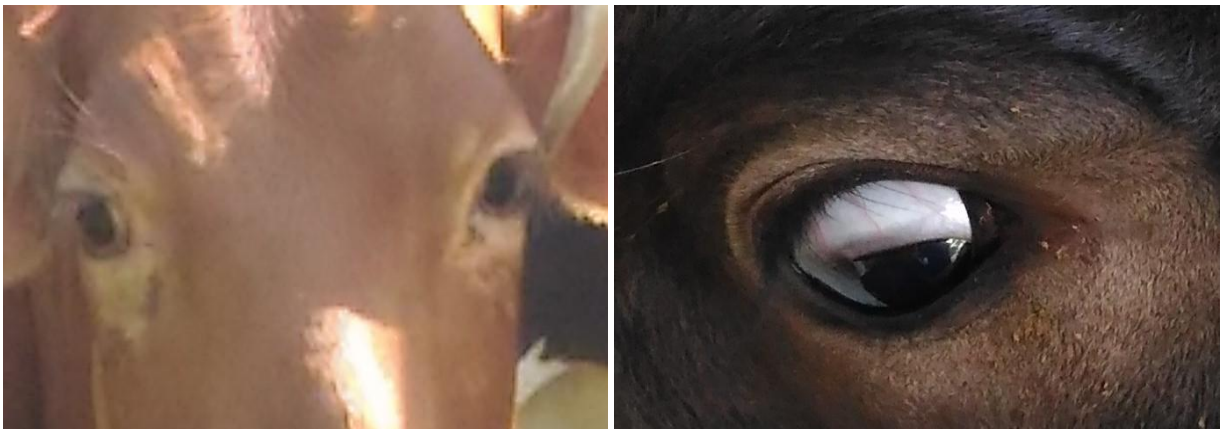

Fig 3: spectacled eye appearance due to copper deficiency.

Conclusion

In conclusion, we found significant seasonal changes in mineral concentrations in healthy animals. The present study postulates seasonal variation in mineral status of animals. Our finding proposes the differential mineral supplementation in different seasons of years and season specific mineral mixture should be prepared for animals in different seasons of year. Season specific mineral supplementation can be both economical and can be advantageous in prevention of deleterious effects of abnormal or unscientific mineral supplementations

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