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Investigation of Calcium, Phosphorous and Magnesium Status of Grazing Sheep in Sabalan Region, Iran

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ABSTRACT

This study was conducted to evaluate seasonal concentrations of macro-minerals in serum or plasma of sheep under grazing conditions to establish mineral deficiencies and excesses over a two-seasonal grazing period (fall-winter and spring-summer corresponding to the rainy and dry seasons) according to variations of year, region and animal classes in Sabalan, Iran. In each flock, ten sheep (38-50 kg body weight, three females groups: yearlings, growing and mature, in addition to one male group) were selected for the study purpose. Approximately 10 ml blood samples were collected in two stages by jugular vein, which were then centrifuged, and plasma or serum were obtained. Plasma phosphorous (P) was determined by the colorimetric method. Serum Ca and Mg were measured by atomic absorption spectrophotometry. Overall, means of serum Ca, Mg and plasma P concentrations were 11.73, 3.24 and 4.92 mg dl^{-1} in dry season and 12.01, 3.17 and 5.3 mg dl^{-1} in rainy season, respectively. Year, season and region significantly affected ($P< 0.001$) serum concentration of Ca but region had no effect ($P> 0.05$) on Plasma P. Unlike season, year effect on Mg concentration was significant ($P< 0.05$). These macro-mineral concentrations of serum and plasma were not different ($P> 0.05$) between animal classes. Ca and P deficiencies were higher in rainy than the dry season (6.24 *vs.* 3.38 and 16.52 *vs.* 15.2 percent, respectively). Although overall mean of serum Ca and P had higher values than the critical levels of these minerals in sheep blood, our results indicate that sheep in Sabalan need a common salt Ca and P supplement to enhance the blood level of these essential minerals in some sheep.

Keywords: Grazing sheep, Iran, Macro-mineral, Status.

INTRODUCTION

An organism needs optimum concentrations of both macro and micro-elements to maintain its life (Littledike and Goff, 1987; Khan *et al.,* 2009). The assessment of mineral needs of animals has come to include determination of them in the tissues, fluids, and products as well as such gross criteria as weight gains, milk yield, etc (Khan *et al.,* 2005). Blood is the most important bio-substrate for the estimation of mineral status of an animal (McDowell, 1985; Khan *et al.,* 2009, 2006). Similar to soil and plants, animal tissue or fluid mineral concentrations are influenced by many factors, including kind and levels of production, age, level and chemical form of elements, breed, mineral intake, interrelationships with other nutrients and animal adaptation (Underwood and Suttle, 1999; Mc Dowell, 1997, 2003). Other factors such as animal physiological status, seasons, region, sex, year and forage species affect the mineral status of animal tissues or fluids (Mc Dowell, 2003; Khan *et al.,* 2009, 2004; Asif *et al.,* 1996; Gartenberg *et al.,* 1999; Romero *et al.,* 2007; Otto *et al.,* 2000;

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Kargin *et al.,* 2004; Espinoza *et al,* 1991a, b). In Sabalan region of Iran, ruminant production largely depends on the use of natural pastures throughout the year. There is no information on mineral concentration of grazing sheep or other ruminants in different regions of Sabalan. On the other hand, weak growth and reproductive problems are common in some years. The objective of this study was to evaluate seasonal concentrations of macro-minerals in serum or plasma under grazing conditions to establish mineral deficiencies and excesses over a two-seasonal grazing period (fall-winter and spring-summer corresponding to the rainy and dry season) according to variations of year, region and animal classes (yearlings, growing ewes, mature ewes and males) in Sabalan, Iran.

MATERIALS AND METHODS

Study Regions

The study was conducted at five regions of Sabalan by using three flocks of Moghani sheep in four animal classes at each region during three years (2004–2006), corresponding to the rainy (fall–winter) and dry (spring–summer) seasons. The pastures of Sabalan (spring–summer pastures) are located in central Ardabil province of Iran and divided into five regions including Sarab (Pastures were predominantly composed of *Festuca sp, Bromus sp, Alopecurus sp, Astragalus sp, Poa sp, Hordeum sp and Agropyron sp,* respectively. Samples of blood in this region were obtained between latitudes 37 °, 59′ , 58.6″ - 38 °, 37′ , 27.9″N and longitudes 47 °, 45′ , 51″ - 47 °, 47′ , 53″ E), Sardabeh (Pastures were predominantly composed of *Hordeum. Sp, Koeleria. Sp, Trisetum flavescens, Festuca. Sp, Trifolium .Sp, Perennial grasses, Bromus sp, Astragalus sp, Alopecurus sp, Poa sp, Lotus sp, Carex sp, and Agropyron sp,* respectively. Samples of blood in this region were obtained between latitudes 38 °, 16′ , 58.3″ - 38 °, 23′ , 04.4″ N and longitudes 47 °, 59′ , 25.2″ - 48 °, 02′ , 08.2″ E), West Meshkinshahr (Pastures were predominantly composed of *Festuca ovina, Trifolium sp, Alopecurus sp, Bromus sp, Poa sp, Perennial grasses, and Agropyron sp,* respectively. Samples of blood in this region were obtained between latitudes 38 °, 23′ , 03.8″ - 38 °, 16′ , 57.1.8″ N and longitudes 47 °, 46′ , 00.4″ - 47 °, 46′ , 36.2″ E), East Meshkinshahr (Pastures were predominantly composed of *Carex sp, Trifolium sp, Rununclus sp, Festuca ovina, Koeleria sp, Astragalus sp, Alopecurus and Textltis*, respectively. Samples of blood in this region were obtained between latitudes 38°, 17′ , 52.7″ - 38°, 26′ , 28.7″ N and longitudes 47°, 48′ , 14.6″ - 47 °, 51′ , 09.2″ E) and Nir (Pastures were predominantly composed of *Festuca sulkata, Festuca ovina, Alopecurus sp, Astragalus sp, Trifolium sp, Bromus sp, Achillea, Acantholimon sp, Thymus sp and Poa sp,* respectively. Samples of blood in this region were obtained between latitudes 38 °, 0 7′ , 11.2″ - 38 °, 0 9′ , 22.6″ N and longitudes 47 °, 54′ , 24″ - 47 °, 52′ , 04.8″ E). In addition, area of Moghan (fall-winter pastures) including Germi (Pastures were predominantly composed of *Artemizia siberie, Agropyron spp, and Astragalus spp,* respectively), Bilesovar (Pastures were predominantly composed of *Artemizia siberie, Caparis spinosa, Salsola. sp* respectively) and Borran (Pastures were predominantly composed of *Artemizia siberie and Salsola sp,* respectively) is located in the north of Ardabil province. Properties of these regions are given in Table 1. Approximately, the different regions of Moghan have similar climate conditions and plants populations. Each pasture maintained four animals /ha/ year under a continual grazing system. In addition, all animals on the pasture had occasional access to a few common salts (rock salt crushed and placed in mangers in the rest stall freely available to sheep).

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Table 1. Geographical characteristics of Sabalan and Moghan regions.

Region [a]	Altitude (m)	Mean of annual temperature (°C)	Annual precipitation (mm)	Soil type
Nir	2150-2950	6.5 [b]	400 – 600 [c]	Clay–sandy
Sarab	1850 - 3000	7.5 [b]	350- 600 [c]	Clay–sandy
Sardabeh	2050 - 3050	8 [b]	350- 620 [c]	loam–clay-sandy
West Meshkinshahr	1400 - 3200	7 [b]	400 – 700 [c]	Clay –sandy–loam
East Meshkenshahr	1850 - 3400	7.5 [b]	350 – 700 [c]	loam–clay–sandy
Germi	600 - 1000	12	280 - 320	Clay–sandy
Bilesovar	40 - 200	14	250 - 350	Clay–sandy–loam
Borran	200 - 400	13	240 - 280	Clay–loam

[a] In order to estimate dominant index of plant pastures in all regions, a 1×1 m^2 quadrate was used. Sampling was done throughout each area of all regions in **W** or **S** shape according to the region's topographical condition. [b] In these regions, temperature decreases about 1 °C per 350 meters altitude increase. [c] In these regions, annual precipitation increases about 100 mm per 300 meters altitude increase.

Animals

In each flock, ten apparently healthy sheep were selected for the study purpose. Average body weights were in the range of 38 to 50 kg. These animals were predominantly of the Moghan breed. The sheep were divided into three female age groups: yearlings, growing ewes (1-2 years old) and mature ewes (≥ 3 years old) and one male group. All experimental animals were different throughout the study period and randomly selected, however with the same body weights and age. Yearlings were non-pregnant at first (May 5^{th}-8^{th} i.e., the end of fall-winter grazing and beginning of spring-summer grazing, referred to as rainy season) but were pregnant in the second stage of blood sampling (September 22^{nd} -25^{th} i.e., the end of spring-summer grazing and beginning of fall-winter grazing, referred to as dry season). Growing and mature ewes were lactating, non-pregnant at the rainy and non- lactating, pregnant in the dry season. Total number of sheep sampled was 300 heads in each year, 10 sheep/flock (3 heads of each female groups and 1 head male/flock) at two stages of three flocks in each of five regions.

Animal Management and Nutrition

Sheep were being reared in the nomadic system. Herdsmen accompanied flocks at all times. Animals were housed in a barnyard (rest stations) with stonewalls and had access to fresh clean water and grasses mixed fecal bed 25 cm thickness during the night, and they were taken out for grazing every day. Sheep were under only one feeding system, which was continual or the same perennial grazing conditions throughout the spring-summer. The pastures or flock grazing sites of regions were away from each other at a distance of 2.5–3 km. The grazing density was four sheep/ha and the time of grazing was 8 h day^{-1} at all regions and years. The animals were on the grass pasture *ad libitum*. All hygienic measures such as lack of epidemic diseases, parasites, clean water were good for the pastures. Sheep were fed during fall–winter season with continual grazing and hand feeding in barn or barnyard at Moghan plain.

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Near end of September, sheep were transported from Moghan to Sabalan by truck for spring-summer grazing.

Sampling and Measurement

The blood samples were collected in rest stations at 7 am before daily grazing. Approximately 10 ml blood samples were collected in two stages, just before and after grazing in spring - summer pastures during three years at five regions of Sabalan from three flocks, by jugular vein puncture with a syringe and needle. Blood samples divided into two parts then transferred into evacuated tubes containing sodium heparin as an anticoagulant to obtain plasma and without heparin for serum. Thus, the total number of samples was 300/year. The sheep were receiving no mineral supplements at the time of investigation and no powder salt or mineral brick was added to the veldt pastures. Blood samples were centrifuged at a speed of 3,000 rpm for 5 minutes. After that, the obtained plasma or serum were transferred into polyethylene tubes and frozen at -20ºC for subsequent analysis. Plasma phosphorous (P) was determined by the colorimetric method (Parsazmun kit, Iran) and by using an auto-analyzer apparatus (Seal Analytical, LTD; Germany). In this method, inorganic phosphorus reacts with ammonium molybdate to form a phosphomolybdate complex. The reduction of this complex produces heteromolybdane, which is blue in color. The intensity of color was assayed by the auto-analyzer (Endres and Rude, 1999). Serum calcium (Ca) and Magnesium (Mg) were measured by an atomic absorption spectrophotometer (Perkin-Elmer. Corporation, USA) using direct sample dilution (Bowers and Pybus, 1972).

Statistical Analysis

Data were analyzed by using the statistical analysis system (SAS, V9). Plasma and serum samples were analyzed in a completely randomized design with factorial arrangement, with year, region, animal class and season as the variables. Significance levels ranged from 0.05 to 0.001 percent of probability. Differences among means were ranked using Duncan's Multiple Range Test.

RESULTS AND DISCUSSION

There were obvious year ($P< 0.001$), season (Ca: $P< 0.01$, P: $P< 0.001$) and interaction of season and year ($P< 0.001$) effects for Ca and P concentrations. Differences between years ($P< 0.05$) were for serum Ca and plasma P during the rainy and dry seasons and for Ca between the rainy and dry season at the Year 2 and P for all years. Calcium had a higher value ($P< 0.05$) in the first year (12.7 mg dl^{-1}) than at both Year 2 (11.14 mg dl^{-1}) and Year 3 (12.1 mg dl^{-1}) during the rainy season and only between Year 1 and Year 3 with Year 2 during the dry season (12.8 and 12.37 *vs.* 9.51 mg dl^{-1}, respectively). Phosphorus had a higher value ($P< 0.05$) in Year 1 (5.74 mg dl^{-1}) than in both Year 2 (4.9 mg dl^{-1}) and Year 3 (4.13 mg dl^{-1}) during the dry season, and only between Year 1 with Year 2 and Year 3 during the rainy season (7.3 *vs.* 4.54 and 4.44 mg dl^{-1}, respectively) (Table 2).

There were significant year ($P< 0.001$) and interaction of season and year ($P< 0.01$) effects for serum Mg concentration, but season was found to be not significant ($P> 0.05$). There were no differences between the Year 2 and Year 3 in terms of serum Mg in each of rainy and dry seasons (Table 2). Magnesium in both seasons in Year 1 had a higher value ($P< 0.05$) than the other years, and there was a significant difference ($P< 0.05$) for Mg between the rainy and dry seasons (3.7 vs. 3.56) (Table 2). Overall, serum Mg almost did not show a deficiency in neither season in any year.

The general tendency was so that for both seasons in Year 1 higher values ($P< 0.05$) of Ca, P and Mg were observed; nevertheless, Ca was not the only element statistically affected

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Table 2. Level of calcium, inorganic phosphorus and magnesium in sheep blood by season and year.

Variable	Critical levels [a]	Season	Year 1		Year 2		Year 3		Overall	
			Mean±SE [b]	% D [c]	Mean±SE	% D	Mean±SE	% D	Mean±SE	%D
Serum										
Ca (mg dl^{-1}) [d]	8	Rainy	12.7 ± 0.16^{c}	1.92	11.14 ± 0.29^{be}	6.82	12.1 ± 0.14^{a}	0.52	12.01 ± 0.11^{d}	3.38
		Dry	12.8 ± 0.13^{a}	0.26	9.51 ± 0.36^{bd}	11.21	12.37 ± 0.13^{a}	0	11.73 ± 0.14^{e}	6.24
Mg (mg dl^{-1}) [e]	2	Rainy	3.7 ± 0.07^{ad}	0.44	2.9 ± 0.04^{b}	0.66	2.92 ± 0.036^{b}	0.44	3.17 ± 0.034	0.77
		Dry	3.56 ± 0.05^{ae}	0	3.1 ± 0.04^{b}	0.44	3.06 ± 0.04^{b}	0.66	3.24 ± 0.03	0.55
Plasma										
P (mg dl^{-1}) [f]	4.5	Rainy	7.3 ± 0.12^{ad}	0.72	4.54 ± 0.12^{bd}	14.71	4.44 ± 0.1^{bd}	15.94	5.3 ± 0.09^{d}	15.2
		Dry	5.74 ± 0.1^{ae}	2.14	4.9 ± 0.12^{be}	4.22	4.13 ± 0.09^{ce}	14.53	4.92 ± 0.07^{e}	17.52

[a] Critical levels (Mc Dowell *et al.,* 1984); Serum (mg dl^{-1}). [b] Means based on the following number of samples: serum 150, 150 for rainy and dry season in each year; [c] % D: deficiency percent; [d] Significance of season or year: S** ; Y*** , SY*** ; S: Season; Y: Year; SY: Season×Year interaction; ns: Non-significant; * Significant at 0.05 level; ** Significant at 0.01 level; *** Significant at 0.001 level; [e] S^{ns} ; Y*** ; SY** ; [f] S*** ; Y*** ; SY*** .
[a,b,c] Means among years in a row with different superscripts differ (P< 0.05); [d,e] Means between seasons in a column with different superscripts differ (P< 0.05).

(P> 0.05) by season (12.7 *vs.* 12.8 mg dl^{-1}). Calcium below critical levels (McDowell *et al.,* 1984) in Year 2 was 6.82% for the rainy and 11.4% for the dry season, also at this year the mean of Ca was lower than the other years and was statistically different within seasons (P< 0.05). Sever deficiencies of P were observed in both seasons in Year 3 and in the rainy season in Year 2 (Table 2). The amount of phosphorous with elapsed years indicates a decreasing trend, as well as showing an uptrend with deficiency percent (Table 2). Overall, plasma P and serum Ca were higher (P< 0.05) in rainy than dry season. Herbivorous animals under natural grazing conditions obtain their minerals from forage plants. Only rarely, however, can pasture forages completely satisfy all minerals requirements (McDowell *et al.,* 1993). Their concentrations in the body fluids will therefore depend on the mineral contents of feed and forage, the level of dietary sources intake, and the availability of minerals (Kamalu *et al.,* 2006; Khan *et al.,* 2007). The concentrations in the body fluids will then depend on absorption from the gut, metabolic usage, hormones and excretion (Littledike and Goff, 1987). Many environmental and plant factors affect the mineral concentration of forage plants; these include, species or strain or variety, soil type, climatic and seasonal conditions during plant growth, stage of maturity of forage plants and other management practices. In Sabalan, stable forages for ruminants are mostly native grasses. Poor animal growth and reproductive problems are common even when forage supply is adequate, and this can be directly related to mineral deficiencies caused by low mineral concentration in soils and associated forages (McDowell, 1997; Tiffany *et al.,* 2000).

Results indicate (Table 2) that Ca and P are higher in the rainy season in the animal blood compared to the dry season. It has been reported that the distribution of different essential minerals and ions between the alimentary fluid and particles of diet is greatly influenced by acidity and that the stage of

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plant maturity adversely affects most minerals in forage (Littledike and Goff, 1987; Khan *et al.,* 2005). The lower concentration of Ca and P in the sheep during the dry season might have been as a result of decreased levels in forage plants due to the advanced level of maturity during this season. This can be supported by the fact that when this study was carried out, the forage plants had dry, brown and withered leaves, and therefore had lower levels of mineral content. In this study, the concentrations of Ca and P were higher in rainy season as compared with those in the dry season, which was probably related to a higher amount available for absorption during this season from forages consumed by the animals studied (Dziuk, 1984). Other factors may possibly be involved as a result of hormonal control of the animals' body metabolism (Dziuk, 1984).

There were obvious region (P< 0.001), season (P< 0.01) and interaction of season and region (P< 0.001) effects for Ca concentration. There were no obvious region (P> 0.05) or interaction of season and region effects for plasma P concentration for sheep, but season was found to be significantly effective (P< 0.001). Also only interaction of season and region (P< 0.05) for serum Mg concentration was significant (Table 3). No differences were found (P> 0.05) among regions for serum Ca (except West Meshkinshahr) and plasma P (except Sardabeh) during the rainy season, similarly P for all regions in the dry season. Calcium had a lower value (P< 0.05) in East Meshkinshahr (11.05, mg dl^{-1}) than in other regions (except Sarab with 11.12, mg dl^{-1}, P> 0.05) during the dry season, and was numerically lower than in both seasons in all regions.

Except Ca concentration in the serum of sheep from Nir region and P concentration in the plasma of sheep from Sardabeh, there were significant differences (P< 0.05, Table 3) between dry and rainy seasons in all regions with regard to calcium and phosphorus in the blood,. Between the two seasons, only in East Meshkinshahr region, sheep blood Mg had significant differences (P< 0.05). Generally, Mg concentration in the blood serum of sheep

Table 3. Levels of calcium, inorganic phosphorus and magnesium in sheep blood by season and regions.

Variable	critical levels [a]	Season	Sardabeh		Sarab		Nir		West Meshkenshahr		East Meshkenshahr		Overall	
			Mean [b]±SE	%D [c]	Mean±SE	%D	Mean±SE	%D	Mean±SE	%D	Mean±SE	%D	Mean±SE	%D
Serum														
Ca (mg dl^{-1}) [d]	8	Rainy	12.32 ± 0.18^{ad}	0	12.25 ± 0.2^{ad}	0.26	12.27 ± 0.22^{a}	0.52	11.33 ± 0.33^{bd}	3.38	11.85 ± 0.35^{ad}	2.6	12.01 ± 0.12^{d}	3.38
		Dry	11.55 ± 0.33^{be}	3.9	11.12 ± 0.26^{bce}	2.34	12.7 ± 0.4^{a}	2.08	12.45 ± 0.18^{ae}	0	11.05 ± 0.34^{ce}	4.16	11.74 ± 0.14^{e}	6.24
Mg (mg dl^{-1}) [e]	2	Rainy	3.04 ± 0.06^{c}	0.22	3.06 ± 0.06^{c}	0.88	3.25 ± 0.07^{ab}	0.22	3.2 ± 0.1^{bc}	0.22	3.38 ± 0.1^{ad}	0	3.18 ± 0.037	0.77
		Dry	3.1 ± 0.04^{b}	0	3.32 ± 0.05^{a}	0	3.25 ± 0.05^{ab}	0	3.36 ± 0.09^{a}	0	3.15 ± 0.07^{be}	1.1	3.24 ± 0.03	0.55
Plasma														
P (mg dl^{-1}) [f]	4.5	Rainy	4.84 ± 0.16^{a}	8.1	5.5 ± 0.19^{bd}	5.96	5.23 ± 0.23^{bd}	5.49	5.57 ± 0.21^{bd}	5.27	5.3 ± 0.18^{bd}	6.93	5.3 ± 0.09^{d}	15.2
		Dry	5.05 ± 0.16	5.33	4.98 ± 0.18^{e}	7.56	4.8 ± 0.1^{e}	7.36	4.83 ± 0.17^{e}	7.77	4.9 ± 0.11^{e}	6.69	4.92 ± 0.07^{e}	17.5

[a] Critical levels (Mc Dowell *et al.,* 1984); Serum (mg dl^{-1}); [b] Means based on the following number of samples: serum 30, 30 for rainy and dry seasons in each region; [c] % D: Deficiency percent; [d] Significance of season or region: S^{**}; R^{***}, SR^{***}; S: Season; R: Region; SR: Season×Region interaction; ns: Non-significant; * Significant at 0.5 level; ** Significant at 0.01 level; *** Significant at 0.001 level; [e] S^{ns}; R^{ns}; SR^{*}; [f] S^{***}; R^{ns}; SR^{ns}.

[a,b,c] Means among regions in a row with different superscripts differ (P< 0.05); [d,e] Means between seasons in a column with different superscripts differ (P< 0.05);

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showed deficiency in both seasons and in all regions. In most of the regions and in the dry season, higher percents of Ca deficiency were obtained than the rainy season. Phosphorus below critical levels (McDowell et al., 1984) was higher in the rainy season than dry season in all regions. Pastrana *et al.* (1991) reported that region has no effect on serum Ca and P concentration in sheep. In this study, the average serum Ca in various regions was 11.33–12.32 in the rainy and 11.05–12.7 mg dl^{-1} in the dry season. These values were higher than those reported (6.1-6.5 mg dl^{-1} in the dry season and 10–10.4 mg dl^{-1} in the rainy season) by Pastrana *et al.* (1991) in various regions of Paramo, Colombia. In addition, P and Mg concentrations were higher as well (5.3 *vs.* 4.7 mg dl^{-1} in the rainy and 4.92 *vs.* 4 mg dl^{-1} P in the dry season; 3.18 *vs.* 2.1 mg dl^{-1} in the rainy and 3.24 *vs.* 2 mg dl^{-1} Mg in the dry season) compared with those reported by Pastrana *et al.* (1991). Romero *et al.* (2007) reported that serum levels of Ca and P in breeding beef cows of Northeastern Mexico changed statically according to season, which were 8.9, 8.5, 9.6 and 9 mg $dl^{-1}$for Ca and 5.9, 6.2, 7.8 and 7.5 mg dl^{-1} for P in the summer, spring, fall and winter, respectively. In this study, altitude, dominant grasses, rainfall, temperature of corresponding altitude and topography of the pastures in various regions were different as well (see Materials and Methods), which might have affected the macro minerals in the serum concentration of the sheep. Broucek *et al.* (2009) reported that mean concentrations of phosphorus ($P< 0.001$) and calcium ($P< 0.01$) significantly differed by the factor of season of year; In addition, the phosphorus concentration was affected by altitude changes ($P< 0.001$). Vitamin D is the major dietary component that influences Ca absorption, and it can be used to explain causes of observed differences in serum Ca between seasons in ruminant animals (Smith and Wright, 1981). It may be helpful to explain that the seasonal changes of Ca occurred in this study in most of regions. When sheep were exposed to a temperature of +8°C (instead of +30°C), a 12% reduction in plasma Mg levels was caused, but it had no effect on Ca levels. Acute cold exposure caused reductions in plasma Mg and Ca levels and an increase in plasma P levels of sheep (Sykes *et al.,* 1969). Hidiroglou (1983) compared the effect of constant (air–conditioned barn, 19±1°C) and fluctuating (conventional barn with free access of barnyard) environments on the plasma macro mineral concentration of ewes from 24th May to 15th November (spring, summer and fall) and reported that calcium, iron, and plasma magnesium differed for both environment and season. Seasonal variations in temperature profoundly affect physiological activity of cattle (Hidiroglou *et al.,* 1977) and sheep (Hidiroglou, 1983).

There were obvious animal classes ($P> 0.05$) and interaction of season and animal classes ($P> 0.05$) effects but no effects for serum Ca, Mg and plasma P concentration were observed. However, season was found to have a significant effect on Ca ($P< 0.01$) and P ($P< 0.001$). The amount of serum Mg in the animal classes with changes in seasons did not change in any way ($P> 0.05$). However, these values in all classes of sheep in both seasons were higher than the standard Mg reference value (2 mg dl^{-1}) (McDowell *et al.,* 1984) (Table 4). Serum Mg concentration in this study was not in line with some investigations (Pastrana *et al.,* 1991; Khan *et al.,* 2005, 2009), who reported plasma Mg concentrations lower than the present findings. This may be related to different management, nutrition, animal type, and various other factors involved in the mineral absorption from the gastro-intestinal tract of animals (McDowell, 1985).

The general tendency was for the dry season to have higher values of percent deficiency of Ca, P and Mg in all animal classes. Calcium below critical levels (McDowell *et al.,* 1984) was 3.38% for the rainy and 6.24% for the dry season. For yearlings, growing ewes, mature ewes and males, Ca values were 3.38, 3.9, 3.65 and 1.56 percent deficient, respectively during the dry season. Sheep and cattle have hormonal mechanisms that maintain blood Ca concentrations within narrow limits by adjusting the proportion of dietary Ca absorbed and, when dietary Ca is inadequate,

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Table 4. Levels of calcium, phosphorus and magnesium in sheep blood by season and animal classes.

Variable	Critical levels[a]	Season	Yearlings		Growing ewes		Mature ewes		Males		Overall	
			Mean[b] ±SE	%D[c]	Mean±SE	%D	Mean±SE	%D	Mean±SE	%D	Mean±SE	%D
Serum												
Ca (mg dl^{-1})[d]	8	rainy	2.38± 0.19^{ad}	1.56	12.05± 0.2^{abd}	1.82	11.68± 0.24^{b}	3.38	11.79 ± 0.38^{b}	0.52	12.01± 0.12^{d}	3.38
		dry	1.75 ± 0.28^{e}	3.38	11.67± 0.25^{e}	3.9	11.85 ± 0.25	3.65	11.53 ± 0.39	1.56	11.74 ± 0.14^{e}	6.24
Mg (mg dl^{-1})[e]	2	rainy	3.16± 0.06	1.56	3.15± 0.05	1.82	3.2± 0.08	3.38	3.29 ± 0.1	0.52	3.18± 0.037	0.77
		dry	3.26 ± 0.06	3.38	3.2± 0.04	3.9	3.26 ± 0.05	3.65	3.15 ± 0.08	1.56	3.24 ± 0.03	0.55
Plasma												
P (mg dl^{-1})[f]	4.5	rainy	5.37± 0.17^{d}	6.69	5.2± 0.16^{d}	11.4	5.38± 0.15^{d}	10.24	5.16 ± 0.24^{d}	3.11	5.3 ± 0.09^{d}	15.2
		dry	5.12 ± 0.12^{ae}	8.91	4.97± 0.12^{abe}	10.7	4.78 ± 0.12^{bce}	11.36	4.57 ± 0.25^{ce}	3.77	4.92 ± 0.07^{e}	17.52

[a] Critical levels (Mc Dowell *et al.,* 1984); Serum (mg dl^{-1}); [b] Means based on the following number of samples: serum 135, 135 in each animal class (female) and 45, 45 in males; [c] % D: Deficiency percent; [d] Significance of season or animal class: S*** , A^{ns} , SA^{ns}; S: Season; A: Animal class; SA: Season×Animal class interaction; ns: Non-significant; * Significant at 0.5 level; ** Significant at 0.01 level; *** Significant at 0.001 level; [e] S^{ns}; A^{ns}; SA^{ns} ; [f] S*** ; A^{ns}; SA^{ns}.
[a,b,c] Means among animal classes in a row with different superscripts differ (P< 0.05); [d,e] Means between seasons in a column with different superscripts differ (P< 0.05).

by desorbing Ca from body reserves in the skeleton (Rowlands, 1980). Stevens *et al*. (1971) found that serum Ca is more affected

by the amounts of P and Mg in the diet than by Ca itself. However, Serum Ca is only affected by severe deficiency, and Ca dietary level may be a better criterion for assessing the status of Ca (CMN, 1973). Plasma inorganic P was deficient in 15.2% of the samples during the rainy and 17.52% during the dry season, i.e., below 4.5 mg dl^{-1} (McDowell *et al.,* 1984). Deficiencies of plasma P for yearlings, growing ewes, mature ewes and males were 6.69, 11.43, 10.24 and 3.11% during the rainy season and 8.91, 10.7, 11.36 and 3.77% during the dry season, respectively. Plasma inorganic P concentrations are maintained by the absorption of P from the gut, and there is no specific mechanism for bone P reabsorption (Jacobson *et al.,* 1972). Positive relationships between dietary P intake and plasma inorganic P have been observed (Rowland's, 1980). Both Yearling and growing ewes serum Ca concentrations were affected by season (P< 0.05), while mature ewes and males were not affected (Table 4). Serum Ca (except mature ewes) and plasma P from all classes of sheep were higher in the rainy season (P< 0.05), but yearlings ewes contained higher (P< 0.05) Ca during the rainy season than that mature ewes and males (12.38 *vs.* 11.68 and 11.79 mg dl^{-1}, respectively). The serum Ca concentration in mature ewes was numerically higher than in other classes in the dry season. Similar results were found for the plasma P concentration in the rainy season (Table 4). The Plasma P in males was lower than that in females for the dry season (P< 0.05) and also the rainy season but not significantly (P> 0.05) (Table 4). Serum Ca concentration in males had a lower value than young ewes in rainy season (P< 0.05). Significant differences (P< 0.05) of serum Ca and plasma P were found between mature and yearling ewes (Table 4), i.e. mature ewes had lower values of serum Ca than yearlings in the rainy season and similarly, plasma P in the dry season. In contrast to our findings, Khan *et al.* (2007) found that males had a higher serum Ca value

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(6.45 mg dl^{-1}) than both lactating (6.05 mg dl^{-1}) and non-lactating (6.36 mg dl^{-1}) ewes during winter. This value was higher (8.99 mg dl^{-1}) than lactating ewes (7.67 mg dl^{-1}) and lower than non-lactating ones (9.18 mg dl^{-1}) during summer. However, our observations of variations in males' serum Ca in both seasons are in line with some investigations (Khan *et al.,* 2009; Otto *et al.,* 2000). We conclude that the males contained lower P and Ca than females in both seasons; also mature ewes had lower Ca and P than young ewes which was in accordance with Otto *et al*. (2000). In this study, the means of serum Ca in the yearlings, growing ewes and mature ewes were 12.38, 12.05 and 11.68 mg dl^{-1} in the rainy season and 11.75, 11.67 and 11.85 mg dl^{-1} in the dry season, respectively. These values are higher than those reported by Pastrana *et al.* (1991) (for yearlings and ewes in the rainy and dry seasons being 10.53 and 9.6 and 6.05 and 5.95 mg dl^{-1}, respectively). In addition, Phosphorus concentration was higher compared with that reported by Pastrana *et al.* (1991) (for yearlings and ewes in the dry season being 5.12 *vs.* 4, 4.78-4.97 *vs.* 2.54, respectively and for ewes in the rainy season being 5.2-5.37 vs. 3.62 mg dl^{-1}. In contrast, yearlings in the rainy season had lower values, i.e., 5.37 *vs.* 5.58 mg dl^{-1}).

Romero (2007) reported that serum Ca and P concentrations during fall-winter were higher than spring-summer, which was in accordance with our findings for all animal classes except mature ewes for Ca. Khan *et al.* (2005, 2007) reported that plasma Ca from both non-lactating and lactating sheep were higher in summer than winter. Also, this element in non-lactating sheep was higher during both seasons than that in lactating animals.

In this study, in the rainy season, yearlings (with non-lactating and non-pregnant physiological conditions) and mature ewes (lactating, non-pregnant), respectively, had higher and lower of serum Ca concentration than yearlings (pregnant) and mature ewes (non-lactating, pregnant) in the dry season. This observation was in agreement with those made by Kulkarni and Talvelker (1984) and Khan *et al,(* 2005, 2007). Otto *et al*. (2000) reported that lactating cows had lower serum Ca and P than pregnant and non-pregnant cows. Non-pregnant cows contained higher levels of these elements than pregnant ones. In lactating sheep, the Ca homeostasis is maintained by an increased rate of dietary Ca absorption, decreased rate of urinary Ca excretion, and mobilization of bone Ca under the complex physiological action of parathyroid hormone, calcitonin, and vitamin D (McDowell, 2003).

Variations in plasma or serum macro-mineral status in different animal classes may be attributed to many factors affecting mineral requirements which include nature and level of production, age levels and chemical forms of elements, interrelationships with other nutrients, mineral intake, class of animals, and animal adaptation (McDowell, 1985). Mineral requirements and status are highly dependent on the level of productivity and physiological state of the animal (NRC, 1985). There is extensive evidence showing marked variations within breeds and between animal classes, in the efficiency of absorption of minerals from the diet (Field, 1984). The most possible causes for this apparent variation in dietary requirements, for some macro- minerals between breeds and classes could be genetic differences in the efficiency of absorption (Field, 1984). Animals most susceptible to some macro-element deficiencies are young rapidly growing animals and those during lactation. Dietary requirements may decline with age, because the major requirement for growth often remains constant, while appetite increases in proportion to body size (Khan *et al.,* 2005). Specific mineral requirements are difficult to pinpoint since exact needs depend on chemical form and numerous mineral interrelationships. With some elements, the chemical form has a major impact on the availability of the element (Towers and Grace, 1983; Grace and Lee, 1990). Age and class of animals can affect requirements of minerals through changes in efficiency of absorption. Overall, young animals absorb minerals more efficiently than older animals (McDowell, 2003). Grazing habits and preferences for different grasses by ewes in various

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physiological states may be a cause of different mineral concentrations found in the present investigation such as growing ewes (two-year-old ewes) with mature ewes and or between lactating and non-lactating as well as pregnant and non-pregnant ewes (San Martin and Bryant, 1989).

CONCLUSION

Results from the investigation on the status of some macro-minerals of grazing sheep in the specified region of Iran (Sabalan) suggest that calcium and particularly phosphorous were indefinitely deficient during both seasons in all regions for all animal classes. Furthermore, the results showed that continual grazing by sheep under spring-summer conditions in various regions of Sabalan is unable to prevent deficiencies of Ca and P concentration in the serum or plasma completely. Therefore, supplementation of these ruminant groups with a bioavailable mineral mixture will probably increase the blood level of these minerals. However, more studies should be carried out to determine requirements and economic benefits of mineral supplementations.

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بررسی وضعیت کلسیم، فسفر و منیزیوم گوسفندان در حال چرای منطقه سبلان ایران

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چکیده

این مطالعه در دو فصل (پاییز– زمستان و بهار– تابستان، منطبق با فصول بارانی و خشک) بر اساس متغیرهای سال، منطقه و کلاس حیوانی به منظور ارزیابی فصلی غلظت، مازاد و کمبودهای عناصر معدنی عمده در سرم یا پلاسمای گوسفندان در حال چرای منطقه سبلان انجام گردید. برای این منظور، از هر گله ۱۰ راس گوسفند (۳ گروه ماده: یکساله، در حال رشد و بالغ ؛ یک گروه نر) انتخاب گردید. در دو مرحله، مقدار ۱۰ میلی‌لیتر خون از ورید وداج گرفته شد که پس از سانتریفوژ کردن، سرم و پلاسمای نمونه‌ها استحصال گردید. فسفر به روش کالریمتری و کلسیم و منیزیوم به شیوه جذب اتمی اندازه‌گیری شدند. در کل، میانگین غلظت کلسیم، منیزیوم وفسفر به ترتیب ۱۱/۷۳، ۳/۲۴ و ۴/۹۲ میلی‌گرم بر دسی‌لیتر در فصل خشک و ۱۲/۰۱، ۳/۱۷ و ۵/۳ میلی‌گرم بر دسی‌لیتر در فصل بارانی بود. سال، فصل و منطقه اثر معنی‌داری روی غلظت کلسیم سرم داشت($P< ۰/۰۰۱$) ولی منطقه تاثیر معنی‌داری بر غلظت فسفر پلاسما نداشت($P>۰/۰۵$). بر عکس فصل، تاثیر سال بر روی غلظت منیزیوم معنی‌دار بود($P<۰/۰۵$). کلاس‌های حیوانی اثر معنی‌داری روی غلظت عناصر معدنی عمده در سرم و پلاسما نداشتند($P>۰/۰۵$).کمبود کلسیم و فسفر در فصول بارانی بیشتر از فصول خشک بود(به ترتیب ۶/۲۴ در مقابل ۳/۳۸ و ۱۶/۵۲ در مقابل ۱۵/۲ درصد). اگرچه میانگین کل غلظت کلسیم و فسفر بیشتر از سطوح بحرانی این مواد معدنی در خون گوسفند بود، نتایج نشان می‌دهند که برخی از گوسفندان منطقه سبلان نیازمند یک مکمل رایج کلسیم و فسفر برای بالا بردن سطوح خونی این عناصر ضروری می‌باشند.

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