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MINERAL STATUS OF LACTATING EWES IN THE ARID AND SEMI-ARID ZONES OF NORTH-CENTRAL MEXICO

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ABSTRACT

Profitable sheep production depends greatly on the mineral content of range forages, as adequate nutrition is a prerequisite for reaching the animal's optimum performance. The objective was to determine the contents of calcium (Ca), phosphorus (P), copper (Cu), zinc (Zn), and manganese (Mn) in range forages and blood serum of grazing, lactating ewes in the arid and semi-arid zones of North-Central Mexico. Samples of forages and blood of lactating ewes were collected in two seasons (spring and winter) from four randomly selected sheep farms located in the state of Aguascalientes. The P content was analyzed using the colorimetrical method, while levels of Ca, Cu, Zn, and Mn were determined by spectrophotometry. Forage mineral content averaged 0.20% P, 0.34% Ca, 8 ppm Cu, 483 ppm Mn, and 29 ppm Zn. In blood serum, mineral content reached 20 ppm P, 147 ppm Ca, 0.52 ppm Cu, 2.9 ppm Mn, and 0.80 ppm Zn in winter, and values of 20.5 ppm P, 177 ppm Ca, 0.57 ppm Cu, 3.13 ppm Mn, and 0.89 ppm Zn in spring. Overall, in both spring and winter seasons, 58-100% of sampled forages showed Ca, P, Cu, and Zn deficiencies, but all of them fitted the optimal content of Mn. Blood samples showed similar patterns in winter and spring seasons, Ca was not deficient (145-180 ppm vs. optimal range: 90-120 ppm), but P, Cu, and Zn were deficient (P: 9-21 ppm vs. optimal range: 50-65 ppm; Cu: 0.51-0.58 vs. optimal range: 0.60-1.5 ppm; and Zn: 0.71-0.92 ppm vs. optimal range: 0.50-1.2 ppm); Mn was slightly over the optimal range (2.89-3.2 μg/L vs. optimal range: 2 to 40 μg/L). In some cases, forage mineral content might not be related to blood serum contents. However, it should be desirable to understand all the uncontrolled factors in addition to the mineral interactions that could interfere with further studies.

Keywords: Grazing sheep systems, sheep nutrient requirements, mineral deficiencies, mineral supplementation strategies, semi-arid ecosystems.

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INTRODUCTION

At a global scale, ruminants are primarily raised under grazing systems, at least in the largest livestock-producing countries (Hedge, 2019). Sheep are affected by climatic variations and are highly vulnerable to extreme droughts (Partida et al., 2013).In North-central México (Aguascalientes and Zacatecas), most sheep are raised under extensive farming conditions (Vázquez -Martínez et al., 2018), even under arid and semi-arid environmental conditions that limit forage availability (BSh and BSk, according to Köppen climate classification; precipitation from 410 to 530 mm/year) (Medina-Cuéllar et al., 2018a). Deficiencies of Cu, Fe, P, and Mg have been found in many soils of semi-arid zones of Mexico. At the same time, forages present moderate levels of Ca, Zn, Mn, and K; for example, in Zacatecas, the soils are deficient in P, Ca, Mg, Na, and K, while low levels of P, Ca, Mg, and Na have been found in range forages. There is a lack of information about the relationship between soils and forages regarding mineral contents, but they might correlate in other types of plants (Gallegos and Bautista, 2022). Previous studies have described that forages in Central-North Mexico have enough Ca, Mg, K, Fe, and Mn to meet the nutrient requirement of livestock, but P, Na, Cu, and Zn could be deficient (Gerrero-Cervantes et al., 2012).

Regardless of the marginal economic incomes of grazing production systems, if grassland areas are not limited, grazing might reduce production costs, increasing the cost/benefit ratios (Islam et al., 2018).

Due to global concerns about the amount of greenhouse gases (GHG) emissions from ruminants mostly fed with fibrous forages, novel studies have focused on the chemical compounds that could enhance or limit the potential ruminal degradability of feedstuff (Miranda-Romero et al., 2020). Additionally, determinations of the mineral concentrations in forages and animal samples help diagnose mineral deficiencies and look for solutions (Huerta-Bravo, 2016). The mineral content of range forages plays a crucial role in ruminant production systems (Islam et al., 2018). The type of soil and forage, rainfall, pasture management, and the use of low-quality mineral supplements cause, in many livestock farms, deficiencies or imbalances of minerals in the soil-plant-animal chain (Khan et al., 2017).

Several studies have mentioned that low levels of Cu, Zn, and Co in the soil were strongly correlated to trace mineral deficiencies in farm animals (Khan et al., 2017). For example, Zn and Mn deficiencies can influence reproductive functions and P (Khan et al., 2009). In sheep, Zn is an essential nutrient in ruminants, and its deficiency produces parakeratosis, low conception rates, and depressed growth (Nielsen, 2012; Maqsood et al., 2022). Similarly, P deficiency in cattle is associated with poor growth and problems in reproduction, while both P and Ca play a vital role in forming bones and teeth.

The objective of the present study was to determine the contents of calcium, phosphorus, copper, zinc, and manganese in range forages and blood serum of grazing, lactating ewes in the arid and semi-arid zones of North-central Mexico.

MATERIALS AND METHODS

Location

The study was conducted in the arid and semi-arid zones of North-Central Mexico (longitude 102° 5′ 21″ W, latitude 21° 52′ 47″ N; Aguascalientes, Mexico). The zone is located at an altitude of 2168 meters above sea level, with a mean annual temperature of 18.2°C, a mean yearly rainfall of 442 mm from June to August, and a semi-dry climate (INEGI, 2017) (BS1kw according to Köppen climate classification (Kottek et al., 2006)).

Farm selection criteria and sheep management

Four farms located in the northeast of Aguascalientes (Mexico), where sheep are raised on grass in the form of pasture/ grazing combined with feed lotting, were randomly selected ("La Providencia," "Buena Vista," "El Arco, and "El Tepetate"; total sampled surface=548 km^2). In the selected farms, producers combine sheep raising with the production of fruit trees such as peaches and vines and corn crops, using minimal technology and mineral supplementation management. The predominant vegetation in the experimental area corresponds to xeric shrublands (basically/mesquite) with associations of annual grasses, mainly of the genus *Bouteloua*. The present study involved 9750 sheep heads (30% of the total sheep population in Aguascalientes, Mexico).

Sample collection

Thirty-four types of forages were sampled (Table 1) in the selected farms' two seasons: spring (starting of the rainy season, 17 samples) and winter (dry season, 17 samples). Samples were taken using a scraper in the different pastures by cutting approximately four cm from the ground (500 g were taken and stored in paper bags). The forages of the farms were pooled to obtain mixed spring and winter samples.

On the other hand, 80 blood samples were

Table 1. Botanical composition of forage samples.

Common name	Scientific name
Bermuda Grass	*Cynodon dactylon* (L.)
Johnson Grass	*Sorghum halepense*
Puncher Grass	*Cenchrus echinatus*
Razor Grass	*Bouteloua gracilis*
Crow's foot grass	*Chloris submutica*
Flag Grass	*Bouteloua curtipendula*
Whitetip Grass	*Digitaria califórnica*
Guia Grass	*Hopia obtusa*
Pajita Grass	*Setaria grisebachii*
Pink Grass	*Rhynchelytrum repens*
Mesquite	*Prosopis laevigata*
Mallow	*Malva sylvestris*
Sunflower	*Helianthus annuus*
Sunflower weed	*Tithonia tubiformis*
Tumble weed	*Salsola tragus L*
Dog quelite	*Chenopodium murale L*
Clover	*Trifolium* spp.

taken in spring and winter from 20 adult lactating ewes of crossed breed (Black belly and Katahdin) in each farm and season, grazing for at least one year at the farm. The ewes were randomly selected from the flock in each season. Blood samples were taken from the jugular vein using vacutainer tubes without anticoagulant, collecting approximately 20 mL of blood per ewe, and centrifuged at 3,000 g for 15 minutes. Subsequently, blood serum was collected in 15-mL tubes and frozen until analysis (Fick et al., 1979).

Chemical composition analysis

The collected forages were oven-dried (Felisa, model FE-293D) at a temperature of 60 °C for 48 h to obtain dry matter (D.M.), which was then ground through a 1-2 mm screen in a mill (Homemade) and stored in plastic bags and labeled including according to de season, farm, and repetition.

Cu, Zn, and Mn contents were determined using ash obtained from 5 g of dry forage. In contrast, Ca and P (AOAC, 2012) were determined by using ash from 1 g of the material, placed into porcelain crucibles, and taken to the muffle furnace (Linderberg, Model 51848) at 600^o C for six hours. Subsequently, acid digestion was performed using a 20% hydrochloric acid solution for 40 min and filtered through Whatman No. 41 filter papers to obtain a mother solution prepared in a 50-mL flask. After this, dilutions were done in triple distilled water; after that, samples were processed according to the specific technique for each element, using standard solutions to get a standard curve to compare each sample and obtain the mineral concentration in ppm, considering the sample weight and the dilutions made.

Mineral contents in forage and blood serum samples were determined by atomic absorption spectrophotometry, except for P, which was obtained by the colorimetric technique of Fiske and Subbarow (1925).

In blood serum: 1) To determine Ca, 1 mL of blood serum was taken and placed into a 50-mL flask, which was brought to the mark with a 0.1% (W/V) lanthanum solution. Subsequently, a sample was analyzed using an atomic absorption spectrophotometer, and a standard curve was prepared. For Cu, Zn, and Mn, 2 mL of blood serum were taken and placed in centrifuge tubes, and 2 mL of 20% (W/V) trichloroacetic acid was added. Subsequently, the tubes were placed in a water bath for 15 min and centrifuged at 3,000 g for 10 min, and the elements were analyzed from the supernatant in the atomic absorption spectrophotometer; 2) To determine P, 1 mL of serum was taken and mixed with 9 mL of a 5% trichloroacetic acid solution, centrifuged at 3,000 g and filtered; then, 3 mL of the filtrate was placed in a 15-mL tube, and then 0.5 mL of 2.5% (W/V) ammonium molybdate, 0.5 mL of a solution of amino 2-naphthol 4-sulphonic acid, and 4 mL of distilled water were added and left in the darkness for 10 min. Subsequently, absorbance was read in a UV-visible spectrophotometer (Cintra 10 MCA GBC) at 660 nm, and then a standard curve was prepared.

Determinations

The Ca and P requirements of 40 kg lactating ewes, which produced an average milk yield of 2 kg/d (MY) and had a D.M. intake (DMI) of 2500 g /d, were calculated according to the NRC (2007) (Models 1 and 2):

$$\text{Ca requirement (g/d)} = \frac{(0.623DMI+0.228)+(1.6MY)}{0.50} \quad \text{Model (1)}$$

$$\text{P requirement (g/d)} = \frac{1.6(0.693DMI-0.06)+(1.3MY)}{1.60} \quad \text{Model (2)}$$

Statistical analysis

Data were analyzed using the Statistical Analysis System software (SAS, 2013; V. 9.2) and assessed for normality using Proc Univariate (T-student and Chi-square tests). Additionally, the probability distribution of mineral contents was analyzed using non-parametrical tests (Shapiro-Wilk et al.).

Comparisons between the observed contents and their requirements were performed per mineral at 95% intervals of confidence (I.C.) for samples with unknown variance (T-student). The seasonal or locality (farm) fixed effects were tested through analysis of variance (ANOVA) using general model linear procedures (Proc GLM; SAS, 2013) according to Model (3). Means and standard errors (S.D.) were obtained through L.S. Mean instructions (SAS, 2013) and compared by Tukey tests (P<0.05).

$$Y_{ij} = \mu + SL_i + \varepsilon_{ij} \quad \text{Model (3)}$$

Where: Y_{ij} = Cu, Ca, Mn, P, and Zn; μ=general mean; SL_i is the effect of the ith season (spring or winter) or ith locality; and ε_{ij}= = random error.

RESULTS AND DISCUSSION

Table 1 shows the botanical composition of the forage samples. Overall, most of the species were grasses from the Poaceae family (Gramineae) and also included species from the Fabaceae family (Leguminosae) such as mesquite (*Prosopis laevigata*), sunflower (*Helianthus annuus*), mallow (*Malva sylvestris*), tumbleweed (*Salsola tragus* L.), and clovers (*Trifolium* spp.), which are more abundant in spring than in winter.

Semi-arid zones of central Mexico may exhibit a mix of grasses, shrubs, and other vegetation, contributing to the area's overall biodiversity. Specifically, in Aguascalientes, shrubs play an important role in maintaining the natural ecosystem and pollinators such as honey bees (Medina-Cuéllar et al., 2018a, b).

Although the production of meat and milk from ruminants under grazing systems is not the most efficient, the sector contributes to food security, with minimal damage to the natural semi-arid landscapes, in turn contributing to reducing the impact of climate change given the fact that natural vegetation is one of the central carbon pools in the global carbon cycle. Its modification contributes to the fluctuation of atmospheric carbon concentration (IPCC, 2013). Agricultural activities in arid riparian ecosystems significantly alter carbon storage by removing native vegetation and engaging in tradeoff cycles, given the storage capacity of crops.

The benefits of sheep farming systems include low investments and ecological improvement derived from carbon storage, soil mycorrhizal recruitment (which also prevents the overuse of chemical fertilizers), biodiversity conservation, and landscape connectivity (Cornejo-Denman et al., 2023). Furthermore, the minimal management requirements for grain and forage production can help reduce the overexploitation and pollution of aquifers.

Like traditional agricultural systems, correct management of grasslands (by avoiding early grazing and overgrazing practices) in extensive livestock systems could contribute to maintaining the natural balance of semi-arid ecosystems and improving the chemical and mineral composition of shrubs (Islam et al., 2018). Because of the nature of the semi-arid ecosystems in Mexico, shrubs can be deficient in N, P, and K (deficiencies detected in *Opuntia* spp., *Prosopis* spp., and *Acacia* spp.). However, using and maintaining naturally adapted plants or shrubs can reduce the need for external inputs and promote water conservation (Bard, 2024a, b).

Mineral content in forages in spring and winter seasons

Table 2 shows the mineral contents of Ca, P, Mn, Cu, and Zn in the sampled forages across the spring and winter seasons. Overall, the obtained values were below the minimum requirement reported by the NRC (2007). Similar studies conducted in the north of Mexico analyzed composite samples that included different proportions of trees (*Quercus et al. grisea*), shrubs (*Atriplex canescens, Acacia constricta, Acacia shaffneri, Cassia wislizeni, Celtis pallida, Condalia lycioides, Cordia parvifolia, Flourencia cernua, Larrea tridentata, Mimosa biuncifera*, and *Prosopis leavigata*), forbs (*Coldenia greggii, Dalea bicolor, Jatropha dioica,* and *Parthenium incanum*), cacti (*Opuntia imbricata, Opuntia leptocaulis,* and *Opuntia leucotricha*); red and white fruits (*O. leucotricha; fruits from O. leptocaulis, O. imbricata, and A. canescens*), pods (*P. leavigata, A. shaffneri*), and flowers (*Yucca* spp.) reported sufficient amounts of Ca, Mg, K, Fe, and

Mn, but marginal P, Na, Cu and Zn deficiencies (Guerrero-Cervantes et al., 2012).

Mineral deficiencies can reduce the growth and productivity of forage species and increase their susceptibility to stress. Common symptoms of nutrient deficiency are poor flowering (N deficiency)), stunted growth caused by low energy transfer (P deficiency), reduction of disease resistance (K deficiency), poor root development and weak stems (Ca deficiency), and yellowing and leaf drop (Mg deficiency) (Bard, 2024a). In addition, mineral deficiencies in forages can lead to economic and environmental negative impacts on livestock production because of reduced dry matter intake (DMI) and D.M. ruminal degradability, livestock immune function, animal growth and development, and milk and meat yield and quality (Bard, 2024a).

Calcium. Deficiency of this mineral was observed in 84% of the forage samples analyzed in the winter season, indicating that Ca intake was below the minimum requirement of 0.40% for medium-sized lactating ewes, as suggested and calculated by the NRC (2007) (Table 2). In spring, the average Ca content was higher (0.47%), but only 52% of the samples reached levels that met the requirement. On average, Ca contents in forage were lower in winter than in spring (0.22% *vs.* 0.47%), showing that winter forages are below the critical level of 0.40% for lactating ewes (NRC, 2007). Legumes are rich in calcium, and this might explain the higher Ca content in spring when there are more legumes growing (Muñoz-González et al., 2017). Additionally, the forage Ca content can decrease from the vegetative period to the post-productive period; for example, the Ca concentration of *Alopecurus myosuroides* grasses decreased from 0.40% (in the vegetative period) to 0.17% (in the post-productive period) (Khan et al., 2017).

Phosphorous. The P content in the sampled forages was, on average, 0.20% (0.19% for winter and 0.22% for spring), being below the nutrient requirements for medium-sized ewes in reproduction and lactation (0.35%) recommended by the NRC (2007). Lower P values were recorded in winter compared to spring values ($P<0.05$). Higher P levels were reported by Domínguez-Vara and Huerta (2008) in a similar study conducted in Toluca Valley, Mexico. The authors found adequate P contents in forages and sheep blood serum in most sampled farms, reporting deficient values in 2 farms in autumn.

In contrast, a study conducted by Marquez-Madrid et al. (2017) revealed deficient P levels in the soil and from deficient to limiting levels in range forages in Zacatecas, Mexico. In the present study, P levels were under the percentile 34 (Deficiency=66%). Soto and Reynoso (2012) have indicated that P deficiency in ruminants is related to the decrease in voluntary DMI, which leads to worse productive performance, especially in first-calving females, who are still growing. Accordingly, the P levels reported in the present study might be explained by reduced economic incomes in other farms in central Mexico's semi-arid zones. However, the results obtained herein are similar to those of Muñoz-Gonzalez et al. (2019), who conducted a study in the south of Mexico (Palenque, Chiapas state) and reported forage P contents that are considered deficient for ruminants (average of 0.19%), with 70% of the samples showing P concentrations below the recommended requirements.

Copper. Even when the overall average was higher than the reference (Table 2), Cu content was highly variable (average=8.15, SD=4.2-5.6); 75% of the forage samples analyzed in the four farms presented deficient or limiting Cu contents compared to the recommended amount by the

Table 2. Mean mineral content in range forages of the northeast of Aguascalientes, Mexico.

Mineral	Season		Average	Calculated requirements for lactating ewes[1,2]	Criteria (I.C. %)
	Winter	Spring			
Calcium[1] (%)	0.22±0.18b	0.47±0.33a	0.34	0.40	66% Def or Lim
Phosphorus[1] (%)	0.19±0.04b	0.22±0.06a	0.2	0.35	100% Def
Manganese[1,2] (ppm)	460±115a	507±107a	483	20	100% High
Copper[1,2] (ppm)	7±4.2a	9.3±5.6a	8.15	6-10	75% Def or Lim
Zinc[2] (ppm)	23±10.0a	36±18.1a	29.5	30	58% Def or Lim

*Different letters in the row indicate significant differences between seasons: Def = Deficient; Lim=Limiting; [1] compared to the calculated requirements by the NRC (2007); [2] compared to the calculated requirements by Khan et al. (2009).

NRC (2007) and Khan et al. (2009) for lactating ewes. The mean Cu content found in spring was 9.3 ppm, a value above the minimum Cu requirement and considered limiting since only 37% of the forages met the recommended requirement for lactating ewes. In winter, the mean content was seven 7 ppm above the minimum requirement. However, 88% of the samples presented deficient to critical levels of Cu in both spring and winter seasons (P>0.05), which can be partly explained by the considerable variation in Cu content in the different forage samples. A previous study by Muñoz-González et al. (2017) reported average values of 9 ppm in different forages in Tlaxcala, Mexico. The authors observed higher Cu contents in legumes than in grasses and deficient to critical levels (0.35 to 0.92 ppm) of this element in the blood serum of ewes. Cu deficiency has also been reported in other semi-arid or arid zones. Studies conducted in Pakistan have reported a mean Cu value of 6 ppm was reported in forages, which is considered deficient for cattle, except if the molybdenum concentration is deficient (Shah and Hussain, 2014); Cu values ranging from less than 1 to 14 ppm in grasses (Khan et al., 2017); Cu contents of 7 ppm in winter and 19 in summer, with adequate levels in the soil and with most of the forages exceeding 10 ppm, a value considered adequate for both cattle and sheep, while all the blood serum samples analyzed from sheep and goats showed average Cu values higher than 0.65 ppm (Khan et al., 2008).

Manganese. The forage samples analyzed in the four farms exceeded 20 ppm Mn, a value suggested as the requirement of ewes (NRC, 2007) (Table 2). The mean contents of forage Mn of the four farms were 507 ppm in spring and 460 in winter. These values are considered high and do not differ between the evaluated seasons. In Pakistan, a study conducted in Mastuj Valley (Chitral District) revealed that mean contents of Mn of 731, 664, and 790 ppm were reported in *Cynodon dactylon* (L.) pers. The pre-flowering, flowering, and post-reproduction stages, respectively, show no significant differences between the phenological stages of the plant (Shah and Hussain, 2014). Another study in the same country reported Mn contents in forages in Central Punjab that exceeded the critical level of 20 ppm recommended for grazing cattle, recording 115 ppm in November and 76 ppm in January (Khan et al., 2009).

Zinc. 71% of forage sampled in spring met the requirement of 30 ppm suggested as adequate by Khan et al. (2009) for breeding ewes. Conversely, 79% of the forage sampled in winter was below the recommended value (Table 2). The average Zn of forages varied from 17.9 to 54.1 ppm and 13 to 33 ppm in spring and winter, respectively. Therefore, shrubs had adequate Zn contents in spring, sufficient for growing lambs, whereas winter shrubs were insufficient for breeding and lactating ewes. According to Domínguez-Vara and Huerta-Bravo (2008), average Zn contents under 30 ppm might cause Zn reduction in the blood serum of sheep (0.28 to 0.62 ppm) in some zones of Central-South Mexico (Toluca, State of Mexico). Khan et al. (2009) described that Zn contents of 25-42 ppm (from October to January) were limiting to optimal for sheep productive performance, even when 7 ppm was enough to allow the growth of lambs, 15 ppm to maintain normal Zn blood levels, and 32 ppm for normal testicular development. Overall, the authors agree that 30 ppm of Zn could be a critical value for sheep (Khan et al., 2008).

Mineral content in blood serum of lactating ewes

Tables 3 and 4 show the contents of Ca, P, Mn, Cu, and Zn in blood samples of lactating ewes in winter and spring in the different localities (farms) included in the present study. Overall, farms had no differences in any mineral content (P>0.14).

Winter. Ca content ranged from 144 to 149 ppm, being higher than the reference values for other forages (90 to 120 ppm) (Table 3); P and Cu were more deficient, being 100 and 80% lower than other forages, respectively (P ranged from 19 to 21 ppm *vs.* 50-65 ppm, and Cu ranged from 0.51 to 0.54 *vs.* 0.60-1.5 ppm); Zn was barely deficient (ranged from 0.71 to 0.82 ppm *vs.* 0.50-1.2 ppm); and Mn levels were slightly over the optimal reported levels that vary from was deficient (ranged from 2.89 to 3.1 μg/L vs. the optimal 2 to 40 μg/L). In this regard, it is difficult to determine any criteria to define the percentile where samples could be associated due to the differences among reported data for optimum contents.

Spring. Blood samples showed similar results in both seasons (Table 4). Ca contents were 100% higher than reference values, ranging from 174 to 179 ppm; P, Cu, and Zn were deficient and lower than reference values (20-21, 0.56-0.58, and 0.88-0.92 ppm for P, Cu, and Zn, respectively). At the same time, Mn showed undetermined deficiencies (criteria non-reported), ranging from 3.03 to 3.2 μg/L.

The Ca deficiencies in forages reported in the present study did not negatively affect the blood

Table 3. Average values of minerals in blood serum (ppm) of adult lactating ewes in the winter season.

Mineral	Localities (farms) L1	L2	L3	L4	SD	P-Value	Reference value[1, 2]	Criteria
Calcium (ppm)	145	148	144	149	22.9	0.8524	90- 120 ppm	100% High
Phosphorus (ppm)	21	21	20	19	3.12	0.2003	50 - 65 ppm	100% Def.
Manganese (µg/L)	2.94	3.1	2.89	2.96	0.45	0.6674	2-40 µg/L	NR
Copper (ppm)	0.51	0.54	0.51	0.52	0.18	0.9681	0.6 - 1.5 ppm	80% Def.
Zinc (ppm)	0.82	0.81	0.79	0.79	0.31	0.9926	0.5 - 1.2 ppm	5% Def.

Def = Deficient; Lim=Limiting; N.R., non-reported; L1, L2, L3, and L4: "La Providencia," "Buena Vista," "El Arco, and "El Tepetate," respectively (Aguascalientes, Mexico); S.D., standard deviation; P-Value, probability value; Reference value [1]Compared to the calculated requirements by the NRC (2007) and [2]Compared to the calculated requirements by Khan et al. (2009); Criteria, interval of confidence 95%; N.R., non-reported.

Table 4. Average values of minerals in blood serum (ppm) of adult lactating ewes in the spring season.

Mineral	Localities (farms) L1	L2	L3	L4	SD	P-Value	Reference value[1, 2]	Criteria
Calcium (ppm)	175	174	179	180	22.8	0.8628	90- 120 ppm	100% High
Phosphorus (ppm)	20	21	21	20	2.59	0.1399	50 - 65 ppm	100% Def.
Manganese (µg/L)	3.15	3.2	3.03	3.14	0.52	0.4078	2-40 µg/L	NR
Copper (ppm)	0.58	0.57	0.56	0.56	0.27	0.9891	0.6 - 1.5 ppm	74% Def.
Zinc (ppm)	0.88	0.92	0.88	0.88	0.35	0.9817	0.5 - 1.2 ppm	5% Def.

Def = Deficient; Lim=Limiting; N.R., non-reported; L1, L2, L3, and L4: "La Providencia," "Buena Vista," "El Arco, and "El Tepetate," respectively (Aguascalientes, Mexico); S.D., standard deviation; P-Value, probability value; Reference value [1]Compared to the calculated requirements by the NRC (2007) and [2]Compared to the calculated requirements by Khan et al. (2009); Criteria, interval of confidence 95%; N.R., non-reported.

serum of lactating ewes in any of the seasons since blood samples showed higher contents than the normal Ca levels. On the contrary, Sowande et al. (2008) reported that the sampling season significantly influences blood serum in sheep and goats. However, many uncontrolled factors, such as Ca content in the soil and Ca interactions with other mineral proportions like P and D_3 available sources, can increase levels of blood serum Ca in sheep.

In other animals, meaningful relationships have been found between Ca/P ratios and Ca absorption, and Ca deficiencies in blood serum could limit nitrogen and P utilization (Li et al., 2022). Márquez-Madrid et al. (2017) found low Ca levels in the soil and forages and attributed these results to the soil Ca, influencing the forage P content.

The present study found P deficiencies in both forage and blood serum, suggesting a relationship between forage and blood serum mineral contents. This agrees with Muñoz-González et al. (2017I), who found that optimal P content in forages might be reflected in normal levels in the blood serum of sheep (69 to 126 ppm). In addition, it has been described that P contents in blood serum are highly affected by seasonal environmental changes, with optimal to limiting levels in spring but suboptimal values in winter (Xin et al., 2011), which agrees with the present study's findings.

Mn contents in the blood serum of lactating ewes were lower in the present study than in reference values. However, Page et al. (2018) reported an average Mn content of 1.7 ng/mL in the blood plasma serum of ewes. Kincaid (2000) suggested implementing strategies to maintain Mn from 5 to 10 micrograms/kg, considering the ability of the liver to remove excesses of this element, even with diets with 40-1000 ppm that exceed the adequate Mn level of 20-25 ppm for ewes according to the NRC (2007). In addition, blood serum Mn could not be a reliable indicator of overall Mn status in all production systems (Spears et al., 2022).

In the present study, the forage samples varied widely in terms of Cu content. On average, however, Cu values were suboptimal, resulting in different levels of deficiencies in sheep blood serum across the sampled seasons. Cu requirements depend on the forage content of other minerals such as Fe, S, and Mo (González-Reyna et al., 2016; Muñoz-González et al., 2017; Page et al., 2018).

Despite the Zn deficiencies in forages observed in the present study, 95% of the blood serum samples from lactating ewes were under a normal range (average=0.31). However, a recent study by Maqsood et al. (2022) reported that levels ranging from 32 to 45 ppm were suboptimal even when blood levels of ruminants ranged from 1.06 to 2.97 ppm. Similarly, in other semi-arid zones (Punjab, Pakistan), suboptimal forage Zn contents (25 ppm in winter and 28 ppm in summer) promoted normal levels of blood serum Zn (Khan et al., 2008).

Limits and perspectives

Since almost 50% of grasslands are under semi-arid conditions, there is a need to maintain extensive systems that reduce the negative impact on soil, water, carbon cycle, and overall biodiversity. Therefore, studies considering economic, social, and environmental implications (FAO, 2024) should be considered when planning strategies for sustainable production systems. The need for more information about the optimal supplementation and normal blood serum ranges, as well as the effects of excessive or insufficient mineral levels on the overall animal body condition and animal productive performance, limits adequate mineral management in ruminants under grazing conditions. More studies on the relationships among all environmental mineral statuses are necessary. Additionally, the interpretation of results from studies performed under uncontrolled systems is limited by factors such as unknown interactions among different minerals.

CONCLUSIONS

In North-Central Mexico, the evaluation of mineral concentrations in forages and serum blood in uncontrolled sheep grazing systems under semi-arid environmental conditions revealed that Mg levels in forages sampled in spring and winter exceeded the normal range of this element in ewes, but with suboptimal levels of Ca, P, Cu, and Zn. Regarding blood serum of lactating ewes, P and Cu levels were below the normal range; Zn and Mn contents were almost optimal; and Ca levels exceeded the normal range. The results suggest no relationships between the mineral contents of forage and blood serum in terms of Ca, Mn, and Zn. However, uncontrolled factors such as interactions between elements may have interfered. Further studies are required to understand better mineral interactions that could help plan supplementation strategies for sheep grazing in semi-arid zones.

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Author contributions

IMH, designed and supervised the study; DNTG, IMH, and GTE decided the references that would be included; IMH, VMMP, MRD, JMMM, and CFAF designed the methodology and performed the study; DNTG and GTE formally statistically analyzed and discussed the results; IMH, DNTG, and GTE designed and wrote the draft paper.

Conflict of interest statement

All authors have revised and approved the publication of the final version of the manuscript. Financial and technical support sources have not interfered in any phase of the results presented in this paper. There are no conflicts of interest to declare.

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