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Article

Mineral evaluation of the components of the intensive silvopastoral system with *Leucaena leucocephala* in three seasons of the year

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Abstract:

A mineral evaluation of the components of the intensive silvopastoral system, soil, drinking water, forage (*Leucaena leucocephala*, *Megathyrsus maximus*) and blood serum of calves and dairy cows was performed. Three samplings were carried out in the cold, dry and rainy seasons. Cu, Fe, Zn, Ca, Mg, K, Na and P were determined and analyzed. Elevated levels of Fe, Ca, K and Mg were found in the soil, while minerals from drinking water remained within adequate ranges, with the exception of Fe (0.61 and 0.57 mg kg^{-1}) at the ranches El Vivero and Los Huarinches, respectively. The concentration of Ca, Mg, K and Na was higher in *Leucaena leucocephala* than in *Megathyrsus maximus*, while the content of Cu (6.16 and 5.66 mg kg^{-1}), Zn (17.9 and 24.4) and P (2,584.5 and 2,682.8 mg kg^{-1}) in both ranches do not meet the requirements of the cows, which could generate low levels of these elements in blood serum, in both cows and calves: Cu (0.64 and 0.54 mg kg^{-1}), Zn (0.74 and 0.60 mg kg^{-1}) and P (49.24 and 39.43 mg kg^{-1}), respectively.

Key words: Minerals, Animal nutrition, *Megathyrsus maximus*, Agroforestry, Silvopastoral system.

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Introduction

The basic components of a silvopastoral system, pastures, trees, animals and soil, interact with each other under a constant flow of elements[1] in such a way that the production levels and nutritional status of the animals depend on the degree to which nutritional requirements are met. This is directly related to the concentration of nutrients present, both in pastures and in the foliage of forage trees, and these in turn are influenced by soil fertility and the amount of minerals that forage plants can absorb[2].

Normally, forage grasses do not provide enough macronutrients (N, Ca, Mg, K and P), micronutrients (Cu, Zn, Fe) and other elements[3,4] required by animals to achieve certain productive parameters, for this reason, the establishment of intensive silvopastoral systems (more than 7,000 trees ha^{-1}) with legumes such as *Leucaena leucocephala* (LL) has been promoted[5]. The cultivation of LL associated with forage grasses is a strategy that, in addition to increasing the supply of feed for ruminants in grazing, contributes to improving its quality, and to correct possible nutritional imbalances of pastures alone. However, despite the fact that legumes are normally richer in macro and microelements than forage grasses[6], various factors affect the content of each element in LL plants. Among these factors are the species, genotype, parts of the plant, growth status and soil fertility[7]. Similarly, serum mineral concentrations in animals are affected by interactions between the amount of each element the animal ingests in the feed and drinking water. Some minerals may interact in ways that can trigger the correct absorption of other minerals in the digestive tract and jointly fulfill various metabolic functions[8], or they may inhibit the absorption of one or more elements and produce antagonistic effects by forming non-absorbable complexes, through competition between cations and anions[6], which can generate a decrease in the expected productive parameters.

Given the above, the mineral state of an intensive silvopastoral system is determined by the contribution of mineral elements of each factor that makes up the system, over time. For this reason, the objective of this research was to evaluate the mineral content of the components of intensive silvopastoral systems (animal, pasture, tree foliage, soil and water) in three seasons of the year, in two cattle ranches located in Apatzingán and Tepalcatepec, Michoacán, Mexico, to determine the contribution of minerals and nutrients and propose alternatives to correct possible nutritional imbalances.

Material and methods

The research was carried out in two cattle ranches (El Vivero and Los Huarinches) located, respectively, in the municipalities of Apatzingán and Tepalcatepec, in the Tierra Caliente region, in the State of Michoacán, Mexico. Both ranches are pioneers in the implementation of intensive silvopastoral systems (ISPS) with *Leucaena leucocephala* and Tanzania grass, with experience of more than 10 years of establishing the grazing system and in the production of milk for the elaboration of cotija cheese (ranch Huarinches) and more recently in the maintenance and development of bovines of the tropical dairy Creole breed and Romosinuano (ranch El Vivero). The study area is located at 350-370 masl, has a warm subhumid climate with rains in summer, with average annual temperature of 28.5 °C and average annual rainfall of 822 mm, the pH of the soil (7.34) is between neutral to alkaline(9,10).

Intensive silvopastoral system

In the two cattle ranches, ISPS consists of *Leucaena leucocephala* bushes in rows every 1.60 m, with densities of 34,500 plants ha^{-1}, in association with Tanzania grass (*Megathyrsus maximus*), which make up the food supply of 60 % grass and 40 % legume. Grazing is carried out following a rotating scheme of 4 d x 40 d of rest, with irrigation in dry seasons.

Samplings

Three samplings corresponding to the most decisive agroecological periods for agricultural production in the area(10,11,12) were carried out. Rains (August), Cold (January) and Dry (May) for a total of three collections.

Grasses and trees

Samplings were carried out in each cattle ranch in the established seasons, adapting the methodology used by Bacab-Pérez *et al*[13], quadrants of 1.60 x 1.60 m were implemented, which were located on the LL furrow, which was considered as the middle line of each quadrant; eight quadrants were randomly distributed on the paddocks that the next day would be used by the animals, and that in turn fulfilled 40 d of regrowth. Tanzania grass was harvested 30 cm from the ground and LL was defoliated manually by taking tender leaves and stems, simulating grazing and browsing carried out by animals; the plant material was homogenized and a subsample of 1 kg of each plant species was selected. The samples were dried in a forced air oven at 60 °C until constant temperature and taken to the laboratory for subsequent analysis.

Blood serum

Blood samples were collected from 8 cows and 8 calves of each breed present on the ranches (Tropical Dairy Creole, Brown Swiss and commercial cross). In adult animals, the blood sample was drawn from the coccygeal vein, and in young animals from the jugular vein. The blood was centrifuged at 3,000 rpm for 15 min for the separation of the blood serum and its conservation at -20 ºC.

Soil and water

Eight soil samples were collected in order to cover the greatest variety of forage supply levels present in each paddock, at depths of 0 to 15 and 15 to 30 cm, in each cattle ranch and in each season of the year, which were dried and sieved with a 0.2 mm mesh. Three water samples were taken directly from the drinking troughs of each paddock of each cattle ranch and each season of the year.

Mineral analysis

The concentrations of Cu, Fe, Zn, Ca, Mg, K and Na, in forage, blood serum, soil and water, were determined by the procedures described by Fick *et al*[14], using an atomic absorption spectrophotometer model AAnalyst 700 from PerkinElmer. The concentration of P was determined by colorimetry[14].

Statistical analysis

For the data on the mineral content of the soil samples, the following statistical model was used:

$$Y_{ijk} = \mu + P_i + S_j + R_k + (SR)_{jk} + \varepsilon_{ijkl}$$

Where

Y_{ijk}= concentration of the mineral;

P_i= effect of the *i-th* depth (0-15, 15-30 cm);

S_j= effect of the *j-th* season of the year (rainy, cold, dry);

R_k= effect of the *k-th* ranch (Los Huarinches, El Vivero);

SR_{jk}= effect of the interaction between the season of the year and the cattle ranch.

For the analysis of the data on the mineral composition of the water, the following statistical model was used:

$$Y_{ijk} = \mu + S_j + R_k + (SR)_{jk} + \varepsilon_{ijkl}$$

Where

Y_{ijk}= concentration of the mineral in water;

S_j= effect of the *j-th* season of the year (rainy, cold, dry);

R_k= effect of the *k-th* cattle ranch (Los Huarinches, El Vivero);

SR_{jk}= effect of the interaction between the season of the year and the cattle ranch.

For the analysis of the data on the nutritional content of the foliage of LL and Tanzania grass, the following statistical model was used:

$$Y_{ijk} = \mu + E_i + S_j + R_k + (SR)_{jk} + \varepsilon_{ijkl}$$

Where

Y_{ijk}= concentration of the nutrient;

S_i= effect of the *i-th* season (rainy, cold, dry);

E_j= effect of the *j-th* forage species (Tanzania grass, leucaena);

R_k= effect of the *k-th* cattle ranch (Los Huarinches, El Vivero).

For the analysis of the mineral concentration of the blood serum samples, the following statistical model was used:

$$Y_{ijkl} = \mu + E_i + S_j + R_k + (SR)_{jk} + (ER)_{ik} + (ES)_{ij} + (ESR)_{ijk} + \varepsilon_{ijkl}$$

Where

$\mathbf{Y_{ijkl}}$= concentration of the mineral in the blood serum;

$\mathbf{E_i}$= effect of the *i-th* physiological stage of the animal (cow, calf);

$\mathbf{S_j}$= effect of the *j-th* season of the year (rainy, cold, dry);

$\mathbf{R_k}$= effect of the *k-th* ranch (Los Huarinches, El Vivero);

$\mathbf{ER_{ik}}$= effect of the interaction between the physiological stage of the animal and the cattle ranch;

$\mathbf{ES_{ij}}$= effect of the interaction between the physiological stage of the animal and the season of the year;

$\mathbf{ESR_{ijk}}$= effect of the interaction between the physiological stage of the animal, season of the year and cattle ranch.

The data were analyzed using the GLM procedure of the SAS statistical software(15) and the comparison of means between the treatments was made using the Tukey test with a significance level of 0.05.

Results and discussion

Soil and water

The concentration of Cu (14.73 *vs* 14.04 mg kg^{-1}), Zn (49.07 *vs* 47.37 mg kg^{-1}), Fe (1661 *vs* 1672 mg kg^{-1}), Ca (9412 *vs* 9679 mg kg^{-1}), K (1963 *vs* 1870 mg kg^{-1}) and Mg (5275 *vs* 5328 mg kg^{-1}) was similar (P>0.05) at the two soil depths (0 to 15 and 15 to 30 cm), respectively. This is probably due to the fact that in both cattle ranches the soil is deep, which facilitates the transport of water and nutrients to the deep roots(16), in addition, silvopastoral systems can maintain and improve the porosity, infiltration and aeration of the soil(17,18). However, the mineral concentration of the soil showed differences between the cattle ranches studied, evidencing different soil conditions at the evaluation sites (Table 1). In the soils of both ranches, there are adequate levels of Cu and Zn for the development of plants; while, the levels of Fe are high, since in soils with neutral or alkaline pH, the fixation of these minerals is favored(19); while the levels of Ca, K and Mg, despite being high, especially in the ranch El Vivero, agree with the availability generated by the pH of the soil. The high content of minerals in the soil of both ranches may be influenced by the proximity of the study area to other agricultural properties dedicated to the production of lemon, which demands constant fertilization with macro and microelements such as N, P, K Ca, Mg, S, Mn, Fe, Zn, Cu and B(20). However, in soils with high content of organic matter, nitrogen and phosphorus, as is the case of soils with silvopastoral management, the availability of Cu can be hindered, which can create induced deficiency of that element in pastures and in the animal that consumes those pastures, and these deficiencies of Cu can be accentuated by an excess of zinc or manganese[21]. It should be noted that several mineral elements, including zinc, increase bioavailability in the soil in a range between 5 to 77, but outside this range they change their ionic state and precipitate as hydroxide, carbonate or sulfide, so the solubility, mobility of these compounds decrease as the pH increases or decreases in the soil[22].

Table 1: Effects of cattle ranch and season of the year on the mineral concentration (mg kg^{-1}) of the soil in the intensive silvopastoral system

Ranch effect	**Cu**	**Zn**	**Fe**	**Ca**	**Mg**	**K**
Los Huarinches	16.2 ^{a}	64.5 ^{a}	1,858 ^{a}	5,042 ^{b}	4,637 ^{b}	2,460 ^{a}
El Vivero	12.5 ^{b}	31.8 ^{b}	1,478 ^{b}	14,049 ^{a}	5,965 ^{a}	1,373 ^{b}
SEM^{y}	0.34	1.36	75.59	541.8	111.8	319.8
Season effect × ranch El Vivero						
Cold	12.85 ^{a}	31.43 ^{a}	1,711 ^{a}	12,875 ^{b}	6,970 ^{a}	1,926 ^{a}
Rainy	11.76 ^{b}	32.55 ^{a}	1,606 ^{a}	12,368 ^{b}	5,378 ^{a}	1,712 ^{a}
Dry	13.04 ^{a}	31.65 ^{a}	1,115 ^{b}	16,903 ^{a}	5,547 ^{a}	482.6 ^{b}
SEM	0.31	1	97.25	875.2	616	136.28
Season effect × ranch Los Huarinches						
Cold	16.06 ^{a}	57.5 ^{b}	1,928 ^{b}	4,540 ^{a}	4,976 ^{a}	2,219 ^{b}
Rainy	17.9 ^{a}	78.74 ^{a}	2,245 ^{a}	5,585 ^{a}	4,241 ^{a}	3,231 ^{a}
Dry	14.84 ^{b}	57.45 ^{b}	1,400 ^{b}	5,001 ^{a}	4,693 ^{a}	1,930 ^{b}
SEM	0.65	2.03	152.3	997.7	482.2	482.2
Appropriate level	5-30^{x}	20-150^{x}	50-500^{x}	1,000 – 2,000^{w}	80-200^{v}	60-180 ^{v}

SEM= standard error of the mean; x(25) v(26) w(27).

ab Means in the same column with different literal show differences (P<0.05).

The interaction between cattle ranch and season of the year in the concentration of Cu, Zn, Ca and K in the soil was important (P<0.05, Table 1). The highest concentration of total Cu in soil in the ranch El Vivero was higher in the dry season, while in Los Huarinches it was in the rainy season; in the case of Zn, the concentration in soil was higher in the ranch Los Huarinches, where the highest level of the element occurred during the rains, while in the ranch El Vivero, there were no significant differences between the seasons of the year (P>0.05); the opposite occurred in the ranch El Vivero for the Ca content in the soil, since during the dry season, the level of this element was higher, while in Los Huarinches, there were no significant differences in the concentration of Ca in the soil between the different seasons of the year (P>0.05). These results show that in the face of similar environmental conditions (temperature and precipitation), specific particularities of each ranch can modify the degree of influence on the mineral concentration in the soil; for example, the availability of Cu can be affected by soil moisture and texture, competition with elements such as Fe and Zn and high levels of organic matter (OM), on the contrary, Zn, in addition to competing with Cu, can decrease its availability due to low levels of OM[23]. Roberts[24] found different modifications in the concentration of minerals in two regions of New Zealand, in the same seasons of sampling, attributed, among other things, to the ability of the silvopastoral system to reincorporate nutrients into the soil, through contributions of biomass or animal excreta. It should be noted that the ranches analyzed work with different stocking rates and have different objectives of production, so the differences in the management of the animals (according to each objective of production) could affect changes in the concentration of minerals in the soil.

The variations in the mineral concentration in the soil of the ranches evaluated, in relation to the season of the year, may be caused by aspects inherent in the management of each production system and environmental conditions of each place, although the similarities present in temperature and precipitation are not sufficient to explain the behavior of the mineral concentration in the soil. Minerals in soil have complex interactions with pH, which control ion mobility and exchange, their precipitation and dissolution, oxide-reduction reactions, microbial activity and nutrient availability[28]. There are also strong interactions with soil organic matter (OM); an excess of organic matter in the soil reduces the absorption of various minerals by plants[29]. For this reason, it is important to note that the management of production methods will be decisive for the accumulation of mineral elements, rather than aspects of environmental condition, as can happen in intensive silvopastoral systems that modulate the content of organic matter, pH and N contributors to the soil.

Mineral concentrations, except Fe, in drinking water on both cattle ranches and in the different seasons evaluated, were below the suggested adequate levels[30]: Cu (<1 mg L^{-1}), Zn (<8 mg L^{-1}), Fe (<0.4 mg L^{-1}), Ca (<1,000 mg L^{-1}), Mg (<1,000 mg L^{-1}) and K (<20 mg L^{-1}). However, the levels of Ca and Mg registered in drinking water were higher than those required ($P<0.05$) at the ranch El Vivero (30.55 and 46.15 mg L^{-1} for each element, respectively) compared to the status of those elements at the ranch Los Huarinches (10.35 and 9.01 mg L^{-1} for each element, respectively). Similarly, the level of Fe in drinking water for the cattle at the ranch El Vivero and the ranch Los Huarinches was 0.61 and 0.57 mg L^{-1}, respectively, concentrations higher than the maximum tolerable level suggested by Puls[30] (<0.4 mg L^{-1}), from which symptoms of Fe poisoning may appear in animals. These data are consistent with the high concentration of Fe present in the soils of both cattle ranches.

Forage

Concentrations of Ca, Mg, K, and Na were higher in LL foliage than in Tanzania grass (Table 2); however, the concentration of Zn was higher in grass than in legume. Both forage species had concentrations of Cu, Zn and P below those required for bovines. These results are consistent with those already reported(31), where it is mentioned that P deficiency is a predominant condition in grazing systems in the tropics. Additionally(32), average values of Zn and Cu, lower than the requirement of bovines for different species of grasses and legumes, are reported showing that grazing production systems, including SPSs, may be limited to meet the minimum requirements of these elements.

The contents of Ca, Mg, K and Na of leucaena were higher than those required for dairy cows, which is consistent with the high concentration of Ca, Mg and K in the soils of both cattle ranches, also evidencing the ability of the legume over the grass to absorb more of these elements from the soil, since the species develops better in soils with higher content of exchangeable Ca(33). Thus, levels of Ca, Mg and K in leucaena of up to 30,000, 23,000 and 11,000 mg kg^{-1}, respectively, have been reported(34,35,36).

Table 2: Effects of the forage species on the mineral concentration of leucaena and Tanzania grass in the intensive silvopastoral system (mg kg^{-1})

Nutrient	Leucaena	Tanzania	SEM	Requirement[u]
Copper	6.1 [a]	5.6 [a]	0.35	10 – 11
Iron	94.1 [a]	83.9 [a]	4.85	12 – 18
Zinc	17.9 [b]	24.4 [a]	0.89	43 – 55
Calcium	11,569 [a]	3,320 [b]	426.7	5,700 – 6,700
Magnesium	2,532 [a]	1,858 [b]	136.5	1,800 – 2,100
Potasium	16,411 [a]	9,981 [b]	1,203	11,000 – 11,900
Sodium	4,595 [a]	2,409 [b]	337.8	2,000 – 2,200
Phosphorus	2,585 [a]	2,683 [a]	132	3,200 – 3,700
Ca:P	4.5 [a]	1.2 [b]	0.20	1.5 – 2[t]

SEM = Standard error of the mean; [u(37) t(38)].

[ab] Means in the same row with different literal show differences ($P<0.05$).

The contents of Ca, Na and P, as well as the Ca:P ratio in Tanzania grass were different between the two cattle ranches (Table 3); these, except for Na, are below the requirement for dairy cows in grazing, showing that, regardless of the specific conditions of each region, the grass alone does not provide these minerals for the maintenance and production of animals, especially Ca. This may occur because grasses of warm climates usually have lower mineral contents than grasses of temperate climate(32), and because of the environmental conditions of each region, which is also observed in the results of the work carried out by Morales *et* *al*[39], who recorded maximum concentrations of Ca and P in *Lolium perenne*, in the Central Valley of Mexico, up to 5,830 mg kg^{-1} and 4,400 mg kg^{-1} and minimum concentrations of 2,540 mg kg^{-1} and 2,400 mg kg^{-1}, respectively, evidencing the influence of the environment on the concentration of these elements in grasses.

On the contrary, the contents of Cu, Mg, K and Na of LL showed differences (P>0.05) between the ranches studied (Table 3), possibly due to the ability of the trees to store more minerals and to extract them from deeper horizons of the soil[40,41].

Table 3: Effects of the cattle ranch on the mineral concentration of leucaena and Tanzania grass in the intensive silvopastoral system (mg kg^{-1})

Leucaena				
Mineral	**El Vivero**	**Los Huarinches**	**SEM**	**Requirement**^{x}
Copper	6.8 ^{b}	5.39 ^{a}	0.38	10 – 11
Iron	96.3 ^{a}	91.2 ^{a}	5.7	12 – 18
Zinc	17.1 ^{a}	18.3 ^{a}	0.49	43 – 55
Calcium	12,257 ^{a}	10,908 ^{a}	830	5,700 – 6,700
Magnesium	2,943 ^{a}	2,075 ^{b}	122.5	1,800 – 2,100
Potasium	18,560 ^{a}	13,984 ^{b}	1,490	11,000 – 11,900
Sodium	3,452 ^{b}	5,604 ^{a}	421.9	2,000 – 2,200
Phosphorous	2,542 ^{a}	2,630 ^{a}	85.6	3,200 – 3,700
Ca:P	4.46 ^{a}	4.41 ^{a}	0.044	1.5 – 2^{w}
Tanzania				
Copper	5.8 ^{a}	5.5 ^{a}	0.22	10 – 11
Iron	82.7 ^{a}	86.7 ^{a}	3.62	12 – 18
Zinc	25.4 ^{a}	23.4 ^{a}	1.29	43 – 55
Calcium	2784 ^{b}	3,894 ^{a}	135.4	5,700 – 6,700
Magnesium	1,925 ^{a}	1,839 ^{a}	92.7	1,800 – 2,100
Potasium	9,340 ^{a}	10,386 ^{a}	964	11,000 – 11,900
Sodium	2,806 ^{a}	2,095 ^{b}	155.8	2,000 – 2,200
Phosphorous	2,540 ^{a}	2,822 ^{b}	70.6	3,200 – 3,700
Ca:P	1.14 ^{b}	1.37 ^{a}	0.06	1.5 – 2^{w}

SEM = Standard error of the mean; $^{x(36)}$, $^{w(37)}$.

^{ab} Means in the same row with different literal show differences (P<0.05).

The concentrations of Ca for LL are higher than those obtained in another study[42] in the Huasteca potosina of Mexico, where Ca levels of 2,300 mg kg^{-1} and a Ca:P ratio of 0.81 were recorded; the higher concentration of Ca in LL obtained in the present study led to the increase in the Ca:P ratio (4.5), which is higher than recommended.

The season of the year influenced the mineral content of leucaena and Tanzania grass, in such a way that the levels of Cu during the dry period were lower than in the rainy season, this was contrary to what was reported by other researchers[43], who found higher concentration of Cu in the dry season (9.4 mg kg^{-1}) compared to the rainy season (8.9 4 mg kg^{-1}) in the warm humid region of Pangasinan, Philippines; however, for Tanzania grass, there was lower Cu content in the cold season. Potassium registered higher concentration in leucaena during the cold season, while the grass showed maximum concentrations of K of 14,823 mg kg^{-1} during the rainy season, in accordance with the fluctuations of that element in the soil. The concentrations of Mg and P in LL were similar (P>0.05) between the three seasons of the year, contrary to what was observed in the Tanzania grass, in which these elements registered greater concentration in the cold season, while the levels of Fe, K and Ca in LL were higher than in star grass during the cold season, probably due to the reduction in the growth rate of the legume in the fresh season of the year (Table 4).

Table 4: Effect of the season of the year on the mineral concentration (mg kg^{-1}) of leucaena and Tanzania grass in the intensive silvopastoral system

Season	Cu	Zn	Fe	Ca	Mg	K	Na	P	Ca:P
Leucaena									
Cold	6.8 ^{a}	21.1 ^{a}	114.4 ^{a}	13,094 ^{a}	2,758 ^{a}	20,927 ^{a}	3,196 ^{b}	2,759 ^{a}	4.8 ^{a}
Rainy	7.4 ^{a}	16.3 ^{b}	86.2 ^{b}	9,673 ^{a}	2,295 ^{a}	15,782 ^{b}	3,000 ^{b}	2,411 ^{a}	3.88 ^{a}
Dry	4 ^{b}	16.4 ^{b}	80.4 ^{b}	11,979 ^{a}	2,476 ^{a}	12,106 ^{b}	7,521 ^{a}	2,588 ^{a}	4.9 ^{a}
SEM	0.47	0.60	7.04	1,025	151.2	1,839	520.7	105.6	0.54
Tanzania									
Cold	4.2c	24.3 ^{a}	100.9 ^{a}	4,245 ^{a}	2,799 ^{a}	5,590 ^{b}	3,501 ^{a}	2,891 ^{a}	1.47 ^{a}
Rainy	6.9 ^{a}	22.2 ^{a}	67.2 ^{b}	2,363 ^{c}	1,091 c	14,823 ^{a}	1,769 ^{b}	2,584 ^{b}	0.95 ^{b}
Dry	5.9 ^{b}	26.6 ^{a}	86.1 ^{a}	3,409 ^{b}	1,755 ^{b}	9,176 ^{b}	2,081 ^{b}	2,570 ^{b}	1.34 ^{a}
SEM	0.27	1.6	4.4	164.8	112.8	1,173	189.7	86	0.07
Req.	10 – 11	43 - 55	12 – 18	5,700 – 6,700	1,800 – 2,100	11,000 – 11,900	2,000 – 2,200	3,200 – 3,700	1.5 – 2^{w}

SEM = Standard error of the mean; Req= requirement [36] [w][37].

[abc] Means in the same column with different literal show differences (P<0.05).

Blood serum

There was no effect of the interaction between cattle ranches and the physiological stage of the animal (P>0.05) on the concentration of the minerals analyzed. However, the interaction between the season of the year and the physiological stage was important (P<0.05) in the content of Zn, Ca, and Na, since the concentration of Zn in the blood serum of the cows was lower than that of the calves, and lower in the rainy and dry season than in the cold season; similarly, Ca levels were lower in the dry season, although serum concentrations of Ca remained within adequate ranges. The triple interaction (cattle ranch, season of the year and physiological stage of the animal) was important (*P*<0.05) for serum concentrations of Cu and Mg. Likewise, the individual effects of cattle ranch, physiological stage of the animal and season of the year influenced the concentrations of most minerals in blood serum (Table 5).

Table 5: Mineral concentration (mg kg^{-1}) in blood serum of cows and calves grazing in the intensive silvopastoral system on two ranches, in three seasons of the year

	Cu	Zn	Fe	Ca	Mg	K	Na	P	Ca:P
Ranch									
Huarinches	0.6 [a]	0.7 [a]	2.7 [a]	110.1 [a]	19.7 [a]	238.3 [a]	2,791 [a]	45.4 [a]	2.6 [a]
El Vivero	0.5 [b]	0.6 [b]	1.7 [b]	109.0 [a]	19.6 [a]	196.7 [b]	2,371 [b]	43.2 [a]	2.5 [a]
SEM	0.01	0.018	0.15	2.42	0.50	6.88	62.24	1.01	0.06
Season									
Cold	0.6 [a]	0.7 [a]	2.2 [a]	130.1 [a]	19.7 [a]	231.4 [a]	2,575 [a]	39.2 [b]	3.4 [a]
Rainy	0.5 [a]	0.6 [a]	2.4 [a]	100.7 [b]	20.9 [a]	192.4 [b]	2,741 [a]	48.8 [a]	2.1 [b]
Dry	0.5 [b]	0.6 [a]	2.1 [a]	97.8 [b]	18.5 [b]	228.6 [a]	2,427 [b]	44.8 [a]	2.2 [b]
SEM	0.02	0.21	0.20	3.07	0.6	8.5	77.6	1.23	0.08
Physiological stage									
Calf	0.6 [a]	0.7 [a]	2.5 [a]	108.7 [a]	17.4 [b]	219.1 [a]	2,634 [a]	49.2 [a]	2.2 [b]
Cow	0.5 [b]	0.6 [b]	2.0 [b]	110.4 [a]	21.9 [a]	215.8 [a]	2,528 [a]	39.4 [b]	2.9 [a]
SEM	0.017	0.019	0.16	2.50	0.51	7.03	63.50	1.03	0.06
Appropriate range	0.8 - 1.5	0.8-1.4	1.3-2.5	80-110	18-35	159-198	3,015-3,450	45-60	1.3-2.7
Effects and interactions									
Ranch	**	.**	***	NS	NS	**	***	NS	NS
Season	*	NS	NS	***	*	.**	***	***	***
Stage	***	***	*	NS	***	NS	NS	***	***
R*S	NS	**	***	***	NS	***	**	NS	**
R*T	NS	NS	NS	NS	NS	NS	NS	NS	NS
S*T	NS	**	NS	**	NS	NS	*	NS	NS
R*S*T	**	NS	NS	NS	*	NS	NS	NS	NS

SEM = Standard error of the mean; Appropriate range[26]; R=ranch, S=season, T=physiological stage. [ab] Means in the same column with different literals show differences (*P*<0.05). NS= Not significant; *= (*P*<0.05); **= (*P*<0.01); *= (*P*<0.001).

In none of the ranches evaluated, the level of Cu and Zn is sufficient to meet the recommended[27], which is consistent with the low levels of these mineral elements in the forage, both in leucaena and in Tanzania grass. Likewise, the serum concentration of P in the animals of the ranch El Vivero is below the recommended level[27], both for adult and young bovines (45-60 and 60-90 mg kg^{-1}, respectively); while in the ranch Los Huarinches, the serum content of P barely meets the minimum recommended for adult bovines, which is consistent with the low P content in the two forage species of the intensive silvopastoral system. Serum levels of Na are also deficient in the animals of both ranches, despite the fact that, in both leucaena and Tanzania, this element is in an acceptable range to meet the requirement of cows in production[27].

Although the season of the year influenced the serum concentration of almost all minerals, the concentrations of Zn, Cu and Na did not reach the adequate minimum, contrary to what happened with P during the rainy season, which reached only the minimum level required. Ca and Mg concentrations in the three seasons of the year are within adequate ranges, despite the fact that these minerals in leucaena were above the requirement for dairy cows, although they could be compensated by the Tanzania grass forage. This shows that both the grass and the legume contribute to correct mineral imbalances generated by their biochemical properties. Contrary to the above, the levels of K in blood serum were higher than the appropriate range, both in the cold and rainy seasons, which is consistent with the contributions of K in Leucaena and Tanzania grass in both ranches. According to some reports[26], excesses of K in the soil lead to increase the content of this element in pastures, which can subsequently have negative effects on the animal health when the tolerable maximum is exceeded.

Both the calves and the adult cows presented serum levels of Cu, Zn and Na below the adequate levels, while the adult animals showed deficiencies of P and, despite the fact that the level of this element in the calves was higher than that of the cows, the deficiency was persistent, since the adequate range of P for young bovines is 60 - 90 mg kg^{-1}[24]. Similarly, the calves presented slight deficiency of Mg, probably due to the milk having a low content of this element (0.1 to 0.2 g L^{-1}; [6]); whereas, for both types of animals, the concentration of K in blood serum was higher than the appropriate ranges.

Conclusions and implications

The variations in the mineral concentration in the soil of the evaluated ranches, in relation to the season of the year, can originate from the management of each production system and from the environmental conditions, so the similarity of temperature and precipitation is not enough to explain the differences in the concentrations of the minerals evaluated, therefore, additional studies are recommended. Except for Fe, the concentrations of the minerals dissolved in drinking water do not meet the requirements of animals. The association of *Leucaena leucocephala* and *Megathyrsus maximus* var. Tanzania complement each other and contribute to improving the mineral balance of the diet of dairy cows, however, edaphic differences of each cattle ranch, the forage species and season of the year are the factors responsible for the deficiencies of Cu, Zn and P of the animals. Serum levels of Ca, Mg and the Ca:P ratio were adequate, while levels of Cu, Zn, Na and P are lower than normal concentrations. However, the serum concentration of K is above the normal range. Because the concentrations of Cu, Zn, Na and P in forage and blood serum are low, it is convenient to implement mineral supplementation strategies to cattle that allow increasing the availability of these minerals in the diet, to meet the requirements for maintenance and production of dairy cows and their calves.

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