



## Mineral diagnosis of grazing beef cows



Ruth Manzayani García-Fuerte <sup>a\*</sup>

Maximino Huerta Bravo <sup>a</sup>

José Ricardo-Álvares Rodríguez <sup>a</sup>

Enrique Cortés Díaz <sup>b</sup>

Laura Haydée Vallejo Hernández <sup>a</sup>

Gustavo Sosa Pérez <sup>c</sup>

<sup>a</sup> Universidad Autónoma Chapingo. Posgrado en Producción Animal, Departamento de Zootecnia. Carretera México-Texcoco km 38.5, C.P. 56230, Chapingo, Estado de México, México.

<sup>b</sup> Universidad Autónoma Chapingo. Centro Regional Universitario Anáhuac. Chapingo, Estado de México, México.

<sup>c</sup> Universidad Autónoma Chapingo. Departamento de preparatoria agrícola. Chapingo, Estado de México, México.

\* Corresponding author: manzayani28@gmail.com

### Abstract:

The present research aimed to determine the mineral concentrations in forage, water, and blood serum, and the clinical signs of grazing cows. Twenty-five (25) Angus and Hereford cows of different physiological states were analyzed, from which blood samples were taken by venipuncture. Forage samples were collected using the hand-plucking technique and water was collected from available sources. The determination of minerals was performed by atomic absorption spectrophotometry and visible ultraviolet spectrophotometry. The blood serum of the cows showed deficient concentrations of copper (100 %), zinc (88 %), sodium

(52 %), potassium (36 %), and phosphorus (16 %). Iron and manganese were in concentrations above the normal range. A copper deficiency classification index (CUDI) was generated: severe 0.41-0.48 mg L<sup>-1</sup>, moderate > 0.48-0.54 mg L<sup>-1</sup>, and mild > 0.54 -0.79 mg L<sup>-1</sup>. Sixty-eight, 20, and 12 % of the cows corresponded to the given classification, respectively. The following signs were identified: abortions, lameness, dull, shaggy, and discolored hair, tearing, steely hair, goiter, and pica. For forage, deficient levels of copper (1.27 mg kg<sup>-1</sup>) and zinc (24.5 mg kg<sup>-1</sup>) were found. In water, copper and zinc were not detectable, and there was excess iron 0.345 (mg L<sup>-1</sup>). In conclusion, the primary deficiency is copper, caused by deficiencies in forage, water, and possible imbalances caused by other minerals.

**Keywords:** Bovines, Minerals, Deficiency, Copper, and Zinc.

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## Introduction

Livestock farming in Mexico is an activity that is carried out in approximately 60 % of the national territory with a wide diversity of production systems<sup>(1)</sup>. The cow-calf system is the most important in Mexico, but it is also the most vulnerable since it is usually implemented under grazing conditions, which makes it dependent on environmental factors<sup>(2)</sup>. In these production systems, the quality of forages can be fluctuating and, consequently, nutritional contributions are variable<sup>(3)</sup>.

Forage generally fails to meet the nutritional requirements of livestock. These forages are insufficient in minerals, which are essential nutrients for development, growth, and reproduction<sup>(4)</sup>. Mineral deficiency in cattle causes some alterations in reproductive and metabolic activity; to avoid these alterations, it is advisable to provide mineral supplementation<sup>(5,6)</sup>. Grazing animals are mainly deficient in phosphorus, copper, and zinc; for this reason, several studies have been carried out on supplementation in this type of production systems<sup>(7,8,9)</sup>.

There are currently several direct and indirect mineral supplementation methods and the choice of the most appropriate method will depend on the type of production system, economy, and availability<sup>(9,10,11)</sup>. In order to carry out effective and accurate supplementation, it is necessary to know the state of the production system through a complete mineral

diagnosis that includes soil, water, forage, blood serum, production objective, season of the year, and animal management. Implementing biochemical and mineral diagnostics helps better assess nutritional status<sup>(10-13)</sup>. The present research aimed to determine the mineral concentrations in forage, water, and blood serum, and the clinical signs of grazing cows in Lagos de Moreno, Jalisco, Mexico.

## Material and methods

The present research was conducted in a cow-calf production system located in the municipality of Lagos de Moreno, Jalisco, Mexico, 21° 21' 24" N, 101° 56' 16" W. The climate is semi-arid temperate, the average annual temperature is 15.1 °C, and its average minimum and maximum temperature ranges between 5.4 °C and 28.5 °C. The average annual rainfall is 665 mm, whereas the average accumulated precipitation is 536.31 mm<sup>(14)</sup>.

Cattle management. Blood samples were taken from 25 animals of the Angus and Hereford breeds in different physiological states: pregnant, empty, recently calved, and heifers, with various body conditions, and they were handled in a cattle chute and press. The presence of signs associated with mineral deficiencies was also recorded.

Blood sample management. Blood samples were collected using the jugular venipuncture technique, extracting 10 ml per cow following the procedures defined by Fick *et al*<sup>(15)</sup>. These samples were placed in vacuum tubes without anticoagulants and centrifuged at 2,500 rpm for 15 min. The blood serum was then obtained, which was refrigerated and stored at -4 °C until the time of analysis.

Forage sample management. Twenty-four (24) forage samples were collected in a rangeland composed of sideoats grama (*Bouteloua curtipendula*), blue grama (*Bouteloua gracilis*), Bermuda grass (*Cynodon dactylon*), and Guinea grass (*Panicum maximum*) in an area of 50 ha; these samples were collected by the hand-plucking technique<sup>(16)</sup> and kept in paper bags. They were then dried in a forced-air oven at 60 °C for 48 h. Subsequently, they were ground, and dry matter (DM), crude protein (CP), etheral extract, and ash were determined by proximate analysis<sup>(16)</sup>. In addition, neutral detergent fiber (NDF) was determined according to Van Soest's<sup>(16,17)</sup> methodology. Mineral determinations were carried out according to Fick *et al*'s<sup>(15)</sup> methodology.

Water sample management. Three water samples were collected from each source available to the animals (2 earthen dams and a drinking trough that was inside the handling pen); these samples were stored in 50 ml plastic cups previously demineralized. These samples were

filtered before their determination following the methodology described by Fick *et al*<sup>(15)</sup>. The sampling was carried out in May (dry season).

Laboratory tests. The determination of serum, water, and forage concentrations for the minerals Cu, Fe, Zn, Ca, Mg, Na, K, and Mn was performed by atomic absorption spectrophotometry. The P concentration of the samples was determined by means of visible ultraviolet spectrophotometry; both determinations followed the methodology described by Fick *et al*<sup>(15)</sup>.

Statistical analysis. The data were analyzed with the GLM procedure in SAS<sup>(18)</sup>. Since the concentration of copper in blood serum was the most deficient of the minerals studied, a copper deficiency classification index (CUDI) was generated: severe (0.41-0.48 mg L<sup>-1</sup>), moderate (0.48-0.54 mg L<sup>-1</sup>), and mild (0.54 -0.79 mg L<sup>-1</sup>). CUDI was considered as the dependent variable and the concentrations of the rest of the minerals as independent variables.

The statistical model included the following parameters:

$$Y_{ijk} = \mu + A_j + e_{ij}$$

Where Y = dependent variable,  $\mu$  = overall mean,  $A_j$ = mineral concentrations, and  $e_{ij}$  = residual error. The comparison of means was performed using Tukey's test.

## Results

### Mineral concentration in blood serum

Deficient concentrations of most of the minerals tested were found in blood serum. Table 1 shows that the most deficient minerals in cows are copper (100 %), zinc (88 %), and sodium (52 %). For potassium and phosphorus, there are some deficient animals; however, the general mean is within the appropriate levels. It should be noted that iron and manganese in blood serum were found in concentrations above those appropriate according to Puls<sup>(19)</sup>.

**Table 1:** Mineral concentration in blood serum of beef cows

Mineral	Mineral concentration (mg L <sup>-1</sup> )	Normal range	Deficient cows (%)
Copper	0.48 ± 0.07	0.8 - 1.5	100
Iron	2.58 ± 0.57	1.3 - 2.5	0
Zinc	0.70 ± 0.09	0.8 - 1.4	88
Calcium	65.28 ± 10.30	37.6 - 48.8*	0
Magnesium	34.65 ± 10.53	18 - 30	0
Sodium	2879.80 ± 744.30	3100 - 3450	52
Potassium	167.54 ± 35.07	160 - 215	36
Manganese	2.40 ± 22.51	0.006 - 0.070	0
Phosphorus	56.38 ± 0.91	40 - 60	16

(19,20\*)

### Copper deficiency classification index (CUDI)

One of the most deficient minerals was Cu, for which a classification index was generated according to the severity of the deficiency based on the concentrations of Cu in blood serum: severe, moderate, and mild. Since it is a primary deficiency, it causes various effects, such as decreased feed intake and alteration between interactions, which in turn affects the metabolism of other elements. However, after analyzing all the elements, no significant effect was found, which could be attributed to the number of animals present in each group.

Most of the animals sampled were in the severe deficiency category (68 %). Twenty percent of the animals were classified in the moderate deficiency category (> 0.48-0.54 mg L<sup>-1</sup>) and finally, only 12 % of the cows were detected with mild deficiency (> 0.54-0.79 mg L<sup>-1</sup>) (Table 2). Blood serum concentrations of zinc, iron, calcium, magnesium, sodium, potassium, phosphorus, and manganese were not affected by CUDI ( $P>0.05$ ).

**Table 2:** Copper deficiency classification index in relation to the different mineral concentrations in blood serum of beef cows

CUDI	Range (mg L <sup>-1</sup> )	DA (%)	Mineral concentrations according to each classification (mg L <sup>-1</sup> )							
			Zn	Fe	Ca	Mg	Na	K	P	Mn
Severe	0.41-0.48	68	0.68	2.8	65.5	34.6	2750	166	53	2.36
Moderate	0.48-0.54	20	0.74	2.3	63.2	34.0	3154	159	66	2.70
Mild	0.54-0.79	12	0.71	1.9	67.8	36.1	3357	204	67	2.00
Probability	-	-	0.47	0.30	0.85	0.97	0.37	0.28	0.42	0.63

CUDI= copper deficiency index; DA= deficient animals; Zn= zinc; Fe= iron; Ca= calcium; Mg= magnesium;

Na= sodium; K= potassium; P= phosphorus; Mn= manganese.

The most characteristic signs of mineral deficiency were identified in the copper deficiency index, where dull, shaggy, and discolored hair was observed in 100 % of the animals in the severe and mild classification. Pica is present in all categories: 50 to 60 % of the animals had it. In the case of tearing, it only occurs in the severe and mild deficiency index. It should be noted that a cow can show more than two signs and all signs are observed in the classification of severe (Table 3).

**Table 3:** Copper deficiency index in relation to the percentage of animals showing characteristic signs of mineral deficiency

CUDI	Range (mg L <sup>-1</sup> )	A	B	C	D	E	F	G	H	I
Severe	0.41-0.48	33	17	100	39	29	39	33	50	22
Moderate	0.48-0.54	20	20	80	0	20	20	20	60	0
Mild	0.54 -0.79	0	0	100	50	19	50	0	50	0

CUDI= copper deficiency index; A= abortions; B= lameness; C= dull, shaggy, and discolored hair; D= tearing; E= steely hair or wool; F=goiter, G= udder inflammation; H= pica; I= excessive salivation.

### Forage composition

The forage analyzed was sideoats grama (*Bouteloua curtipendula*), blue grama (*Bouteloua gracilis*), Bermuda grass (*Cynodon dactylon*), and Guinea grass (*Panicum maximum*) in a total area of 50 ha, and it was found dry and ripe and had deficient concentrations of CP (<6 %), high levels of NDF (71.97 %), which may be an indication that the available forage is highly lignified, and low concentrations of ethereal extract (0.93 %). Deficient levels of Cu (1.27 mg kg<sup>-1</sup>) were identified; the opposite occurred for the other minerals (Table 4).

**Table 4:** Mineral concentrations of forage on a dry basis (mg/kg for microminerals and percentage for macrominerals)

	Cu	Fe	Zn	Mn	Na	K	Ca	Mg	P
Critical level	10	20	30	40	0.06	0.8	0.3	0.1	0.25
Average + SD	1.275 ± 0.33	23 ± 8.25	24.5 ± 2.65	176 ± 76.15	0.03 ± 0.01	0.33 ± 0.16	0.40 ± 0.10	0.20 ± 0.03	0.38 ± 0.05

Cu= copper; Fe= iron; Zn= zinc; Mn= manganese; Na= sodium; K= potassium; Ca= calcium; Mg= magnesium; P= phosphorus.

Critical levels of forage minerals based on ruminant needs<sup>(21)</sup>.

## Minerals in drinking water

The minerals copper and zinc in the water were undetectable. The rest of them were within the normal range suggested by Puls<sup>(19)</sup>, except for iron, which exceeds the maximum tolerable levels (349 mg L<sup>-1</sup>) (Table 5).

**Table 5:** Mineral concentration in the different water sources

Minerals	Cu	Fe	Zn	Mn	Na	K	Ca	Mg	P
Maximum tolerable	>1.0	<0.3	<25	<0.05	<800	<20	<1000	<1000	<0.7
Average ± SD	ND	0.35 ± 0.05	ND	57.76 ± 20.24	1.71 ± 0.34	37.5 ± 12.1	18.84 ± 3.25	4.82 ± 0.06	711 ± 277

Cu= copper; Fe= iron; Zn= zinc; Mn= manganese; Na= sodium; K= potassium; Ca= calcium; Mg= magnesium; P= phosphorus.

ND= not detectable; SD= standard deviation.

Maximum tolerable levels in water<sup>(19)</sup>.

## Discussion

In systems where the components of the diet are properly analyzed to define mineral supplementation, favorable responses in production are achieved, with increases of up to 16 % in daily weight gain<sup>(22)</sup>. According to the results of this work, copper is a mineral that is deficient in all the animals analyzed ( $0.48 \pm 0.07$  mg L<sup>-1</sup>); this deficiency is relevant because it is involved in various enzymatic functions as a cofactor, one of the most important being ceruloplasmin, a copper-dependent enzyme<sup>(23)</sup>.

Copper is also related to embryogenesis since it promotes early embryo development and increases the percentage of blastocysts, and it is mentioned that Cu transporters could exist in the cumulus-oocyte complex<sup>(24)</sup>. Hence, the effects caused by hypocupremia are associated with decreased conception, anovulatory cycles, and anestrus, which causes an increase in open days, reproductive tract integrity, and embryonic death<sup>(25)</sup>. Several studies in humans have demonstrated the function of copper in the central nervous system and its relationship with various diseases<sup>(26)</sup>.

Arthington *et al*<sup>(27)</sup> defined how copper interferes with immune responses, but when there is a deficiency, haptoglobin and fibrinogen are suppressed, and the immune response is provoked<sup>(28,29,30)</sup>. Phillippo *et al*<sup>(30)</sup> described how delayed puberty was found in heifers supplemented with molybdenum, this being a copper antagonist. Some authors mentioned that manganese may have effects on Cu-dependent enzymes, such as lysyl oxidase, causing negative effects on reproduction<sup>(31,32)</sup>.

Copper deficiency has been reported in most parts of the world and is considered to be the second most relevant deficiency of grazing animals<sup>(33)</sup>. In a study on the prevalence of Cu deficiency in Canada, it was found that the deficiency ranged from 24 to 43 % of the population studied<sup>(34)</sup>. Another study<sup>(35)</sup> diagnosed 80 cows with copper deficiency, which after supplementation showed favorable reproductive responses and a decrease in reproductive pathologies, such as placental retention, metritis, pyometras, and cysts. Ramírez *et al*<sup>(36)</sup> found more than 50 % of animals with severe copper deficiency.

## Zinc

Arthington and Ranches<sup>(37)</sup> mentioned that zinc is the third element with the highest deficiency in grazing animals; the most notable function of this mineral is based on the synthesis of DNA and RNA, processes such as spermatogenesis, and immune function. Multiple studies indicate that zinc is essential in animal development<sup>(38,39,40)</sup>. This nutrient is closely related to the synthesis of proteins and carbohydrates and other biochemical functions<sup>(41)</sup>. Davy *et al*<sup>(42)</sup> found deficiencies ranging from 23 % to 47 % in the region studied.

## Sodium

Sodium is a macromineral considered an electrolyte mineral because it is responsible for maintaining the balance of body fluids, muscles, and nerves<sup>(43)</sup>. It also interacts in the digestion and denaturation processes of tannins<sup>(44)</sup>. Hyponatremia is defined as sodium deficiency and deficiency is considered when serum concentration levels  $< 3,139.5 \text{ mg L}^{-1}$ <sup>(45)</sup> are present. Davy *et al*<sup>(42)</sup> found some areas of California with sodium deficiencies and Olson *et al*<sup>(46)</sup> found cows with levels below  $3,523 \text{ mg L}^{-1}$ . Sodium deficiency can present some clinical signs, such as loss of body condition, decrease in weight gain, and pica, which is a disorder where the animal licks, chews, and consumes objects<sup>(47)</sup>.

## Copper deficiency index

The most deficient mineral in this diagnosis was copper. Its deficiency was classified as severe ( $0.41\text{-}0.48 \text{ mg L}^{-1}$ ), moderate ( $0.48\text{-}0.54 \text{ mg L}^{-1}$ ), and mild ( $0.54\text{-}0.79 \text{ mg L}^{-1}$ ). Several characteristic signs of mineral deficiency were identified, which occurred when the deficiency was severe: abortions, lameness, dull, shaggy, and discolored hair, tearing, steely hair or wool, goiter, udder inflammation, pica, and excessive salivation. The more severe the deficiency, the more signs are present. When the deficiency is mild, the signs found are fewer.

**Dull, shaggy, and discolored hair.** In the severe deficiency classification, 100 % of the animals had shaggy, dull, and discolored hair. Copper is involved in the transformation of melanin through polyphenol oxidase; melanin is responsible for providing coloration to the skin and hair<sup>(48,49,50)</sup>. Some studies<sup>(52,53)</sup> describe that black coat tones tend to become reddish, and reddish coats acquire yellowish tones.

**Lameness and claudication.** This sign is also considered characteristic of Cu deficiency, and the diagnosis showed that it is present for both severe and moderate deficiency, and it is due to the interaction that copper has with the lysyl oxidase enzyme; this enzyme is an important part of the formation of collagen polypeptide chains<sup>(49,50)</sup>. Copper has also been associated with inflammation and hardening of joints; others describe that the keratin defect causes soft nails, a situation also observed in zinc deficiencies<sup>(51,52)</sup>.

**Abortions and reproductive disorders.** The role of Cu in reproduction is still not very clear; some authors describe that Cu deficiency can cause abortion, embryonic resorption, anestrus, alteration in the estrous cycle, decrease in the number and frequency of LH pulses<sup>(52,53,54)</sup>. Copper participates in the reduction of oxidative stress in the ovary; it has also been mentioned that copper participates in the synthesis and secretion of gonadotropins and follicular growth<sup>(55-58)</sup>.

**Steely hair.** The curly appearance of the hair is due to the disulfide that is present in keratin, but to convert it, it necessary to transform sulfhydryl into disulfide, and this is carried out with copper-dependent enzymes; when there is a copper deficiency, the hair is dull, rough, and very fragile<sup>(59,52)</sup>. Other signs of copper deficiency are diarrhea, neonatal ataxia, anemia, and decreased body condition<sup>(52,60)</sup>.

**Pica.** Pica is a disorder that occurs in animals and humans, in which they consume, lick, and chew on objects<sup>(61)</sup>. It can be caused by a deficiency of one or more minerals, but some studies describe that it is a deficiency of sodium, a mineral that was deficient in the sampled animals<sup>(62)</sup>. Sodium deficiency also causes languor, dry hair, weakness, and decreased milk production<sup>(52,62)</sup>. In a study conducted in Saskatchewan, Canada, between 2003 and 2012 to see the prevalence of mineral deficiency, the following signs were found: abortions, deaths, downer cows, neonatal losses, diarrhea, weak calves, and the most common mineral deficiency was Cu (47.2 %)<sup>(63)</sup>.

## Forage and water

Forages had low protein concentrations (< 6 %) and high concentrations of NDF (71.97 %), which are considered typical forage concentrations<sup>(64)</sup>. Muñoz-González *et al*<sup>(65)</sup> found NDF levels of 64.6 to 66.4 %. Cu and Zn deficiencies were also identified. In a study conducted

in the tropics, they found that almost 91 % of forages had copper deficiency and 16 % had zinc deficiency and none of the forages analyzed reached the levels required by cattle<sup>(66)</sup>. In water, iron ( $349 \text{ mg L}^{-1}$ ) was found to be above the maximum tolerable levels and according to some studies, there is a contamination of this mineral, which can cause antagonism with some others<sup>(67)</sup>.

### **Antagonism of iron and copper**

Iron is one of the essential minerals for animals and one of the most abundant on earth; it is considered to be present in all food sources, so grazing animals consume large amounts of it<sup>(68,69)</sup>. Iron is antagonistic to some minerals, such as Cu, Mn, and Zn<sup>(70)</sup>. Copper metabolism is affected by elevated concentrations of Fe, Zn, and S; it has been described that cattle that had iron levels above the normal range showed a copper deficiency, which triggered hypocupremia, a situation reported in Fe-rich waters<sup>(70,71)</sup>.

### **Mexico's previous results found**

A study assessed blood serum and found that the concentration of Cu was deficient, below the critical level, mainly in the localities of Benito Juárez  $0.36 \text{ mg dL}^{-1}$  and Jilotepec  $0.46 \text{ mg dL}^{-1}$ , in the state of Mexico; in the latter, Cu was also low in forage<sup>(72)</sup>. Likewise, in the same locality, zinc deficiencies in blood serum were found:  $0.55 \text{ mg dL}^{-1}$ . As can be identified, it was the same situation that happened with the sampled cattle<sup>(72)</sup>. In Chihuahua, a study carried out in several localities found phosphorus and iron deficiency and copper deficiency in three localities<sup>(73)</sup>. Cu deficiency is widespread throughout the country in cattle, sheep, and goats. In a study conducted in the state of Yucatan, it was found that, in some areas, up to 99 % of the animals had this deficiency, with an average Cu content of less than  $2 \text{ ppm}$ <sup>(74)</sup>.

When analyzing the forage in San Luis Potosí, it was found that there was more Fe ( $P \leq 0.05$ ) in the dry season, which coincides with what was found, and it is agreed that the concentrations of minerals found in the forage do not meet the requirements of grazing cattle<sup>(72)</sup>. Muñoz-González *et al*<sup>(75)</sup> found that 100, 28, and 72 % of forage was below the minimum level for Cu, Zn, and P<sup>(75)</sup>, respectively.

### **Conclusions and implications**

The cattle of the ranch studied, which is located in the arid and semi-arid zone of the country, have copper deficiency, which is related to the existing deficiency of this mineral in forage and water. When copper deficiency is severe, more clinical signs appear in the animal, such as abortions, lameness, dull, shaggy, and discolored hair, tearing, steely hair or wool, goiter,

and pica. Another problem in cattle is zinc deficiency, which can also contribute to the presence of clinical signs and mineral imbalances. Excess iron in forage and water contributes to copper deficiencies because of the antagonism that exists between them.

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