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# ORGANIC MICROMINERALS IN THE DIET OF WEANED CALVES ON PRODUCTIVE PERFORMANCE AND BLOOD PARAMETERS

Pablo Mancillas<sup>1\*</sup>, Daniel Díaz<sup>2</sup>, Perla Ordoñez<sup>3</sup>, Nahomi Rodríguez<sup>4</sup>, Andrea González<sup>5</sup>, José Espinoza<sup>6</sup>, Dána Gonzalez<sup>7</sup>, Eduardo Santellano<sup>8</sup>

<sup>1</sup>Autonomous University of Chihuahua, Faculty of Zootechnics and Ecology, Chihuahua, Mexico.  
Email: [dplascencia@uach.mx](mailto:dplascencia@uach.mx), ORCID iD: <https://orcid.org/0000-0001-8093-6623>

<sup>2</sup>Autonomous University of Chihuahua, Faculty of Zootechnics and Ecology, Chihuahua, Mexico.  
ORCID iD: <https://orcid.org/0000-0002-8309-8597>

<sup>3</sup>Autonomous University of Chihuahua, Faculty of Zootechnics and Ecology, Chihuahua, Mexico.  
ORCID iD: <https://orcid.org/0000-0003-3705-4195>

<sup>4</sup>Autonomous University of Chihuahua, Faculty of Zootechnics and Ecology, Chihuahua, Mexico.  
ORCID iD: <https://orcid.org/0000-1111-2222-3333>

<sup>5</sup>Autonomous University of Chihuahua, Faculty of Zootechnics and Ecology, Chihuahua, Mexico.  
ORCID iD: <https://orcid.org/0000-1111-2222-3333>

<sup>6</sup>Autonomous University of Chihuahua, Faculty of Zootechnics and Ecology, Chihuahua, Mexico.  
ORCID iD: <https://orcid.org/0000-0003-0697-0852>

<sup>7</sup>Autonomous University of Chihuahua, Faculty of Zootechnics and Ecology, Chihuahua, Mexico.  
ORCID iD: <https://orcid.org/0000-0001-8761-6294>

<sup>8</sup>Autonomous University of Chihuahua, Faculty of Zootechnics and Ecology, Chihuahua, Mexico.  
ORCID iD: <https://orcid.org/0000-0003-0884-0971>

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Corresponding Author: Pablo Mancillas  
([dplascencia@uach.mx](mailto:dplascencia@uach.mx))

## ABSTRACT

*This study investigated the benefits of incorporating organic microminerals into the diet of weaned calves, focusing on their productive performance and blood parameters. A total of 108 calves, approximately six months old and weighing around 133.70 kg, were divided into two groups: a control group (T1) receiving no organic minerals and an experimental group (T2) receiving 7 grams of organic microminerals per animal daily. Both groups were fed isoprotein and isoenergetic diets. Key variables such as body weight, daily weight gain, and feed conversion were monitored at 20, 60, and 90 days. Blood analyses, including cell counts and cortisol levels, were also conducted at these intervals. The results clearly demonstrated a significant positive impact of the organic micromineral supplementation, with the T2 group showing superior values ( $P < 0.05$ ) in body weight, daily weight gain, and feed conversion by day 90. Furthermore, an increase in the general average of white blood cells was observed in the T2 group, alongside specific average values for leukocytes, neutrophils, mixed cells, and cortisol. This comprehensive evaluation concludes that including organic microminerals in the diet of weaned calves positively influences both their productive performance and immune system function.*

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**KEYWORDS:** Organic Microminerals, Weaned Calves, Blood Counts.

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## 1. INTRODUCTION

The human population's primary needs, which include sustenance, have given rise to diverse systems of domestic and wild animal production across the globe. Among these, cattle are particularly noteworthy for their contribution to meat production. In the northern states of Mexico, cattle ranching is a lucrative industry that plays a pivotal role in the regional economy, contributing significantly to foreign exchange earnings. A predominant agricultural activity in this region is the export of calves to the United States of America. This process is facilitated by a production system centered on the breeding of cows and calves.

Preconditioning is a feed management strategy that enables calves to regain weight and prepare for export, thereby facilitating their adaptation to grazing in pastures and feeding in pens (Piñón et al., 2015). This process entails the provision of sufficient feed and the implementation of specific handling procedures for pre-weaning calves, with a weight range of 150 to 180 kilograms. During the process of export, animals undergo a reduction in body mass due to the stressors associated with transport, temperature fluctuations, and altered feeding regimens. This stress can result in an approximate loss of 10 kilograms per animal.

As Underwood and Suttle (1999) have noted, the provision of trace elements through nutrition has been demonstrated to support physiological functions related to growth, reproduction, and immunity in animals. However, factors such as weaning, transport, and vaccination trigger normal physiological responses that activate the immune system to stressful stimuli (Arthington et al., 2013). This reaction is characterized by an increase in the concentration of circulating proinflammatory cytokines (Klasing and Korver, 1997) and acute-phase proteins (Petersen et al., 2004), both of which have been demonstrated to affect nutrient metabolism and animal growth (Carroll and Forsberg 2007).

In the state of Chihuahua, Mexico, the cattle production system oriented to the export of calves is based on diets consisting of fodder and agricultural shear. This diet has been shown to limit the digestibility of fiber and, consequently, the productive response of the animals (Dado and Allen, 1996). In the pursuit of cost-effective production strategies, producers are exploring the potential of organic microminerals as a means to enhance animal productivity (Gunn et al., 2010).

Current trends in the field of bovine mineral nutrition indicate that minerals constitute approximately 4-5% of the animal's live weight (Ciria et al., 2005). These minerals are acquired primarily

through food and drinking water (Coria, 2020). Maintaining an adequate mineral balance is contingent upon the intake of minerals that meet the specific requirements of each element. This is necessary to prevent imbalances or alterations caused by deficiencies or toxicities (Beede et al., 2017).

In light of the aforementioned points, the present study endeavors to evaluate the impact of organic microminerals in the diet of weaned calves on their productive performance and the response of blood parameters.

## 2. MATERIALS AND METHODS

### 2.1 Location of the Study Area

The study was conducted at the facilities of the Grupo Agropecuario Gran Visión SPR ranch of RL de CV, located in the municipality of Cuauhtémoc, Chihuahua, Mexico. The site's geographical coordinates are 28° 25' 28" N and 107° 07' 59" E, with an altitude of 2,214 meters above sea level (Google, n.d. 2024).

The region's climate is classified as semi-humid to dry, with an average annual temperature of 18°C, ranging from a minimum of -15°C to a maximum of 38°C. The mean annual precipitation is 400 millimeters. The experiment was initiated on December 26, 2022, and concluded on March 24, 2023.

### 2.2 Animal Descriptions and Treatments

The study's sample population comprised 108 weaned Angus calves, with an average age and live weight (BW) of six months and 133.70 ± 1.01 kg, respectively. The calves were randomly assigned to two treatments: T1 (n = 53): control, 0 g of organic minerals (OM) per animal per day; T2 (n = 55): The daily dosage of OM was set at 7 g per animal (Table 1).

**Table 1: Diet offered to calves weaned from the two treatments<sup>1</sup>.**

| Ingredients                     | Kilograms, BS |
|---------------------------------|---------------|
| Alfalfa hay                     | 33.00         |
| Oat hay                         | 6.00          |
| Concentrate 20.99%              | 61.00         |
| <b>Total, kg</b>                | <b>100.00</b> |
| <b>Calculated Diet Analysis</b> |               |
| M. S. %                         | 88.00         |
| T.N.D. %                        | 60.00         |
| P.C. %                          | 15.00         |
| E.M.Mcal/kg <sup>-1</sup>       | 2.28          |
| E.N.m.Mcal/kg <sup>-1</sup>     | 1.49          |
| E. N. g. Mcal/kg <sup>-1</sup>  | 0.92          |
| Ca %                            | 0.96          |
| P %                             | 0.40          |

<sup>1</sup>The animals of each treatment received the same diet per period (0, 20, 60 and 90 d), the difference was the addition of organic microminerals at the time of offering the feed in the feeder, T1: control (0 g-1) and T2: OM (7.5 g-1).

### 2.3 Animal Management

Prior to the initiation of the experiment, the animals were weighed and identified with the plastic earring. The following vaccines were administered: Bobact® 8 (5 mL/animal), intramuscularly; and Bovilis® Nasalgen™ IP (IBR and PI3), intranasally (2 mL/animal). The animals were also treated with vitamins and dewormer subcutaneously: Iverfull® Macrovit ADE (1 mL/75 kg of BW) and a Ralgro® implant (36 mg/animal). Subsequently, the subjects were confined to pens equipped with access to uncontaminated water. This confinement persisted for a duration of 90 days, with the initial 20 days allocated for acclimatization. The dietary regimen comprised alfalfa hay, oat hay, and a commercially available concentrate with 20.99% CP, in accordance with the recommendations of the NRC (1996) for beef cattle. The provision of feed was scheduled at 8:00 a.m. and 4:00 p.m. Every 20 days, the dry matter intake (DMI) was adjusted by weighing the consumed and rejected food for five consecutive days, ensuring the availability of the diet throughout the day.

### 2.4 Ethical Aspects

The study was conducted in accordance with the internal code of bioethics. It took into consideration the animal welfare regulations of the FZyE of the UACH and the regulations of the Official Mexican Standard on animal care, which were published by the Ministry of Agriculture and Rural Development (SAGARPA 1999).

### 2.5 Sample Sampling

Ambient temperature (TA). The DATA ACCESS VIEWER tool was used to measure the maximum and minimum BP per day over the course of the test.

Productive behavior. To analyze the variables of the behavior test, individual weighing was carried out at the beginning, at 20, 60, and 90 days during the experiment (0, 20, 60, and 90 days) after the animals were previously fed. To measure the live weight (BW), a REVOLTA® scale (capacity of 1,000 kg) was used. Daily weight gain (GDP) was measured by calculating the difference between consecutive weights and dividing the result by the number of days between weighings. The data collected on the basis of the Cost-to-Animal Ratio (CA) and the Gross Domestic Product (GDP) was utilized to ascertain feed conversion, defined as the ratio of feed consumed to weight gain over a given period.

**Blood samples.** Fifteen animals were utilized for each treatment, with two tubes collected from each animal via direct puncture of the jugular vein. This procedure was performed after individual weighing

and prior to the animals receiving their diet during the morning. The samples were then preserved in a cooler that was stocked with ice.

A blood count (BH) was performed. To analyze BH, a Vacutainer® tube (containing 7.2 mg of EDTA as an anticoagulant) was utilized for each sample. Additionally, a cortisol measurement was recorded for each sampling period. In BH, the quantity, percentage, and weight of different blood components were determined, and the following results were obtained: The following components were analyzed: leukocytes (Leu), neutrophils (Neu), lymphocytes (Lin), mixed cells (Cm), erythrocytes (Er), hemoglobin (Hem), hematocrit (Hto), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), and platelets (Pq).

The samples for BH and cortisol were dispatched to the facilities of ASSAY Laboratorio Clínico S. de R. L. de C. V., where their analysis was performed using a Beckman Coulter®/act/dif/1al cytometer.

### 2.6 Statistical analysis

The behavioral test's variables, including PV, GDP, CA, BH, and cortisol, were analyzed using the MIXED procedure. This procedure incorporated a fixed effect for treatment and the date of sampling, as well as their interaction. Additionally, the initial sampling was incorporated as a covariate within a completely randomized design (SAS, 2004). All analyses were conducted with a confidence level of 95% or  $\alpha = 0.05$ , employing Tukey's test to ascertain potential disparities in the means of the treatments (Steel & Torrie, 1997).

## 3. RESULTS AND DISCUSSION

### 3.1 Live Weight

The inclusion of organic microminerals (OM) in the diet of weaned calves exhibited a substantial impact ( $P < 0.05$ ) on PV, GDP, and AC. In the initial two periods of the experiment, the animals in Treatment 1 (T1) and Treatment 2 (T2) exhibited no statistically significant differences ( $P > 0.05$ ). The mean body weights of the animals in T1 and T2 were  $123,430 \pm 0.90$  kg and  $121,700 \pm 0.90$  kg at 20 days, and  $148,170 \pm 2.42$  kg and  $147,630 \pm 2.00$  kg at 14 days, respectively.

In the initial 60-day period, the mean body weight (BW) of the T2:MO calves (42 kg) was not significantly different from the T1: control calves (41 kg). However, by 90 days, the T2:MO calves exhibited a significantly higher BW (170.170 kg) compared to the T1: control calves (166.830 kg), as indicated by a P-value of less than 0.05 (Figure1).

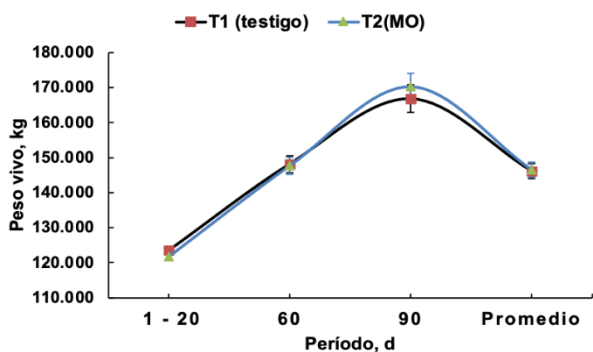


Fig.1. Mean ( $\pm$  E.E.) of the live weight of weaned calves fed a traditional diet (T1: control) and a diet that included trace minerals (T2:MO).

Pérez et al. (1986) posited that the supplementation of grazing heifers with microminerals enhances daily weight gain and meat production at a minimal cost, without compromising management conditions. Similarly, the mixture of microminerals with common salt yields superior outcomes, as it can augment meat production by up to 10%. The findings of the present study demonstrate that the animals that received OM in their diet exhibited an increase in PV at 90 days post-test. This phenomenon may be attributed to the influence of trace minerals on the activity and enzymatic efficiency responsible for energy and protein metabolism, cellular repair and integrity, as well as immune system functionality (Gómez, 2016).

Conversely, Geary et al. (2021) reported that the source of trace minerals does not affect the live weight gain of the mature bull or reproductive parameters. However, hydroxychloride-based trace minerals have been shown to improve copper and zinc concentrations in the liver, with the latter exhibiting a positive correlation with reduced acrosomal damage. In a separate study, it was documented that a group of heifers fed a mineral nutrition regimen comprising a proportion of organic trace minerals for a period of six months exhibited equivalent height and weight measurements in comparison to those of a control group that received an inorganic trace mineral source exclusively. This observation was made with a dry matter intake of less than 1.4 kilograms per day (Gómez, 2016).

### 3.2 Daily Weight Gain

The animals of the T1:control and T2:MO groups exhibited no significant difference ( $P > 0.05$ ) in the periods of 20 ( $0.623 \pm 0.05$  and  $0.557 \pm 0.05$  kg) and 60 d ( $0.568 \pm 0.04$  and  $0.699 \pm 0.04$  kg) of the test; however, at 90 d, the animals of T2:MO ( $0.801 \pm 0.05$ ) demonstrated higher ( $P < 0.05$ ) GDP than the calves of T1:control ( $0.572 \pm 0.05$ ), as illustrated in Figure 2.

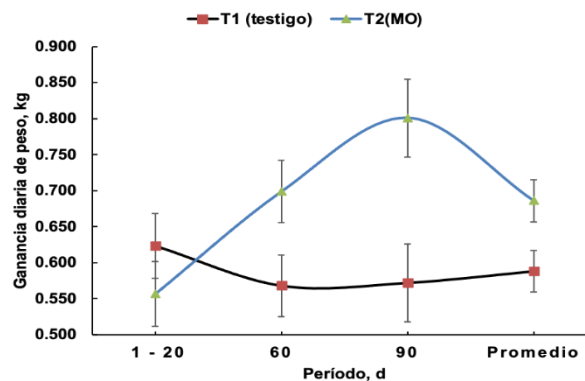


Fig. 2. Means ( $\pm$  E.E.) of daily weight gain of weaned calves fed a traditional diet (T1: control) and a diet that included trace minerals (T2:MO).

León-Cruz et al. (2020) published that intraruminal boluses with controlled release of trace minerals in Holstein-Friesian weaned calves improved GDP (0.590 kg) compared to control animals (0.530 kg). In a separate study, an injection of Cu (25 mg of Cu ml<sup>-1</sup>) and 20 g boluses with CuO micropellets were utilized. The authors observed no discrepancy in GDP (0.564 g d<sup>-1</sup>) in calves of the Aberdeen Angus and Hereford breeds. Nevertheless, they recommended that CuO boluses serve as a preventative measure against Cu deficiencies.

In contrast, Hernández (2020) reported that the addition of high and low concentrations of microminerals to the diets of calves of the Simmental and Simbrah breeds resulted in a diet high in microminerals exhibiting improved growth performance (1,330 kg) compared to the diet low in trace minerals (1,190 kg). This finding aligns with the results of the present experiment, wherein calves that received the OM diet demonstrated enhanced growth performance (0.801 kg) in comparison to the control group (0.572 kg).

Concurrently, Ward and Spears' (1997) findings indicated a substantial discrepancy in the Gross Domestic Product (GDP) and Ca levels in cattle administered concentrations of 5 milligrams per kilogram of Cu in their diet. Conversely, Ahola et al. (2005) documented enhancements in calf development during their initial year of age, attributable to the provision of inorganic microminerals through dietary supplementation (GDP 1,460 kg). However, these outcomes did not exhibit statistical significance among the various treatment groups.

### 3.3 Feed Conversion

Upon statistical analysis, no significant difference ( $P > 0.05$ ) was observed between the T1:control and T2:MO groups over the 20-day period, with mean

weights of  $7,611 \pm 0.46$  and  $8,263 \pm 0.46$  kilograms, respectively. A similar observation was made for the 60-day test, with mean weights of  $7,593 \pm 0.46$  and  $7,152 \pm 0.46$  kilograms recorded for the T1:control and T2:MO groups, respectively. However, at 90 d, the animals of T2:MO ( $3,786 \pm 0.46$ ) exhibited greater efficiency ( $P < 0.05$ ) in the AC compared to the calves of T1:control ( $4,685 \pm 0.46$ ), as illustrated in Figure 3.

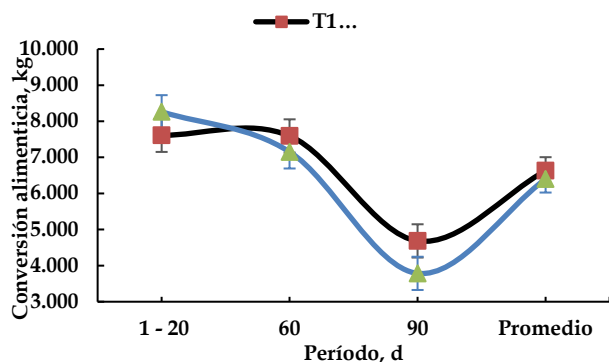


Fig. 3. Means ( $\pm$  E.E.) of feed conversion of weaned calves fed a traditional diet (T1:control) and a diet that included trace minerals (T2:MO).

Hernández (2020) observed that, in the context of comparing high and low concentrations of microminerals in the diet of calves, animals receiving high amounts exhibited greater efficiency in CA (6,190 kg) compared to those receiving low amounts (7,240 kg). These results are consistent with those of the present study, in which animals that received OM in their diet exhibited higher efficiency compared to those in the control group.

Lee et al. (2020) reported that the supply of trace minerals in rumen boluses in beef-producing cattle did not observe a difference between treatments in the adaptation, transition, and termination phases in the PV, CMS, GDP, and CA. However, upon analysis of the data with interaction in the completion phase, it was observed that the animals that received the rumen boluses exhibited superior performance in the CMS (10.2 and 9.6 kg) and GDP (1.48 and 1.45 kg), respectively, in comparison to the control group.

In a separate study, sheep were provided with diets containing Se and Cr. No alterations in GDP, CMS, or CA were observed. However, the sheep that received the nuclei with Cr and Se exhibited enhanced partial efficiency of feed utilization compared to the control group (Rodríguez et al., 2011). In 2009, an experiment was published that examined the effects of a completion diet on carcass measurements and productive variables. The experiment involved the administration of doses of Cr (0, 0.25, and 0.35 mg) and Se (0 and 0.3 mg) chelated in yeast. The inclusion of Se did not affect the productive variables or carcass measurements.

However, Cr exhibited a quadratic response in the accumulation of protein and fat in the carcass and a positive linear response in GDP. The aforementioned authors further noted that the variability of the response is contingent upon the Se status of the animal and the baseline level of the supplemented diet.

The efficiency of feed utilization is a metric that quantifies the biological relevance of the regression slope between consumption and gain. In contrast, feed conversion is a statistical metric that assumes all feed is used for production, thus ignoring variations in maintenance efficiency and weight gain.

### 3.4 Blood Biometry

**White Blood Cells.** The results of the study demonstrated a statistically significant effect ( $P < 0.05$ ) of OM supplementation on the concentration of leukocytes, neutrophils, and mixed cells in weaned calves.

**Leukocytes.** In the initial phase of the experiment, there was no statistically significant difference observed between the T1:control and T2:MO groups ( $P > 0.05$ ). However, the calves of T2:MO exhibited a reduced concentration of leukocytes ( $8.65 \pm 0.58$ ,  $9.08 \pm 0.58$ , and  $8.79 \pm 0.45$   $10^3/\mu\text{L}$  at 60 and 90 d) and the overall mean of the test (Figure 4) when compared to the T1:control.

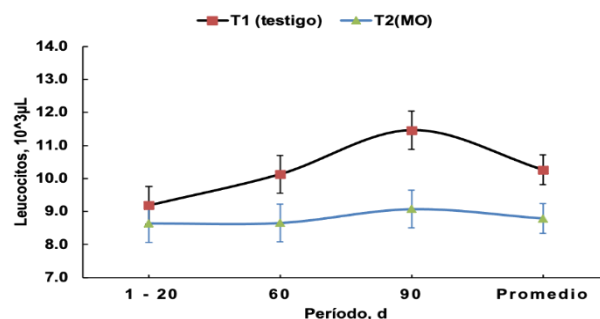


Fig. 4. Means ( $\pm$  E.E.) of leukocytes in weaned calves fed a traditional diet (T1:control) and a diet that included trace minerals (T2:MO) Leukocyte values in cattle (4 to 12  $10^3/\mu\text{L}$ )

Gupta et al. (2007) published the relationship between leukocyte profile and plasma glucocorticoid level during physiological stress. These hormones have been shown to increase neutrophil concentration and decrease lymphocyte cells (Blanco et al., 2009). In the present study, it was observed that the calves of the T1:control group remained close to the upper limit of leukocyte values in cattle (4 to 12  $10^3/\mu\text{L}$ ), while the animals that received OM in their diet exhibited mean values for these parameters.

Conversely, Vidal (2006) noted that zinc is a mineral present in high concentrations in bovine hair

(201 ppm in DM), while its concentration in leukocytes is 21 ppm. Its function is to act as a cofactor or regulator of different enzymes, such as carbonic anhydrase, DNA and RNA polymerases (enzymes involved in cell replication and protein synthesis), and superoxide dismutase (an antioxidant enzyme).

In the event of zinc deficiency, a reduction in the number of leukocytes, total gamma-globulin concentration, and production of specific antibodies is observed. Furthermore, there is a marked decrease in the size of the thymus, an increase in the number of immature forms of neutrophils, and an increased susceptibility to disease (Spears & Weiss, 2008).

**Neutrophils.** The animals in both the T1:control and T2:MO groups exhibited no statistically significant differences ( $P > 0.05$ ) during the initial 1–20-day period of the test. However, in the second and third periods (60 and 90 d), as well as in the general average of the test, the calves of T2:MO presented a lower concentration of neutrophil cells in quantity and percentage. Specifically, the concentrations were  $1.77 \pm 0.22$ ,  $2.31 \pm 0.22$ , and  $1.98 \pm 0.15$   $10^3/\mu\text{L}$ , and the percentages were  $20.80 \pm 1.44$ ,  $25.33 \pm 1.44$ , and  $22.33 \pm 0.93$ , respectively, with respect to T1: witness (Figures 5 and 6).

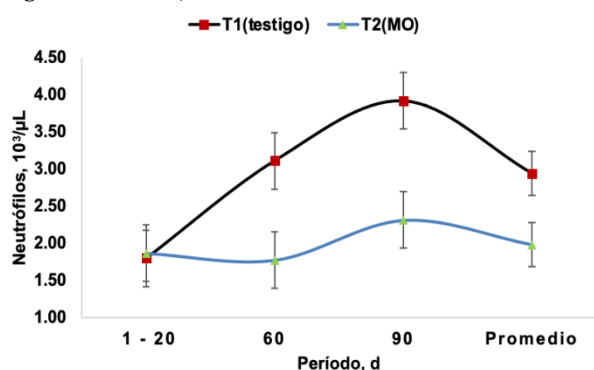


Fig. 5. Means ( $\pm$  E.E.) of neutrophils in number of weaned calves fed a traditional diet (T1:control) and a diet that included trace minerals (T2:MO).

Neutrophil values in cattle (0.6 to 4.0  $10^3/\mu\text{L}$ ).

Alarcón-Corredor's 2009 publication posits a correlation between Cu and the body's defense mechanisms against inflammatory and infectious processes. The study suggests that Cu modulates the activity of neutrophils, exerts bactericidal action, demonstrates anti-inflammatory effects, and plays a role in the inflammatory process. In addition, Romero et al. (2011) stated that, in response to infections, inflammation, and stress, there is an increase in the number of neutrophils in the bloodstream. Conversely, there is a decrease in the number of neutrophils in the bloodstream in certain

infections and states of anaphylaxis. In the present study, the animals that received MT in their diet exhibited levels and percentages that fell below the mean of the parameters observed in cattle. This finding suggests that MT has a beneficial effect on the immune system. In contrast, the calves of the T1:control group demonstrated higher concentrations of neutrophils, approaching the upper limit. This observation may be indicative of stress processes experienced by the calves.

In contrast, glucocorticoids derived from stressed animals have been shown to stimulate the migration of neutrophils from the bone marrow into the bloodstream. This process has been observed to reduce the migration of neutrophils to other anatomical compartments, resulting in an increase in both mature and immature neutrophils within the bloodstream (Buckham *et al.*, 2008).

**Lymphocytes.** The animals of the T1:control and T2:MO groups exhibited no statistically significant difference ( $P > 0.05$ ) in the number of lymphocytes. However, in the third period (90 d), the calves of T2:MO exhibited a lower ( $P < 0.05$ ) concentration of cells ( $5.78 \pm 0.38$   $10^3/\mu\text{L}$ ) when compared to the T1:control. In addition, the T2:MO calves exhibited a downward trend in cell count ( $P > 0.06$ ) when compared to the T1:control calves ( $6.55 \pm 0.30$   $10^3/\mu\text{L}$ ), as illustrated in Figure 6.

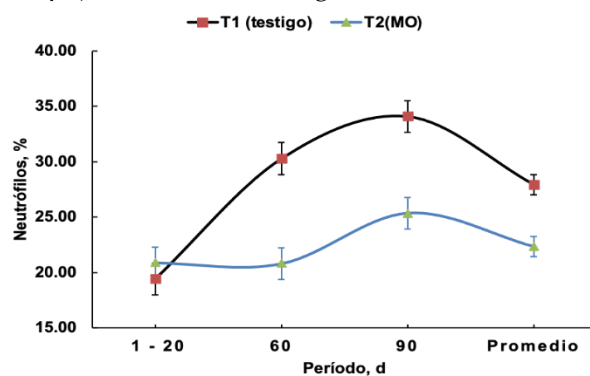


Fig. 6. Means ( $\pm$  E.E.) of neutrophils as a percentage of weaned calves fed a traditional diet (T1:control) and a diet that included trace minerals (T2:MO).

Neutrophil values in cattle (15 to 45 %).

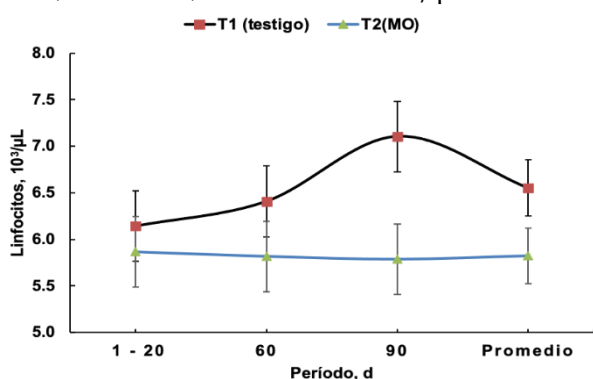
As Shankar and Prasad (1998) have previously indicated, the impact of zinc on the immune system is multifaceted, extending from the skin barrier to the genetic regulation within lymphocytes. This element is imperative for the development and normal function of cells that mediate nonspecific immunity, including neutrophils and natural killer cells. Zinc deficiency has also been demonstrated to impede the development of acquired immunity by hindering the growth and certain functions of T lymphocytes, such

as activation, the production of Th1 cytokines, and B lymphocytes. Consequently, this results in the compromise of B lymphocytes and the production of antibodies, particularly immunoglobulin G.

In the present study, the calves that received OM in their diet demonstrated lymphocyte parameters that remained at the mean for cattle. In contrast, the animals in the T1:control group exhibited lymphocyte parameters at the upper limit. Therefore, it can be hypothesized that the calves in the control group experienced a stress process, which was more evident in the third period (90 d) and followed the same trend in the average values of the test.

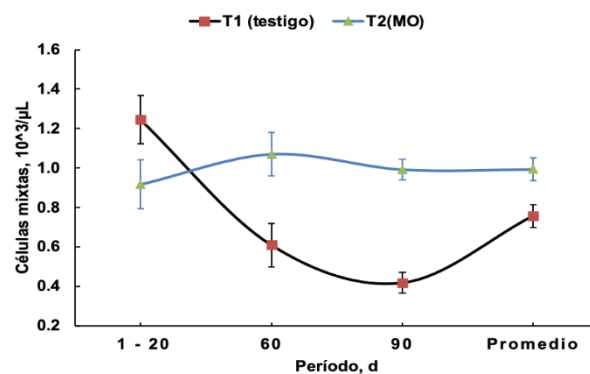
As Calamari *et al.* (2004) have previously indicated, an increase in the ratio of neutrophils to lymphocytes can be regarded as an indicator of stress. In a similar vein, Pagliasso *et al.* (2023) have demonstrated that the initial physiological response to stress in animals is characterized by a transient increase in glucocorticoids and catecholamines. These hormones can modulate a series of biological effects that affect immune functions. Therefore, it is imperative to regulate the actions that prevent an excessive release of proinflammatory cytokines (IL-6, IFN- $\gamma$ , and TNF- $\alpha$ ) and other components that lead to severe inflammation and tissue damage (Hughes *et al.*, 2014).

**Mixed cells (SC).** Calves that received OM in their diet exhibited a substantial impact ( $P < 0.05$ ) on the concentration of CM in percentage and quantity during the test when compared to the T1:control. The T2:MO animals exhibited a higher ( $P < 0.05$ ) concentration of CM ( $11.03 \pm 1.17$ ,  $12.23 \pm 0.64$ ,  $11.03 \pm 0.44$  and  $11.43 \pm 0.38$  %; Figure 7) in the three sampling periods and in the general average of the test. This observation was replicated in Figures 8 and 9, which depicted the same trend in  $0.92 \pm 0.12$ ,  $1.07 \pm 0.11$ ,  $0.99 \pm 0.05$ , and  $0.99 \pm 0.06$   $10^3/\mu\text{L}$  of CM.



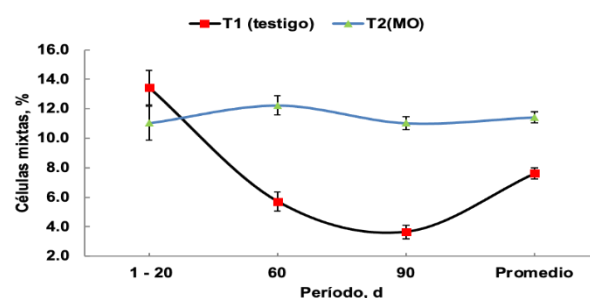
**Fig. 7.** Means ( $\pm$  E.E.) of lymphocytes in weaned calves fed a traditional diet (T1:control) and a diet that included trace minerals (T2:MO).

Lymphocyte values in cattle (2.5 to 7.5  $10^3/\mu\text{L}$ )



**Fig. 8.** Means ( $\pm$  E.E.) of mixed cells as a percentage of weaned calves fed a traditional diet (T1:control) and a diet that included trace minerals (T2:MO).

Mixed cell values in cattle (2.0 to 20.0 %)



**Fig. 9.** Means ( $\pm$  E.E.) of mixed cells in number of weaned calves fed a traditional diet (T1:control) and a diet that included trace minerals (T2:MO).

Mixed cell values in cattle (0.0 to 2.4  $10^3/\mu\text{L}$ )

SCs are a differential count of leukocytes consisting of five parts: polymorphonuclear (neutrophils, eosinophils, and basophils), lymphocytes, and monocytes (Bain *et al.*, 2006). In the present study, the calves of the T2:MO remained in the mean of the CM values in quantity and percentage, while the animals of the T1:control were at the lower limit of the parameters.

Shamri *et al.* (2011) noted that the majority of mammals have evolved immune responses involving eosinophils to protect themselves from pathogens, utilizing their cytotoxic effector functions. However, paradoxically, eosinophils can also trigger respiratory inflammation, a phenomenon that is particularly evident in weaned calves (Hulbert & Moisés, 2016). Conversely, experts in immune interactions due to stress have concluded that short-term changes in immunity due to stressors do not necessarily harm the immune system of animals in the long term (Sorrells and Sapolsky, 2007).

Furthermore, eosinophils possess a wide range of receptors to microbe-associated molecular patterns (MAMPs). Once activated, these cells degranulate, providing other leukocytes with a medium of

cytokines, chemokines, mediators, and other factors during an innate immune response (Shamri et al., 2011). In a similar manner, eosinophils and neutrophils have been demonstrated to exhibit active responses to inflammatory stimuli.

### 3.5 Red Blood Cells

The inclusion of OM in the diet of weaned calves exhibited a substantial impact ( $P < 0.05$ ) on hemoglobin and mean corpuscular hemoglobin. At 60 days post-test, it was observed that the calves of T2:MO exhibited an increase in hemoglobin concentration of  $12.86 \pm 0.21$  g/dL in comparison to the T1:control group, which registered at  $12.00 \pm 0.21$  g/dL. This increase followed a parallel trend ( $P < 0.05$ ) for HCM of  $14.90 \pm 0.20$  and  $14.29 \pm 0.20$  pg for T2:MO and T1:control, respectively (see Table 2 for details).

In the event of blood parameter deficiencies,

nutritional problems may be indicated, given the potential for decreased iron intake that falls below the animal's requirements. Such deficiencies may also be associated with intestinal issues that impede absorption or with excessive fecal losses (Quiroz & Bouda, 2001). HCM and HCCM are used to indicate the concentration of hemoglobin based on the number of red blood cells; however, the latter is considered the most appropriate (Aiello and Mays, 2000).

According to Stanger et al. (2005), exposure of animals to stress conditions has been demonstrated to result in the stimulation of the hypothalamic-pituitary-adrenal axis. This stimulation leads to the release of various hormones, including catecholamines and glucocorticoids. These hormones have the potential to modify the biochemical and cellular composition of the blood, energy metabolism, and immune response.

**Table 2: Red blood cell stockings ( $\pm$  E.E)<sup>a</sup> in weaned calves fed a traditional diet (T1:control) and a diet that included trace minerals (T2:MO).**

| Periods, d   | T1 (witness)       | T2(MO)             |                    | USA  | P Value |
|--|--------------------|--------------------|--------------------|------|---------|
| <sup>2</sup> Erythrocytes, 10 <sup>6</sup> / $\mu$ L |                    |                    |                    |      |         |
| 1 - 20   | 8.61               | 8.38               |                    | 0.16 |         |
| 60   | 8.29               | 8.75               |                    | 0.18 | 0.221   |
| 90   | 8.98               | 9.12               |                    | 0.28 | 0.840   |
| Average  | 8.63               | 8.75               |                    | 0.16 | 0.968   |
| <sup>2</sup> Hemoglobin, g/dL                        |                    |                    |                    |      |         |
| 1 - 20   | 12.12              | 12.06              |                    | 0.20 |         |
| 60   | 12.00 <sup>b</sup> | 12.86 <sup>a</sup> |                    | 0.21 | 0.027   |
| 90   | 13.30              | 13.24              |                    | 0.39 | 0.912   |
| Average  | 12.47              | 12.72              |                    | 0.18 | 0.385   |
| <sup>2</sup> Hematocrit, %                           |                    |                    |                    |      |         |
| 1 - 20   | 37.95              | 36.21              |                    | 0.92 |         |
| 60   | 37.51              | 38.72              |                    | 0.92 | 0.153   |
| 90   | 41.28              | 40.48              |                    | 0.92 | 0.747   |
| Average  | 38.92              | 38.47              |                    | 0.67 | 0.826   |
| <sup>2</sup> VCV, fL                                 |                    |                    |                    |      |         |
| 1 - 20   |                    | 43.21              | 43.82              | 0.65 |         |
| 60   |                    | 44.26              | 45.29              | 0.65 | 0.196   |
| 90   |                    | 45.00              | 45.49              | 0.65 | 0.475   |
| Average  |                    | 44.15              | 44.87              | 0.63 | 0.327   |
| <sup>2</sup> HCM, Pg                                 |                    |                    |                    |      |         |
| 1 - 20   |                    | 13.92              | 14.48              | 0.20 |         |
| 60   |                    | 14.29 <sup>b</sup> | 14.90 <sup>a</sup> | 0.20 | 0.043   |
| 90   |                    | 14.60              | 14.76              | 0.20 | 0.606   |
| Average  |                    | 14.27              | 14.71              | 0.19 | 0.111   |
| <sup>2</sup> CHCM, g/dL                              |                    |                    |                    |      |         |
| 1 - 20   |                    | 32.31              | 33.01              | 0.28 |         |
| 60   |                    | 32.35              | 32.88              | 0.28 | 0.224   |
| 90   |                    | 32.50              | 32.43              | 0.28 | 0.824   |
| Average  |                    | 32.39              | 32.77              | 0.24 | 0.302   |

<sup>ab</sup> Means with different literal in a row, are different ( $P < 0.05$ ) between treatments.

<sup>1</sup> Means without a literal in a row and column showed no difference ( $P > 0.05$ ).

<sup>2</sup> Blood count values in cattle, Erythrocytes (5 to 10 10<sup>6</sup>/ $\mu$ L); Hemoglobin (8 to 15 g/dL); Hematocrit (24 to 46%); Mean corpuscular volume (MCV, 40 to 60 fL); Mean corpuscular hemoglobin (HCM, 11 to 17 Pg); Mean corpuscular hemoglobin concentration (HCCP, 30 to 36 g/dL).

The results of the present study were consistent with those published by Sena et al. (2018), who

evaluated the biochemical changes and blood cells of beef-producing cattle subjected to varying degrees of

stress. The stress levels were assessed based on the duration of transport (24, 48, and 72 hours). As the transport duration increased, the animals exhibited signs of increased stress, manifesting alterations in blood cell concentrations. The aforementioned authors have indicated that an increase in the values of VCP and Hg can be indicative of dehydration in animals, a condition that is more pronounced in animals experiencing elevated stress levels.

In a separate study, the researchers noted that a comparison of the values of VCP and Hg during the animals' embarkation and disembarkation revealed higher values upon arrival. This observation is likely attributable to the water imbalance resulting from previous stress and the duration of the journey (Bernardini *et al.*, 2012).

**Platelets.** In calves that received OM in the diet (T2:MO), a lower number of platelets ( $P < 0.05$ ) was observed:  $3.90 \pm 0.24$ ,  $5.02 \pm 0.23$ , and  $4.77 \pm 0.21$   $10^5/\mu\text{L}$ , respectively, compared to the T1:control ( $7.16 \pm 0.24$ ,  $5.71 \pm 0.23$ , and  $5.79 \pm 0.21$   $10^5/\mu\text{L}$ ) at 60 and 90 d, as well as in the general average of the test (Figures 10).

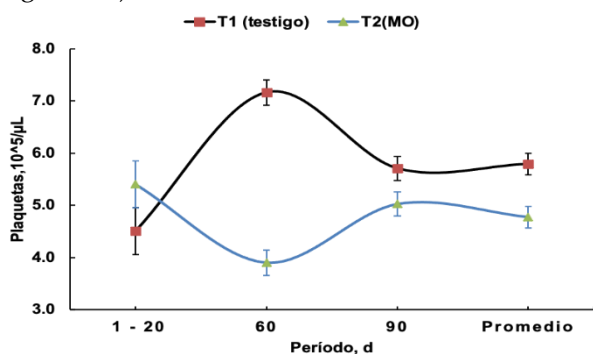


Fig. 10. Means ( $\pm$  E.E.) of platelets in weaned calves fed a traditional diet (T1:control) and a diet that included trace minerals (T2:MO).

Platelet values in cattle ( $1.0$  to  $8.0$   $10^5/\mu\text{L}$ ).

Jones and Allison's (2007) seminal study published that the increase in platelets in cattle is associated with situations of exercise, stress, or inflammatory states. This phenomenon is part of the physiological response to the release of adrenaline that occurs due to poor handling, affecting the physical, behavioral, and psychological state of the animals. It can be posited that the calves of the T1: control group may have been subject to elevated levels of stress due to the transport process and the subsequent abrupt fluctuations in temperature that were documented during the experimental procedure. Furthermore, these calves exhibited elevated concentrations of leukocytes and neutrophils, as indicated by the values of white blood cells for cattle.

Lopes and Cunha (2002) published a study that examined the utilization of white blood cell evaluation in the detection of inflammation, the presence of toxins, and emotional changes in animals. Conversely, Jain (2003) posited that the uncontrolled release of neutrophils is triggered by the presence of glucocorticoids, whether they are endogenous or exogenous, resulting in the mobilization of marginal neutrophils from the microvasculature and the subsequent induction of a heightened release of these cells from the bone marrow.

In his experiment, Holguín (2017) utilized 80 bulls of the Brahman/Charolais (BhXCh) and Brahman/Brown Swiss (BhXPs) breeds, which were assigned to two feeding systems: intensive silvopastoral (SSPi) and confinement (SC). The study concluded that the number of platelets differed between the two systems ( $P < 0.05$ ). The presence of a correlation between the number of platelets in the animals and the percentage of leukocytes was observed during the sampling period. This correlation was identified on days 71 and 132 of the sampling period. In accordance with the findings of Aiello and Mays (2000), the decline in platelet production may be attributable to the administration of pharmaceutical agents, the synthesis of toxins resulting from an infection, and, in certain instances, to immunological activity involving the production of antibodies capable of binding to the surface of these platelets.

**Cortisol.** The cortisol results indicate that the T2:MO calves exhibited numerically lower concentrations ( $P > 0.05$ ) with the exception of the adaptation period ( $2.51 \pm 0.31$   $\mu\text{g}/\text{dL}$ ) and the overall test average ( $3.11 \pm 0.26$   $\mu\text{g}/\text{dL}$ ), as compared to the T1:control animals ( $3.15 \pm 0.31$  and  $3.54 \pm 0.26$   $\mu\text{g}/\text{dL}$ , Figure 11). However, these differences did not achieve statistical significance ( $P < 0.05$ ).

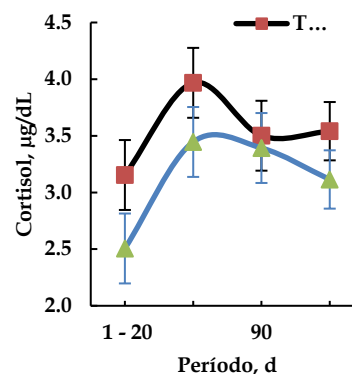


Fig. 11. Means ( $\pm$  E.E.) of cortisol in weaned calves fed a traditional diet (T1: control) and a diet that included trace minerals (T2:MO).

Dantzer et al. (2014) published that animals exposed to situations that cause fear, anxiety, or restlessness, and that alter their homeostasis, perceive these situations as a threat to their survival. In such individuals, stress is the adaptive physiological response that includes the activation of the hypothalamic-pituitary-adrenal axis and increased secretion of glucocorticoids (cortisol or corticosterone). This response is intended to address the threat to re-establishing homeostasis.

The present study's findings are consistent with those documented by Sena et al. (2018). The researchers' findings indicated a mean concentration of cortisol in the blood plasma of cattle of 4.02 and 4.24 µg/dL at 24 hours, as well as 4.0 and 4.87 µg/dL at 72 hours. The administration of cattle with elevated stress factors resulted in increased cortisol levels when compared to the professional handling of animals.

In other studies, it has been reported that intensive fattening of cattle favors the appearance of stressful situations to which animals have to adapt (Mobligia et al., 2014). Cattle that are fattened in pens are exposed to various factors, including transport, handling, changes in social structure, and reduced space or access to feed, water, and shade. These factors can trigger a stress response that affects animal welfare (Solano et al., 2004), reduces live weight gain and carcass performance (Nanni Costa et al., 2006), and alters the organoleptic characteristics of meat by shortening its shelf life (Amtmann et al., 2006).

In contrast, Campos-Granados (2015) reported that the supplementation of chromium during

periods of stress led to a reduction in cortisol concentration, an increase in antibody production, and proliferation of white blood cells, thereby strengthening the immune system. Furthermore, trace elements have been demonstrated to play a pivotal role in the immune system's response to potential pathogens (Palomares, 2022). These elements are also crucial for sustaining the health and productive efficiency of animals (Galyean et al., 1999). Smart et al. (1981) published that trace elements, although required in minimal amounts, are indispensable for numerous biological processes, including energy production, signal transduction, nucleic acid replication, transcription, and translation, as well as antioxidant protection against cell damage.

#### 4. CONCLUSIONS AND RECOMMENDATIONS

The findings of the present study demonstrate that the incorporation of organic minerals into the diet of weaned calves results in an increase in live weight, an enhancement in daily weight gain, and an improvement in feed conversion. Conversely, a favorable impact was noted on the concentration of blood cells and cortisol, which remained within the average range according to the established parameters for cattle. It is recommended that further research be conducted on the conditioning of weaned calves. This research should include an evaluation of different levels of inclusion of organic minerals in the diet, as well as enzymatic metabolites. In addition, further evidence of their effect on the blood parameters of the animals should be obtained.

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