Organic and inorganic supplementation of Cu and Se in the diets of Normande dairy cows

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Received: April 2020; Accepted: August 2020; Published: November 2020.

ABSTRACT

Objective. To evaluate the effects of organic and inorganic copper (Cu) and selenium (Se) on milk production, milk quality and reproductive parameters of Normande dairy cows. Materials and methods. 36 multiparous cows (BW = 600 ± 32 kg) were used, distributed in completely randomized blocks. Treatments were: Control: without mineral supplementation, IS: inorganic mineral supplementation with Cu and Se, IS+OS: 50% Inorganic supplementation + 50% organic supplementation, with an experimental period of 150 days (30 days before calving and 120 days of lactation). The variables evaluated were milk production and quality (total solids, protein, fat, somatic cells and MUN), blood metabolites (glucose, betahydroxybutyrate and BUN) and reproductive parameters (open days, days to first service and services per conception). Results. No differences were observed in milk production and quality (p>0.05). However, open days and services per conception were shorter when cows were supplemented with organic minerals (p<0.05). Conclusions. In this study milk production, milk quality and blood metabolites were not affected by supplementation with Se and Cu. However, the reproductive parameters of the cows improved when supplemented with organic Se and Cu.

Keywords: Microminerals; mineral mixture; ruminants; trace minerals (Source: CAB).
nitrógeno ureico en leche), metabólitos sanguíneos (glucosa, Betahidroxibutirato, nitrógeno ureico en sangre) y parámetros reproductivos (días abiertos, días al primer servicio y servicios por concepción).

**Resultados.** No se observaron diferencias en la producción y composición de la leche ($p>0.05$). Sin embargo, los días abiertos y los servicios por concepción fueron menores cuando las vacas fueron suplementadas con minerales orgánicos ($p<0.05$). **Conclusiones.** La suplementación con minerales orgánicos (Cu y se) no mejora la producción y composición de la leche. No obstante, si mejoró los parámetros reproductivos de las vacas suplementadas con Se y Cu orgánicos.

**Palabras clave:** Mezcla mineral; microminerales; minerales traza; rumiantes (*Fuente CAB*).

**INTRODUCTION**

Mineral deficiency and imbalance in the diet are of the main causes of low productivity in grazing cattle, since pasture-based feeding is insufficient to meet the nutritional requirements of minerals (1). However, in order to reach the mineral requirements in animals, inorganic sources have been used in the formulation of salts and supplements such as oxides and sulfates (2). None less, at the end of the 1980s, chelated organic minerals were developed as an alternative to traditional sources of minerals (2,3). The main advantages of the use of chelates is greater bioavailability, less toxicity and little antagonism with different sources of minerals, fibers or fats (4,5,6).

Changes in physiological status of dairy cattle during the transition period are the most critical and stressful time faced by a dairy cow during her lifetime (7,8). During the transition period, dairy cattle are more susceptible to a variety of metabolic and infectious diseases, due to multiple physiological and environmental factors that occur at this moment, this leads to the development of an oxidative stress process in the animals. Oxidative stress has an important role in tissue damage and leads to pathological conditions. Tissue defense mechanisms against free-radical damage generally include vitamins, and several metalloenzymes which include glutathione peroxidase (Se) and superoxide dismutase (Cu, Zn, Cr and Mn) are also critical in protecting the internal cellular constituents from oxidative damage. Only when these microminerals are offered in the diet in sufficient amounts, the animal body can synthesize these antioxidant enzymes (9,10).

In contrast, copper and selenium deficiency has been associated with a decrease in the reproductive parameters, as well as a decrease in milk production, lower daily weight gain and greater susceptibility to developing diseases (11,12). Due that, selenium and copper act as cofactors in numerous biochemical reactions (13). Selenium plays an important role in the regulation of various metabolic processes and is an integral part of selenoproteins (14). However, the physiological role of selenium is mainly concentrated in the activity of glutathione peroxidase (GSHPx), therefore, it has been suggested that selenium could improve immunity, growth, reproductive performance (15). On the other hand, copper is essential for erythropoiesis, transport and use of iron in the biosynthesis of hemoglobin and osteogenesys (13). It is also required as a cofactor in the enzyme superoxide dismutase ceruloplasmin and cytochrome C oxidase (16). However, the studies that evaluate the effect of organic Cu and Se supplementation in grazing conditions in the high tropics are scarce. Thus, the objective of this study was to evaluate the effects of organic copper (Cu) and selenium (Se) on production and milk quality and reproductive parameters of Normande dairy cows.

**MATERIALS AND METHODS**

**Local.** The experiment was carried out on the farm “Las Mercedes” in the rural area of Bogotá savanna; located at the geographical coordinates 4°-75’6644’ North Latitude and 74°-08’4010’ West Latitude, with an average temperature of 13.6ºC, a height of 2542 meters above sea level, annual precipitation of 1000 mm and relative humidity between 78–83%.

**Cows and treatments.** There were used thirty-six multiparous Normande cows averaging (3.2±1.5 births) and 600 ± 32 kg body weight, in a design of completely randomized block. Treatments were: Control: without supplementation of Cu and Se, IS: Inorganic supplementation (Copper Sulfate and Sodium Selenite) IS+OS: 50% Inorganic supplementation and 50% organic supplementation (Copper B-traxim® and Seleno-methionine Selemax®), cows were assigned to each block according to the probable date of birth.
Cows were kept in a rotational grazing system of *Pennisetum clandestinum* and *Lolium multiflorum* with formulated concentrate supplementation to fulfill nutritional requirements according to NRC, (17). 30 days before calving the cows received 1 kg of concentrate and 70 gr of the mineral supplement. After delivery and until 120 days of lactation the cows received 4 kg of concentrate and 150 gr of mineral supplement. The composition of diets, ingredients and supplements are shown in Table 1.

**Table 1.** Analyzed nutrient composition of diets and ingredients used in the experiment (DM basis).

| Item       | Forage Concentrated | Treatment Control | IS | IS+OS |
|------------|---------------------|-------------------|----|-------|
| DM %       | 20.30               | 89.77             | -  | -     |
| OM %       | 90.01               | 81.71             | -  | -     |
| CP %       | 16.13               | 14.50             | -  | -     |
| NDF %      | 58.21               | 25.43             | -  | -     |
| ADF %      | 23.34               | 14.80             | -  | -     |
| Ashes %    | 10.10               | 8.06              | -  | -     |
| EE %       | 2.10                | 5.38              | -  | -     |
| Ca %       | 0.24                | 1.92              | 7.98| 7.98  |
| P %        | 0.28                | 0.72              | 2.28| 2.28  |
| Mg %       | 0.11                | 0.37              | 0.27| 0.27  |
| K %        | 1.37                | 1.06              | 0.24| 0.24  |
| S %        | 0.42                | 0.22              | 12.00| 12.00 |
| Fe mg/Kg   | 236.00              | 20.00             | 2.00| 2.00  |
| Zn mg/kg   | 37.32               | 41.10             | 45.22| 45.22 |
| Mn mg/kg   | 43.08               | 76.80             | 21.35| 21.35 |
| Cu mg/kg   | 13.54               | 7.16              | 325.00| 325.00|
| Se mg/kg   | 0.30                | 0.21              | 32.00| 32.00 |

Control: without Supplementation of Cu and Se, IS: Inorganic Supplementation, IS+OS: 50% Inorganic supplementation 50% organic supplementation

Samples of concentrated and forage were pre-dried for 72 hours in an air circulation stove at 55°C and ground in Willey type mills (1 mm) and were analyzed in the animal nutrition laboratory of the University of Tolima. Dry matter (DM), crude protein (CP), ether extract (EE), ashes were determined using the methods of (18) NDF and ADF according to (19).

**Milk production and quality.** Production data were collected during 120 days after calving of each cow (Kg / day) in the two milking daily periods (5:00 a.m. - 2:00 p.m.) was taken into account. Milk samples were taken at 30, 60 and 120 days in the morning milking, 40 mL of milk were collected per cow in plastic bottles with preservative (Proponol), and were identified, then stored at 4°C and sent to the milk quality laboratory of the University of Antioquia, where it was determined: total solids, protein, fat, somatic cells count (SCC) and urea nitrogen in milk (MUN).

**Blood Metabolites.** Blood samples were collected 15 days before calving and 30, 60, 90 and 120 days after calving, blood samples were obtained by puncturing the jugular vein and the blood were transport in vacutainer® tubes without anticoagulant. Then samples were centrifuged for 10 minutes at 3500 rpm for serum separation, subsequently packed in 1 ml eppendorf tubes, which were stored at -20°C and then sent to the veterinary diagnostic laboratory of the University of Tolima (LADIVE), for the determination of glucose, beta-hydroxybutyrate and blood urea nitrogen (BUN) using commercial kits and a Biosystems® A15 automatic system.

**Mineral excretion.** Samples of feces were collected per animal (500 g) every 15 days directly from the rectum twice a day, in the morning and in the afternoon after feeding; the samples were duly stored in bags, identified with the animal ID number, treatment, and stored in the freezer at -20°C; They were then sent to the LASEREX Laboratory of the University of Tolima, where the minerals Ca, Mg, K, Mn, Zn, Cu and Se were determined by atomic absorption spectrophotometry (24, Method. 968.08) and P by Ultraviolet spectrophotometry visible (24, Method. 965.17).

**Reproductive Parameters.** There were calculated: Days at first service: from calving to the presentation of the first fertile heat; Open days: Days from calving to the day of pregnancy (confirmed); Services per conception: the number of inseminations made from the calving day to the confirmed pregnancy and Pregnancy rate: Taking into account the number of pregnant cows in relation to the total number of animals per treatment.

**Statistical analysis.** A completely randomized block design was performed; the data was analyzed using the SAS 9.2 MIXED procedure. Shapiro Wilk test were used to evaluate normality. An ANOVA analysis of variance and a Tukey mean comparison test were performed, with a 95% level of significance. The model used in the experiment was as follows:

\[ Y_{ijk} = U + T_i + B_j + E_{ijk} \]

Where: \( Y_{ijk} \): Dependent variable; \( U \): Medium, \( T_i \): Fixed effect of the treatment (i = 0, 1, 2), \( B_j \): Fixed effect of the Block, \( E_{ijk} \): Residual error.
RESULTS

Milk production and quality. Average milk production during the experiment was 23.52; 25.25 and 23.46 for Control, IS and IS+OS, respectively. Milk production was not affected by treatment (p>0.05). Similarly, there were not differences in total solids, fat, protein, and lactose in milk (Table 2).

Table 2. Milk production and quality in Normande dairy cows supplemented with Cu and Se organic and inorganic.

| Variable       | Treatment | Control | IS | IS+OS | SE | p-value |
|----------------|-----------|---------|----|-------|----|---------|
| Milk production Kg/day |           | 23.52   | 25.25 | 23.46 | 0.95 | 0.3290  |
| Milk Protein %   |           | 3.03    | 3.30 | 2.96  | 0.12 | 0.1670  |
| Milk Fat %       |           | 3.82    | 3.70 | 3.80  | 0.50 | 0.9830  |
| Total Solids %   |           | 12.79   | 13.06 | 11.14 | 0.74 | 0.1660  |
| Lactose%         |           | 4.92    | 5.17 | 4.66  | 0.18 | 0.1570  |
| MUN mg/dl       |           | 14.45   | 15.60 | 13.38 | 0.85 | 0.2330  |
| SCC (x10⁹/ml)    |           | 96.73   | 81.71 | 51.94   | 15.01 | <0.0010 |
| Acetone          |           | 0.21    | 0.24 | 0.22  | 0.02 | 0.4080  |
| BHB              |           | 0.03    | 0.04 | 0.03  | 0.01 | 0.5240  |

Control: without Supplementation of Cu and Se, IS: Inorganic Supplementation, IS+OS: 50% Inorganic supplementation 50% organic supplementation, MUN: milk urea nitrogen, SCC: somatic cell count, BHB: Beta-hydroxybutyrate SE: standard error of the mean.

Blood Metabolites. The plasma levels of BUN, glucose, and BHB were also not affected by supplementation or the source of minerals (p>0.05) (Table 3). However, there were differences (p<0.05) for Cu in plasma, between IS (inorganic) and IS+OS (organic) treatment 0.79 and 0.72 mg/L respectively. Se plasma there was similar between treatments (table 3).

Table 3. Blood metabolites in Normande dairy cows supplemented with organic and inorganic minerals.

| Variable       | Treatment | Control | IS | IS+OS | SE | p-value |
|----------------|-----------|---------|----|-------|----|---------|
| BUN mg/dl      |           | 15.08   | 14.52 | 13.32 | 0.80 | 0.2974  |
| Glucose mg/dl  |           | 67.06   | 72.74 | 65.80 | 4.19 | 0.4675  |
| BHB mmol/L     |           | 0.45    | 0.38  | 0.40  | 0.04 | 0.5491  |
| Copper mg/L    |           | 0.76a   | 0.79a | 0.72a | 0.02 | 0.0290  |
| Selenium mg/L  |           | 0.030   | 0.033 | 0.025 | 0.059| 0.6501  |

Control: without Supplementation of Cu and Se, IS: Inorganic Supplementation, IS+OS: 50% Inorganic supplementation 50% organic supplementation, BUN: Blood urea nitrogen, BHB: Beta-hydroxybutyrate SE: Standard error of the mean.

Minerals excretion. In the measurements made to Calcium (Ca), Magnesium (Mg), Manganese (Mn), Selenium (Se), Phosphorus (P), Potassium (K) and Zinc (Zn) were also not affected by supplementation or the source minerals (p>0.05) (Table 4). However, there were differences (p<0.05) for Cu in feces, excretion was lower in IS+OS (organic) treatment 22.52 mg/Kg.

Table 4. Minerals excretion in Normande dairy cows supplemented with organic and Inorganic minerals.

| Variable       | Treatment | Control | IS | IS+OS | SE | p-value |
|----------------|-----------|---------|----|-------|----|---------|
| Ca (%)         |           | 1.32    | 1.21 | 1.45  | 0.10 | 0.1385  |
| P (%)          |           | 0.90    | 0.92 | 0.97  | 0.05 | 0.6892  |
| Mg (%)         |           | 0.43    | 0.40 | 0.55  | 0.14 | 0.6321  |
| K (%)          |           | 0.60    | 0.54 | 0.66  | 0.17 | 0.5469  |
| Mn (mg/kg)     |           | 232.63  | 211.01 | 198.67 | 17.13 | 0.3380  |
| Zn (mg/kg)     |           | 117.05  | 127.99 | 106.97 | 12.59 | 0.5921  |
| Se (mg/kg)     |           | 509.21  | 547.89 | 605.33 | 76.05 | 0.2227  |
| Cu (mg/kg)     |           | 35.91ab | 30.68ab | 22.52ab | 3.73 | 0.0001  |

Control: without Supplementation of Cu and Se, IS: Inorganic Supplementation, IS+OS: 50% Inorganic supplementation 50% organic supplementation. SE: standard error of the mean. Values with different letters within a line differ significantly (p<0.05).

Reproductive Parameters. Days at the first service decreased in the cows of IS+OS, from 61.18 and 62.94 days in groups Control and IS respectively to 54.58 days (p<0.05), however the open days and services per conception did not show differences between the different treatments (p>0.05), although there was a tendency with respect to IS+OS (organic) (Table 5).

Table 5. Reproductive Parameters in Normande dairy cows supplemented with organic and inorganic minerals.

| Variable       | Treatment | Control | IS | IS+OS | SE | p-value |
|----------------|-----------|---------|----|-------|----|---------|
| % of pregnancy |           | 41.70   | 58.30 | 58.30 | - | -       |
| Days at first service |       | 61.18ab | 62.94ab | 54.58ab | 1.73 | 0.0040  |
| Open days      |           | 72.56   | 83.38 | 74.00 | 4.72 | 0.2390  |
| Services / Conception |       | 2.20    | 2.43  | 1.57  | 0.26 | 0.0740  |

Control: without Supplementation of Cu and Se, IS: Inorganic Supplementation, IS+OS: 50% Inorganic supplementation 50% organic supplementation. SE: standard error of the mean. Values with different letters within a line differ significantly (p<0.05.).
DISCUSSION

In this study, milk production and quality were not affected with the supplementation of Cu and Se, in this sense, Bacnicka et al (20) found that when supplementing Holstein dairy cows with sodium selenium (5.88- 7.43 mg / cow / day) and Selenium-yeast (6 mg / cow / day) between 150 and 240 days of lactation, no differences were found in milk production and quality. Regarding the appropriate concentration in the diet Sun et al (21) evaluated the effect of different doses of sodium selenite and hydroxy-Selenomethionine (0; 0.1; 0.3 and 0.5 mg/kg DM), observed that neither the level of supplementation nor the source of Se were not affected of milk production and quality, except percentage of fat, that increased linearly as the level of organic Se supplementation increased. This is consistent with was reported by many authors who found that there were no differences in milk production and quality when using organic or inorganic sources (22,23,24) or a total mixed ration (TMR). However, several authors have reported an increase in milk production by supplementing with organic Se in goats (20,25).

The somatic cell count (SCC) was affected by supplementation. Phipps et al (26), reported that supplementation with Se increases the concentration of GSH-Px, contributing to reduce the incidence of mastitis (lower number SCC). Regarding Copper, Yamamoto et al (27), concluded that supplementation with 125 mg of Copper daily, contributed to increase milk production by 5% compared to treatment without supplementation, however no effect was found on SCC versus the Control group, which differs from those reported by Griffiths et al (28), who observed that dietary supplementation with a combination of organic trace minerals increased milk yield, but did not affected the composition of the same.

Blood metabolites measured in this study (BUN, Glucose and BHB) were not affected by supplementation with Cu and Se (organic and inorganic). The values of biochemical indicators obtained in this study are within normal reference ranges (20). However, Juniper et al (29) and Phipps et al (26), indicate that equal amounts of Se in organic or inorganic form do not cause differences in chemical composition of blood vs a non-supplemented cow; likewise, Yamamoto et al (27) report that the dietary combination of copper, zinc, manganese and cobalt does not affect blood parameters of BUN and Glucose in Holstein cows in lactation. Similarly, in other studies they report that supplementation of organic trace minerals or the replacement of inorganic form of trace minerals with an organic form in the diet does not affect biochemical parameters of plasma (30,31).

Although there were differences between the amount of copper from organic and inorganic sources, it is found that these are within the established reference values for lactating animals, in the same way NRC (17) describes that copper has an absorption that ranges between 1 and 5% of dietary copper and that inorganic sources such as copper sulfate which was used in this experiment as an inorganic source have a high bioavailability.

In another study conducted by Cetz et al (32) reported the decrease of minerals in feces with the use of chelated minerals in the supplementation of heifers, since these present a better use or absorption in the body of ruminants.

Organic Se and Cu supplementation in this study showed differences in the reduction of days at first service compared to inorganic Se and Cu treatment and Control group. Griffiths et al (28), reported that increased copper intake in dairy cattle through supplementation of complex trace mineral sources increases fertility and decreases the days at first serviced compared to Control cows. On the other hand, Campbell et al (30) observed that supplementing the cows with Zn, Mn, Cu and Co in the form of Co glucoheptonate and specific amino acid complexes of Zn, Mn and Cu, from birth, reduced the days to first estrus and tended to reduce the days to the first luteal activity. Although there are no significant differences in the open days in the present study, if a numerical difference is found, with fewer days being open in the treatment of organic and inorganic Se and Cu, correlated with that reported by Uchida et al (33), who, when replacing inorganic sources of trace minerals with organic ones, resulted in the reduction of open days, days at first service and fewer services per conception; This was because both copper and selenium are closely related to reproduction through their physiological and metabolic mechanisms of action (34).

Copper and selenium are involved in the synthesis of prostaglandins, including prostaglandin F2 alpha, which is the one in the corpus luteum to perform luteolysis and restart the estrus cycle, thus avoiding delay or cancellation of
jealousy (35). Also, copper is reported to work together with a large number of proteins such as ceruloplasmin, which is essential for the absorption and transport of iron, just as this is necessary for the synthesis of hemoglobin and superoxide dismutase, the latter responsible for protecting cells from the toxic effects of reactive oxygen metabolites; by decreasing this activity, the ability of cells to kill and phagocyte the organisms decreases, which favors the presence of infectious problems and this in turn is reflected in fetus abortions or placental retention (36).

In addition to this, selenium is also related to placental retention, endometritis and low conception. According to Todorović et al. (37) and Balamurugan et al. (36), this mineral is a fundamental part of the enzyme Glutathione Peroxidase, which, like, superoxide dismutase, is responsible for protecting the cells from over-oxidation produced by peroxides. The muscle cells are highly oxidative due to the constant activity to which they are subject, therefore Selenium is important to strengthen them, among them the myometrium, favoring postpartum uterine involution. Similarly, the decrease in glutathione peroxidase activity can lead to immunological problems, favoring the presence of infections (38,39,40).

Finally, the tendency to decrease those by conception with the use of organic Cu and Se, being more marked with the use of organic minerals, without having a significant statistical result, the economic difference in the value of a day is recognizable opened in the specialized dairy, as well as the value of the insemination service.

In conclusion, Supplementation with organic minerals (Cu and Se) does not improve milk production and composition. However, the reproductive parameters of the cows improved when supplemented with organic Se and Cu. Therefore, it is necessary to carry out more studies that help to understand the exact mechanism by which these trace minerals act positively on reproduction.

Interest conflict

The authors have no conflicts of interest.

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