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Effects of copper, zinc, selenium and manganese parenteral supplementation on reproductive performance of Holstein cows

Efectos de la suplementación parenteral de cobre, zinc, selenio y manganeso sobre el desempeño reproductivo de las vacas Holstein

Efeitos da suplementação parenteral de cobre, zinco, selênio e manganês no desempenho reprodutivo de vacas Holstein

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ABSTRACT

The objective of this investigation was to study the supplementation of injectable trace minerals (ITM), based on Cu, Zn, Mn, and Se on reproductive performance of dairy cows from Torreon, Mexico, a geographical region characterized by a semi-desertic climate. The study consisted of 2 field trials. Trial 1 compared the application of 2 doses of ITM during the dry period (at dry-off and at prepartum) (n= 75) with a control group (n=79). Trial 2 compared the application of 1 dose of ITM at 35 d postpartum (n=258) with a control group (n=258). In both trials, conception rate at first service (CRFS) and days to conception were evaluated. In Trial 1, CRFS was not different between groups, but calving to conception interval tended to be shorter in 10 days in the treated than the control group (P=0.14). In trial 2, CRFS was not different between groups either, but time to pregnancy was significantly 14 days earlier than the control group (P ≤ 0.05). It is

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concluded that although CRFS was similar between groups, the application of an ITM reduced the time to pregnancy in treated than the control groups, which have a great impact on herd's fertility and profit.

Keywords: Copper; Zinc; Selenium; Manganese; Fertility; Days Open; Dairy Cattle

RESUMEN

El objetivo de esta investigación fue estudiar la suplementación de oligoelementos inyectables (ITM), con base en Cu, Zn, Mn y Se sobre el desempeño reproductivo de vacas lecheras de Torreón, México, una región geográfica caracterizada por un clima semidesértico. El estudio consistió en 2 ensayos de campo. El ensayo 1 comparó la aplicación de 2 dosis de ITM durante el período seco (al secarse y antes del parto) (n = 75) con un grupo de control (n = 79). El ensayo 2 comparó la aplicación de 1 dosis de ITM a los 35 días después del parto (n = 258) con un grupo de control (n = 258). En ambos ensayos, se evaluaron la tasa de concepción en el primer servicio (CRFS) y los días hasta la concepción. En el Ensayo 1, el CRFS no fue diferente entre los grupos, pero el intervalo entre el parto y la concepción tendió a ser más corto en 10 días en el grupo tratado que en el grupo de control (P = 0,14). En el ensayo 2, la CRFS tampoco fue diferente entre los grupos, pero el tiempo a la preñez fue significativamente 14 días antes que en el grupo control (P ≤ 0,05). Se concluye que aunque la CRFS fue similar entre los grupos, la aplicación de un ITM redujo el tiempo a la gestación en los grupos tratados que en los de control, lo que tiene un gran impacto en la fertilidad y las ganancias del hato.

Palabras clave: Cobre; Zinc; Selenio; Manganeso; Fertilidad; Días Abiertos; Vacas Lecheras

RESUMO

O objetivo desta pesquisa foi estudar a suplementação de oligoelementos injetáveis (ITM), à base de Cu, Zn, Mn e Se no desempenho reprodutivo de vacas leiteiras de Torreón, México, uma região geográfica caracterizada por um semidesértico. clima. O estudo consistiu em 2 ensaios de campo. O ensaio 1 comparou a aplicação de 2 doses de MIT durante o período de seca (na secagem e antes do parto) (n = 75) com um grupo controle (n = 79). O ensaio 2 comparou a aplicação de 1 dose de ITM 35 dias após o parto (n = 258) com um grupo controle

(n = 258). Em ambos os ensaios, a taxa de concepção do primeiro serviço (CRFS) e os dias para a concepção foram avaliados. No ensaio 1, o CRFS não foi diferente entre os grupos, mas o intervalo entre o parto e a concepção tendeu a ser menor em 10 dias no grupo tratado do que no grupo controle (P = 0,14). No ensaio 2, o CRFS também não foi diferente entre os grupos, mas o tempo até a gravidez foi significativamente 14 dias antes do grupo controle (P ≤ 0,05). Conclui-se que embora o CRFS tenha sido semelhante entre os grupos, a aplicação de um MIT reduziu o tempo a gestação nos grupos tratados do que nos grupos controle, o que tem grande impacto na fertilidade e no ganho de rebanho.

Palavras-chave: Cobre; Zinco; Selênio; Manganês; Fertilidade; Dias Abertos; Gado Leiteiro

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Introduction

Milk production, health status and fertility of dairy cows are strongly related each other, significantly accounting for of the system's profitability. Moreover, these three variables are the expression of an adequate cow-comfort, a consistent nutritional program, a high genetic merit of the animals, adequate herd health protocols and an efficient human resources team. [1]

One of the most critical times in the productive cycle of the dairy cow is the transition period (21 d before parturition to 21 d postpartum). Most diseases occur at that moment, primarily due to the lower dry matter intake and the typical immunosuppression that cows experience. [2] During the transition period, cows undergo a negative energy and protein balance, which must be managed properly. In addition, the characteristic immunosuppression taking place during this time should be attenuated, and the severity of hypocalcemia diminished to its minimum expression. If body condition score (BCS) at calving and its loss during the first month of lactation can be targeted efficiently, the cow will be healthier and will produce more milk. In fact, cows that lose BCS during prepartum and cows that lose excessive BCS during the postpartum period will have a higher incidence of periparturient diseases. [1, 2] Furthermore, cows with one or more disease events during the postpartum period will be affected by poor fertility and lower milk yield. [3]

The use of additives in diets fed during the transition period may ameliorate the nutritional burden and metabolic imbalances of transition dairy cows, including certain microminerals that are essential for life, since they are constituents of several enzymes, hormones and important proteins. [1, 4]

The high metabolic demands associated with milk production induce oxidative stress, which is an imbalance between the levels of oxidation-derived free radicals (known as reactive oxygen species, ROS) and antioxidant molecules. [5] Trace minerals such as selenium, copper, zinc and manganese play a major role in the antioxidant systems, as they are part of the structure and function of enzymes necessary to reduce the high levels of ROS during oxidative stress in dairy cows. For instance, Cu, Zn and Mn act as cofactors for the enzyme superoxide dismutase, which catalyzes the chemical transformation of superoxide anions into hydrogen peroxide. Then, the enzyme glutathione peroxidase a metallo-protein containing selenium, metabolizes the reduction of hydrogen peroxide into hydroxyl radicals and H_2O. Copper interacts at the mitochondrial level with enzymes such as cytochrome oxidase, which is essential for energy production. In addition, zinc plays important role in more than 2,500 enzymatic reactions. [4, 5] Oocyte zinc reserves and release (known as zinc sparks 2 hours after fertilization) is associated with the oocyte ability to undergo fertilization and further embryo development. Oocytes store, compartmentalize, and then release zinc to control maturation, fertilization, and the development of a healthy embryo. Zinc is highly associated with physiologic process that involve active cell replication such as mitosis during embryo growth and development [6] In addition, the supplementation with injectable trace minerals (ITM) has improved leukocyte function, antioxidant response, [7, 8] and total pregnancy rate [9] in dairy cows.

Accordingly, the hypothesis of this investigation was that the cows receiving ITM supplementation have greater fertility during lactation. Consequently, the objectives of this research were (i) to compare the effect of a subcutaneous ITM supplement containing Se, Cu, Zn and Mn applied at dry-off and at prepartum versus a selenium/vitamin supplement on reproductive performance of Holstein cows (Trial 1) and (ii)to evaluate the effect of a single dose of ITM at 35 days postpartum versus a non-treated control group on reproductive performance of Holstein cows (Trial 2).

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Vol. 23, Nº 2, Sección: Artículos de investigación , pp. 101-118

Materials and methods

2.1 ETHICAL STATEMENT

This investigation consisted of 2 field trials conducted in several farms from Torreon city, Coahuila, Mexico; therefore, the parenteral application of microminerals was part of the common routine of these dairies.

2.2 FARMS AND LOCATION

The research was conducted under commercial settings of dairy farms from Torreon, Coahuila, Mexico. The investigation consisted of two field trials considering the application of a commercial micromineral mix composed of selenium (5 mg/mL), copper (15 mg/L), manganese (10 mg/mL) and zinc (60 mg/mL), for intramuscular injections (Multimin 90, Multimin USA, Inc., Fort Collins, CO). Trial 1 consisted of the application of 2 doses of the ITM during the dry period, and Trial 2 evaluated a single dose of a subcutaneous ITM during the postpartum period.

Farms that participated in this investigation belong to a dairy Mexican basin with 185,000 milking cows, called "Comarca Lagunera", located around Torreón city, capital of the state of Coahuila, Mexico. This dairy zone has an altitude of 1,100 meters above sea level, with geographical coordinates of 25 ° 31 ' N, 103 ° 25´ W. The predominant topography is flat, the climate is dry (desertic) with annual relative humidity of 50%, an average annual rainfall of 240 mm and an average annual temperature of 22 ° C (range: 14.7 ° C in January, 28.2 ° C in June) [10].

This Mexican macrozone is characterized by having large dairy systems with average herd size of 2,000 lactating cows. Major breed is Holstein Friesian with an average mature equivalent 305 days milk yield of 10,000 kg. Most dairies milk the cows twice a day and feed total mixed rations based on corn silage, alfalfa hay, and concentrates (corn grain, soybean meal, cotton seed, cotton meal, DDGS, canola meal, mineral, vitamins and additives). Most herds are housed in dry-lot systems with roof and fans at the feed-bunk area.

Reproductive management primarily consists of ovulation synchronization protocols and timed artificial insemination combined with heat detection protocols. Pregnancy diagnosis is carried out by ultrasonography between 28 to 35 days post service. Non-pregnant cows are resynchronized immediately, and pregnant cows are reconfirmed

for gestation between 60 to 70 days post service. Pregnant cows are reconfirmed for gestation between 50 and 75 days before expected parturition (BEP). If the cow remains pregnant, she is dried-off and handled in a dry cow group until 28 to 35 days BEP where the cow is moved to a prepartum group and fed a total mixed ration with anionic products to prevent hypocalcemia. Cows are consistently monitored for signs of parturition. They deliver at the same prepartum lot and calving assistance is carried out as needed. After birth, the calf is immediately separated from the dam and the cow is milked and assessed for reproductive tract lacerations, intrapelvic trauma, peripheral nerve inflammations and clinical hypocalcemia (downer cow syndrome) within the first 6 hours postpartum. Cows are treated according to veterinary standard operating procedures. After that, cows are subjected to a postpartum health monitoring program until 10 to 15 days post calving. Cows are evaluated for retained fetal membranes, puerperal metritis, mastitis, ketone bodies and left displacement of the abomasum. Body condition score (BCS) is assessed at dry-off, at prepartum, and at parturition, using a scale 1 to 5 in a ¼ units of increment [11].

Th herd health program was based on vaccinations and biosecurity measures to prevent common diseases that affect dairy herds at the Comarca Lagunera in Torreon, Mexico, including brucellosis, leptospirosis, IBR, BVDV, and clostridial diseases. Additionally, it also included a mastitis preventive program based on cleaning, disinfection and adequate milking procedures.

2.3 STUDY DESIGN

2.3.1 TRIAL 1

The study 1 was conducted at the dairy "Mapulas", consisting of 3,800 lactating cows, milked three times a day, average milk yield per cow of 38.2 kg/day, fed a TMR, and housed in a dry lot system.

The trial consisted of comparing one group that enrolled randomly 75 cows (32 primiparous, 43 multiparous), receiving 2 doses of ITM during the dry period. First dose of ITM (6 mL, subcutaneous, 1 mL per 100 kg of body weight) was injected at dry off and the second dose (6 mL, intramuscularly) when cows were moved to the prepartum lot. The other group enrolled 79 cows (33 primiparous, 46 multiparous) and treated with a commercial product based on vitamin E and selenium, providing sodium selenite 10.95 mg/ml and tocopherol 50 mg/m, injecting 10 mL subcutaneously (Se-Ve®, LAPISA, S.A., La Piedad, Guadalajara, Mexico).

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Vol. 23, Nº 2, Sección: Artículos de investigación , pp. 101-118

Both groups were compared for BCS at calving, conception rate at first service (CRFS), and calving to conception interval.

2.3.2 TRIAL 2

The second study was carried out at 3 dairy farms (La Gloria, El Clavel, La Luna) from the "Comarca Lagunera". The three dairies belonged to a large dairy corporation, utilizing the same feeding and reproductive management.

"La Gloria" dairy consisted of 3,200 lactating cows, milked three times a day, average milk yield per cow of 35.1 kg/day, fed z TMR, and housed in a dry lot system. "El Clavel" dairy consisted of 3,300 lactating cows, milked three times a day, average milk yield per cow of 37.8 kg/day, fed z TMR, and housed in a dry lot system, and "La Luna" dairy consisted of 11,000 lactating cows, milked three times a day, average milk yield per cow of 37.7 kg/day, fed a TMR, and also housed in a dry lot system. After parturition, cows were subjected to a synchronization of ovulation timed artificial insemination protocol (Presynch-Ovsynch) [12, 13].

This trial compared two experimental groups. Treatment group enrolled at random 258 Holstein cows (99 primiparous, 159 multiparous), receiving 1 dose of ITM (7 mL intramuscularly) between 35 and 38 days in milk (DIM). Control group (without treatment) randomly assigned 258 cows (98 primiparous, 160 multiparous). Both groups were compared for CRFS, and time to pregnancy. Treated and control cows were housed and handled homogeneously in the same lactating group.

2.3.3 STATISTICAL ANALYSIS

Body condition score at calving (trial 1) was analyzed by using the non-parametric method Kruskal-Wallis test. Conception rate at first service (trial 1 and 2) was analyzed by a logistic regression model, considering the group effect as main effect, and parity and farm effect as covariates (trial 2). Calving to conception interval (trial 1) was compared by a one-way ANOVA. Days to pregnancy (trial 2) was assessed by survival analysis, constructing Kaplan -Meier Survival Curves. Statistical analysis was conducted using the corresponding computer procedure of SAS for Windows 9.4 [14].

Results

3.1. TRIAL 1

In Table 1, descriptive statistics (median) and P-value (Kruskal-Wallis test) for BCS at dry-off, at prepartum, and at calving for ITM and Se-Ve groups are shown. There was not statistical differences between groups for BCS at dry-off, meaning an adequate randomization process for experimental animal assingment. Body condition score at prepartum and at calving did not differed statistically between experimental groups either. In Figure 1, CRFS (%) in cows treated with ITM during the dry period and controls is shown. In Figure 2, calving to conception interval (days) in cows treated with ITM during the dry period and controls is shown.

3.2. TRIAL 2

In Figure 3, CRFS (%) in cows treated with ITM during the postpartum period and controls is shown. In Figure 4, Time to Pregnancy Survival Curves (Kaplan-Meier statistics) in cows treated with ITM during the postpartum period and controls are illustrated.

3.3 FIGURES, TABLES AND SCHEMES

Table 1: Median (range) and Kruskal-Wallis test P-values results for Body Condition Score (BCS) at dry-off, prepartum and calving in Holstein cows (Trial 1)

Item	ITM	Se-Ve	P-value
BCS at dry-off	3.50 (2.75-4.75) (n=71)	3.25 (3.0-4.0) (n=73)	0.51
BCS at prepartum	3.50 (2.75-4.25) (n=71)	3.50 (3.0-4.5) (n=73)	0.84
BCS at calving	3.50 (1.50-4.50) (n=71)	3.50 (3.0-4.0) (n=73)	0.74

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Vol. 23, Nº 2, Sección: Artículos de investigación , pp. 101-118

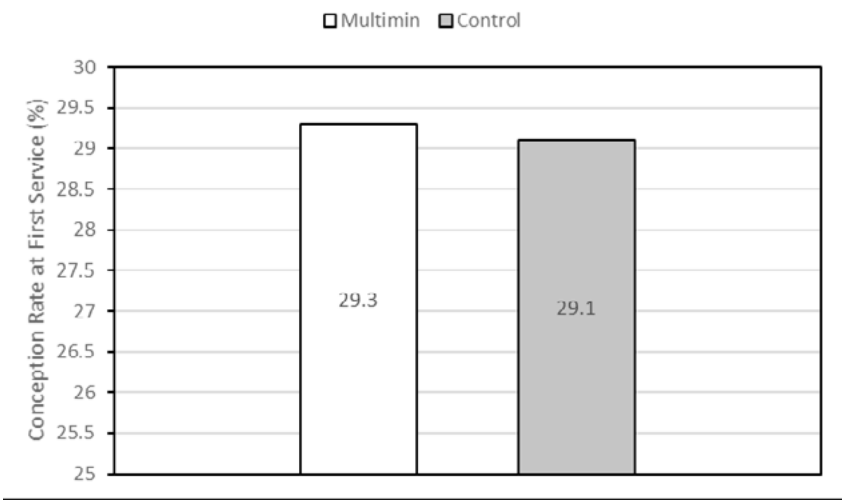

Figure 1: Conception Rate at First Service (%) in cows treated with ITM during the dry period and controls (Trial 1). No statistical differences (P =0.95)

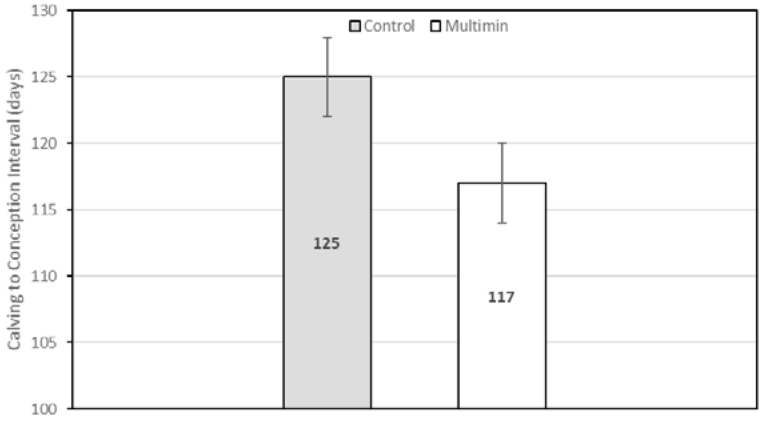

Figure 2: Calving to Conception Interval (days) in cows treated with ITM during the dry period and Controls (Trial 1). Statistical tendency (P=0.14).

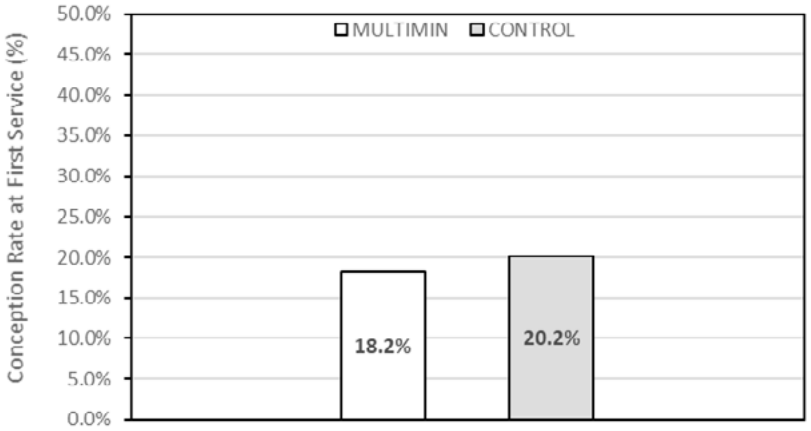

Figure 3: Conception Rate at First Service (%) in cows treated with ITM during the postpartum period and controls (Trial 2). No statistical differences (P =0.75)

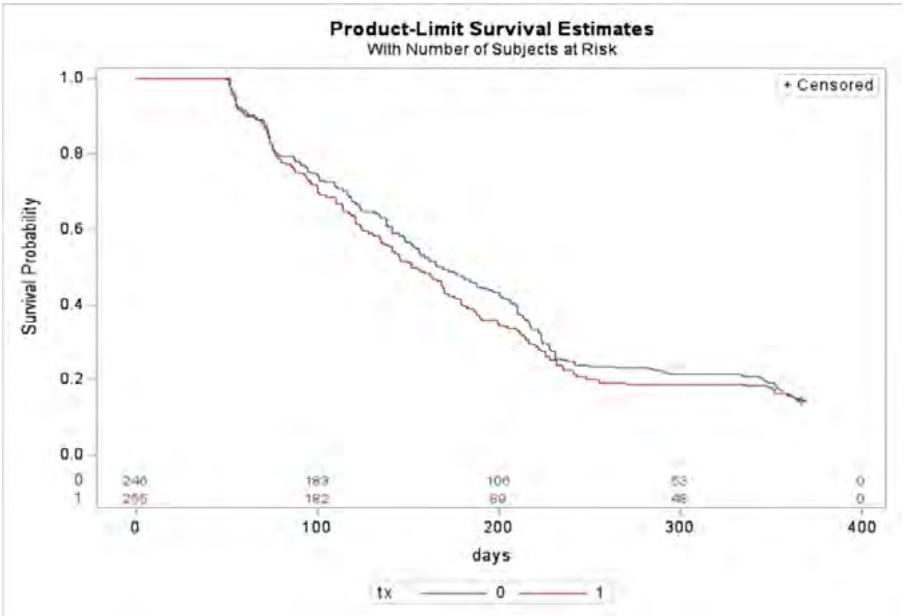

Figure 4: Time to Pregnancy Survival Curves (Kaplan-Meier statistics) in cows treated with ITM during the postpartum period and Controls (Trial 2). Fifty percent of treated animals became pregnant at 152 days (CI 95% = 136-170 days) (red line) and 50% of control animals became pregnant at 167,5 days (CI 95% = 151-194 days) (line blue) (P < 0.05).

Discussion

This research comprises 2 field trials conducted under commercial conditions in Mexican herds from one of the most important dairy regions of the country (Torreon, Coahuila). The strength of these field trials is that they were carried out under 'real-farming' conditions, with natural development of the outcome of interest, in this case "fertility responses". However, one of the major concerns of these type of studies is the randomized allocation of the animals. Proper randomization and an adequate sample size help to prevent systematic bias, avoiding the negative impact of confounding variables. In both trials, the selection of animals was at random, and after the treatment application both groups (control and experimental) were housed in the same group, handled homogeneously, without more interventions and reproductive variables were recorded consistently and blind to the investigators.

TRIAL 1

In this trial, the effects of two injectable doses of micro minerals during the dry period (at dry-off and at prepartum) on reproductive performance was evaluated. In this case, an experimental commercial product was compared with another supplement based on vit E/Se, which was the standard routine for this dairy. Consequently, it was no feasible to have a control group without supplementation. BCS at calving was within expected values (median=3.5) and similar between both groups. The application of ITM during the dry period did barely modified BCS, with similar results for both groups. If dry cows are properly fed, it is unlikely that a supplementation with injectable minerals and/or vitamins can change subcutaneous fat deposition. On the other hand, BCS is a subjective assessment of energy nutrition, because adipose tissue accretion not only occur at the subcutaneous level, but also inside of abdominal cavity and omental tissue, with genetic differences in their distribution [15]. In other words, BCS may be similar between two animals, but the amount of deposited abdominal fat may considerably fluctuate. Unfortunately, visual assessment of abdominal fat accumulation is not feasible, which would either require a blood biomarker evaluation or an invasive surgical technique to approach the abdominal cavity.

Regarding reproductive responses, both groups were supplemented with minerals, and CRFS was similar between them, but calving to conception interval was 8 days shorter (117 days) in the ITM group

than the control group (125 days) (P=0.14). This slight improvement might be explained due to the control group received only vitamin E and selenium unlike the treatment group receiving four microminerals, also including selenium, which may boost immunity and fertility better than a single dose of vitamin E and selenium. A study, that also injected 2 doses of the same mineral supplement [7] during the dry period (dry-off and prepartum), found that treated cows had significantly less stillbirth and endometritis, but similar conception rate and calving to conception interval than control cows. Unfortunately, based on the nature of the data set of our trial, health events were not accurate enough to be recorded and analyzed. Although in our study groups were balanced for parity number and BCS at calving, occurrence of diseases could be a major confounding. Perhaps treated group experienced less diseases than the control group and indirectly tended to become pregnant earlier.

TRIAL 2

The four microminerals of the supplement used in the present investigation are involved in several cell processes that affect the normal function of tissues, organs, and systems, improving immunity and fertility of animals. As previously reviewed by Goff [4], briefly, selenium form part of glutathione molecule, a powerful antioxidant system in many cells, which has been demonstrated to reduce the incidence of mastitis and retained fetal membranes in dairy cows. Copper is a structural component of the superoxide dismutase, another antioxidant; cytochrome oxidase, which is part of electron transport system in mitochondria; lysil oxidase, involved in collagen and elastin synthesis and bone formation; ceruloplasmin, related to hemoglobin synthesis; and tyrosinase, involved in melanin production. Manganese is a component of pyruvate carboxylase, a key enzyme of carbohydrate metabolism; Mn superoxide dismutase, which is an important antioxidant inside of mitochondria; acts in the conversion of mevalonic acid to squalene, which is part of the pathway of cholesterol synthesis; and essential for the synthesis of polysaccharides, glycoproteins, and sulphate chondroitin (organic matrix of bone). Finally, Zn is necessary for the activity of over 300 enzymes. It is component of Cu-Zn superoxide dismutase, controlling oxidative stress; carbonic anhydrase, helping in the acid-base homeostasis; and alkaline phosphatases, which are a group of isoenzymes, located on the outer layer of the cell membrane, catalyzing the hydrolysis of organic phosphate esters present in the extracellular space. In addition, Zn forms part of calmodulin, protein kinase C, inositol phosphate, thymosin, and participates in the

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Vol. 23, Nº 2, Sección: Artículos de investigación , pp. 101-118

synthesis of prostaglandins. Consequently, these four microminerals are extremely important for several metabolic and cell vital processes, also including reproductive physiology.

Strategic administration of ITM during the transition period in dairy cows is becoming a popular management tool in dairy operations in the USA. This practice is aimed to provide a boost with these important elements during critical points of the production cycle (when animals have greater demands) mainly due to the higher levels of oxidative stress [5]. Administration of ITM supplements is not intended to substitute the oral mineral supplementation provided within the TMR diet on a daily basis. The major constraints of these microminerals when they are supplemented orally as a unique source is their low digestive absorption coefficient (< 10%) and great variability in their bioavailability due to multiple factors such as inadequate forage mineral levels, variability in free choice mineral intake, management that does not allow adequate feeding, extremely antagonistic interaction with elements in feed, water and forage, imbalances in feedstuffs, and variability in animal mineral requirements over lactation. This feature makes these microminerals poorly available in the digestive tract of ruminants [4].

In this trial, the effect of one dose of ITM (Se, Mn, Cu, Zn) at around 35 DIM on reproductive performance was compared against a control group without any supplementation. A strength of this trial was that experimental cows from both groups were housed, fed and handled homogeneously in the same lot. In addition, parity number of cows from treated group (99 primiparous, 159 multiparous) was similar to cows from the control group (98 primiparous, 160 multiparous). This kind of randomized field trial design, using an injectable product, reduces the variability of the model, making the source of variation for reproductive responses linked mostly to the parenteral supplementation of microminerals. Although the CRFS was similar between groups, the speed on how cows became pregnant was 14 days earlier in treated than control cows ($P \leq 0.05$). Survival analysis is a very powerful statistical tool to determine how quick cows become pregnant over time. It is a reflex of pregnancy rate, where eligible cows to be bred (heat detection rate) and cows that conceive after breeding (conception rate) are in conjunction analyzed [16]. Conception rate in dairy cattle is affected by several factors including the incidence of postpartum diseases [3]. Cows with one postpartum health episode became pregnant at 160 DIM, cows with several postpartum health episodes became pregnant beyond 200 DIM, while cows with no postpartum diseases became pregnant at 125 DIM. Unfortunately, the nature of health records

from the dairies used in this second trial did not allow for a consistent analysis of incidence of postpartum diseases either, consequently, we must acknowledge that the lack of accurate assessment for health conditions might be an important confounding and sort of variation for our results. In addition, mineral supplement was injected (35 DIM) way beyond the high-risk period for periparturient diseases. Hence, if the randomization process for animals assigned to both groups was consistent, we must assume the incidence of postpartum diseases was similar between groups, impacting fertility evenly. Although mineral supplementation was after than the disease risk period, treated group still had better reproductive responses than the control group. The question arising is what would have happened if the mineral application had been at the time of parturition? Would there have been a positive impact on the incidence of postpartum diseases? Would there have been a complementary improvement on herd's fertility?

A similar study compared cows that were also treated with the same trace mineral supplement versus a control group. Unlike our study, the experimental group received 3 injections of the supplement: at approximately 230 days of gestation, 260 days of gestation, and 35 DIM. Overall results found no significant differences in reproductive performance (similar pregnancy rate between groups). Median calving to conception interval for the control and treated group was 110 and 111 days, respectively (P = 0.61). Interestingly, the same study reported a lower incidence of stillbirth (P=0.039), and endometritis (P=0.028) for treated than controls cows [7]. This suggests, mineral supplementation could boost the immune system of cows, reducing endometritis occurrence. In addition, cows with less stillbirth are less likely to develop uterine infections. Endometritis is extremely linked to poor immunity, but not necessarily to poor fertility. In fact, a recent study demonstrated that genes associated with subclinical endometritis and neutrophil function had lower capacity of determining reproductive responses, accordingly, the authors suggested the presence of different genetic expression patterns that are better predictors of reproductive efficiency [17]. Contrary to previous results, a meta-analysis study, reported that endometritis increased significantly calving to conception interval by 15 days, and decreased the likelihood of pregnancy by 31% [18]. On the other hand, in the study of Machado et al [7], calving to conception interval in treated and control group was around 110 DIM, while in our study the same interval was around 120 DIM. A large data set [19] reported that the mean calving to conception interval for Holstein cows was 101 days. This suggests that when days open are shorter and show a value close to the normal average of the

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Vol. 23, Nº 2, Sección: Artículos de investigación , pp. 101-118

population, it is more difficult to improve this characteristic through nutritional strategies (e.g. mineral supplementation), because of a normal constraint of genetic and physiological mechanisms. This could be an explanation why in Machado's study [7] the mineral booster did not improve calving to conception interval, unlike our results in which a positive effect of the same mineral injection was observed on the same reproductive interval.

Overall, both field trials had similar conception rates between treated and control groups, but cows receiving an ITM exhibited a time to pregnancy shorter than the control group. Although they showed the same risk for conception, becoming pregnant earlier has a great impact on herd's fertility and profit. In fact, the cost of an extra day open over the ideal interval in a herd has been estimated to range from 1.4 to 5.4 US$ [20, 21]. If we extrapolate our findings to a herd of 1,000 cows reducing its calving to conception interval by 10 days, there will be a saving between 14,000 to 54,000 US$ per year.

Conclusions

In trial 1 the parenteral application of 2 doses of a commercial multimineral product based on Cu, Se, Zn and Mn during the dry period did not affect BCS at calving and tended to decrease the days from calving to conception, although CRFS was similar between groups. In trial 2, the application of the same mineral product at 35 DIM did not affect the CRFS, but it reduced significantly the time to pregnancy (survival curves) by 14 days when compared with a control group.

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