

Effect of Parenteral Supplementation with a Mineral Compound of Cu, Zn, Mn on Bovine Mastitis

Insua AD*, Noval-Artiles E, Garcia-Díaz R, Naranjo-Quintero Y and Nickli-Domer R

Department of Veterinary Medicine and Zootechnics, Plant and Livestock Sciences Faculty, Central University Marta Abreu of Las Villas, Santa Clara (54830), Villa Clara, Cuba

*Corresponding author: Alfonso-Insua D, Department of Veterinary Medicine and Zootechnics, Plant and Livestock Sciences Faculty, Central University Marta Abreu of Las Villas, Camajuani Road 5.5 km, Santa Clara (54830), Villa Clara, Cuba, Tel: +5342281421; E-mail: danielai@uclv.edu.cu

Rec date: May 08, 2017; Acc date: December 06, 2017; Pub date: December 07, 2017

Copyright: © 2017 Insua AD, et al. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

Abstract

The objective of this investigation was to evaluate the effect of supplementing an injectable compound of Cu, Zn and Mn on bovine mastitis. The investigation was carried out in the dairy farms of San Quintín (Lat. 22.4070, Long. 80.0448) and Tres Caminos (Lat. 22.4019, Long. 80.0563) of "Desembarco de Granma", Plant and Livestock Company, Yabu of Santa Clara during the dry period. The animals used were crossbreeds of Holstein and zebu, these animals were milked two times daily and fed with CT-115 and natural grasses. 5 mL of the injectable compound (50 mg Cu, 100 mg of Zn and 50 mg Mn) was administered parentally to the cows in production and the cows in the last trimester of gestation, with repetitions every two months until three applications were completed. In the animals treated the prevalence and indice of subclinical mastitis was reduced significantly ($P < 0.05$) from 67.9% to 40.3% y 1.48 to 0.81; 62% to 33.3% y 1.27 to 0.55, in San Quintín and in Tres Caminos respectively. The risk index was estimated and it demonstrated that in the group of cows which was given the mineral supplement was affected 0.34 times less with mastitis subclinical with each cow that was not supplemented. The incidence rate was evaluated and it was discovered that the group of animals exposed to the mineral supplement was 6.3%, meanwhile the animals not exposed to the mineral supplement was 18.5%. It can concluded that the supplementation of Cu, Zn and Mn via parenteral reduced the presentation of mastitis subclinical, demonstrating a protective effect to reduce the relative risk for this pathology.

Keywords: Bovine mastitis; Minerals; Zn; Cu; Pathology

Introduction

In milk production, the most important element is the cow. According Barlow, the term "mastitis" is a non-specific descriptor of the inflammatory changes in the mammary gland, for which there are numerous etiology. From a practical perspective, mastitis is the response of the gland to infection commonly acquired by invasion of pathogenic bacteria through the orifice and teat canal. This disease is an inflammatory process of the mammary gland that causes changes in the composition of milk, the secretion of the epithelial cells and reduced functional capacity of the udder [1]. It is the disease with the most prevalence in dairy herds [2], with a great economic impact on milk production [3]. Overall, between 15 and 40% of the milking cows have one or more mammary quarters with subclinical mastitis; while between 1 to 8% may be going through the stage of clinical mastitis [4].

For infection (mastitis) to occur in the udder many factors are involved. In some dairy herds in the incidence of mastitis may be greater due to certain nutritional deficiencies. If cows are not consuming or absorbing enough minerals and vitamins needed to have a functional immune system may have a higher incidence of mastitis. This could be due to a weakened immune system that cannot successfully fight bacteria that invade the udder [5]. O'Rourke, stated that there is a relationship between nutrition and mastitis in dairy cows and nutritional deficiency is linked to the quality of soil [6], in the tropics 66% of soils are found between very little productive and unproductive, with the following constraints: salinity and sodium

14.9%, 43.3% erosion, poor drainage 40.3%, 44.8% low fertility and low organic matter content of 69.6% [7].

Recent studies on brown soils with carbonates in animal units of the southern province of Villa Clara showed Cu deficiency in 100% of samples of soil and grass, 75% of serum samples and 72% of liver tissue [8]; deficiencies in this ecosystem of Zn and Mn were detected in the soil plant animal axis [9].

The main impact of nutrition on udder health is by suppressing the immune system. Cows with negative energy balance are at increased risk of developing ketosis, and clinical ketosis is associated with a twofold increase in the risk of clinical mastitis. The trace minerals and vitamins that can affect the health of the udder are selenium and vitamin E, copper, zinc, manganese and vitamin A and β -carotene.

Mastitis is a constant challenge for all dairy farmers. There is a positive genetic correlation between mastitis and milk production, as milk production increases, susceptibility to mastitis also increases. Mineral deficiency is rarely a primary cause of mastitis. Certain trace elements (Cu, Se, Zn, Co) and vitamins (E and A) are known to influence the immune response and its deficiency may be associated with a high incidence of clinical or subclinical infection.

General objective

To assess the effect of an injectable composite Cu, Zn and Mn, on mastitis in dairy cows.

Specific objectives

To evaluate the effect of supplementation of a mineral compound on the epizootic bovine mastitis structure before and during the administration of the compound.

To determine the prevalence of subclinical mastitis and infectious pathogens involved as causes of the disease, before and during the administration of the compound:

Determining the level of subclinical mastitis before and during the administration of the compound.

Determine cumulative incidence and incidence rate of subclinical mastitis in the course of the investigation.

Evaluate the economic feasibility of the results obtained in the investigation.

Materials and Methods

The research was conducted in the San Quintín (Lat 22.4070, Long. 80.0448) and Tres Caminos (Lat 22.4019, Long. 80.0563) dairy farms of Plant and Animal Production company "EL YABU" of the "Desembarco de Granma" branch in Santa Clara, Villa Clara, Cuba during the dry period.

The animals are crossbred Holstein and Zebu undergoing double milking which takes place in the early morning hours (2:30 am) and evening (2.30 pm) and a feeding system using CT-115, natural pasture grasses, supplementation of cane, commercial feed (north gold), common salt and urea. A parenteral doses of 5 mL of injectable compound (50 mg Cu, 100 mg Zn and 50 mg Mn) was administered to the cows in production and those in the last trimester of pregnancy in the farms under study, repeating the injection every two months to complete three applications, monitoring and evaluation of variables to investigate (before and during) the application of the compound in the cows are and those which were incorporated.

Variables research

Investigative variables evaluated were:

Prevalence of subclinical mastitis using California Mastitis Test (CMT)-(NC 118:01) [10].

Prevalence of clinical mastitis [11].

Prevalence of atrophied quarters [11].

Total prevalence of intra-mammary infection.

Prevalence of intra-mammary infections according to the predominant organism [12,13].

Subclinical Mastitis index (IMSC).

Economic analysis of losses by the prevalence of subclinical bovine mastitis.

Mathematical design and statistical analysis

Prevalence: An observational study with a transversal design was done for processing of electronic data tab by using Microsoft - Excel to determine the prevalence of different clinical forms of mastitis and infections caused by microorganisms that produces the disease, such epizootiological indicators are generally determined by the following expression:

Prevalence=Total of positive quarters/Total of quarters exposed to the risk of infection × 100

Subclinical Mastitis Index (SCMI)

To calculate the SCMI: which considers all cows that are lactating and excludes the teats having clinical mastitis during the time of evaluation and the blind or dysfunctional teats, to determine the subclinical mastitis index (SCMI), values are assigned following, according to the results:

N=0, T=1, (+)=2, (++)=3, (+++)=4

N- Negativo, T- Trace, +- Positive, ++ - Weak Positive, +++ - Strong Positive.

To calculate the index of subclinical mastitis the following formula was used:

SCMI=(N × 0)+(T × 1)+((+ × 2)+((++ × 3)+((+++ × 4)/No. of functional teats.

Statistical analysis

Relative risk (RR) was estimated by forming contingency tables 2 × 2 [14], applying a prospective observational cohort study of the analytical type, to determine cumulative incidence and incidence rate, with the following scheme (Table 1):

| Classification | Affected | Not affected | Total |
|----------------|----------|--------------|-------------|
| Exposed | A | B | (A+b) |
| No exposed | C | D | (C+d) |
| Total | (A+c) | (B+d) | (A+c)+(b+d) |

Table 1: 2 × 2 Contingency table.

Further analysis of comparing proportions between the different samples with different forms of presentations of the disease was done, using the statistical package STAGRAPHS * - Centurion XV, version 6.0 for Windows.

The processing of the data was done using the program for epidemiological analysis of tabulated data EPIDAT. Version 3.1. (Contingency test, type of study: Cohort) to assess the cumulative incidence and incidence rate.

Economic analysis

To determine the loss of production the relationship between the degree of positive reaction and production losses was taken into consideration; Trace (8%); Weak positive (9 to 18%); Positive (19 to 25%) and Strong positive (over 25%), where losses due to grade of reaction to the detection of the test (CMT) was calculated, which was estimated the losses from the percent that is not produced from the actual production from the beginning of the investigation [15].

Results and Discussion

Tables 2 and 3 shows the results of the epizootic characterization of the dairy farm Tres Caminos, where a prevalence of 62% bovine clinical mastitis was found before the application of the injectable compound and a prevalence of detecting pathogens 90%, while for the dairy farm San Quintín was 67.9% and 45% respectively (Figure 1), in

a comparison of proportions between the different degrees of reaction in the samples that were collected, showed that there was a significant decrease in quarters that had reaction, increasing the negative quarters as the injectable complex was applied to cows, with statistically

significant differences ($p \leq 0.05$) for both units under study. It clearly shows that there is a decrease in somatic cells count and in turn this results in an improvement of the health of the udder.

| Grades of Reaction | Before | | I | | II | | III | | IV | |
|--------------------|--------|-------------------|-----|-------------------|-----|--------------------|-----|--------------------|-----|--------------------|
| | N | % | n | % | n | % | n | % | n | % |
| N | 71 | 38.0 ^b | 127 | 56.2 ^b | 131 | 62.4 ^{ab} | 132 | 61.7 ^{ab} | 138 | 67.1 ^a |
| T | 46 | 24.6 ^a | 22 | 9.7 ^b | 25 | 11.9 ^b | 28 | 13.1 ^b | 30 | 14.6 ^b |
| + | 28 | 15.0 ^b | 54 | 23.9 ^a | 38 | 18.1 ^{ab} | 36 | 16.8 ^{ab} | 30 | 14.6 ^{ab} |
| ++ | 33 | 17.6 ^a | 23 | 10.2 ^b | 16 | 7.6 ^b | 16 | 7.5 ^b | 8 | 4.1 ^c |
| +++ | 9 | 4.8 ^a | 0 | 0.0 ^b | 0 | 0.0 ^b | 2 | 0.9 ^b | 0 | 0 ^b |
| Clinical | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Atrophied | 5 | | 2 | | 6 | | 6 | | 6 | |
| Total | 192 | | 228 | | 216 | | 220 | | 212 | |
| Quarters tested | 187 | | 226 | | 210 | | 214 | | 206 | |
| Total reacted | 116 | 62 | 99 | 43.8 | 79 | 37.6 | 82 | 38.3 | 68 | 33.3 |

Table 2: Epizootic characterization of bovine subclinical mastitis in Tres Caminos during the investigation. (a y b): different letters in columns differ statistically with value ($p \leq 0.05$) *.

It is estimated that 18.20% of herds specialized in milk production in western and central Cuba are affected by chronic mastitis, clinical mastitis shows 3.02% and 45.1% subclinical; while 76.5% were positive

to CMT. Where most frequent microorganisms isolated were *Staphylococcus aureus*, *Corynebacterium bovis* and *Streptococcus agalactiae* with a prevalence of 30.5%, 9.2% and 8.3% respectively.

| Grades of Reaction | Before | | I | | II | | III | | IV | |
|--------------------|--------|-------------------|-----|-------------------|-----|--------------------|-----|-------------------|-----|--------------------|
| | N | % | N | % | n | % | n | % | n | % |
| N | 62 | 32.1 ^c | 107 | 63.3 ^b | 105 | 55.6 ^{ab} | 125 | 62.5 ^a | 111 | 59.7 ^{ab} |
| T | 49 | 25.4 ^a | 22 | 13.0 ^b | 30 | 15.9 ^b | 33 | 16.5 ^b | 18 | 9.7 ^c |
| + | 32 | 16.6 ^a | 25 | 14.8 ^a | 42 | 22.2 ^a | 31 | 15.5 ^a | 39 | 20.9 ^a |
| ++ | 28 | 14.5 ^a | 12 | 7.1 ^b | 11 | 5.8 ^b | 11 | 5.5 ^b | 13 | 7 ^b |
| +++ | 22 | 11.4 ^a | 0 | 0.0 ^c | 0 | 0.0 ^b | 0 | 0 ^b | 4 | 2.2 ^c |
| Clinical | 0 | 0 | 3 | 1.8 | 1 | 0.5 | 0 | 0 | 1 | 0.5 |
| Atrophied | 11 | | 11 | | 7 | | 8 | | 6 | |
| Total | 204 | | 180 | | 196 | | 208 | | 192 | |
| Quarters tested | 193 | | 169 | | 189 | | 200 | | 186 | |
| Total reaction | 131 | 67.9 | 59 | 36.7 | 83 | 44.4 | 75 | 37.5 | 74 | 40.3 |

Table 3: Epizootic characterization of bovine subclinical mastitis in San Quintín during the investigation. (a y b): different letters in columns differ statistically with value ($p \leq 0.05$) *.

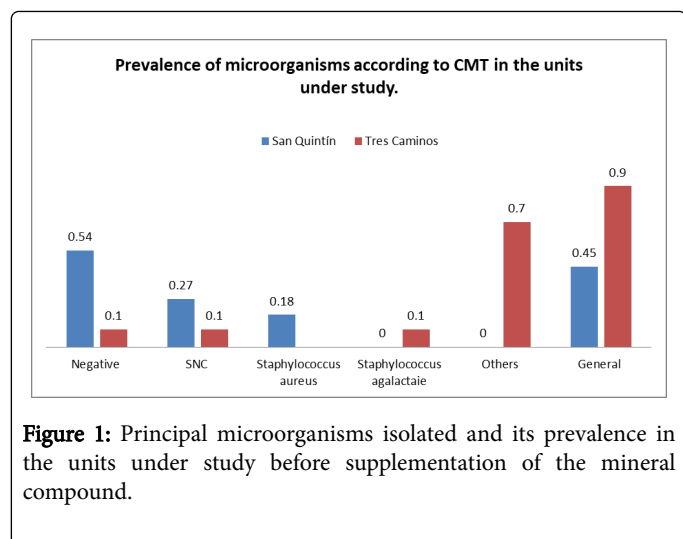


Figure 1: Principal microorganisms isolated and its prevalence in the units under study before supplementation of the mineral compound.

A study of the tendency in relation to the percent of the prevalence and the period of application of the injectable compound showed that the cows that were exposed to the mineral compound for a longer period, the prevalence of subclinical mastitis decreased (Figures 2 and 3).

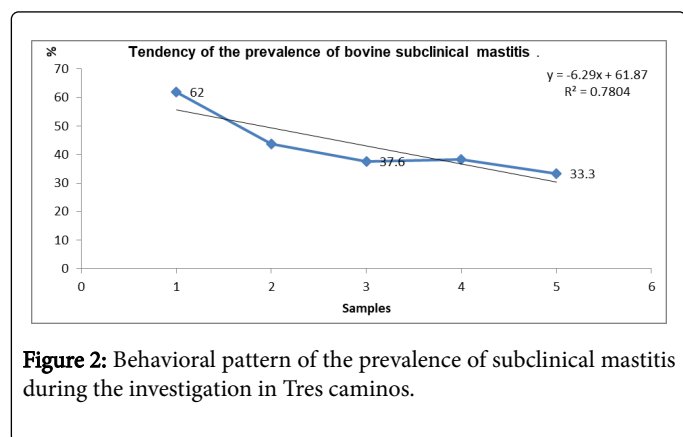


Figure 2: Behavioral pattern of the prevalence of subclinical mastitis during the investigation in Tres Caminos.

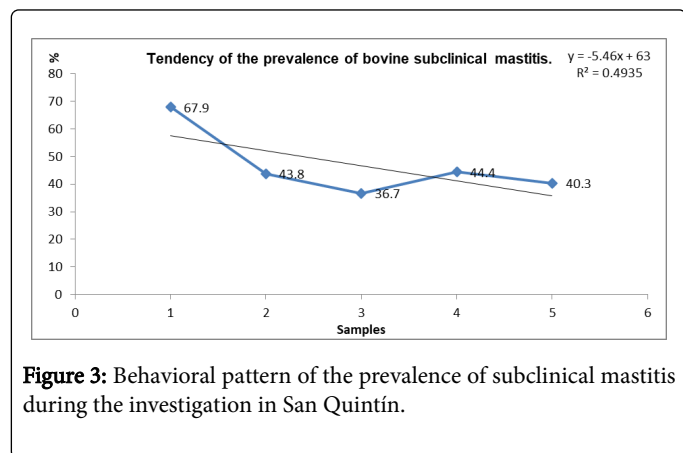


Figure 3: Behavioral pattern of the prevalence of subclinical mastitis during the investigation in San Quintín.

Figure 4 relates the behavior of the index of subclinical mastitis in the two units under study, where according Alfonso [16] reported index values of 1.42 and 1.62 respectively as results in other studies, then 1.27 to 1.48 before application of the mineral compound,

achieving a reduction in the indices to 0.81 and 0.55, respectively, considering them as desirable indices according to Castle et al. [17]. These results may be influenced by the prevalence of pathogens isolated *Staphylococcus aureus* and *Staphylococcus coagulase* negative (25 and 27%) respectively in the unit of San Quintín, unlike Tres Caminos where the overall prevalence is greater than 100% in the isolates, with 37% and 47% (*Streptococcus agalactiae* and others).

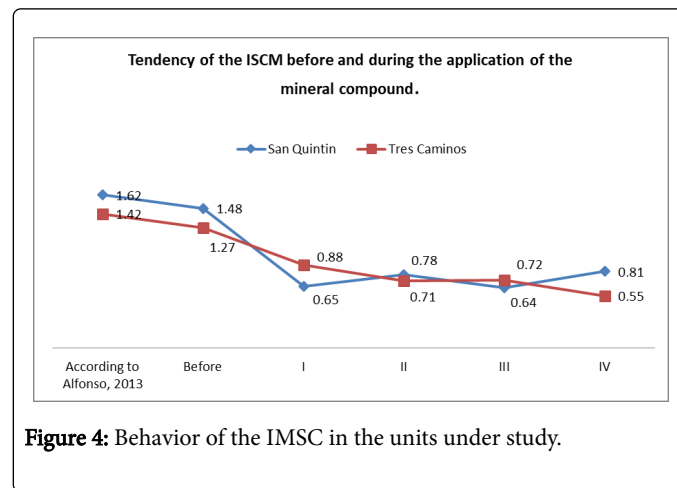


Figure 4: Behavior of the IMSC in the units under study.

When we estimated the risk index (hazard ratio) in the animals evaluated according to the presence or absence of the parenteral supplementation with the mineral compound (Table 4) and taking into account the odds ratio, it was demonstrated, that in the group of cows where the mineral compound was applied, cows were affected 0.34 times less with subclinical mastitis per cow that was not supplemented with the mineral compound; this association is significant (CI. 95% [0.27, 0.23] and statistically significant ($p \leq 0.05$). According to Novoa et al. undernourishment is a risk factor in the health of the udder; with decreased intake of natural pasture grasses in quantity and quality, characteristic during the dry season rain, causes a low intake of Cu, Zn, Mn, Se and Vitamin E to body, compounded by the absence of supplementation of these minerals, which act on the immune function, so that its administration is associated with a better activation of circulating white blood cells in response to microbial invasion, destruction of bacteria and maintaining a low rate of colony forming units, short duration of clinical signs and reducing the incidence of environmental mastitis, thus faster removal of udder infections. The prevalence of subclinical mastitis episodes in cows in the control group was 65%, while in cows supplemented with the mineral compound were affected only by 38%. As a result of this association, it was determined that the attributable fraction in cows exposed is 0.40, i.e., if these cows had been supplemented with mineral complex could have prevented the disease in 40% of the cows in this group and 35% in the entire population (attributable fraction in population of 0.35).

| Prevalence | Estimation | CI (95.0%) | |
|---------------|------------|------------|------|
| With INYT. | 0.38 | - | - |
| Without INYT. | 0.65 | - | - |
| Odds ratio | 0.34 | 0.27 | 0.43 |
| AFE | 0.4 | 0.34 | 0.45 |

| | | | |
|-----|------|------|------|
| AFP | 0.35 | 0.27 | 0.36 |
|-----|------|------|------|

Table 4: Study of the relative risk regarding the supplementation or not of the mineral compound and prevalence of subclinical bovine mastitis. AFE=attributable fraction in exposed AFP=attributable fraction in the population.

For the assessment of the study of the incidence rate (Figure 5), we can say that the group of animals exposed the incidence was 6.3%, and for the unexposed 18.5%. In all cases the confidence intervals (CI) is less than (1), of which there was association, thus existing statistically significant differences ($p \leq 0.05$), we can infer that the application of the mineral compound exerts a protective effect on incidence of bovine mastitis, considering the application of injectable compound, a factor for the prevention of this disease.

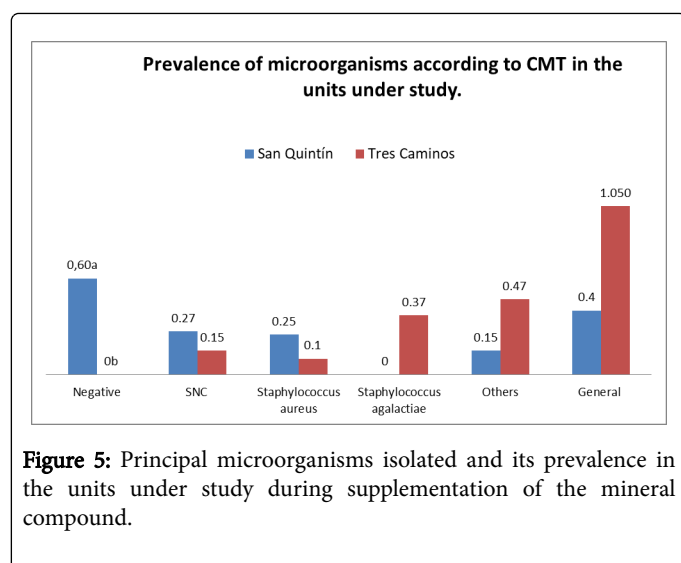


Figure 5: Principal microorganisms isolated and its prevalence in the units under study during supplementation of the mineral compound.

Note that the incidence of bovine mastitis, affects not only paying for quality, but a considerable amount is lost in individual production, such as the high prevalence in cows affected with subclinical bovine mastitis in our dairy and causes greater economic losses in dairy herds specialized herds, as is the case of our research, where according to Hernández et al. [15] there is a relationship between positive degrees of infection and losses in production (Trace (8%), weak positive (9 to 18%), positive (19 to 25%) and strong positive (over 25%), where losses were calculated for the degrees of reaction to the detection of the test (CMT), which stresses that losses from the percent that is left to produce in a day before the application of mineral compound is 73.22 and 46.78 liters of milk, which is making a conservative estimate; would be losing 2197 liters in San Quintin and 1403 liters of milk in Tres Caminos monthly (Figure 6). This would represent a refund for our economy \$ 2,040, knowing the price of powder milk in the international market 5100 USD/MT according to FAO, for the purchase of 400 Kg milk powder to restore the alleged losses, the concept of reduced production, if we apply the mineral compound it can reduce losses by more than 50% of the initial losses [18]. The study has enabled the production of a product in quantity and best quality for obtained for every peso invested 446 pesos was gained (1:446), giving the measure of efficiency for the 6 months of research.

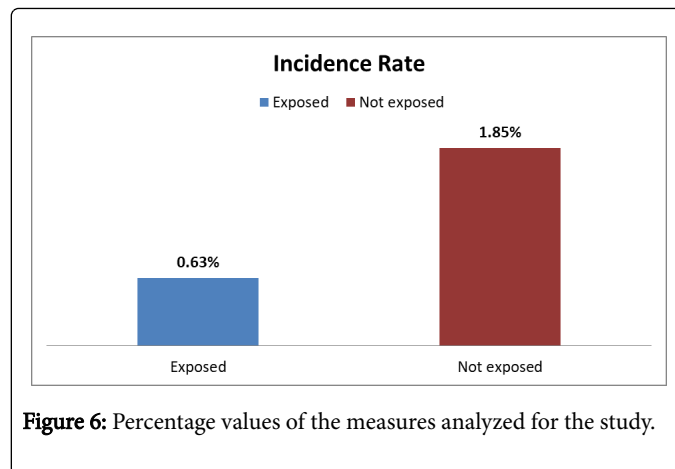


Figure 6: Percentage values of the measures analyzed for the study.

According to Ponce et al. mammary gland can resist most infections if they are provided with the nutrients necessary to maintain this resistance to new infections by acting as a prophylactic measure against bovine mastitis [19]. The important minerals and vitamins are Selenium, Copper, Zinc, Manganese, Vitamins A and E. When these are not provided in adequate amounts, the rate of new infections may increase. According to Garcia in animal units in the central province of Villa Clara deficiencies Cu, Zn and Mn were detected in the soil - plant - animal axis, therefore the mineral supplementation is necessary to decrease the incidence rate mastitis [8,9].

According to the results, it is evident that the mineral complex has a positive effect on bovine subclinical mastitis in the two farms, saying there was a significant decrease in the prevalence of bovine mastitis when prevalence is compared before and during mineral supplementation. Before supplementation the prevalence of subclinical mastitis was 67.9% in San Quintin and 62% in Tres Caminos and in the last application of the compound (sample IV), the prevalence decreased to 40.3% in San Quintin and 33.3% in Tres Caminos. It is shown that these trace minerals are beneficial in increasing the immune response in dairy cows (Berger) and according to Scaletti et al. at 34 days of lactation decreases the severity of intramammary infection and cell counting somatic (CCS) supplementing the diet with copper.

The strong positive relationship between the state of trace minerals and the immune response is well known. Research showed a correlation between the Cu, Zn, Mn, and is in the blood and reduced the incidence of bovine mastitis. Recent research from the University of Missouri showed that organic zinc supplementation increases the synthesis of keratin in the teat, which provides a physical barrier to bacteria. This physical barrier of keratin is the first line of defense before the entry of bacteria into the udder, thus acting as a bactericide in the teat canal, thus decreasing the incidence of bovine mastitis and CCS. Copper and manganese also play a key role in immune function. The increase in trace elements in dairy cows with the application of an injectable product could increase immune function, right after birth and therefore reduce mastitis infections [20]. However keep in mind that in addition to inadequate nutrition are other factors that increase the incidence of bovine mastitis, the main ones are; correct milking procedure, proper hygiene and injury to the mammary gland.

Conclusion

According to the results obtained and the literature consulted:

In the treated animals the prevalence rate of subclinical mastitis was significantly decreased in the farms under study.

It was shown that the risk index in the group of cows that the mineral compound was applied, were less affected with subclinical mastitis.

Supplementation of trace minerals complex had a protective effect in reducing the relative risk for this disease.

Reducing the prevalence and incidence of bovine mastitis also reduced economic and production losses.

For every Cuban Pesos invested 446 Cuban Pesos was gained.

Recommendations

Continue strategic studies with supplementation of these minerals. Include in the mineral compound selenium and vitamin E.

References

1. Souto LIM, Minagawa CY, Telles EO, Garbuglio MA, Amaku M, et al. (2010) Correlation between mastitis occurrence and the count of microorganisms in bulk raw milk of bovine dairy herds in four selective culture media. *J Dairy Res* 77: 63-70.
2. Klostermann K, Crispie F, Flynn J, Meaney WJ, Ross RP, et al. (2009) Efficacy of a teat dip containing the bacteriocin lacticin 3147 to eliminate Gram-positive pathogens associated with bovine mastitis. *J Dairy Res* 29: 1-8.
3. Huijps K, Lam TJGM, Hogeveen H (2008) Costs of mastitis: facts and perception. *J Dairy Res* 75: 113-120.
4. Araúz EE (2012) Subclinical mastitis and its influence on production, quality and dairy economy and strategic management measures for its prevention and appropriate control. Department of Animal Husbandry, Center for Teaching and Agricultural Research of Chiriqui. Panama.
5. Pritchard DE (2011) Trace Minerals, Immune Function and Mastitis-What Is Their Relationship? North Carolina State University, USES.
6. O'Rourke D (2011) Nutrition and udder health in dairy cows: a review. Ortec Consultancy. Canterbury, United Kingdom.
7. ONE (2007) Agro-productive classification of the soils of Cuba. Accessed on 20-02-2011.
8. García JR (2008) Relationship between the cupremia and the reproductive indicators of the bovine female. Thesis presented as an option to the scientific degree of doctor of veterinary sciences. National Center for Agricultural Health (CENSA) - Agrarian University of Havana -Fructuoso Rodríguez Pérezl (UNAH).
9. García JR, Cuesta M, Quiñones M, Figueredo JM, Mollineda, et al. (2010) Characterization of the content of microelements in the soil system of the animal plant and its influence on bovine reproduction in the central zone of Cuba. *Rev Cubana Cienc Agric* 44: 233-237.
10. NC 118: 01 (2001) Milk California test for mastitis diagnosis.
11. Philpot WN, Nickerson SC (1993) Mastitis: The counterattack. A strategy to combat mastitis. Published by Babson Bros Co.
12. National Mastitis Council (1987) Laboratory and Field Handbook on bovine Mastitis.
13. Harmon RJ, Eberhat RJ, Jasper DE, Langhois BE, Wilson PJ (1990) Microbiological procedures for use in the diagnosis of bovine udder infections. *Nat. Mastitis Counc*, Arlington, USA.
14. Thrusfield M (2007) *Veterinary Epidemiology*. 3rd edn, Blackwell Publishing, pp: 269-270.
15. Hernández R, Armentero A (2011) Milk productive chain. Editorial: Cuban Association of Animal Production. p: 114.
16. Alfonso D, Cuéllar, Mirta, Cepero O, Hernández M (2013) Producing milk with excellent quality standards is a challenge for the food sovereignty of our country. XXIII Meeting of the Latin American Association of Animal Production. Palace of Conventions, Havana from November 18 to 22. Cuba.
17. Castle M, Suniaga J, Rojas G, Hernández J, Janeth C, et al. (2009) Study of prevalence of subclinical mastitis in the upper area of the state of Merida. University of the Andes, Mexico.
18. <http://www.fao.org/home/en/>
19. Ponce P, Armentero P, Noa M (1992) Automation of milk analysis, needs and possibilities. CENLAC/CENSA, p: 76.
20. Mitchell K (2003) Trace Minerals and Dairy Cattle. Valley Veterinarians, Inc., Tulare, California. USES.