Revista de Medicina Veterinaria

Volume 1 | Number 41 Article 8

2020-11-24

Relación entre la concentración sérica de calcio, beta-hidroxibutirato y glucosa y la ocurrencia de trastornos mamarios y uterinos durante la lactancia temprana en ganado lechero

Gabriel Esnaola
Federal University of Rio Grande do Sul, gabrielesnaola@yahoo.com.br

Waldemir Santiago Neto
Plataforma de Salud Animal, INIA La Estanzuela, Uruguay, wal_sanet@hotmail.com

Félix González
Federal University of Rio Grande do Sul, felix.gonzalez@ufrgs.br

Citación recomendada

Esnaola G, Neto WS y González F. Relación entre la concentración sérica de calcio, beta-hidroxibutirato y glucosa y la ocurrencia de trastornos mamarios y uterinos durante la lactancia temprana en ganado lechero. Rev Med Vet. 2020;(41): 83-90. doi: https://doi.org/10.19052/mv.vol1.iss41.8

https://doi.org/10.19052/mv.vol1.iss41.8

Relationship of Serum Concentrations of Calcium, Beta-hydroxybutyrate and Glucose to the Occurrence of Mammary and Uterine Disorders during Early Lactation on Dairy Cattle*

Gabriel Esnaola[1] / Waldemir Santiago Neto[2] / Félix González[3]

Abstract

This study aimed to relate the serum levels of energy indicators (glucose and beta-hydroxybutyrate) and calcium to the occurrence of uterine and mammary diseases in the transition period of Holstein cows. One hundred and fifteen Holstein cows from a commercial herd were monitored starting one week before calving and during the first 2 weeks after calving. The presence of subclinical mastitis was monitored for 3 weeks postpartum using the California Mastitis Test (CMT) and somatic cell counting. Positive samples of subclinical mastitis were analyzed for bacterial identification. Clinical mastitis was monitored for 6 weeks postpartum, using data that were recorded by the farmer based on some established definitions. The presence of uterine disorders was identified for 3 weeks postpartum by clinical examination. Blood collections were made from all cows for determining calcium, glucose, and β-hydroxy-butyrate (BHB) the week before calving and twice during the postpartum (days 5 and 15 postpartum). A group of 70 healthy cows was used as control. Clinical mastitis was identified in 20% of the cows, subclinical mastitis in 10.4% and clinical metritis in 8.7% of the cows. The bacterial agents with the highest occurrence in subclinical mastitis cases were coagulase-negative *Staphylococcus* and *Streptococcus*. No relationships were observed between any of the biochemical metabolite studied and the uterine and mammary diseases in the cows during the studied period.

Keywords: metabolic profile, metritis, subclinical mastitis.

* Research article.

1 DVM, MSc. Postgraduate Program in Veterinary Science, Federal University of Rio Grande do Sul.
gabrielesnaola@yahoo.com.br
https://orcid.org/0000-0003-1913-2276

2 Plataforma de Salud Animal, INIA La Estanzuela, Uruguay
wal_sanet@hotmail.com
https://orcid.org/0000-0001-7099-546X

3 DVM, MSc, PhD. Full Professor, Faculty of Veterinary, Federal University of Rio Grande do Sul.
felix.gonzalez@ufrgs.br
https://orcid.org/0000-0001-9438-6888

How to cite this article: Esnaola G, Neto WS, González F. Relationship of Serum Concentrations of Calcium, Beta-hydroxybutyrate and Glucose to the Occurrence of Mammary and Uterine Disorders during Early Lactation on Dairy Cattle. Rev Med Vet. 2020;(41):83-90. https://doi.org/10.19052/mv.vol1.iss41.8

Relación entre la concentración sérica de calcio, beta-hidroxibutirato y glucosa y la ocurrencia de trastornos mamarios y uterinos durante la lactancia temprana en ganado lechero

Resumen

Este estudio tuvo por objetivo relacionar valores séricos de indicadores energéticos (glucosa y beta-hidroxibutirato) y calcio con la ocurrencia de trastornos uterinos y mamarios durante el periodo de transición en vacas holstein. Ciento quince vacas holstein de un rebaño comercial fueron monitoreadas en la semana previa al parto y durante las primeras dos semanas después del parto. La presencia de mastitis subclínica fue monitoreada por tres semanas posparto mediante la prueba de mastitis California (CMT) y conteo de

Received: 14 March, 2020. Approved: 2 October, 2020. Online First: 18 November, 2020. Final publication: 1 December, 2020

células somáticas. Las muestras positivas a mastitis subclínica fueron analizadas para identificación bacteriana. La mastitis clínica fue monitoreada durante seis semanas posparto usando datos registrados en el rebaño. La presencia de trastornos uterinos fue identificada durante tres semanas posparto mediante examen clínico. Se realizaron muestras de sangre de todas las vacas para determinar calcio, glucosa y β-hidroxibutirato la semana anterior al parto y dos veces en el posparto (días 5 y 15). Un grupo de 70 vacas sanas fue usado como control. Un 20% de las vacas presentó mastitis clínica, el 10,4% mastitis subclínica y el 8,7% metritis clínica. Los agentes bacterianos con mayor ocurrencia en los casos de mastitis subclínica fueron *Staphylococcus* coagulasa-negativos y *Streptococcus*. No se observó relación de ninguno de los metabolitos bioquímicos con los trastornos uterinos o mamarios en las vacas durante el periodo estudiado.

Palabras clave: mastitis subclínica, metritis, perfil metabólico.

Introduction

The transition period in dairy cows, which comprises 3 weeks before and 3 weeks after calving (1) is a time of important metabolic and endocrine changes in the physiological response to lactation challenge, when feed intake can decrease up to 25-35% (2). The calving and early lactation impose great physiological challenges for energy and calcium balances and immunosuppression may be a common feature (3).

Clinical hypocalcemia is a metabolic disorder resulting from the sudden change in the demand for calcium of body compartments to supply the production of colostrum and milk during early lactation. This metabolic change is regulated by compensatory mechanisms, as increased intestinal absorption, renal reabsorption and bone mobilization of calcium (4). However, when the regulation mechanisms fail, animals can develop clinical manifestations of milk fever in the first 72 hours after calving (5). Subclinical hypocalcemia is less serious for cow´s health, but it causes a decrease in the herd productivity and reduces dry matter intake during early lactation (6). The prevalence of subclinical hypocalcemia is about 25-54% depending on the number of lactations (7). Studies indicate that subclinical hypocalcemia increases cow´s susceptibility to secondary diseases such as downer cow syndrome, dystocia, mastitis, retained placenta, metritis and displaced abomasum (3, 8). Hypocalcemia reduces the responsiveness of immune cells to stimuli, thereby contributing to the occurrence of infections (9, 10).

High yielding dairy cows in early lactation have high requirements in glucose and fatty acids that cannot be achieved by dietary intake, which is evidenced by a sharp increase in the mobilization of fatty acids and a significant rise in ketone bodies (11). Various authors have found a relationship between increases in serum beta-hydroxybutyrate (BHB) and non-esterified fatty acids (NEFA) with higher incidence of metritis and mastitis in dairy cows (12).

Udder infections are common in early lactation and have a great economic impact on the dairy industry. The economic benefits of identifying and eliminating infections in early lactation are significant, including the prevention of chronic mastitis, less discarded milk and reduction in somatic cells counts (13). The uterine health in dairy cows is often compromised due to contamination after calving by opportunistic bacteria that cause clinical diseases. One third of dairy cows may be affected by some kind of metabolic or infectious disorder in early lactation (14). Diet manipulation and appropriate herd management during late pregnancy and early lactation might reduce to a minimum the incidence of metabolic disorders with the potential of increasing milk yield, promote health and improve herd reproductive efficiency (6). Many epidemiological studies on the relationship between udder and uterine disorders and metabolic disorders in early lactation have been designed based on large populations (8, 15). However, in individual herds, the relationship between some blood indicators and the susceptibility to develop uterine or mammary diseases is difficult to establish. Therefore, the present study aimed to associate serum levels of energy indicators (glucose and beta-hydroxybutyrate) and calcium with the occurrence of uterine and mammary diseases.

Materials and methods

All the procedures involving animals were approved by the Ethics Committee of the Federal University of Rio Grande do Sul, Brazil (Project no. 28158).

The study was conducted on a commercial dairy farm in southern Brazil, with 500 lactating Holstein cows under a semi-confinement system. The average milk yield of the herd was 8,500 kg/cow/year. One hundred and thirty-three cows (102 multiparous and 31 primiparous) were selected one week before calving based on the estimated calving date, considering an gestation average of 283 days, using the Delpro™ management software (DeLaval). All cows were housed in prepartum paddocks approximately 25 days prior to calving.

During the postpartum period, diets were formulated using Spartan Dairy 3 software. The paddocks were held with scheduled mowed so that the pasture intake was restricted. The animals were fed 22.4 kg of dry matter per day of TMR diet (7.2 kg corn silage, 6.63 kg predried ryegrass, 3.98 kg of extruded soybean meal, 2.96 kg ground corn, 0.8 kg wheat straw, 0.08 kg shell flour, 0.12 kg sodium bicarbonate, and 0.63 kg supplement of minerals and vitamins). The estimated intake of pasture was 3.47 kg of Piatan/Papua grass. The forage: concentrate ratio was 66.6:33.4. The bromatological analysis of the ration was (dry matter basis): neutral detergent fiber 34.2%, net energy 1.6 Mcal/kg, and crude protein 15.6%.

All the cows were selected from normal calving without any complication such as dystocia or twin calving. The selected cows were weekly subjected to a complete physical examination. The diagnosis of clinical metritis was accomplished based on the presence of red-brown foul uterine discharge (16) during the first 21 days postpartum. The vaginal discharge was obtained by transrectal palpation and retraction of both the cervix and uterus to obtain a uterine fluid source. Clinical mastitis was diagnosed by the stripping test identifying altered milk with a strip cup and by observing signs of inflammation in any udder quarter up to 60 days postpartum. Subclinical mastitis was identified weekly during the first 21 days of lactation through the CMT test performed in milk samples aseptically collected in individual mammary quarters. In addition, chilled samples were sent to the laboratory to do a somatic cell count (SCC) by a flow cytometric method (CA3A5 SomatoScop, Dairy Equipments, USA) and total bacterial counts (Bactocount IBC Dairy Equipments, USA). Animals were taken as positive to subclinical mastitis when CMT samples showed a reaction greater than zero and SCC was higher than 100×10^3 cells/mL (17). Positive samples in the CMT test were frozen for later analysis of bacterial identification in a referenced laboratory. The microbiological culture was done with sowing fresh milk (10 μL) in bipartite plates on blood agar and MacConkey agar and incubated in an aerobic environment for 72 hours at 37.5 °C.

The body condition score (BCS) was evaluated with a scale of 1 through 5 (1 = thin, 5 = fat), always evaluated by the same veterinarian. The daily milk yield was measured using a scale that estimates the individual production of animals (DeLaval, Brazil). Body condition and milk yield were monitored weekly for 21 days postpartum.

Blood samples were collected from coccygeal vein into plain tubes (Vacutainer, Becton Dickinson, Brazil) once before calving (5 days prepartum) and twice after calving (5 and 15 days postpartum). Blood sampling was always done at 06:30. The samples were sent to the laboratory within two hours after collection. In the laboratory, the samples were centrifuged (2,500 rpm, 10 minutes) to obtain serum, and stored at -20°C in eppendorf tubes. Serum samples were analyzed for glucose and total calcium by spectrophotometry (Architect C8000, Abbott, USA) using commercial kits (Labtest, Brazil). Serum concentrations of BHB were measured by a digital meter (Ketovet, Korea).

Data were entered in Excel sheet and then exported to SPSS program v.18.0 for statistical analysis. Categorical variables were expressed as frequencies and percentages and compared using the chi-square test. Quantitative variables with symmetrical distribution were described by the mean, standard error of the mean and 95% Confidence Interval. Comparison among groups (clinical metritis, clinical mastitis, subclinical mastitis, and healthy cows) and along the sampling periods (pre and postpartum) were done using the model of generalized estimation equations. A 5% significance level was considered.

Results

Eighteen cows out of the 133 participating in the study were excluded due to premature calving, death or culling. Out of the remaining 115 cows analyzed, 23 were diagnosed with clinical mastitis (20%), 12 with subclinical mastitis (10.4%) and 10 showed clinical metritis (8.7%). The control group consisted of 70 healthy cows.

Serum levels of BHB did not differ among groups (Table 1) but differences along the periods were observed in cows with clinical mastitis and in healthy cows with higher concentrations at the postpartum as compared to the prepartum period. No differences in serum calcium values were observed among groups of cows with postpartum disorders or in healthy cows, neither along the periods. Serum glucose levels were not different among the groups. However, glucose concentrations in cows with clinical metritis were lower during the postpartum as compared to the prepartum period, while in healthy cows the opposite was observed. Although without significant differences, the groups of cows with mastitis (clinical and subclinical) had a trend similar to healthy cows with higher glucose values at the postpartum period. The body condition score (BCS) did not differ among groups of cows with disorders or healthy ones, but the BCS in the postpartum periods was lower in all cases.

Table 1. Mean and standard error (SE) of BHB, calcium, glucose, BCS among groups along the sampling periods

	Sampling period	Clinical mastitis	Subclinical mastitis	Clinical metritis	Healthy cows
BHB (mmol/L)	N	23	12	10	70
	Prepartum (5 days)	0.91[b] (0.06)	1.03 (0.11)	1.06 (0.04)	0.93[b] (0.04)
	Postpartum (5 days)	1.24[a] (0.10)	1.33 (0.19)	1.09 (0.13)	1.17[a] (0.05)
	Postpartum (15 days)	1.16[a] (0.11)	1.26 (0.13)	1.32 (0.30)	1.14[a] (0.05)
Calcium (mmol/L)	N	23	12	10	70
	Prepartum (5 days)	1.89 (0.11)	1.99 (0.15)	1.6 (0.33)	2.07 (0.05)
	Postpartum (5 days)	2.06 (0.09)	2.0 (0.23)	1.85 (0.14)	2.0 (0.04)
	Postpartum (15 days)	1.88 (0.11)	2.2 (0.08)	1.59 (0.25)	2.04 (0.04)

	Sampling period	Clinical mastitis	Subclinical mastitis	Clinical metritis	Healthy cows
Glucose (mmol/L)	N	23	12	10	70
	Prepartum (5 days)	2.45 (0.25)	2.19 (0.43)	3.23[a] (0.2)	2.61[c] (0.12)
	Postpartum (5 days)	2.94 (0.25)	3.01 (0.16)	2.83[b] (0.3)	2.60[b,c] (0.13)
	Postpartum (15 days)	3.17 (0.22)	2.50 (0.34)	2.89[b] (0.35)	3.05[a] (0.13)
BCS (1-5)	N	23	12	10	70
	Prepartum (5 days)	3.29[a] (0.1)	3.53[a] (0.2)	3.1[a] (0.12)	3.2[a] (0.05)
	Postpartum (5 days)	3.19[a,b] (0.09)	3.36[a,b] (0.13)	2.93[b] (0.14)	3.1[b] (0.05)
	Postpartum (15 days)	3.03[b] (0.11)	3.0[b] (0.13)	2.75[b] (0.13)	3.06[b,c] (0.05)

BHB: serum beta-hydroxybutyrate, BCS: body condition score. Different letters indicate significant differences ($p < 0.05$) among sampling periods (columns).

Milk yield showed a mean of 25.9 ± 6.9 L/cow/day without any differences observed among the groups of cows. Cows with clinical mastitis had a higher number of lactations as compared to the other groups of cows ($p = 0.03$). The range of lactation number was 1-3 for cows with metritis, 1-8 for cows with clinical mastitis, 1-6 for cows with subclinical mastitis and 1-5 for healthy cows. Clinical metritis showed more cases in primiparous cows, while subclinical and clinical mastitis occurred more in multiparous cows ($p = 0.07$).

The bacterial agents with the highest prevalence in subclinical mastitis cases were coagulase-negative *Staphylococcus* (36%), *Streptococcus* (36%), coagulase-positive *Staphylococcus* (18%) and *Staphylococcus* sp. (10%).

Discussion

Many studies concerning the relationships between blood metabolic indicators and puerperium disorders have been conducted under an epidemiological approach using various herds and individuals (18). On a herd health approach, it may be difficult to correlate metabolic profiles as a diagnostic tool to identify individuals with uterine and mammary disorder susceptibility. However, the aim of this study was to observe how the serum concentrations of calcium, glucose and beta-hydroxybutyrate are related to the most frequent disorders at early postpartum in one dairy herd.

Metritis may affect about 20% of lactating cows, with an incidence ranging from 8 to 40% according to the husbandry adopted in the herd (19). There are scarce studies concerning mastitis incidence in Brazilian dairy herds. In our study, the occurrence of clinical metritis observed in the first three weeks postpartum was 8.7%, which means that the farm has good sanitary measures to control the disease. Metritis showed a trend to occur in primiparous cows, which may compromise the productive life of the animal due to the risk of premature culling (20).

No relationship was observed between the cows with clinical metritis and the serum levels of glucose, calcium or BHB, although it has been mentioned that cows with metritis have lower serum calcium concentration as compared to healthy animals (21) and that subclinical hypocalcemia increases the risk to develop clinical metritis (3). Also, animals with higher concentrations of

BHB both before and after calving have greater risk of developing clinical metritis as compared to normal animals (22). Although no differences in serum glucose and BHB concentrations were noted between healthy cows and cows with uterine disorders in our study, a significant decrease in glucose was observed at the postpartum period in the cows affected by metritis, which might affect their defenses by limiting the fuel required for populations of immune cells (23).

Mastitis is worldwide the costliest production disease in dairy herds (24), which may affect around 23% of milking cows in a herd (25). Moreover, the incidence of subclinical mastitis may reach almost half of the cows in high-yielding dairy herds (26). In our study, clinical mastitis was observed in 20% of the cows during 60 days postpartum while subclinical mastitis occurred in 10.4% of the cows during 21 days postpartum. These differences in the periods of monitoring the mastitis was due to the concept that defines clinical mastitis as a cow having affected quarters during at least a period of 14 days (27). Thus, a period of 21 days may result being too short for considering a more comprehensive clinical case.

Although no differences in serum BHB concentrations were seen when comparing cows with mastitis and healthy cows, a significant increase in BHB was observed in clinical but not in subclinical mastitis cows and in healthy cows. This finding reveals a higher lipomobilization condition in cows suffering the clinical condition. Nevertheless, the same observation in healthy cows shows that higher BHB values may be related to normal adjustment in the energy balance of the cows during early postpartum period (28), which is consistent with the loss in body condition in all groups of animals. Cows with higher lactation numbers are more susceptible to mastitis (29) and this was observed in our study when cows with clinical and subclinical mastitis had a mean of 3 lactations as compared to a mean of 2 lactations for healthy cows.

The bacterial identification results found in our study from cows with subclinical mastitis are in line with other studies. Paape et al. (30) found that the most frequent Gram-positive agents of mastitis in dairy cows include *Staphylococcus aureus,* coagulase-negative *Staphylococcus*, *Enterococcus, Streptococcus agalactiae, Streptococcus uberis* and *Streptococcus dysgalactiae*.

Conclusion

The results showed no significant association of calcemia, glycemia and ketonemia with uterine and mammary gland disorders in the early puerperal period analyzed on a herd basis. From an epidemiological approach, the limitation of our study is the low number of cows used in only one herd. However, from a diagnosis approach it is useful to establish the weak association of the studied parameters.

References

1. Grummer RR. Impact of changes in organic nutrient metabolism on feeding the transition dairy cow. J Anim. Sci. 1995;73:2820-33. https://doi.org/10.2527/1995.7392820x
2. Drackley JK. Biology of dairy cows during the transition period: the final frontier? J Dairy Sci. 1999;82:2259-73. https://doi.org/10.3168/jds.S0022-0302(99)75474-3
3. Martinez N, Risco CA, Lima FS, Bisinotto RS, Greco LF, Ribeiro ES, Maunsell F, Galvão K, Santos JE. Evaluation of peripartal calcium status, energetic profile, and neutrophil function in dairy cows at low or high risk of developing uterine disease. J Dairy Sci. 2012;95:7158-72. https://doi.org/10.3168/jds.2012-5812
4. Goff JP. Major advances in our understanding of nutritional influences on bovine health. J Dairy Sci. 2006;89:1292-01. https://doi.org/10.3168/jds.S0022-0302(06)72197-X
5. Goff JP. The monitoring, prevention, and treatment of milk fever and subclinical hypocalcemia in dairy cows. Vet J. 2008;176:50-7. https://doi.org/10.1016/j.tvjl.2007.12.020

6. DeGaris PJ, Lean IJ. Milk fever in dairy cows: a review of pathophysiology and control principles. Vet J. 2009;176:58-69. https://doi.org/10.1016/j.tvjl.2007.12.029
7. Reinhardt TA, Lippolis JD, McCluskey BJ, Goff JP, Horst RL. Prevalence of subclinical hypocalcemia in dairy herds. Vet J. 2011;188:122-4. https://doi.org/10.1016/j.tvjl.2010.03.025
8. Chapinal NM, Carson TF, Duffield M, Capel S, Godden M, Overton TR, Santos JE, LeBlanc SJ. The association of serum metabolites with clinical disease during the transition period. J Dairy Sci. 2011;94:4897-903. https://doi.org/10.3168/jds.2010-4075
9. Kimura K, Reinhardt TA, Goff JP. Parturition and hypocalcemia blunts calcium signals in immune cells of dairy cattle. J Dairy Sci. 2006;89:2588-95. https://doi.org/10.3168/jds.S0022-0302(06)72335-9
10. Martinez N, Sinedino LD, Bisinotto RS, Ribeiro ES, Gomes GC, Lima FS, Greco LF, Risco CA, Galvão KN, Taylor-Rodriguez D, Driver JP, Thatcher WW, Santos JE. Effect of induced subclinical hypocalcemia on physiological responses and neutrophil function in dairy cows. J Dairy Sci. 2014;97:874-87. https://doi.org/10.3168/jds.2013-7408
11. Bell AW. Early lactation regulation of organic nutrient late pregnancy metabolism during transition from to early. J Anim. Sci. 1995;73:2804-19. https://doi.org/10.2527/1995.7392804x
12. Ospina PA, McArt JA, Overton TR, Stokol T, Nydam DV. Using nonesterified fatty acids and β-hydroxybutyrate concentrations during the transition period for herd-level monitoring of increased risk of disease and decreased reproductive and mlking performance. Vet Clin N Am Food Anim Pract. 2013;29:387-412. https://doi.org/10.1016/j.cvfa.2013.04.003
13. Dingwell RT, Leslie KE, Shukken YH, Sargeant JM, Timms LL. Evaluation of the California Mastitis Test to detect an intramammary infection with a major pathogen in early lactation dairy cows. Can Vet J. 2003;44:413-6.
14. LeBlanc SJ. Monitoring metabolic health of dairy cattle in the transition period. J Reprod Develop. 2010;56 (Suppl):S29-35. https://doi.org/10.1262/jrd.1056S29
15. Ospina PA, Nydam DV, Stokol T, Overton TR. Association between the proportion of sampled transition cows with increased nonesterified fatty acids and β-hydroxybutyrate and disease incidence, pregnancy rate, and milk production at the herd level. J Dairy Sci. 2010; 93:3595-601. https://doi.org/10.3168/jds.2010-3074
16. Sheldon IM, Lewis GS, LeBlanc S, Gilbert RO. Defining postpartum uterine disease in cattle. Theriogen. 2006;65:1516-30. https://doi.org/10.1016/j.theriogenology.2005.08.021
17. Sargeant JM, Leslie KE, Shirley JE, Pulkrabek BJ, Lim GH. Sensitivity and specificity of somatic cell count and california mastitis test for identifying intramammary infection in early lactation. J Dairy Sci. 2001;84:2018-24. https://doi.org/10.3168/jds.S0022-0302(01)74645-0
18. Moyes KM, Larsen T, Friggens NC, Drackley JK, Ingvartsen KL. Identification of potential markers in blood for the development of subclinical and clinical mastitis in dairy cattle at parturition and during early lactation. J Dairy Sci. 2009;92:5419-28. https://doi.org/10.3168/jds.2009-2088
19. Huzzey JM, Veira DM, Weary DM, Von Keyserlingk MA. Prepartum behavior and dry matter intake identify dairy cows at risk for metritis. J Dairy Sci. 2007;90:3220-33. https://doi.org/10.3168/jds.2006-807
20. Wittrock JM, Proudfoot KL, Weary DM, Von Keyserlingk MA. Metritis affects milk production and cull rate of Holstein multiparous and primiparous dairy cows differently. J Dairy Sci. 2011;94:2408-12. https://doi.org/10.3168/jds.2010-3697
21. Bicalho ML, Lima FS, Ganda EK, Foditsch C, Meira EB, Machado VS, Teixeira AG, Oikonomou G, Gilbert RO, Bicalho RC. Effect of trace mineral supplementation on selected minerals, energy metabolites, oxidative stress, and immune parameters and its association with uterine diseases in dairy cattle. J Dairy Sci. 2014;97:4281-95. https://doi.org/10.3168/jds.2013-7832
22. Giuliodori MJ, Magnasco RP, Becu-Villalobos D, Lacau-Mengido IM, Risco CA, de la Sota RL. Clinical endometritis in an Argentinean herd of dairy cows:

Risk factors and reproductive efficiency. J Dairy Sci. 2012;96:210-8. https://doi.org/10.3168/jds.2012-5682

23. Hammon DS, Evjen IM, Dhiman TR, Goff JP, Walters JL. Neutrophil function and energy status in Holstein cows with uterine health disorders. Vet Immun Immunopathol. 2006;113:21-9. https://doi.org/10.1016/j.vetimm.2006.03.022
24. Hortet P, Seegers H. Loss in milk yield and related composition changes resulting from clinical mastitis in dairy cows. Prev Vet Med. 1998;37:1-20. https://doi.org/10.1016/S0167-5877(98)00104-4
25. Olde Riekerink RG, Barkema HW, Kelton DF, Scholl DT. Incidence rate of clinical mastitis on Canadian dairy farms. J Dairy Sci. 2008;91:1366-77. https://doi.org/10.3168/jds.2007-0757
26. Gianneechini R, Concha C, Rivero R, Delucci I, Moreno-López J. Occurrence of clinical and subclinical mastitis in dairy herds in the west littoral region in Uruguay. Acta Vet Scand. 2002;43:221-30. https://doi.org/10.1186/1751-0147-43-221
27. Bartlett PC, Agger JF, Houe H, Lawson LG. Incidence of clinical mastitis in Danish dairy cattle and screening for non-reporting in a passively collected national surveillance system. Prev Vet Med. 2001;48:73-83. https://doi.org/10.1016/S0167-5877(00)00192-6
28. Contreras GA, Sordillo LM. Lipid mobilization and inflammatory responses during the transition period of dairy cows. Comp Immun Microbiol Infect Dis. 2011;34:281-9. https://doi.org/10.1016/j.cimid.2011.01.004
29. Curtis CR, Erb HN, Sniffen CJ, Kronfeld DS. Path analysis of dry period nutrition, postpartum metabolic and reproductive disorders, and mastitis in Holstein cows. J Dairy Sci. 1985;68:2347-60. https://doi.org/10.3168/jds.S0022-0302(85)81109-7
30. Paape M, Mehrzad J, Zhao X, Detilleux J, Burvenich C. Defense of the bovine mammary gland by polymorphonuclear neutrophil leukocytes. J Mam Gland Biol Neopl. 2002;7:109-21. https://doi.org/10.1023/A:1020343717817