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Review

Hypocalcemia in the dairy cow. Review

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Abstract:

Calcium (Ca) levels decrease in blood and cytosol at the time of calving, altering nerve impulse transmission, muscle contraction, and immune cell activity. In the nervous system, Ca participates in the conduction of stimuli. In the muscular system, it decreases contractions, causing alterations in smooth muscle, uterus and mammary gland. In the uterus, there is retention and storage of uterine fluids and waste, with bacterial complications. In the immune system, the function of neutrophils is important, and it manifests itself with a decrease in cells engaged in phagocytosis, predisposing to mastitis and metritis. In bovine hypocalcemia, two manifestations are distinguished: clinical and subclinical. In the clinical one (Ca values less than 5.5 mg/dl), homeostasis alters, with loss of appetite, decubitus and lethargy.

Subclinical hypocalcemia is more common (Ca between 8.0 and 5.5 mg/dl), and homeostasis does not alter, but muscle contraction and immune function decrease. The treatment is based on the application of calcium orally in standing cows, and intravenously in prostrate cows. Prevention depends on the inclusion of rations that contain anionic salts, which favors the stimulus to maintain blood Ca levels to control the level of cations and anions. In addition, Ca can be administered orally. Calcium homeostasis in lactation is regulated by the serotonin hormone, which stimulates the parathyroid hormone and bone resorption in osteoclasts.

Key words: Hypocalcemia, Dairy cow, Homeostasis, Calcium, Serotonin, Metritis.

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Introduction

The transition period of the dairy cow comprises 3 wk before and 3 wk after calving[1] and several physiological changes occur in the obtaining of nutrients for the calving process, expulsion of fetal membranes and production of colostrum and milk. Therefore, circulating levels of calcium (Ca) decrease in blood and cytosol[2,3]. Homeostasis or self-regulation of Ca normally uses the following feedback mechanism: it decreases the concentration of ionized calcium (iCa^{2+}), stimulating the parathyroid gland to secrete the parathyroid hormone (PTH). PTH binds to its hormone receptors in kidneys and bone tissue. In the kidneys, PTH increases renal reabsorption of Ca as well as the increase in production of 1,25-dihydroxyvitamin D, the active form of vitamin D[4]. Vitamin D stimulates the epithelial cells of the intestine to increase the active transport of Ca[5]. If the calcium in the diet is insufficient to generate homeostasis, the mechanism is directed to bone tissue[4]. Dairy cows slowly begin the reabsorption of Ca from bone tissue, but the accelerated demand of the mammary gland induces clinical hypocalcemia[2]. The parathyroid gland (PTH) participates in the homeostasis of Ca, but there is also the function of the parathyroid hormone-related protein (PTHrP), secreted in the mammary gland[6]. The serotonin hormone is responsible for stimulating the production of the PTHrP protein[7].

Serum Ca is present in three forms: ionic (iCa^{2+}) or free calcium (50 % of total calcium), bound to proteins (approximately 40 %) and in the form of complexes with anions (10 %). iCa^{2+} is the only biologically active calcium. Calcium participates in nerve, muscle and immune functions[8-10]. At the nervous level, it participates in the conduction of stimuli. At

the muscular level, in muscle contraction, and in the immune part, with the function of immune cells. Therefore, cows with hypocalcemia alter these functions depending on the severity in the decrease in calcium. There are two types of hypocalcemia: 1) clinical and 2) subclinical. The purpose of this review is to succinctly evaluate the incidence of hypocalcemia, as well as its consequences on immune function, metritis and mastitis[11-13].

Hypocalcemia

Hypocalcemia is a metabolic-nutritional disease, caused by the decrease in blood Ca. It usually occurs after calving, its manifestation can be clinical and subclinical.

Clinical hypocalcemia

Clinical hypocalcemia, also known as milk fever or puerperal paresis, is characterized by a momentary imbalance in the regulation of the concentration of calcium (Ca) in the blood between 48-72 h postpartum. Serum Ca levels decrease to 5.5 mg/dL, with the subsequent alteration in homeostasis[14-15]. This disease causes great economic losses in dairy production units, mainly due to the cost of treatments, secondary complications and the deaths it causes[13]. Among the risk factors for hypocalcemia, the following are considered: 1) The age of the cow, 2) The high demand for Ca to produce colostrum and milk, 3) The diet consumed during the transition period. Animals recovered from puerperal hypocalcemia produce 5 to 15 % less milk in that lactation[14-15]. That is, the homeostasis of Ca[16] alters, mainly affecting highly producing cows, showing loss of appetite, decubitus and lethargy. Its incidence varies from 5 to 7 %[14-21] and increases as lactations progress. Calcium is related to muscle contraction. During milk fever, muscle contraction and tone in the gastrointestinal and cardiovascular systems are not maintained, and it can cause the death of the animal. Immune function decreases[2,22], and the risk of postpartum diseases such as mastitis, fetal membrane retention (FMR), metritis and abomasum displacement increases[11-14,23-26]. The clinical signs of hypocalcemia are divided into three phases[11]. In phase I, the cow does not show paresis, it may even go unnoticed, its signs are tenuous and transient; it is hypersensitive, nervous, excitable, with muscle tremors, anorexia, ataxia and general weakness. Some cows lose weight quickly and drag their hind limbs. The animal avoids walking or moving, does not feed, body temperature can be normal, and it can remain several hours in that state. Some cows show clinical signs of hypocalcemia similar to those described, but without having calved. This alteration usually occurs after periods of stress or decrease in dry matter consumption. This condition is more common in cows in estrus or heat, with severe digestive

disorders or severe toxic mastitis. Transient hypocalcemia may occur in cows with anorexia and low intestinal motility[17].

Phase II, (prodromal), it exhibits moderate to severe depression, partial paralysis and the characteristic sign of lying down with the neck bent and with the head directed towards the flank. The tetany observed in the first phase progresses to impossibility to get up, paresis and prolonged decubitus, cold extremities, dry muzzle and temperature higher than normal (36.5 to 38 °C); weak arterial pulse, heart sounds, barely audible, and moderate heart rate (80/min). Absence of rumen movements is detected, which can lead to states of secondary exhaustion.

Phase III is the most severe. The animal exhibits complete lateral decubitus. Severe cardiac depression, irregular pulse (almost imperceptible), decreased shallow breathing. Animals without an established therapy die in a few hours, with the manifestation of a state of shock. The diagnosis of milk fever is based on the history of the animal, age of the mother and serum calcium concentration. The decrease in serum magnesium and phosphorus levels may be associated with eosinopenia and lymphopenia (adrenal hyperactivity), but the latter are not specific. It is necessary to make the differential diagnosis with hepatic steatosis, septic endometritis, mastitis and acute rumen acidosis.

Subclinical hypocalcemia

It occurs when blood Ca decreases to levels less than 2.00 to 1.38 nM, but homeostasis continues[14]. The normal concentration of Ca is 8.5 to 10 mg/dL (2.1 - 2.5 nM). It can start 12-24 h after calving, when the lowest concentration of Ca is recorded, and increases with a greater number of lactations, affecting up to 50 % of cows[2,14,20,27-28]. That is why subclinical hypocalcemia is more expensive[29-30]. The decrease in blood Ca is related to the transmission of nerve impulses that lead to less muscle contraction; with lower rumen and abomasal motility, with the subsequent displacement of the abomasum and lower food consumption[14,31]. For example, with the reduction of the blood level of Ca to 7.5 and 5 mg/dl, the abomasal motility of cows decreases by 30 to 70 %, respectively[32]. Its effects on muscle contraction also prevent the effective closure of the mammary nipple duct (teat), which contributes to the occurrence of mastitis, and its biological and economic consequences[13-14]. In addition to the relationship of Ca with muscle contraction, Ca also affects immune function and insulin secretion[12]. The function of neutrophils decreases, as the cytosolic concentration of ionized calcium (iCa^{2+}) in peripheral blood mononuclear cells decreases[2,33]. Therefore, the severity of the problem will manifest itself with secondary disorders related to production and reproduction, such as retention of fetal membranes and metritis[12,30-33]. iCa^{2+} corresponds to approximately 50 % of total calcium, the rest is bound

to proteins and is biologically inactive. Cows with subclinical hypocalcemia decrease insulin secretion and increase blood glucose concentration[3,33-35]. Because the entry of glucose into peripheral tissues reduces, as happens in the period of insulin resistance during the postpartum period of the dairy cow[36]. The cytosol of pancreatic cells requires iCa^{2+}, which decreases during hypocalcemia, for the release of insulin[35-37]. The decrease in insulin allows the release of the lipase hormone, responsible for participating in lipolysis. This increases the plasma concentration of non-esterified fatty acids (NEFAs)[12,20,23,27,37-39] with its corresponding risk of ketosis[23-25] due to the increase of ketone bodies: acetone, β-hydroxybutyrate and acetoacetic acid in the bloodstream.

Immune function

The immune system contains cells and molecules with the ability to recognize and eliminate invading or foreign microorganisms; it is regulated by the cytosolic concentration of ionic calcium[8-10]. The [Antigen-Receptor] binding of the immune cell triggers a series of events characterized by the increase of iCa^{2+} in the cytosol and depletion of iCa^{2+} reserves in the endoplasmic reticulum; this continues with the obtaining of additional iCa^{2+} from the extracellular space[40]. Hypocalcemia reduces the cytosolic concentration of iCa^{2+} in the mononuclear cells of the blood, also reducing immune function[2]. The ATPase pumps for iCa^{2+} of the sarcoplasmic and endoplasmic reticula regulate the entry and replacement of iCa^{2+} in the endoplasmic reticulum[41]. The cytosolic increase of iCa^{2+} is needed for the adhesion of neutrophils to endothelial cells, their transmigration into tissues, chemotaxis and phagocytosis[42]. This could be altered in cases of the decrease in extracellular iCa^{2+}. In addition, control in the magnitude, amplitude and duration of the destination of iCa^{2+} in the immune cell is also required for the functions of immune cells[43]. Immunity can be innate and specific.

Innate immunity

Innate immunity is activated quickly and constitutes the first immune defense when the infection begins. It depends on phagocytes such as polymorphonuclear neutrophils, macrophages and mammary epithelial cells. Macrophages identify and recognize foreign pathogens, produce cytokines (interleukin-1γ, interleukin-6 and tumor necrosis factor-α) to begin the immune response, they also recruit polymorphonuclear neutrophils. In addition, they phagocytize and eliminate invading pathogens and constitute a bridge between the innate response and the specific response through the major histocompatibility complex class

II, to prepare T cells[44]. After the start of the inflammatory response, the predominant cells are polymorphonuclear neutrophils, which, through blood circulation, are directed by chemotaxis to locate the site of invasion[45]. Polymorphonuclear neutrophils, as well as macrophages, engulf and eliminate foreign microorganisms. In activated phagocytes, oxidative burst (respiratory burst) is triggered by the activation of the nicotinamide-adenine-dinucleotide phosphate (NADPH) enzyme that catalyzes the reduction of oxygen to the superoxide anion, extremely toxic to foreign microorganisms. Finally, foreign microorganisms are eliminated by exocytosis. In hypocalcemia[12,22], the function of neutrophils reduces, the percentage of neutrophils engaged in phagocytosis decreases[3,12,22,33-34], the mononuclear cellular response to the antigen-activated stimulus weakens[2] and the oxidative burst response reduces after incubation with pathogenic bacteria[3]. In neutrophils of cows with subclinical hypocalcemia, it has been observed that the cytosolic level of iCa^{2+} decreases more rapidly than in normocalcemic cows, therefore the influx of calcium is not sufficient to maintain and use the cytosolic iCa^{2+}, or replenish endoplasmic reticulum deposits, or both. This leads to a decrease in their ability to phagocytize and eliminate pathogenic bacteria[3]. The reduction of the immune response leads to the manifestation of other infections of bacterial origin such as mastitis[23-24] and metritis[12,46-48].

Specific immunity

It depends on antibodies, macrophages and T and B lymphocytes that recognize specific microorganisms[47]. This immunity is activated if the infection persists. T cells are subdivided into helper T lymphocytes and cytotoxic T lymphocytes. Helper cells produce cytokines, such as interleukin (IL-2) and interferon gamma (IFN-γ), crucial in the immune response. Cytotoxic T cells recognize and eliminate cells infected with an antigen, as well as predecessor immune cells or damaged cells, which, when present, increase the susceptibility of infection. B lymphocytes differentiate into plasma cells that produce antibodies or immunoglobulins (Igs): IgG_1, IgG_2 and IgM, or memory cells[47].

Metritis

Metritis (puerperal metritis) is a postpartum bacterial complication that can be caused by less contraction of the uterine muscle (myometrium), facilitating the entry and proliferation of bacteria in the uterus, or by less activity of immune cells. Both factors caused by hypocalcemia. This infection can lead to negative consequences on reproductive function during the postpartum period[48-49]. In the first 65 d postpartum, the percentage of gestation

at first service has been found in 39.4 % in cows diagnosed with metritis, 38.7 % in cows with clinical endometritis and 51.4 % in cows without uterine infection[50]. Immune function is compromised before metritis. Circulating neutrophils in these cows have shown a decrease in glycogen at the time of calving, and monocytes stimulated by the bacterium *Escherichia coli* have reduced the expression of tumor necrosis factor-α[51].

Metritis is characterized by an increase in uterine size, dark red and foul-smelling aqueous uterine discharges, associated with decay, loss of appetite, high heart rate, fever and decrease in milk production[52-54]. There are predisposing factors such as fetal membrane retention (FMR)[55], fetal maceration and dystocia[53-58]. The incidence of metritis ranges from 2.2 % to 37.3 %[59]. At the herd level, the factors of greatest risk for the occurrence of metritis are the size of the herd (greater in large herds), time of year (greater in November and April), number of calving (greater in animals of three calvings or less), dystocia and placental retention[60-61]. The process of infection is as follows: after calving, the cervix and cervical canal remain open for a few days for the expulsion of fluids and waste from the uterus, through the contraction of the uterine muscles[62-65]. This process is more efficient in normocalcemic *vs* hypocalcemic cows[60,66-69]. Hypocalcemic cows are more prone to retention and stagnation of uterine fluids and waste, and therefore to a greater risk of bacterial complications[60,66-69]. Stagnation of fluids and waste is an excellent medium for bacterial multiplication[70-73]. The opening of the cervix allows bacteria to enter the uterus, although their presence will not necessarily develop the infection. Bacteria have been isolated in most cows after calving[60,74-75], but it is controlled by the action of neutrophils and other leukocytes[57-60,67,76-79]. They migrate to the uterine lumen in response to the presence of bacteria and are generally able to control bacterial populations until the infection is eliminated. The cow remains healthy and has a normal postpartum period: milk production, and a new conception and gestation. The above, however, does not always happen. In some cows with subclinical hypocalcemia[12], neutrophils do not stop the infection, bacterial populations grow, and females have purulent and fetid discharges, characteristic of metritis[80-81]. In the diagnosis of gestation by rectal palpation, the uterus presents an increase in size and its inflammation suppresses postpartum follicular growth and development[79-82]. The cows have a fever and remain depressed and inappetent. The lack of adequate food consumption predisposes to the presence of other disorders such as abomasum displacement and the fatty liver complex/ketosis. If the inflammation continues, it usually progresses to endometritis, which greatly compromises the cow's fertility. Cows with subclinical hypocalcemia had a lower gestation rate and a longer interval from calving to conception compared to normocalcemic cows; the risk of metritis decreases with high levels of Ca in the blood[12].

Mastitis

Two models of mastitis transmission are recognized: environmental mastitis and contagious mastitis[83].

Environmental mastitis

Some normal microorganisms in the environment such as *Escherichia coli*, *Klebsiella* spp., *Enterobacter* spp., *Serratia* spp., *Pseudomona* spp., *Proteus* spp., and some gram-positive bacteria such as *Streptococcus uberis* and *Streptococcus dysgalactiae*, are the ones involved in causing environmental mastitis[84-87]. The cow uses innate immunity to combat environmental mastitis, with physical barriers such as the teat sphincter; chemical barriers such as keratin and lactoferrin, and immune system components such as macrophages, dendritic cells, mast cells, neutrophils, eosinophils and natural killer cells (NKCs)[88-93]. Hypocalcemia affects the teat canal and neutrophils. It can influence the immune system through the secretion of cortisol during calving. The teat canal is the first line of defense against mastitis because it is the pathway by which pathogens can enter the mammary gland. The canal is sealed between milkings and during the dry period by a keratin plug derived from the lining of the stratified epithelium of the canal. Probably, the main function of this waxy plug is to establish a physical barrier to prevent bacterial penetration. The teat has muscles in its sphincter that keep it closed between one milking and another. After milking, it takes two hours for the contraction of the sphincter and closure of the teat canal[94]. Calcium decreases at the time of calving, both in the blood circulation and in the internal deposits of blood cells[2]. Normally, the calcium recovers within a few days. In cows with hypocalcemia, this decrease is accentuated, which leads to other alterations linked to Ca. In cows with subclinical hypocalcemia, probably the teat sphincter remains distended for longer due to inefficient muscle contraction caused by Ca deficiency. In addition, when starting lactation, cows remain prostrate for long periods, compared to normocalcemic cows. This facilitates the entry of environmental pathogens through the teat canal, which reach the cistern of the mammary gland, where they proliferate and consequently induce mastitis[95]. Lactoferrin is a protein that exerts different functions related to innate immunity, is synthesized in neutrophils[96], and has a high affinity for iron (Fe; chelating activity), so it binds to free iron and reduces it. Microorganisms require Fe for their growth[97-99], its bacteriostatic effect prevents bacterial proliferation[100-101], although lactoferrin can also act as a bactericide[102]. In hypocalcemia, the function of neutrophils reduces[3,22], and the activity of lactoferrin decreases, generating a higher incidence of mastitis in hypocalcemic cows.

Hypocalcemia can reduce immune function through cortisol at the time of calving. The fetus starts the calving in the cow, stimulating the hypothalamic-pituitary-adrenal axis and increasing the secretion of cortisol. Cortisol changes the steroidogenic pathway, instead of directing it towards the synthesis of progesterone (P4), it directs it towards the synthesis of estradiol (E2). As a result, the synthesis of progesterone reduces and the synthesis of estradiol increases, inducing calving. Cortisol secretion increases considerably in cows with hypocalcemia. Cortisol secretion is higher in hypocalcemic cows than in normocalcemic cows[103]. In addition, cortisol is considered a very potent immunosuppressive agent and probably increases the immunosuppression observed in the cow during peripartum[104], with the subsequent risk of occurrence of mastitis.

Contagious mastitis

The microorganisms involved in contagious mastitis are: *Staphylococcus aureus*, *Streptococcus agalactiae*, *Arcanobacterium pyogenes*, *Mycoplasma* spp.[105-106]. The spread of the bacterium responsible for the infection occurs during milking, due to practices such as the shared use of towels to wash and dry udders, through contaminated hands of milkers and use of teatcups of mechanical milking without disinfecting between cow and cow. The use of individual gloves or towels, as well as the milking separately and milking of infected cows to the end, with prior disinfection of the milking units, helps to prevent infection[107-109].

Treatment

Treatment should be applied immediately. The best option is to apply calcium orally to cows that are still standing. The blood calcium level increases over the course of 30 min after administration[110] and remains elevated for 4 to 6 h[110-111]. Intravenous treatment rapidly increases blood calcium levels, but this increase can be extreme and potentially dangerous, and can cause fatal cardiac complications, so it is not advisable to administer it in cows that are still standing[112]. After intravenous treatment, the level of blood Ca decreases again to lower than normal concentrations; consequently, the cow again shows hypocalcemia in a period of 12 to 18 h[112-113]. Even the dosage of Ca intravenously suspends the animal's ability to mobilize the necessary Ca and meet the requirements at critical times[111-113]. Experimentally, atropine-induced arrhythmia has been reversed by alternating states of hypercalcemia and hypocalcemia in dairy cows[114]. For cows in phases II and III of clinical disease, 500 ml of 23 % calcium gluconate solution should be administered immediately slowly intravenously. This provides 10.8 g of elemental calcium, which is enough to correct

the total calcium deficiency in the cow (4-6 g). The administration of Ca intravenously is little recommended[115]. In cows that respond favorably to treatment, it is important to reinforce it with oral administration 12 h after recovery, to avoid relapses[111].

Prevention

Hypocalcemia is prevented by manipulating the diet and administering calcium orally[113-119].

Diet manipulation

Low-calcium diets (LCDs) are administered, and the ration is adjusted to meet nutritional needs considering the dietary cation-anion difference (DCAD). Feeding with LCDs leads to transient hypocalcemia, with subsequent reabsorption from bone tissue and increase in absorption from the small intestine and increases in calcium availability[120]. Rations with 8 to 10 g of calcium per day produce favorable effects for the aforementioned purpose[120]. The use of anionic salts to reduce hypocalcemia is based on their acidogenic nature, which causes digestive and metabolic acidity, and generates optimal conditions for the circulation of Ca in the body[121-122]. Another dietary strategy to reduce the occurrence of hypocalcemia consists of providing a ration deficient in Ca before calving. This causes a negative Ca balance in the cow before calving and stimulates the secretion of parathyroid hormone (PTH) and 1,25-dihydroxyvitamin D, promoting Ca homeostasis at calving. In the field, it is recommended to provide prepartum rations with reduced levels of Ca (approximately 0.5 % of Ca)[123-124]. The acidification of the pH in the rumen and intestine leads to the increase of the solubilization of Ca; acidosis promotes the activation of parathormone (PTH), and this in turn participates in the absorption of intestinal Ca[124-125]. Acidity increases the function of osteoclasts, responsible for bone resorption, transferring iCa^{2+} from the bones to the blood circulation and increasing the excretion of Ca in the urine[125-127]. Cows fed a negative DCAD diet in the prepartum increase the blood concentration of iCa^{2+}[127]. Under normal conditions, the body maintains a pH between 7.35 and 7.45[128], through various physical-chemical regulatory mechanisms, such as: the buffer systems of the plasma (bicarbonates and proteins) and bone tissue. There are other physiological regulators such as the elimination of CO_2 by respiratory route to the detriment of bicarbonates, the elimination of acids through the kidneys and the reabsorption of bicarbonates. The concentration of ions (milliequivalents) establishes an equality in the different media, the sum of anions (negatively charged ions with acid nature) is equal to the sum of cations (positively charged ions with basic nature). Based on the fact that Na^+, K^+ and Cl^- ions (bioavailable ions that cannot be metabolized in

simpler forms) determine the acid-base balance of the plasma medium and the acidogenic function of sulfates ($SO^{=}$; they directly acidify biological fluids)[129], these four ions are taken into account for the calculation of the cation-anion balance of the raw material and the formulation of diets for dry cows[117-118,125]. By calculating the dietary cation-anion difference (DCAD), the diet can be formulated on the ionic balance and pH of the plasma medium[117-118]. DCAD is defined as the difference between cations and anions. Feeding cows with a negative DCAD diet to dry cows at the end of gestation increases anions ($SO4^{=}$ and Cl^{-}), so the blood buffer capacity alters, and the blood acidifies[125]. In response, the body releases cations (H^{+}) to neutralize anions and maintain electroneutrality. This causes the pH to decrease and generates acidity in the urine and greater excretion of Ca; the level of iCa^{2+} in the blood circulation reduces and stimulates the secretion of PTH and this in turn participates in the active formation of 1,25 dihydroxyvitamin D3 ($1,25(OH)_2$ D3) and in the mobilization of bone Ca[4,130] with the increase in the concentration of iCa^{2+} in the blood[123-125,130]. In addition, cows fed negative DCAD diets increase their concentrations of serotonin[127], which is important for the function of the mammary gland during lactation. Based on the above, the use of DCAD reduces the incidence of hypocalcemia[34,116-118,124,131-132], with the subsequent increase in leukocyte function and reproductive health[34]. A problem with anionic salts is their low palatability, as they reduce feed consumption and predispose to other eating disorders such as low energy intake in the transition period. Fortunately, the new DCAD are more palatable and avoid this situation. Another disadvantage is their cost, but the cost-benefit of their use must be analyzed[133-137].

Oral administration of calcium

The favorable effect of oral calcium for the prevention of hypocalcemia in dairy cows has been demonstrated, even having access to rations with anionic salts or in herds with low incidence of milk fever cases[119]. When the animal consumes less Ca than required, the absorption of Ca increases. On the contrary, when the animal consumes more Ca than required, the absorption of Ca decreases[138]. Events that cause changes in efficient absorption of Ca begin with changes in plasma Ca but depend on the control of the active metabolite of vitamin D_3, known as 1-25 dihydroxyvitamin D3 [1-25-$(OH)_2$ D_3]. Although there is evidence of pre-duodenal absorption of Ca, the greatest absorption of Ca occurs in the duodenum or upper part of the small intestine[139]. The transfer of Ca through the intestinal villi occurs by facilitated transport and is initiated by 1,25-$(OH)_2$ D_3, which enters the enterocyte by means of cell diffusion and binds to its receptor in the cellular cytoplasm[140]. The 1,25-$(OH)_2$ D_3 receptor complex moves to the chromatin fraction in the cell nucleus and this hormone-receptor complex synthesizes more messenger RNA and specific proteins that regulate Ca transport.

There are several components that limit the bioavailability and absorption of Ca. Oxalates, which could reduce the amount of Ca in hays and alfalfas, or low levels of phosphorus (P) in the diet, as well as high levels of magnesium fluoride, concentration of lipids in the diet, or by nucleic acids produced by bacteria or bacterial cell walls[138]. There are compounds such as calcium chloride ($CaCl_3$) that have the ability to maintain the concentration of blood calcium[110-111], this is due to its bioavailability and its ability to stimulate the acid response in the cow, which increases its own mobilization of calcium[110]. Good absorption is obtained with 50 g of elemental calcium dissolved in 250 ml of water. However, care should be taken with the dosage of calcium. There is a risk of inhalation, and it is very caustic for the tissues of the upper airways[110]. Calcium propionate is absorbed slowly, probably because it does not increase acidity. The administration of 75 to 125 g dissolved in water and propylene glycol offers good results[110-111]. Calcium carbonate dissolved in water is another presentation that has been evaluated, without satisfactory results since it does not increase the level of blood calcium[110], probably due to its low bioavailability. In addition, calcium carbonate produces an alkalogenic response, which acts in the opposite way to anionic salts and prevents the mobilization of bone calcium. To facilitate the dosage of calcium, the use of boluses with calcium chloride and sulfate has been studied. The bolus is administered immediately after calving and 12 h after. With this treatment, the ionic concentration of plasma calcium has been increased[134-136]. This bolus has the advantage of being palatable, Ca is not wasted, there are no risks of inhalation, and the release of calcium is slower and more effective. The application of calcium subcutaneously is not recommended because it causes irritation and necrosis in the tissues[11].

Serotonin

Serotonin regulates the physiology of the mammary gland during lactation. It is synthesized in various tissues of the body from the L-tryptophan amino acid, by the action of the tryptophan hydroxylase (TPH) enzyme to transform it into 5-hydroxytryptophan (5-HTP). Decarboxylase converts 5-HTP into serotonin[137-140]. In rodents, serotonin is synthesized in the intestine and other tissues[141-144], travels through the bloodstream and acts on the mammary gland. The mammary gland has receptors for serotonin[145-148]. In addition, it expresses the TPH enzyme[147] and synthesizes serotonin during lactation[148-151]. Serotonin stimulates the synthesis and secretion of parathyroid hormone-related protein (PTHrP) in the mammary gland[145-146,151-157] and participates in the expression of calcium-sensitive receptors (CaSRs) during lactation[158-160]. PTHrP is secreted into the maternal circulation and acts on bone cells to stimulate bone resorption in osteoclasts, releasing calcium into the systemic circulation[142], destined for the mammary gland[160]. The addition of 5-HTP, the precursor of serotonin, to the feed ration, during the gestation-lactation transition period in

rodents, increases the circulating concentration of serotonin, PTHrP and Ca, as well as the content of Ca in milk[151]. PTHrP, unlike PTH, acts as a paracrine regulator and is located in the circulation during lactation or humoral hypercalcemia[160]. Therefore, like PTH, PTHrP acts as a hormonal regulator of Ca and is important for the homeostasis and mobilization of Ca during calving and lactation[161-162]. In addition, PTHrP is detected in blood only during lactation. It decreases its production, plasma concentration and the preservation of bone mass[159]. The zero serum concentration of PTHrP during lactation is restored with the application of 5-HTP over the course of 1 h[146]. This demonstrates the importance of 5-HTP in the production of PTHrP during lactation and in the increase of blood Ca[152-153]. CaSR identifies variations of extracellular free calcium, its ions bind, and the link for cellular response is established in various organs[163-167]. During lactation, the production of PTHrP is inhibited and the transport of calcium to milk is stimulated, it is activated with the increase of calcium in the circulation[158,168]. Therefore, the mammary gland acts as a calcium-sensitive organ during lactation, which responds to changes in its extracellular concentration, mainly through the calcium-sensitive receptor. Which identifies the concentration of Ca in the blood circulation and together with the PTHrP regulates its level in the blood[159]. Serotonin helps maintain calcium homeostasis in lactation. PTHr releases calcium from bone tissue into the circulation, and activates CaSRs, which have negative feedback on PTHrP. Consequently, the stimulation on the osteoclasts is suspended, that is, their release from bone tissue is decreased. CaSR also promotes the transport of blood calcium to milk. Consequently, it reduces Ca in the blood and causes greater secretion of PTHrP in the mammary epithelial cells, increasing calcium reabsorption. Therefore, the mammary gland regulates its own calcium requirements under a negative feedback system[159] that allows it to maintain its calcium requirements during milk production. In dairy cattle, a process similar to that of rodents may occur. There is expression of serotonin receptors in the mammary epithelium[144,169]. The circulating concentration of serotonin on the first day of lactation has been positively correlated with the circulating level of Ca in dairy cows[153-156], as well as throughout most of lactation[170]. The intravenous application of 5-HTP, the precursor of serotonin, administered at the end of lactation in non-pregnant Holstein cows[157] and at the end of gestation in pregnant cows[148-151] has increased the systemic level of serotonin and calcium, and has decreased the elimination of calcium in the urine, increasing the concentration of calcium in milk and colostrum. The effect of serotonin is independent of parathyroid hormone[151]. In addition, PTHrP has been previously identified in the cow's circulatory system[170-172].

Conclusions

Hypocalcemia can occur in the peripartum due to alterations in calcium homeostasis when the blood and cytosolic concentration of Ca decreases. Hypocalcemia can occur clinically and subclinically. The reduction of calcium leads to a decrease in immune function and in smooth muscle contractions, increasing the risk of metritis and mastitis, among other alterations. Clinical hypocalcemia is treated with intravenous calcium and subclinical hypocalcemia with oral calcium. Prevention requires the addition of anionic salts in the ration and the addition of calcium orally. In addition to inducing mild prepartum hypocalcemia to stimulate the secretion of parathyroid hormone (PTH) and of 1,25-dihydroxyvitamin D and thus induce Ca homeostasis after calving. Efficient absorption of Ca depends on the plasma Ca level and on the active metabolite of vitamin D_3, called 1,25-dihydroxyvitamin D_3 [1-25-$(OH)_2$ D_3]. During lactation, serotonin participates in maintaining calcium homeostasis through the synthesis and secretion of parathyroid hormone-related protein (PTHrP), and this effect is independent of the action of parathyroid hormone.

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Table 1: Blood levels of calcium (Ca) in dairy cows exposed to different treatments and conditions of the production system

Country	Treatment	No. of animals	Ca (mg/dl)	Subclinical hypocalcemia	Clinical hypocalcemia	FMR[1] (%)	Fallen cows (%)	Ref[2]
Argentina	Control	240	7.11			9.4	12.5	173
	Treatment[3]	280	9.32			6.0	0.6	
United States of America	Control[4]		3.8		82 %			174
	Treatment[5]		4.3		30 %			
Mexico	Control	10		80 % (8/10)	0 % (0/10)			175
	Treatment	10		40 % (4/10)	0 % (0/10)			
	System:							
Spain and Uruguay	Intensive	256	10.96 ± 0.06					176
	Silvopastoral	354	9.35 ± 0.09					
Chile		76	2.0-2.6 mmol/L		51 %		26	177
	Prepartum:							178
	1986-2002[6]	471	2.37 ± 0.14					
	2003-2011	270	2.29 ± 0.18					
	Postpartum:							
	1986-2002	1041	2.35 ± 0.14					
	2003-2011	766	2.27 ± 0.12					
Costa Rica	Breed:							
	Holstein	49	7.85	27 (55 %)	2 (4 %)			179
	Jersey	62	7.49	31 (50 %)	8 (13 %)			
	Guernsey	41	8.06	18 (44 %)	0 (0 %)			
Colombia	Dairy production:	Stage:						180
	Low	Prepartum	2.14 ± 0.10					
		Postpartum	2.39 ± 0.10					
	High	Prepartum	2.42 ± 0.11					
		Postpartum	2.40 ± 0.12					

[1]FMR= Fetal membrane retention (%).

[2]Ref= Bibliographic references.

[3]Treatment: mixture of mineral salts (150g Cl_2Ca, 150g $NHSO_4$, 29g MgO_2).

[4]Control= Diet with +50 meq/kg;

[5]Diet with -250meq/kg.

[6]Periods (years).

Table 2: Comparison of incidence of cases of subclinical hypocalcemia and clinical hypocalcemia in dairy herds in different countries.

Country	**Breed**	**No. of animals (n=)**	**Plasma levels of calcium (mg/dL)**	**Subclinical hypocalcemia (%)**	**Clinical hypocalcemia (%)**	**Ref.[1]**
Costa Rica	Holstein	49	7.85	27 (55)	2 (4)	180
(Grazing)	Jersey	62	7.49	31 (50)	8 (13)	
	Guernsey	41	8.06	18 (44)	0 (0)	
Costa Rica	:	No. of animals	No. of calvings	Subclinical hypocalcemia (%)	Clinical hypocalcemia (%)	181
(Grazing)	Jersey	454	1	25	1	
		447	2	41	4	
		291	3	49	6	
		166	4	51	10	
		72	5	54	8	
		32	6	42	13	
United States	Holstein		No. of calvings	Subclinical hypocalcemia (%)	Clinical hypocalcemia (%)	182
(Housed)			1	53	6	
			2	42	13	
			3	78	2	
			4	44	29	
			5	47	29	
			6	63	25	
Mexico	Holstein		No. of calvings	Risk indices FMR[2]	Hypocalcemia	183
(Housed)			1-2	0.68	0.31	
			3-4	1.33	0.32	
			5-6	1.57	5.80	
			7-8	1.65	3.37	
			>9	1.12	2.14	

[1]Ref= Bibliographic reference.

[2]FMR=fetal membrane retention.