Review Article

DOI: 10.22067/veterinary.v9i2.69198

Received: 2017-Oct-05
Accepted after revision: 2017-Dec-05
Published online: 2018-Jan-20

Subclinical Hypocalcemia in Dairy Cows: Pathophysiology, Consequences and Monitoring

Hesam A. Seifi[a,b], Samuel Kia[a]

[a] Department of Clinical Sciences, School of Veterinary Medicine, Ferdowsi University of Mashhad, Mashhad, Iran
[b] Center of Excellence on Ruminant Abortion and Neonatal Mortality, Ferdowsi University of Mashhad, Mashhad, Iran

Keywords

milk fever, hypocalcemia, subclinical, dairy cow, monitoring

Abstract

Milk fever and subclinical hypocalcemia are the most important macro-mineral metabolic disorders that affect transition dairy cows. Many studies have shown that cows with subclinical hypocalcemia are also prone to many diseases and disorders. The drain of Calcium (Ca) during early lactation represents a significant increase in Ca demand over that for late fetal growth and physiological maintenance. The requirements of the mammary gland for Ca often exceeds the ability of the cow to replenish the plasma Ca pools. Blood Ca concentrations remarkably decline in dairy cows around calving, with the lowest concentrations occurring about 12 to 24 hours after calving. To maintain Ca homeostasis after calving, at the start of lactation, Ca compensating mechanisms are activated. These mechanisms involve a coordinated effort among the hormones 1,25-dihydroxyvitamin D3, parathyroid hormone (PTH), and calcitonin. Hypocalcemia is associated with an increased risk of several important health conditions such as mastitis, retained placenta, metritis, abomasum displacement and immune insufficiency, particularly in transition period. The incidence of subclinical hypocalcemia approaches 40-50% in multiparous cows after calving in dairy herds. In spite of developments in preventive approaches, tremendous economical impact of hypocalcemia on health, production and fertility of dairy cows is a major concern for dairy herd owners. The paramount advances in dairy health have been the paradigm shift from treatment of clinical illness to disease prevention and redefining disease more broadly, to include subclinical conditions. Herd-based tests are now available for use in routine herd monitoring and for investigating dairy herds with metabolic subclinical problems. This review provides the criteria for hypocalcemia monitoring and interpretation of the results in dairy herds.

IRANIAN JOURNAL OF VETERINARY SCIENCE AND TECHNOLOGY

Tel: +98 51 3880 3742 *Fax:* +98 51 3876 3852
Web: ijvst.um.ac.ir *Email:* ijvst@um.ac.ir

Corresponding author:

Hesam A. Seifi
Department of Clinical Sciences, School of Veterinary Medicine, Ferdowsi University of Mashhad, Mashhad, Iran
Center of Excellence on Ruminant Abortion and Neonatal Mortality, Ferdowsi University of Mashhad, Mashhad, Iran
E.mail: haseifi@um.ac.ir
Tel: +98 51 3880 5623

Abbreviations

Ca: Calcium
PTH: Parathyroid Hormone
DCAD: Diatary Cation-Anion Difference
BHBA: Beta-Hydroxybutyrate;
NEFA: Non-Esterified Fatty Acids

Introduction

During the last decade, dairy industry has undergone a wide range of major changes. Milk production has steadily risen in average production per cow and substantially in greater total annual milk production. The modern high-producing dairy cow will produce about 40 to 50 kg of milk per day in early lactation; and production as high as 60 kg/d is not uncommon [1]. This sharp and tremendous increase in milk yield has imposed great impact on metabolic capacities of dairy cows [2]. Metabolic disease incidence typically escalates as milk production rises and as herds become larger [3]. Metabolic diseases of dairy cows are manifestation of the cow's ability to cope with the metabolic demands of high milk production. The etiology of those metabolic diseases can be traced back to insults that occurr during transition period.

The biggest advance in dairy health in the last 25 years has been the paradigm shift from treatment of clinical illness to disease prevention. Health management has been defined as the promotion of health, improvement of productivity, and prevention of disease in cows within the economic framework of the owner and industry. Another major advance has been redefining disease more broadly, to include subclinical conditions (e.g., subclinical hypocalcemia, subclinical mastitis, ketosis) [4].

Milk fever and subclinical hypocalcemia (total blood Ca ≤ 2.0 mmol/L) are the most important macro-mineral metabolic disorders that affect transition dairy cows [5]. On average 5-10% of dairy cows succumb to clinical milk fever [6], with the incidence rate of subclinical hypocalcemia has been recorded at 30-50% on the day of calving [7, 8]. Both forms are associated with an increased risk of mastitis, retained placenta, endometritis, slower uterine involution, delayed first ovulation after calving, ketosis, displaced abomasum and reduced gastrointestinal motility [5]. Therefore increased incidences of these conditions suggest there may be a Ca problem in transition cows. The objective of this review article is to cast light upon the recent advances and findings in physiopathology and consequences of hypocalcemia and also will focus on strategies for testing and monitoring subclinical subclinical hypocalcemia.

Calcium homeostatic mechanisms

Ca is essential for life in animals. It is involved in many fundamental biological processes in the body, such as bone formation, muscle contraction, nerve transmission, and blood clotting, and it serves as a second messenger regulating the actions of many hormones. Therefore, it is of major importance that Ca concentration is regulated within a narrow range [9, 10]. In mammals, this process involves a coordinated effort among the hormones 1,25-dihydroxyvitamin D3, parathyroid hormone (PTH), and calcitonin [10]. Homeostatic control of Ca concentrations in blood is so strong that variations are small and do not reflect dietary intake at all [11].

The skeleton of a 600-kg cow contains approximately 8.5 kg of Ca. There are 3 g Ca in the plasma pool and only 8 to 9 g Ca in all the extracellular fluids of a 600-kg cow. Blood Ca in the adult cow is maintained between 2.1 and 2.5 mmol/L (8.5 and 10 mg/dL) and is slightly higher in young animals [12]. About 50% of the blood Ca is bound to proteins such as albumin, less than 10% is in mineral complexes with inorganic phosphates and the remainder exists in the ionized form [13]. The ionized Ca concentration is the biologically active form of the Ca in blood [14] and is most important for immediate metabolic function [15]. During acidosis, larger numbers of protons compete with Ca (and with other cations) for binding to anionic sites of plasma proteins such as albumin. This drives more protein-bound Ca into solution, thereby increasing the ionized Ca concentrations. Conversely, alkalosis decreases the ionized Ca concentrations [13]. Cows that are alkalotic from upper gastrointestinal obstruction or other conditions may have normal total Ca while exhibiting clinical signs of hypocalcemia [15]. Under acidic conditions, the ionized portion of Ca in the blood is closer to 48%; under alkaline conditions, it is closer to 42% ionized. The final 3% to 7% of Ca in blood is bound to soluble anions, such as citrate, phosphate, bicarbonate, and sulfate [12]. If total serum proteins are greatly reduced (hypoalbuminemia), it is possible to have low total Ca in the blood and relatively normal levels of ionized

Ca in the blood. Appropriately measured ionized Ca is the gold standard to evaluate physiologically active forms of Ca in a patient. Adjusted formulas were suggested to correct Ca concentrations in regard to albumin or total protein variations in dairy cows, when total Ca is measured [16]. However, serum total Ca concentration has routinely been the primary source for evaluation of Ca abnormalities in dairy cows.

Dairy cows in early lactation producing colostrum (containing 1.7-2.3 g Ca per kg) or milk (containing 1.2 g Ca per kg) [9] typically secrete 30 to 40 g of Ca each day. The total amount of Ca in plasma and extracellular fluids is estimated to be 12 g. Thus, body experiences negative Ca balance with initiation of lactation in dairy cows.

Blood Ca concentration remarkably declines in dairy cows around calving, with the lowest concentrations occurring about 12 to 24 hours after calving [17, 18]. Blood samples obtained at this time can reveal the extent of hypocalcemia experienced by a dairy herd [12,19].

The drain of Ca during early lactation (30–50 g/day) represents a significant increase in Ca demand needed for late fetal growth (10-15 g/day) [20, 21]. During the dry period, the supply of Ca through the diet is usually more than requirements of dam and fetus. Thus, passive Ca transfer from intestine is adequate to maintain homoeostasis without activating the Ca mobilization system, which is usually not activated until parturition [12, 19]. The main site for Ca absorption is assumed to be the small intestines, at least at moderate Ca intakes [22]. The capacity for cows to absorb Ca through the rumen and abomasum is uncertain [23, 24].

The demand of the mammary gland for Ca often exceeds the ability of the cow to replenish the plasma Ca pools, resulting in an acute decrease in the plasma Ca concentration in most cows [20]. To maintain Ca homeostasis after calving, at the start of lactation, Ca-maintaining homeostatic mechanisms are activated. These mechanisms include: increased Ca reabsorption in the kidneys, increased Ca absorption in the intestine and Ca withdraw from bone [17]. Two hormones, 1,25 dihydroxy cholecalciferol ($1,25(OH)_2$ D3) and parathyroid hormone (PTH) are involved in each of these processes (Figure 1).

Parathyroid hormone (PTH) - As Ca concentrations in the plasma decline below 10 mg/dL, the parathyroid glands are stimulated to secrete PTH [10]. PTH is an 84 amino acid peptide that binds to receptors located on the surface of its target tissues. The primary target cells are bone osteoblasts and osteocytes as well as renal tubular epithelial cells [12].

Bone Ca mobilization and renal tubular ab-

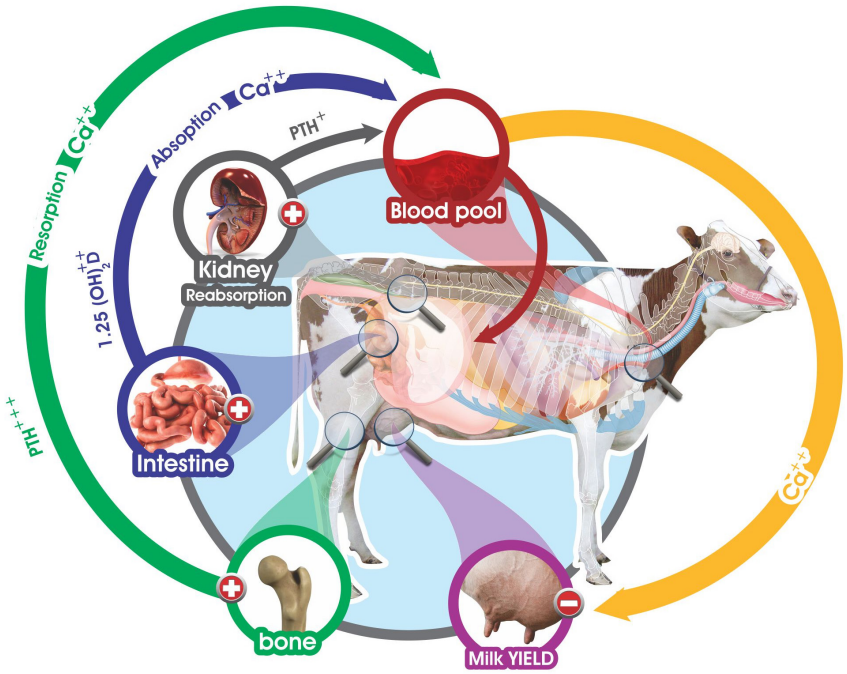

Figure 1
Ca homeostatic mechanisms in dairy cows in response to Ca outflow into milk during very early lactation (illustration by Taraneh Ebnalnassir).

sorption is enhanced by PTH. Release of PTH quickly promotes renal tubular reabsorption of Ca from the glomerular filtrate. But only small amounts of Ca are lost in urine (<1 to 2 g/day in the cow), so this action of PTH is sufficient to restore normal blood Ca concentration only if the Ca deficit is small [17]. PTH receptors are found on the osteoblasts that line all bone surfaces. Larger Ca deficits cause prolonged secretion of PTH (hours to days), which stimulates osteoclastic resorption of bone Ca to use skeletal Ca to make up the deficit [19]. The osteoblasts respond to PTH by secreting cytokine factors [25], which cause preexisting osteoclasts to begin to resorb bone collagen to release Ca and cause osteoclast progenitor cells to differentiate and become new osteoclasts. It generally takes several days for osteoclastic bone resorption to become fully active [26].

The dairy cow is programmed to go into a state of lactational osteoporosis, mobilizing bone Ca to help her achieve normocalcemia in early lactation [17]. This will typically result in loss of 9–13% of her skeletal Ca in the first month of lactation [27], which is reversible in later months of lactation.

Vitamin D - A second hormone, $1,25(OH)_2$ D3, is required to stimulate the intestine to efficiently absorb dietary Ca. Two modes of intestinal Ca absorption have been proposed: one is a passive and paracellular mode that occurs at luminal concentrations of Ca > 2 – 6 mmol/L and the other mode is an active transcellular process via the action of the active form of vitamin D [28]. This hormone is made from vitamin D by the kidneys – but only in response to an increase in blood PTH [17]. The final hydroxylation of vitamin D in the kidney is regulated by PTH [21]. PTH -elevated 1α hydroxylase enzyme activity in kidney and this enzyme have important roles in the conversion of $25(OH)_2$ D3 to $1,25(OH)_2$ D3. In addition to PTH, low blood Ca, can activate mitochondrial 1α-hydroxylase in the kidney to convert $25(OH)_2$ D3 into $1,25(OH)_2$ D3 [19]. On the other hand, high level of blood inorganic phosphorus (IP) inhibits the action of 1α-hydroxylase and production of active form of vitamin D. During late gestation, $1,25(OH)_2$ D3 is elevated 2- to 3-fold in most species [29-31] and, during early lactation in the bovine, can reach concentrations 10-fold above normal for 2-3 days postpartum [32].

Metabolic adaptation mechanisms for Ca are not rapid enough at the onset of lactation; cows require about to 1 to 2 days to maximize Ca inflow from the gastrointestinal tract and from bone to the mammary gland. Consequently, almost all cows experience some degree of hypocalcemia during the first days after calving but plasma Ca concentration returns to normal within 2 to 3 days [9, 12, 19].

Factors disrupting Ca homeostasis

Age - Age has a profound effect on susceptibility of dairy cows to hypocalcemia. Older cows are affected by hypocalcemia more common and more severe than young cows. The Ca output, however, does not explain the increase in prevalence of hypocalcemia within multiparous cows with increasing age as their colostrum yield is not different [33]. The age and parity-associated susceptibility might be related Ca homeostatic mechanisms. With increasing age, the hemostasis process is impeded and results in moderate to severe hypocalcemia. It has been assumed that the number of vitamin D receptors in intestines decline with increasing age [34]. In addition, as animals age increase, the number of receptors for PTH on target tissues decline [35].

Metabolic alkalosis - Current evidence suggests that milk fever and hypocalcemia may occur in cows as a result of excessive dietary cations. The typical ration of dairy cows are rich in cations (primarily potassium and sodium) and has lower amounts of anions (chloride and sulfur). These cations induce a metabolic alkalosis in the cow that impairs Ca homeostatic mechanisms via attenuated responsiveness of tissues to PTH [36-39]. It has been shown that cows in a more acidotic state, which can be measured by the pH in the urine, have a decreased risk of developing milk fever [40].

In cows fed diets rich in cations, a greater number of positively charged cations enter the blood than negatively charged anions and results in an electrical disparity. To restore electroneutrality to this positively charged blood, a positive charge in the form of a hydrogen ion (H^+) is lost from the blood compartment and the pH of the blood is increased. Adding readily absorbable anions to the diet increases the total negative charges in the blood, allowing more H^+ to exist and a decreased blood pH, restoring tissue sensitivity to PTH [9].

Under normal conditions, PTH released in response to hypocalcemia interacts with its receptor, located on the surface of bone and kidney cells. This stimulates G-proteins and adenylate cyclase

resulting in production of cyclic AMP, which acts as a second messenger within the cytosol of target cells. This initiates mechanisms such as bone Ca resorption and renal production of $1,25(OH)_2$ D3 to restore blood Ca concentration to normal levels. In alkalotic conditions induced by high cationic diets, the shape of the PTH receptor protein is changed so that it is less able to recognize and bind PTH, resulting in failure to activate the cell by producing cyclic AMP. Furthermore, Mg is required for full function of the adenylate cyclase complex [17].

Hypomagnasemia - Hypomagnesemia affects Ca metabolism by reducing PTH secretion in response to hypocalcemia, and by reducing ability of PTH stimulated cells to produce cyclic AMP, resulting in failure to activate the target tissues to PTH [9, 17]. On the contrary, serum Ca and magnesium concentrations are negatively associated. Cows suffering from hypocalcemia have higher serum magnesium concentration [33]. In a period of low serum Ca concentration, PTH is secreted into the blood. PTH secretion raises the threshold for renal magnesium excretion, resulting in a higher serum magnesium concentration [17, 41].

High dietary Ca – Before calving, the approximate daily requirement for Ca is only 30 g, comprising 15 g in fecal and urinary loss and 15 g to fetal growth [21]. When supplying Ca far in excess of the daily requirements at dry period, the passive transfer of Ca is sufficient to overcome the needs of the cow and the fetus. Therefore, active hemostatic mechanisms of absorption and resorption of Ca become depressed. As a consequence, at calving when sudden massive demands for Ca occur, the cow is unable to rapidly return to hemostatic mechanisms and is susceptible to severe hypocalcemia until these mechanisms can be activated, which may take several days [42].

High dietary phosphorus – In dry cows, high dietary levels of phosphorus (more than 0.5% dry matter intake) increase the serum level of inorganic phosphorus, which has inhibitory effect on the renal enzyme (1α-hydroxylase) that catalyzes the conversion of vitamin D into its active form ($1,25(OH)_2$ D3) and thereby predisposes cows to hypocalcemia [43-45]. High dietary phosphorus has also been reported to have a negative affect on intestinal magnesium absorption, which further makes periparturient cows susceptible to hypocalcemia [9, 46].

Definition and incidence of hypocalcemia

Hypocalcemia may be clinical or subclinical. Clinical hypocalcemia, also known as milk fever, is a particular concern in the newly calved cow. Most of cases of milk fever occur within 24-48 hours of calving. Generally, cows with milk fever are recumbent and are unable to rise as a result of low blood Ca, whereas cows with subclinical hypocalcemia have no clinical signs [47].

The diagnosis of subclinical hypocalcemia is usually based on serum levels of Ca, and cut-off points at total Ca levels of 2.0 mmol/L (8 mg/dL) [8] and 1.88 mmol/L (7.5 mg/dL) [26] have been suggested. Such levels commonly occur in dairy cows soon after calving [8].

The overall incidence of milk fever found from a national dairy study in United States was 5% [8, 12]. Other field studies reporting incidence of milk fever from 1977 to 2007 found that the incidence in 10 North American studies was 3.45% (range 0–7%), in 10 European studies it was 6.17% (range 0–10%), and for 10 Australasian studies it was 3.5% (range 0–7%) [21]. Clinical milk fever was prevalent in 1, 4, 6, and 10% of first, second, third, and ≥ fourth lactation cows, respectively [48]. Prevalence of clinical milk fever in a recent German study was higher (13.4, 15.0, and 21.7% for fourth, fifth, and sixth parity, respectively) [33]. It is assumed that preventive strategies are more common in the United States. German farmers favored oral Ca supplementation more than anionic salts as a preventive strategy. They supplemented Ca subcutaneously or orally in 46.1% of the herds and only 8.7% of the herds used anionic salts to prevent hypocalcemia [33]. Based on the US agriculture ministry report, 68.9 and 27.6% of the herds used Ca products and anionic salts, respectively. The same report indicated that 20.7% of heifers and 27.6% of cows were fed anionic salts [49].

The incidence of subclinical hypocalcemia – blood Ca values between 2 and 1.38 mmol/L (8 and 5.5 mg/dL) during the periparturient period – is around 50% in older cows [50]. In a recent German study, 47.6% of multiparous cows suffered from subclinical hypocalcemia (less than 2 mmol/L) within 48 h after parturition [33]. This finding is in agreement with previous studies [8, 51, 52]. Subclinical hypocalcemia increased with age and was present in 41%, 49%, 51%, 54%, and 42% of 2nd–6th lactation cows, respectively (Figure 2) [8].

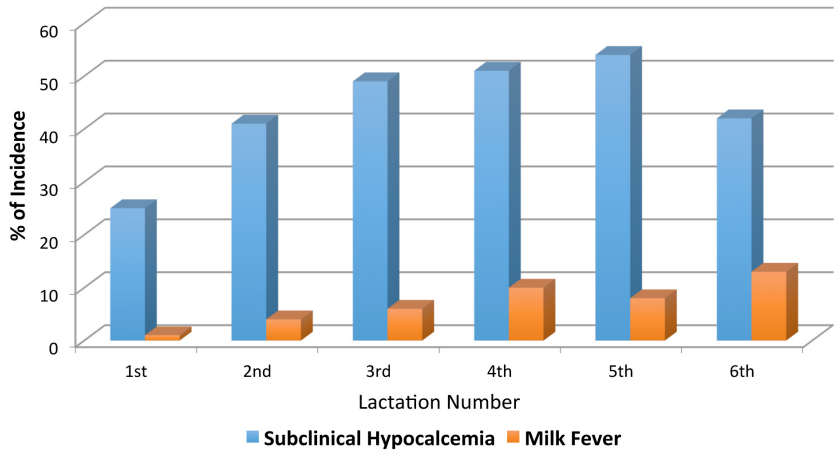

Figure 2
The percent of cows by lactation number that experienced a clinical milk fever or subclinically hypocalcemia are shown in the graph, adapted from Reinhardt et al. (2011) [8].

Forty percent of multiparous cows were subclinical hypocalcemic (blood Ca concentration of 2.0 mmol/L) in a five-year study on commercial dairy farms in Iran, which had annual milk production ranged from 10600 to 13000 kg (Figure 3). All the cows received anionic-supplemented rations in close-up period and sampled between 12 to 48 hours after calving (Seifi, HA; unpublished data).

In contrary to multiparous cows, there are conflicting reports on the prevalence of hypocalcemia in primiparous cows. Reinhardt et al. (2011) observed a prevalence of 25% from 480 herds in the United States [8]. However, hypocalcemia was infrequently found in primiparous cows (5.7%) in a German study [33], which is in agreement with a previously described prevalence of 2% for primiparous cows from 7 herds in Canada using the same threshold [52].

Pathophysiology of hypocalcemia

Ca is necessary for proper function of a wide variety of systems in the body from structural functions such as bone and other tissues to intracellular processes as a second messenger. Extracellular Ca is necessary for muscle contraction, nerve impulses, blood clotting, and is a component of milk and bone. Intracellular Ca is involved in second messenger systems for a wide variety of processes [53]. Hypocalcemia is considered as a gateway disease (Figure 4) and predisposes the cow to various metabolic and infectious disorders in early lactation [17] such as metritis [54], mastitis [55], abomasal displacement [56], and reproduction disturbances [57].

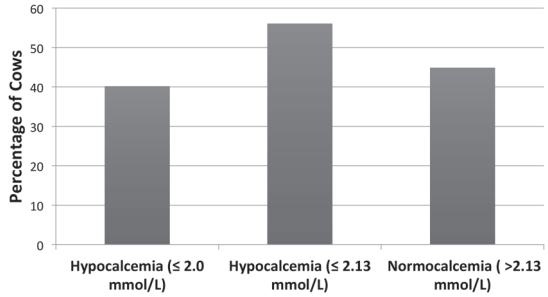

Figure 3
Percentage of subclinical hypocalcemic cows (with two cut-off levels) in Iran diary herds. All the cows received anionic-supplemented rations in close-up period and sampled between 12 to 48 hour after calving (Seifi, HA; unpublished data).

Immune suppression - It is well documented that nearly all dairy cows experience some degree of immune suppression during the transition period [58-61]. Contributing factors for immune suppression in transitional period consist of decreased polymorphonuclear leukocytes, glycogen stores, decreased blood Ca concentration and increased non-estrified fatty acids (NEFA) and ß-hydroxyl butyrate (BHBA) [58].

Ca is critical for proper immune cell function, which is very important in transition dairy cows. In a study by Martinez et al. (2012), numbers of neutrophils were reduced and their ability to undergo phagocytosis and oxidative burst was impaired in cows affected by hypocalcemia, which might in part explain the increased risk for infectious diseases [54]. Hypocalcemia is associated with decreased intracellular Ca stores in peripheral mononuclear cells [18]. This is the cause of a blunted intracellular Ca release response to an immune cell activation signal [18, 62]. Kimura et al. (2006) concluded that intracellular Ca stores decreases in peripheral blood mononuclear cells before parturition and development of hypocalcemia. This decrease contributes to periparturi-

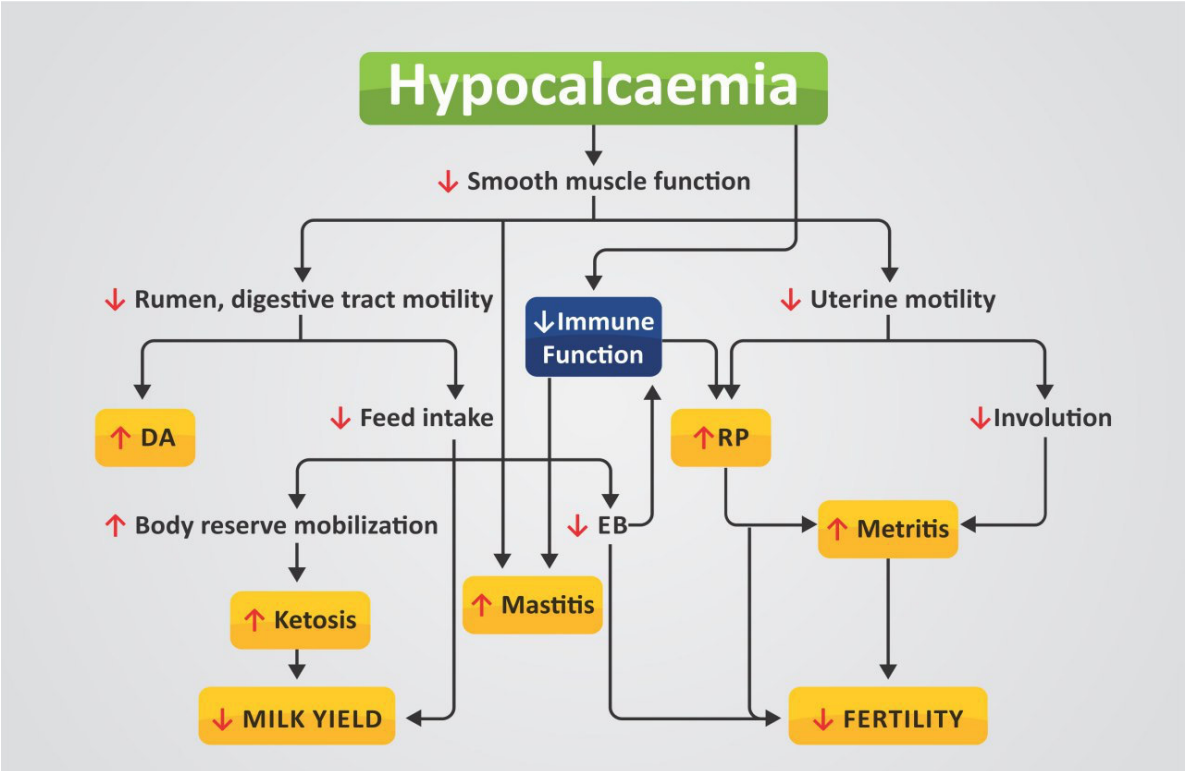

Figure 4
Hypocalcemia as a "gateway disease". Schematic view of consequences of hypocalcemia in dairy cows (modified chart from Howard, JL; Smith RA (1999) Current Veterinary Therapy: Food Animal Practice. W.B. Saunders).

ent immune suppression [18]. Ca also regulates cell polarity, which is required for directional cell killing, and it is also involved in the migration of leukocytes toward chemokines in the area of inflammation [63].

Feed intake and Weight loss - It has been shown that cows with subclinical hypocalcemia have impaired rumen and abomasum motility and depressed feed intake [64-66]. This reduction in ruminal and abomasal motility will likely cause a reduction in feed intake [47] and increased weight loss in early lactation [67]. Therefore, hypocalcemia may well exacerbate negative energy balance in cows that are already underfed [47].

Ketosis – Hypocalcemia has been attributed to the occurrence of ketosis [55]. Rodríguez et al. (2017) showed that hypocalcemic cows demonstrate 5.5 greater odds of having ketosis than normocalcemic cows [68]. The exact mechanism is unknown, however, the hypocalcemia impact on feed intake and resulting negative energy balance may be a factor in promoting of ketosis. Cows with naturally occurring hypocalcemia at parturition and experimentally induced hypocalcemia had elevated concentrations of NEFA and BHBA as indicators of increased lipid mobilization [8, 54, 62].

Lipolysis - Ca is also important in adipocytes for regulating lipid metabolism and triglyceride storage [69]. In studies with rat and human adipocytes, increased intracellular Ca has been shown to have antilipolytic effects [53, 70]. It can be speculated that hypocalcemia may deplete adipocyte Ca stores, resulting in increased lipolysis.

Abomasal displacement - It has been shown that hypocalcemia increases the risk for displacement of abomasum [56, 71]. Abomasal atony due to hypocalcemia seems to be a logical risk factor for abomasal displacement. Hypocalcemia may reduce abomasal tone and result in gas accumulation [65, 72, 73]. It was reported that 82% of the cows with displaced abomasum had Ca values equal or less than 2.0 mmol/L in the first week after calving [74]. There is also a report from one herd that subclinical hypocalcemia at calving was a risk factor for left displacement of abomasum [72]. Seifi et al. (2011) showed that the odds of the development of displacement of abomasum were 5.1 times greater in cows with serum Ca concentrations equal or less than 2.3 mmol/L in the first week post-partum [56]. Reduced Ca concentrations have been

associated with a reduction in rumen and abomasal motility, which in turn is thought to increase the risk of abomasal displacement [65]. It is likely that Ca concentration is an indicator of inadequate dry matter intake, which most likely contributes to the development of displacement of abomasum [71]. However, Leblanc et al. (2005) did not find a direct relationship between Ca concentrations and left displacement of abomasum incidence and suggested that subclinical hypocalcemia may be a function of decreased feed intake, resulting in other diseases such as left displacement of abomasum and subclinical ketosis [75].

Dystocia, uterine prolapse and retained placenta - Cows with clinical and subclinical hypocalcemia are at increased risk of dystocia, retained placenta and metritis [5, 47, 76]. The loss of muscle tone in the uterus due to hypocalcemia increases the incidence of dystocia, uterine prolapse and retained placenta [5]. It has been reported that milk fever affected cows are up to three times more likely to develop dystocia [47]. In some cases the increased odds of dystocia were reported as six times in hypocalcemic cows than that of normal ones [55, 76, 77]. Furthermore, dystocia can increase the risk of occurrence of retained placenta.

The association of retained placenta and hypocalcemia has been reported [5, 78]. Erb et al. (1985) determined that cows with hypocalcemia were two times as likely to have retained placenta [76]. Another study showed that cows with retained placenta had lower plasma concentrations of Ca at parturition and up to 7 days after parturition than cows without retained placenta [79]. In addition, it has been reported that cows suffering from uterine prolapse have a lower serum Ca concentration than normal cows [80].

It is worthy to bear in mind that retained placenta is a multi-etiological condition with many risk factors. Therefore, any association does not mean causal relationship. On the other hand, the pathogenic process leading to retained placenta is initiated before parturition [81] and the serum level of Ca, after parturition cannot be considered a risk factor for retained placenta.

Metritis and endometritis – Subclinical hypocalcemia has been related to metritis [55, 82]. Because under hypocalcemic conditions, immune function may be impaired and muscle contraction diminished [83], metritis is more prone to occur [54]. Martinez et al. (2011) studied 110 cows in one herd in Florida, and demonstrated that cows with Ca <2.14 mmol/L at least once between 0 and 3 days in milk had 4.5-fold increased odds of metritis [54]. In a recent study, multiparous cows with subclinical hypocalcemia had 4.85 greater odds of having metritis compared with normocalcemic multiparous cows [68]. A significantly higher incidence rate of endometritis was observed in UK cows that suffered clinical hypocalcemia in comparison to normocalcemic cows [84].

Mastitis – It was reported that cows with clinical milk fever were eight times more likely to develop mastitis than normal cows [55]. Hypocalcemia reduces teat sphincter contraction, thus, an open teat canal invites environmental pathogens to enter the mammary gland. On the other hand, hypocalcemic cows tend to spend more time lying down than do normocalcemic animals, which could increase teat end exposure to environmental opportunist organisms [47, 57, 85]. In addition, hypocalcemia has deleterious effect on peripheral blood mononuclear cells function and this exacerbates periparturient immunosuppression [18].

Reproduction performance –The association of clinical hypocalcemia and decreased fertility was reported in several studies [47, 68, 86]. Some reports showed that there are no differences in the incidence of uterine diseases, services per conception, or days open when comparing hypocalcemic cows with normocalcemic ones [87]. However, there are plentiful evidence indicated that hypocalcemia may cause infertility.

A UK study reported an increased number of services per conception, an increased calving to first service interval and an increased calving to conception interval for clinical hypocalcemic dairy cows [86]. Martinez et al. (2012) found that pregnancy rate and interval between calving and pregnancy were reduced under hypocalcemia [54]. It has been suggested that clinical hypocalcemia results in reduced fertility in dairy cows due to its effect on uterine muscle function, slower uterine involution [47, 86] and reduced blood flow to the ovaries [88]. Cows with clinical hypocalcemia had a greater diameter of the gravid uterine horn and non-gravid uterine horn between 15 and 45 days post-partum (indicative of slower uterine involution) and a significantly reduced likelihood of having a corpus luteum (indicative of ovulation since parturition) than normal cows [84].

Furthermore, subclinical hypocalcemia affected reproductive performance such as estrous cyclicity [89-90] and pregnancy rate to first AI

[91]. The odds of expressing estrus before 60 days in milk were lower in subclinical hypocalcemic cows than in normocalcemic ones [68]. Caixeta et al. (2017) reported that cows with normocalcemia were 1.8 times more likely to return in estrus by the end of the voluntarily waiting period than cows classified as having subclinical hypocalcemia [90].

Higher Ca concentrations from week -1 through week 3 relative to calving were associated with increased odds of pregnancy. The odds of conceiving was 1.5 times higher for cows with pre-calving Ca >2.3 mmol/L, and 1.3 times higher for cows with Ca >2.2 mmol/L in week 1, >2.3 mmol/L in week 2, and >2.4 mmol/L in week 3 relative to calving [91].

In addition, it was reported that subclinically hypocalcemic cows have fewer ovulatory sized follicles at days 15, 30 and 45 post-partum and smaller follicles at first ovulation than normal cows [92].

It should be emphasized that trying to improve fertility in dairy herds without first having an appropriate hypocalcemia prevention strategy will bring only limited improvements.

Culling - An increased culling risk was reported for cows with hypocalcemia [56, 93]. Cows with serum Ca concentrations less than or equal to 1.8 mmol/L and not diagnosed with milk fever were approximately 3 times more likely to be culled in the first 60 days of lactation [59]. Seifi et al. (2011) also showed that Ca concentrations at weeks 1 and 2 post-calving were associated with subsequent culling during the early lactation period. The odds of culling in early lactation were 2.4 and 5.3 times greater in cows with serum Ca concentrations ≤ 2.2 and ≤ 2.3 mmol/L in the first and second weeks after calving, respectively [56]. Moreover, it was shown that culling risk were 4. 57% and 27.6% for cows fed anionic and control diets, respectively [94].

Milk production - An association between low pre- and post-calving Ca concentrations and milk loss was found. The optimal Ca cut-off level was 2.1 mmol/L at week -1 and 1 relative to calving. It is likely that lower Ca concentrations are an indicator of inadequate dry milk intake rather than metabolic disease [91]. Hutjens (2003) has reported that the average loss due to milk fever per animal was the loss of 500 kg of milk [95].

The relationship between hypocalcemia and milk yield are inconsistent. Rajala-Schultz et al. (1999) found when the cows' own mid-lactation milk yield was used as a reference level, milk fever was associated with milk losses during the first 4-6 weeks of lactation [96]. On the other hand, several studies have indicated that there is no significant difference in milk yield, milk components and somatic cell count between hypocalcemic and normocalcemic cows [97-98].

In addition, it has been shown that milk protein content was lower in hypocalcemic cows at 21 and 35 DIM, but there was no difference in somatic cell count, percent milk fat, solids-non-fat, and milk yield [87].

Herd based monitoring of hypocalcemia

Herd-based tests are now available for use in routine herd monitoring and for investigating dairy herds with metabolic subclinical problems. It has been suggested that 12 multiparous cows to be sampled within 48 hour after calving. And, the results to be interpreted as the proportion of cows below the cut points of Ca [3, 33].

Besides defining the appropriate cut points for tests evaluated as proportion outcomes, it is also necessary to determine the alarm level for the proportion of animals below the described cut point. Because of normal biologic variation, a few individual cows are expected to be below the biologic threshold in any dairy [3].

Ca cut-off levels - Cows with a serum Ca concentration less than 2.0 mmol/L were considered as hypocalcemic [8, 21, 99]. Either pre- or post-partum cows with serum total Ca below 2.0 mmol/L were four times more likely to have post-partum disease problems [100]. Although this is a conservative threshold, it is well accepted in research and clinical practice [8, 21, 99].

Recently higher thresholds were associated with a negative health outcome such as displacement of abomasum and metritis [54, 91] or an increased culling risk [56, 93]. These associations, however, found in a longer risk period before or after calving [54, 56]. Neves et al. (2017) has shown that prepartum cows with Ca concentrations <2.4 mmol/L at approximately 1 week before calving to have an increased risk of being classified as subclinical hypocalcemia at parturition [101]. Martinez et al. (2012) showed that cows with subclinical hypocalcemia, as defined by serum Ca less than 2.15 mmol/L within 72 hour after parturition, had reduced concentrations of neutrophils in the blood, impaired neutrophil function, and increased incidences of metritis and puerperal

metritis compared with normocalcemic cows [54].

Furthermore, Van Saun (2000) considered serum Ca concentration below 2.25 mmol/L as a cut-off level for interpretation of hypocalcemia in a group of fresh cows whereas it would be considered normal in an individual [102]. Further evaluations are necessary to define the most appropriate threshold of hypocalcemia within any given time during transition period [33].

Interpretation of the herd based Ca testing - Because the duration of parturient hypocalcemia is extremely short (about the first 48 hours after calving), its incidence is monitored instead of its prevalence [103]. Limited data are available to assist in determining an alarm level for parturient hypocalcemia [3]. In regard to incidence of hypocalcemia, it was suggested that 30% hypocalcemia is a reasonable alarm level in multiparous Holstein cows [3].

In authors' experience, the best time to collect blood samples is about 12 to 24 hours. Some researchers suggested cows to be sampled within 48 hours after calving [33]. Further studies are needed to determine the best sampling time and the accurate and precise cut-off level for either sampling time.

Herds were categorized based on the proportion of positive samples (i.e., blood Ca below threshold) into negative (0 to 2 out of 12 cows), borderline (3 to 5 out of 12 cows), or positive (= 6 cows out of 12) [3, 33]. Such classification is based on the assumptions provided by Oetzel (2004) using a 75% confidence interval and an alarm level of 30% (Figure 5). The cows sampled within 48 hour after parturition and serum Ca below 2.0 mmol/L were considered as hypocalcemic [33]. It is needed to emphasize that this alarm level is considered to predict clinical hypocalcemia. Therefore, for predicting subclinical hypocalcemia, the alarm level may be lower. Some herds may be classified as borderline. In such cases, Venjakob et al. (2017) advise to draw more samples to classify the herd more appropriately [33].

Conclusion

Clinical and subclinical hypocalcemia are of great economic impact on dairy industry. Recent studies indicated that the incidence of inapparent hypocalcemia is 8-10 times greater than clinical hypocalcemia [8, 33, 48, 52]. The high prevalence of subclinical hypocalcemia should be viewed as a potential health risk to the transition cow. Culling, health problems, complications of parturition, loss of milk production and low fertility are common outcomes of hypocalcemia in dairy cows. Therefore, the economic loss due to subclinical hypocalcemia is estimated to be tremendous.

Postpartum blood Ca is below the normal range in many more cows than we have previously appreciated [8], and this is in spite of recent developments in hypocalcemia prevention. It should be highlighted that trying to improve dairy herds management without an effective prevention strat-

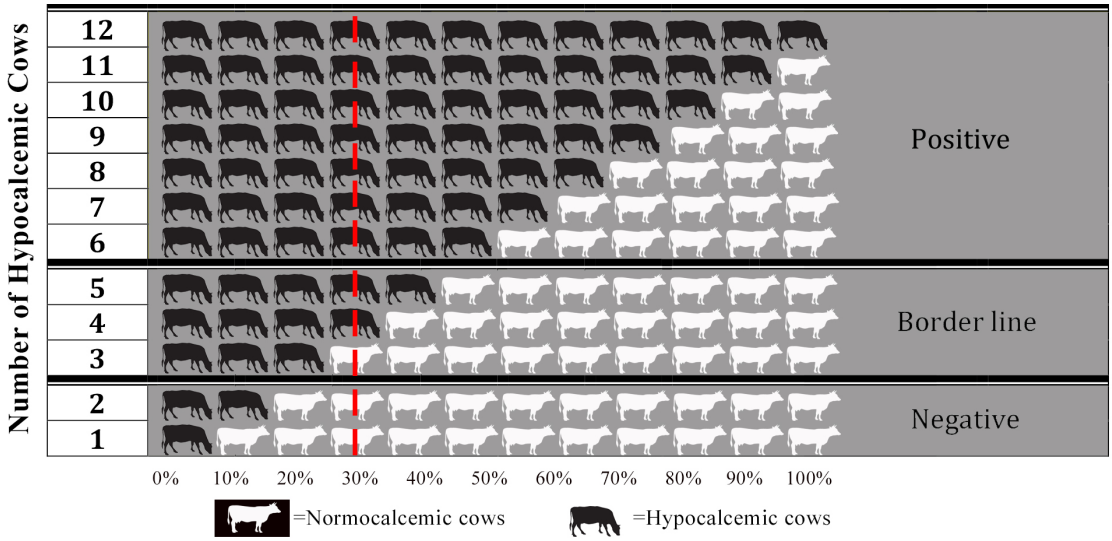

Figure 5
Classification of blood Ca concentrations using 75% confidence interval and an alarm level of 30% for test results from 12 cows sampled from a group of 100 cows. This calculation illustrates the association between positive blood samples in the cohort and prevalence of hypocalcemia in the tested herd, adapted from data of Venjakob et al. (2017) [33].

egy will not be fulfilling.

Milk fever and subclinical hypocalcemia can, to a large extent be prevented by good dry cow management and appropriate nutrition. Appropriate prevention strategy includes precise monitoring of urine pH and adjusting DCAD of diets of close-up cows on the basis of urine pH results. It should be noted that the incidence of hypocalcemia is lower in primiparous than multiparous cows around calving. However, feeding anionic diets to primiparous cows seems to be beneficial, as well.

Acknowledgement

The authors would like to thank Ms. Taraneh Ebnalnassir for graphical designs (Figures 1 & 4) and Dr. Mohammad Riahi for preparation of Figure 5.

Author Contributions....

H.A.S. conceived and designed the paper. S.K. performed bibliography. H.A.S. and S.K. wrote primary drafts and H.A.S. wrote the final version of article.

Conflict of Interest

The authors declare that there is no conflict of interest.

References

1. VandeHaar MJ, St-Pierre N. Major advances in nutrition: relevance to the sustainability of the dairy industry. Journal of Dairy Science. 2006 Apr 30;89(4):1280-91.

2. Seifi HA. Production diseases in dairy herds: monitoring transition cows. In "Dairy cows: nutrition, fertility and milk production". Nova science publishers, Inc. new York. 2011. P: 99-121.

3. Oetzel GR. Monitoring and testing dairy herds for metabolic disease. Veterinary Clinics: Food Animal Practice. 2004 Nov 1;20(3):651-74.

4. LeBlanc SJ, Lissemore KD, Kelton DF, Duffield TF, Leslie KE. Major advances in disease prevention in dairy cattle. Journal of dairy science. 2006 Apr 30;89(4):1267-79.

5. LeBlanc SJ, Lissemore KD, Kelton DF, Duffield TF, Leslie KE. Major advances in disease prevention in dairy cattle. Journal of dairy science. 2006 Apr 30;89(4):1267-79.

6. Houe H, Østergaard S, Thilsing-Hansen T, Jørgensen RJ, Larsen T, Sørensen JT, Agger JF, Blom JY. Milk fever and subclinical hypocalcaemia--an evaluation of parameters on incidence risk, diagnosis, risk factors and biological effects as input for a decision support system for disease control. Acta Veterinaria Scandinavica. 2001;42(1):1-29.

7. Roche JR. The incidence and control of hypocalcaemia in pasture-based systems. Acta veterinaria Scandinavica. Supplementum. 2003;97:141-4.

8. Reinhardt TA, Lippolis JD, McCluskey BJ, Goff JP, Horst RL. Prevalence of subclinical hypocalcemia in dairy herds. The Veterinary Journal. 2011 Apr 30;188(1):122-4.

9. Goff JP. Macromineral disorders of the transition cow. Veterinary Clinics: Food Animal Practice. 2004 Nov 1;20(3):471-94.

10. Horst RL, Goff JP, Reinhardt TA. Calcium and vitamin D metabolism during lactation. Journal of mammary gland biology and neoplasia. 1997 Jul 1;2(3):253-63.

11. Whitaker DA. Use and interpretation of metabolic profiles. The Health of Dairy Cattle. 2000:89-93.

12. Goff JP. Calcium and magnesium disorders. Veterinary Clinics of North America: Food Animal Practice. 2014 Jul 31;30(2):359-81.

13. Thrall MA, Baker DC, Compbell TW, Denicola D, Fettman M, Lassen ED, Rebar A, Weiser G. Veterinary Hematology and Clinical Chemistry. Lippincott Williams and Wilkins. United State of America. 2004;77.

14. Meyer DJ, Rich EH, Meyer LJ, Coles EH, Rich LJ. Veterinary laboratory medicine: interpretation and diagnosis. 1992:199-202.

15. Hunt E, Blackwelder JT. Disorders of calcium metabolism. Large animal internal medicine, 3rd edn. Mosby, St. Louis, MO. 2002:1248-54.

16. Seifi HA, Mohri M, Ehsani A, Hosseini E, Chamsaz M. Interpretation of bovine serum total calcium: effects of adjustment for albumin and total protein. Comparative Clinical Pathology. 2005 Dec 1;14(3):155-9.

17. Goff JP. The monitoring, prevention, and treatment of milk fever and subclinical hypocalcemia in dairy cows. The Veterinary Journal. 2008 Apr 30;176(1):50-7.

18. Kimura K, Reinhardt TA, Goff JP. Parturition and hypocalcemia blunts calcium signals in immune cells of dairy cattle. Journal of dairy science. 2006 Jul 31;89(7):2588-95.

19. Ferneborg S. Calcium homeostasis at calving in cows milked prepartum. Swedish university of agricultural science, department of animal nutrition and management. 2010.

20. Horst RL, Goff JP, Reinhardt TA. Adapting to the transition between gestation and lactation: differences between rat, human and dairy cow. Journal of mammary gland biology and neoplasia. 2005 Apr 1;10(2):141-56.

21. DeGaris PJ, Lean IJ. Milk fever in dairy cows: A review of pathophysiology and control principles. The Veterinary Journal. 2008 Apr 30;176(1):58-69.

22. Schröder B, Breves G. Mechanisms and regulation of calcium absorption from the gastrointestinal tract in pigs and ruminants: comparative aspects with special emphasis on hypocalcemia in dairy cows. Animal Health Research Reviews. 2006 Jun;7(1-2):31-41.

23. Holler, H., Breeves, G., Kocabatmax, M., Gredes, H. Mineral absorption across isolated rumen mucosa. Queensland Journal of Experimental Physiology. 1988. 73:609-611.

24. Khorasani GR, Janzen RA, McGill WB, Kennelly JJ. Site and extent of mineral absorption in lactating cows fed whole-crop cereal grain silage of alfalfa silage. Journal of Animal Science. 1997 Jan 1;75(1):239-48.

25. Hoorn EJ, Zietse R. Disorders of calcium and magnesium balance: a physiology-based approach. Pediatric Nephrology. 2013 Aug 1;28(8):1195-206.

26. Goff JP, Littledike ET, Horst RL. Effect of Synthetic Bovine Parathyroid Hormone in Dairy Cows: Prevention of Hypocalcemic Parturient Paresis1. Journal of Dairy Science. 1986 Sep 1;69(9):2278-89.

27. Ellenberger HB, Newlander JA, Jones CH. Calcium and phosphorus requirements of dairy cows. 2. Weekly balances through lactation and gestation periods. Calcium and phosphorus requirements of dairy cows. 2. Weekly balances through lactation and gestation periods. 1932.

28. Christakos S. Recent advances in our understanding of 1, 25-dihydroxyvitamin D 3 regulation of intestinal calcium absorption. Archives of Biochemistry and Biophysics. 2012 Jul 1;523(1):73-6.

29. Halloran BP, Barthell EN, DeLuca HF. Vitamin D metabolism during pregnancy and lactation in the rat. Proceedings of the National Academy of Sciences. 1979 Nov 1;76(11):5549-53.

30. Kumar R, Cohen WR, Silva P, Epstein FH. Elevated 1, 25-dihydroxyvitamin D plasma levels in normal human pregnancy and lactation. Journal of Clinical Investigation. 1979 Feb;63(2):342.

31. Toverud SU, Boass A, Haussler MR, Pike JW. Circulating levels and function of 1, 25-(OH)2D3 in lactation. Journal of Steroid Biochemistry. 1983 Jul 1;19(1):505-10.

32. Horst RL, Eisman JA, Jorgensen NA, DeLuca HF. Adequate response of plasma 1, 25-dihydroxyvitamin D to parturition in paretic (milk fever) dairy cows. Science. 1977 May 6;196(4290):662-3.

33. Venjakob PL, Borchardt S, Heuwieser W. Hypocalcemia—Cow-level prevalence and preventive strategies in German dairy herds. Journal of Dairy Science. 2017 Aug 31;100(11):9258-9266.

34. Horst RL, Goff JP, Reinhardt TA. Advancing age results in reduction of intestinal and bone 1, 25-dihydroxyvitamin D receptor. Endocrinology. 1990 Feb 1;126(2):1053-7.

35. Hanai H, Brennan DP, Cheng L, Goldman ME, Chorev M, Levine MA, Sacktor B, Liang CT. Downregulation of parathyroid hormone receptors in renal membranes from aged rats. American Journal of Physiology-Renal Physiology. 1990 Sep 1;259(3):F444-50.

36. Dishington IW. Prevention of milk fever (hypocalcemic paresis puerperalis) by dietary salt supplements. Acta Veterinaria Scandinavica. 1974 Dec;16(4):503-12.

37. Gaynor PJ, Mueller FJ, Miller JK, Ramsey N, Goff JP, Horst RL. Parturient Hypocalcemia in Jersey Cows Fed Alfalfa Haylage-Based Diets with Different Cation to Anion Ratios1. Journal of Dairy Science. 1989 Oct 1;72(10):2525-31.

38. Goff JP, Horst RL, Mueller FJ, Miller JK, Kiess GA, Dowlen HH. Addition of Chloride to a Prepartal Diet High in Cations Increases 1, 25-Dihydroxyvitamin D Response to Hypocalcemia Preventing Milk Fever1. Journal of Dairy Science. 1991 Nov 1;74(11):3863-71.

39. Phillippo M, Reid GW, Nevison IM. Parturient hypocalcaemia in dairy cows: effects of dietary acidity on plasma minerals and calciotrophic hormones. Research in Veterinary Science. 1994 May 1;56(3):303-9.

40. Seifi HA, Mohri M, Zadeh JK. Use of pre-partum urine pH to predict the risk of milk fever in dairy cows. The Veterinary Journal. 2004 May 31;167(3):281-5.

41. Martín-Tereso J, Martens H. Calcium and magnesium physiology and nutrition in relation to the prevention of milk fever and tetany (dietary management of macrominerals in preventing disease). Veterinary Clinics: Food Animal Practice. 2014 Nov 1;30(3):643-70.

42. Constable PD, Hinchcliff KW, Done SH, Gruenberg W. Veterinary Medicine: A textbook of the diseases of cattle, horses, sheep, pigs and goats. Elsevier Health Sciences; 2016 Oct 25.

43. Jorgensen NA. Combating milk fever [Dairy cattle, metabolic disorders]. Journal of Dairy Science (USA). 1974;57:933–44

44. Reinhardt TA, Conrad HR. Mode of action of pharmacological doses of cholecalciferol during parturient hypocalcemia in dairy cows. The Journal of Nutrition. 1980 Aug 1;110(8):1589-96.

45. Grünberg W. Treatment of phosphorus balance disorders. Veterinary Clinics: Food Animal Practice. 2014 Jul 1;30(2):383-408.

46. Schnewille JT, Klooster AT, Beynen AC. High phosphorus intake depresses apparent magnesium absorption in pregnant heifers. Journal of Animal Physiology and Animal Nutrition. 1994 Jan 8;71(1-5):15-21.

47. Mulligan F, O Grady L, Rice D, Doherty M. Production diseases of the transition cow: Milk fever and subclinical hypocalcaemia. Irish Veterinary Journal. 2006 Dec 1;59(12):697.

48. NAHMS D. Part III: Reference of dairy cattle health and health management practices in the United States, 2002.

49. USA Department of Agriculture. Dairy 2014, "Dairy Cattle Management Practices in the United States. 2016.

50. Horst RL, Goff JP, McCluskey BJ. Prevalence of subclinical hypocalcemia in US dairy operations. Journal of Dairy Science. 86 (Suppl. 1), 247.

51. Oetzel GR. Minimizing hypocalcemia during early lactation. InTri-State Dairy Nutrition Conference. Fort Wayne, IN, USA: Ohio State University 2013 Apr 23 (pp. 23-32).

52. Miltenburg C. Management of peripartum dairy cows for metabolic health and immune function (DVSc. Thesis). University of Guelph. 2015.

53. Chamberlin WG. Influence of subclinical hypocalcemia on plasma biochemical parameters, liver histologic changes, and common postpartum diseases in dairy cows. University of Missouri-Columbia; 2011.

54. 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. Journal of Dairy Science. 2012 Dec 31;95(12):7158-72.

55. Curtis CR, Erb HN, Sniffen CJ, Smith RD, Powers PA, Smith MC, White ME, Hillman RB, Pearson EJ. Association of parturient hypocalcemia with eight periparturient disorders in Holstein cows. Journal of the American Veterinary Medical Association. 1983 Sep;183(5):559-61.

56. Seifi HA, LeBlanc SJ, Leslie KE, Duffield TF. Metabolic predictors of post-partum disease and culling risk in dairy cattle. The Veterinary Journal. 2011 May 31;188(2):216-20.

57. Goff JP. Transition cow immune function and interaction with metabolic diseases. In Tri-State Dairy Nutrition Conference 2008 Apr 22 (pp. 45-57).

58. Galvão KN. Association between immune function and development of uterine disease in dairy cows. Animal Reproduction. 2012 Jul 1;9(3):318-22.

59. Duffield T. Impact of subclinical metabolic disease on risk of early lactation culling. Journal of Dairy Science. 2005;88(1):199-abstr.

60. Wyle FA, Kent JR. Immunosuppression by sex steroid hormones. The effect upon PHA-and PPD-stimulated lymphocytes. Clinical and Experimental Immunology. 1977 Mar;27(3):407.

61. Franklin ST, Young JW, Nonnecke BJ. Effects of Ketones, Acetate, Butyrate, and Glucose on Bovine Lymphocyte Proliferation1, 2. Journal of Dairy Science. 1991 Aug 1;74(8):2507-14.

62. Martinez N, Sinedino LD, Bisinotto RS, Ribeiro ES, Gomes GC, Lima FS, Greco LF, Risco CA, Galvão KN, Taylor-Rodriguez D, Driver JP. Effect of induced subclinical hypocalcemia on physiological responses and neutrophil function in dairy cows. Journal of Dairy Science. 2014 Feb 28;97(2):874-87.

63. Gallo EM, Canté-Barrett K, Crabtree GR. Lymphocyte calcium signaling from membrane to nucleus. Nature Immunology. 2006 Jan 1;7(1):25-32.

64. Hansen SS, Nørgaard P, Pedersen C, Jørgensen RJ, Mellau LS, Enemark JD. The effect of subclinical hypocalcaemia induced by Na2EDTA on the feed intake and chewing activity of dairy cows. Veterinary Research Communications. 2003 Apr 1;27(3):193-205.

65. Daniel RC. Motility of the rumen and abomasum during hypocalcaemia. Canadian Journal of Comparative Medicine. 1983 Jul;47(3):276.

66. Jørgensen RJ, Nyengaard NR, Hara S, Enemark JM, Andersen PH. Rumen motility during induced hyper-and hypocalcaemia. Acta Veterinaria Scandinavica. 1998;39(3):331-8.

67. Caixeta LS, Ospina PA, Capel MB, Nydam DV. The association of subclinical hypocalcemia, negative energy balance and disease with bodyweight change during the first 30 days post-partum in dairy cows milked with automatic milking systems. The Veterinary Journal. 2015 May 31;204(2):150-6.

68. Rodríguez EM, Arís A, Bach A. Associations between subclinical hypocalcemia and postparturient diseases in dairy cows. Journal of Dairy Science. 2017 Sep 1;100(9):7427-34.

69. Zemel MB. Role of calcium and dairy products in energy partitioning and weight management. The American Journal of Clinical Nutrition. 2004 May 1;79(5):907S-12S.

70. Xue B, Greenberg AG, Kraemer FB, Zemel MB. Mechanism of intracellular calcium ([Ca2+] i) inhibition of lipolysis in human adipocytes. The FASEB Journal. 2001 Nov 1;15(13):2527-9.

71. Chapinal N, Carson M, Duffield TF, Capel M, Godden S, Overton M, Santos JE, LeBlanc SJ. The association of serum metabolites with clinical disease during the transition period. Journal of Dairy Science. 2011 Oct 31;94(10):4897-903.

72. Massey CD, Wang CH, Donovan GA, Beede DK. Hypocalcemia at parturition as a risk factor for left displacement of the abomasum in dairy cows. Journal of the American Veterinary Medical Association. 1993 Sep;203(6):852-3.

73. Doll K, Sickinger M, Seeger T. New aspects in the pathogenesis of abomasal displacement. The Veterinary Journal. 2009 Aug 31;181(2):90-6.

74. Madison JB, Troutt HF. Effects of hypocalcaemia on abomasal motility. Research in Veterinary Science. 1988 Mar;44(2):264-6.

75. LeBlanc SJ, Leslie KE, Duffield TF. Metabolic predictors of displaced abomasum in dairy cattle. Journal of Dairy Science. 2005 Jan 31;88(1):159-70.

76. Erb HN, Smith RD, Oltenacu PA, Guard CL, Hillman RB, Powers PA, Smith MC, White ME. Path Model of Reproductive Disorders and Performance, Milk Fever, Mastitis, Milk Yield, and Culling in Holstein Cows1. Journal of Dairy Science. 1985 Dec 1;68(12):3337-49.

77. Correa MT, Erb H, Scarlett J. Path analysis for seven postpartum disorders of Holstein cows. Journal of Dairy Science. 1993 May 1;76(5):1305-12.

78. Seifi HA, Dalir-Naghadeh B, Farzaneh N, Mohri M, Gorji-Dooz M. Metabolic changes in cows with or without retained fetal membranes in transition period. Transboundary and Emerging Diseases. 2007 Mar 1;54(2):92-7.

79. Risco CA, Drost M, Thatcher WW, Savio J, Thatcher MJ. Effects of calving-related disorders on prostaglandin, calcium, ovarian activity and uterine involution in postrartum dairy cows. Theriogenology. 1994 Dec 31;42(1):183-203.

80. Risco CA, Reynolds JP, Hird D. Uterine prolapse and hypocalcemia in dairy cows. Journal of the American Veterinary Medical Association. 1984 Dec;185(12):1517-9.

81. Melendez P, Donovan GA, Risco CA, Goff JP. Plasma mineral and energy metabolite concentrations in dairy cows fed an anionic prepartum diet that did or did not have retained fetal membranes after parturition. American Journal of Veterinary Research. 2004 Aug 1;65(8):1071-6.

82. Goff JP, Horst RL. Physiological changes at parturition and their relationship to metabolic disorders1, 2. Journal of Dairy Science. 1997 Jul 1;80(7):1260-8.

83. Murray R. RD Murray, JE Horsfield, WD McCormick, HJ Williams, D. Ward. The Veterinary Record. 2008 Nov 8;163:561-5.

84. Whiteford LC, Sheldon IM. Association between clinical hypocalcaemia and postpartum endometritis. Veterinary Record. 2005 Aug 13;157(7):202.

85. Goff J. Managing the transition cow-Considerations for optimising energy and protein balance, and immune function. Cattle Practice. 2003 Apr 1;11:51-63.

86. Borsberry S, Dobson H. Periparturient diseases and their effect on reproductive performance in five dairy herds.

The Veterinary Record. 1989 Mar;124(9):217-9.

87. Chamberlin WG, Middleton JR, Spain JN, Johnson GC, Ellersieck MR, Pithua P. Subclinical hypocalcemia, plasma biochemical parameters, lipid metabolism, postpartum disease, and fertility in postparturient dairy cows. Journal of Dairy Science. 2013 Nov 30;96(11):7001-13.

88. Jonsson NN, Daniel RC. Effects of hypocalcaemia on blood flow to the ovaries of the sheep. Transboundary and Emerging Diseases. 1997 Feb 12;44(1-10):281-7.

89. Ribeiro ES, Lima FS, Greco LF, Bisinotto RS, Monteiro AP, Favoreto M, Ayres H, Marsola RS, Martinez N, Thatcher WW, Santos JE. Prevalence of periparturient diseases and effects on fertility of seasonally calving grazing dairy cows supplemented with concentrates. Journal of Dairy Science. 2013 Sep 30;96(9):5682-97.

90. Caixeta LS, Ospina PA, Capel MB, Nydam DV. Association between subclinical hypocalcemia in the first 3 days of lactation and reproductive performance of dairy cows. Theriogenology. 2017 May 31;94:1-7.

91. Chapinal N, Carson ME, LeBlanc SJ, Leslie KE, Godden S, Capel M, Santos JE, Overton MW, Duffield TF. The association of serum metabolites in the transition period with milk production and early-lactation reproductive performance. Journal of Dairy science. 2012 Mar 31;95(3):1301-9.

92. Kamgarpour R, Daniel RC, Fenwick DC, McGuigan K, Murphy G. Post partum Subclinical Hypocalcaemia and Effects on Ovarian Function and Uterine Involution in a Dairy Herd. The Veterinary Journal. 1999 Jul 1;158(1):59-67.

93. Roberts T, Chapinal N, LeBlanc SJ, Kelton DF, Dubuc J, Duffield TF. Metabolic parameters in transition cows as indicators for early-lactation culling risk. Journal of Dairy Science. 2012 Jun 30;95(6):3057-63.

94. Seifi HA, Mohri M, Farzaneh N, Nemati H, Nejhad SV. Effects of anionic salts supplementation on blood pH and mineral status, energy metabolism, reproduction and production in transition dairy cows. Research in Veterinary Science. 2010 Aug 31;89(1):72-7.

95. Hutjens M. An Alternate to Metabolic Disorders: Looking at Hypocalcaemia, Dairy Decision Column. University of Illinois, Urbana. 2003 Feb;18.

96. Rajala-Schultz PJ, Gröhn YT, McCulloch CE. Effects of milk fever, ketosis, and lameness on milk yield in dairy cows. Journal of Dairy Science. 1999 Feb 1;82(2):288-94.

97. Østergaard S, Larsen T. Associations between blood calcium status at calving and milk yield in dairy cows. Journal of Dairy Science. 2000 Nov 1;83(11):2438-40.

98. Hunter AL. Association of Serum Calcium Status at Calving on Survival, Health, and Performance of Post-partum Holstein Cows and Calves (Doctoral dissertation), The Ohio State University. 2015.

99. Wilhelm AL, Maquivar MG, Bas S, Brick TA, Weiss WP, Bothe H, Velez JS, Schuenemann GM. Effect of serum calcium status at calving on survival, health, and performance of postpartum Holstein cows and calves under certified organic management. Journal of Dairy Science. 2017 Apr 30;100(4):3059-67.

100. Van Saun RJ. Metabolic profiles for evaluation of the transition period. Proceedings of American Association of Bovine Practitioners. 2006;39:130-8.

101. Neves RC, Leno BM, Stokol T, Overton TR, McArt JA. Risk factors associated with postpartum subclinical hypocalcemia in dairy cows. Journal of Dairy Science. 2017 May 31;100(5):3796-804.

102. Van Saun RJ. Blood profiles as indicators of nutritional status. InProc. 18th Annu. Western Canadian Dairy Seminar. Red Deer Alberta, Canada 2000 Mar 1 (pp. 1-6).

103. Oetzel GR. Effect of calcium chloride gel treatment in dairy cows on incidence of periparturient diseases. Journal of the American Veterinary Medical Association. 1996 Sep;209(5):958-61. 5