Methods of measurement and implications of abnormal calcium concentrations in fresh dairy cows

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Abstract

Hypocalcemia is a prevalent disorder in the early postpartum period. Advances in periparturient nutrition and management strategies have decreased the occurrence of clinical hypocalcemia over the past few decades; however, subclinical hypocalcemia remains an important aspect of transition cow health. These proceedings will discuss the diagnostic tools available to bovine practitioners for individual animal calcium concentration determination and herd-level monitoring, as well as current developments in the epidemiology of hypocalcemia and its prevention.

Key words: dairy cow, hypocalcemia, diagnostics, periparturient period

Résumé

L'hypocalcémie est un trouble répandu en période postpartum. Des percées en nutrition périnatale et dans les stratégies de gestion ont réduit la prévalence de l'hypocalcémie clinique au cours des dernières décennies. Toutefois, l'hypocalcémie sous-clinique demeure un enjeu important pour la santé des vaches en transition. Cette présentation discutera des outils de diagnostic que les praticiens bovins peuvent utiliser pour déterminer la concentration du calcium chez un individu et faire la surveillance au niveau du troupeau. Nous détaillerons aussi les derniers développements concernant l'épidémiologie de l'hypocalcémie et sa prévention.

Introduction

One of the challenges in dairy cattle management is the maintenance of optimum blood calcium concentration during the early postpartum period. Postparturient hypocalcemia occurs due to the massive mobilization of calcium needed to initiate and sustain lactation. To maintain calcium concentrations at a level required for life (e.g., thermoregulation, muscle contraction) while transferring a large quantity to colostrum, cows increase secretion of parathyroid hormone which increases release of calcium from bone, decreases calcium excretion by the kidney, and indirectly increases intestinal absorption of calcium. Hypocalcemia results when serum calcium concentration falls faster than homeostatic mechanisms can adapt to the demands of lactation. Clinical hypocalcemia (milk fever) affects approximately 2 to 5% of periparturient dairy cattle.[1] This disease results in weakness that can progress to recumbency and death and is a widely accepted risk factor for subsequent displaced abomasum, ketosis, metritis, retained placenta, mastitis, culling, and decreased milk production.[2-4] Traditionally, subclinical hypocalcemia (SCH) has been defined as a serum calcium concentration <8.0 mg/dL (<2.0 mmol/L). A recent report from Reinhardt and colleagues used this cut-point in samples collected within 48 hours of parturition, as part of the 2007 NAHMS study, and found that SCH is very prevalent in the US, affecting up to 50% of postpartum multiparous cows.[5] Recent evidence suggests SCH is also a risk factor for future adverse health events, lower milk production, and decreased reproductive performance.[3,4,6,7] Surprisingly, little mathematical effort has been placed in the estimation of the economic impact of hypocalcemia, but loose calculations in lay journals by Charles Guard (Cornell University) and Garrett Oetzel (University of Wisconsin) estimate its cost between $125 to $300 per case.

Diagnostics

The majority of calcium in the blood is in a free or ionized form (50 to 60%), which is recognized to be the biologically active form responsible for calcium homeostasis. Approximately 30% is bound to proteins, primarily albumin, with another 10% in a complexed form bound with anions (e.g., phosphate, bicarbonate, sulfate, citrate, and lactate). Although ionized calcium makes up the majority of the blood calcium pool, measurement of the ionized form is expensive and requires special handling of samples, thus most research has been conducted using total calcium measurements. Although some studies show that total calcium is reasonably associated with ionized calcium concentrations in bovine blood, this relationship has been found to change near calving. Work from Leno and colleagues suggests that because of this variation between ionized calcium and total calcium in the few days immediately following parturition, these 2 measurements of calcium status cannot be used interchangeably;[a] this idea requires further investigation to accurately equate cut-point concentrations for these measurements.

Total calcium is more stable than ionized calcium and is currently the easiest and recommended form to analyze. Blood should be collected into lithium heparin (green top)

or preferably non-anticoagulant (red top) tubes; collection into EDTA (purple top), citrate, or sodium fluoride tubes may reduce total calcium concentration to an unmeasurable level due to calcium binding agents found in these tubes. Whole blood can be stored in a green or red top tube for up to 6 hours at 39.2 or 71.6°F (4 or 22°C) with minimal changes in total calcium measurements.[b] For best results, however, plasma or serum should be separated as soon as possible.

Until recently, the only well-validated way to measure total calcium was via benchtop analyzer. Costs per sample range from $5 to $15 at accredited veterinary diagnostic laboratories, which makes this method not economical for individual cow monitoring due to cost and the slow turnaround time; however, herd-level monitoring, while expensive, is more realistic. Recent work from Leno and colleagues shows that the IDEXX VetTest[c], an analyzer found in many veterinary clinics, is an option for on-farm calcium testing at both the individual and herd levels.[a] However, between-machine variation needs to be addressed and each machine's bias adjusted for interpretation of the results. With this adjustment, they found that the VetTest had a sensitivity and specificity of 87% and 89%, respectively, with a total calcium threshold of 8.5 mg/dL (2.125 mmol/dL) via the gold-standard reference method. This makes the VetTest a reasonable method of hypocalcemia diagnosis in a veterinary clinic, which decreases the turnaround time from a diagnostic lab referral; however, the cost of each sample remains expensive at $5 to $7.50 in addition to the cost of the machine itself. In addition, the VetTest still uses dry chemistry (an older technology) compared to the newer, more sensitive and precise method based on wet chemistry analysis that is used in diagnostic laboratories. A few additional machines have recently come on the market advertising their use as "on-farm" total calcium measurement tools, but to date, none of these machines has been well validated.

Ionized calcium is more problematic to measure due to sample collection and handling difficulties. Exposure of a blood sample to air changes the pH, and thus the amount of ionized calcium in the sample; stability over time is questionable, and the sample must be processed as soon as possible. Samples can be submitted to veterinary diagnostic labs or run in veterinary clinics, but results are often not accurate due to the aforementioned handling difficulties and the time it takes for a sample to arrive at a lab or clinic location. Machines targeted for on-farm use (e.g. i-STAT[d], VetStat[e], Stat Profile Prime[f]) cost approximately $5,000 to $15,000 in addition to per-sample costs; the i-STAT is the only machine with any validation using bovine blood, and only 24 samples to that end. At $15 to $20 per sample, ionized calcium is not currently a cost-effective method of individual animal or herd-level hypocalcemia monitoring on farms, in veterinary clinics, or by diagnostic laboratories. Current work is under way to find accurate and cost-effective methods of ionized calcium concentration determination on farms.

Wolfgang Heuwieser's group, well known for their investigation of on-farm methods of disease measurement (remember the Precision Xtra[g] meter?), recently took the historical clinical impression of "cold ears" and applied it to the diagnosis of SCH using an infrared thermometer to determine the skin temperature of ears in fresh cows. Unfortunately, although clinically hypocalcemic cows had lower ear temperatures than cows with SCH, the ambient temperature confounded the results too much to detect SCH via ear temperature alone, making this a non-reliable method of SCH diagnosis. Thus, cold ears do not necessarily mean low calcium!

Current cut-point recommendations for hypocalcemia diagnosis taken within the first 24 hours postpartum range from 8.6 mg/dL (2.15 mmol/L) to 8.0 mg/dL (2.0 mmol/L). As with all tests, a use of a higher cut-point decreases the sensitivity and increases the specificity of the result. Clinical hypocalcemia is much easier to diagnose based on observation alone, and total calcium concentrations are often <6.0 mg/dL (1.5 mmol/L) in animals displaying signs of milk fever. Interestingly, a cow with a total calcium concentration <6.0 mg/dL is not necessarily recumbent; test enough postpartum cows and you will notice cows with completely normal behavior walking around with total calcium concentrations <4.5 mg/dL (1.125 mmol/L). Given the sample handling and cost issues associated with obtaining accurate ionized calcium measurements, not much work has been reported on cut-points for diagnosis of SCH with this analyte. Current cut-point suggestions for diagnosis of hypocalcemia range from 1.00 mmol/L to 1.10 mmol/L.[8]

Epidemiology

Blood calcium concentrations have been well-characterized to reach a nadir between 24 and 48 hours postpartum in dairy cows.[4,9] However, few epidemiological studies have been conducted to determine the cut-point concentration at which a cow should be classified as hypocalcemic, and even the guidelines mentioned above are a bit arbitrary. In addition, the ideal time postpartum at which to classify a cow as hypocalcemic has not been studied.

Interestingly, recent work by Caixeta et al shows that cows with total calcium concentrations ≤8.6 mg/dL (2.15 mmol/L) at days 1, 2, and 3 in milk, which they termed "chronic subclinical hypocalcemia", had a 70% decreased odds of pregnancy to first service compared to normocalcemic cows.[10] Perhaps it is the persistence of hypocalcemia, not the absolute concentration, which is detrimental to the immediate postpartum cow. This study, which involved 97 Holstein cows, also showed that this chronic hypocalcemia occurs in all parity animals with a higher incidence amongst multiparous cows at an incidence of 20%, 32%, and 46% of parity 1, 2, and ≥3 cows, respectively. Further supporting this idea is work from Neves et al which shows that risk factors associated with SCH differ depending on the day of SCH diagnosis.[11] Using 301 Holstein cows and a total calcium cut-point of 8.4 mg/dL (2.1 mmol/L), the prevalence of SCH in a sample

collected within 4 hours of calving was 2%, 40%, and 66% for parity 1, 2, and ≥3 animals, respectively; the prevalence of SCH at 2 days-in-milk for the same animals was 20%, 36%, and 53%, respectively. Risk factors associated with an increased risk of diagnosis of SCH at calving included a lower total calcium concentration 1 week prepartum and increasing parity; risk factors associated with an increased risk of SCH diagnosis at 2 days-in-milk included being diagnosed as SCH at calving and worsening locomotion score. Taken together, these studies suggest that we may need to change the focus of our preventative strategies from cows with low calcium concentrations immediately postpartum to those with persistent hypocalcemia. Multiple groups are currently focused on finding answers to these questions.

We do not yet have great data on herd-level hypocalcemia prevalence and its association with adverse health events and production parameters. Chapinal and colleagues found adverse health and production effects for cows with calcium <8.4 mg/dL (2.1 mmol/L) within the first week of calving; however, a herd-alarm prevalence has yet to be determined.[3,6] In general, routine monitoring is never a bad idea given the economic resources and labor are available to do so. Statistically speaking, a prevalence sample should include blood samples collected from at least 20 cows, with current recommendations having that sample collected within 24 to 48 hours postpartum. As mentioned above, these timing recommendations and cut-point thresholds for diagnosis may change. Current work from Miller and Oetzel suggests that the distribution of SCH prevalence under a range of cut-points may provide information as to calcium equilibrium across the transition period in general, as well as point to areas for targeted nutritional and management bottlenecks.[h] More knowledge in this area is necessary, and research is currently under way to better describe SCH monitoring and response on a herd level.

Prevention

As hypocalcemia increases the risk that a cow will experience adverse health events during her lactation, prepartum management aimed at prevention of hypocalcemia also represents a sizable opportunity for preventing other postpartum diseases. The nutritional strategy of reducing the dietary cation-anion difference (DCAD) is the mainstay of hypocalcemia prevention, although vitamin D supplementation or provision of a calcium-deficient diet are also used.[1,12] Cows fed low DCAD rations develop mild acidosis that is thought to increase tissue sensitivity to parathyroid hormone, allowing faster mobilization of calcium when demand increases. Some evidence suggests that feeding a low DCAD ration can reduce the incidence of periparturient hypocalcemia from 50% to 30%.[9] Current recommendations are to feed a DCAD diet of approximately -10 to -15 mEq/100 g of dry matter for 3 weeks before expected calving.[13] Recent work from Leno et al, showed that plasma calcium concentrations increased linearly in the postpartum period for cows fed a prepartum diet without anion supplementation, partial anion supplementation, or full anion supplementation at +18.3, +5.9, and -7.4 mEq/100g of dry matter, respectively, for 4 weeks before parturition.[14]

The close-up diet can be adjusted for the DCAD level using either commercial products or through the nutritionist's formulation of feed ingredients. While some commercial products and formulations have improved palatability, feeding a low DCAD may decrease intakes, so routine monitoring of dry matter intakes is important. Proper diet mixing and feeding strategies are also imperative! To ensure the diet is being fed appropriately, monitor urine pH in close-up dry cows. Measure at least 12 to 15 cows each week via midstream urine samples. Meters for pH measurement (e.g., LAQUAtwin[i], $175) are quite accurate (and handy for measuring other things on dairies), but litmus paper will give you a reasonable reading for much less money. If you use these pH strips for measuring urine pH, make sure to purchase those with a smaller pH range (e.g. 5.5 to 8.0) so you can really focus on the target range of 5.5 to 6.0 (normal urine pH ~ 8.0). While some cows' urine will be <5.5 or >8.0, it is only important to note that a cow is above or below this range; the exact pH measurement is not necessary. Some dairies will report feeding a low DCAD with resulting normal urine pH values. In this case, cows are likely not consuming their expected dry matter, the TMR is not mixed properly, or there has been improper evaluation and adjustment in the ration for free-choice minerals or other forage content. If there is a large variation in urine pH between cows, this indicates an unequal consumption of the ration within the feeding group. This may be due to social factors or overcrowding or sorting due to poor mixing. Variation in urine pH distribution between weeks may indicate inconsistency in ration mixing or changes in feed ingredients which changes the DCAD. Use your urine pH monitoring information to improve feeding strategies!

Conclusions

Although clinical hypocalcemia is becoming a rare occurrence on modern dairy farms employing good nutritional management strategies, SCH is an often underdiagnosed problem at both the individual-animal and herd levels. Current research is under way to better define the epidemiology of hypocalcemia on today's large commercial herds with high-producing cows. Testing and monitoring options remain expensive although current studies to improve on-farm, economically-viable testing methods are under way. Prevention of hypocalcemia through peripartum nutritional management is key.

Endnotes

[a]Leno BM, Martens EM, Felippe MJB, Zanzalari KP, Lawrence JC, Overton TR. Dissertation, Cornell University. 2017

Acknowledgements

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