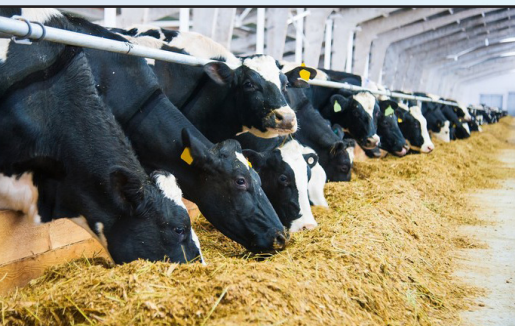


Rapid Screening for Hypocalcemia in Cow's Blood on Dairy Farm

Hypocalcemia, characterized by decrease in blood calcium below normal level, is one of the most common disorders affecting dairy cows at the onset of lactation. Among the three forms of calcium in blood, the ionized calcium (iCa) is the physiologically active. To help dairy farmers and veterinarians measure iCa in cows' blood samples immediately after collection on farms, HORIBA developed and introduced the LAQUAtwin Ca-11C bovine blood calcium meter equipped with a replaceable calcium sensor. It can serve as screening device for detection of hypocalcemia in cows. With its low cost and rapid blood iCa analysis, the meter is ideal for individual animal and herd-level monitoring on farms.



Introduction

Among mammals, the most common cause of acute hypocalcemia [*hypo-* low + *calc(o)-calcium* + *-emia* a blood condition of] is lactation or milk production. The mammary gland responsible for lactation gets calcium from the bloodstream. Shortly after giving birth, a female animal needs to produce enough milk to provide for its offspring. This sudden calcium demand for milk production can rapidly deplete calcium from the blood and can make the animal critically ill. In this state, the parathyroid gland stimulates parathyroid hormone (PTH) secretion which in turn responds by stimulating osteoclastic activity or calcium release from the bone. This activity takes time. Calcium is more rapidly obtained from extracellular and interstitial fluids. When calcium is taken from muscle tissues, the muscles can't contract and become too weak to support body weight. This is why cows often go down ("downer cows") in "milk fever" (hypocalcemia). Severe hypocalcemia is life-threatening and requires intervention.¹

Calcium is important in clotting, maintaining healthy bones, bone growth and repair, nerve transmission, muscle contraction, and milk production. Too much or too little calcium in the blood and tissues can create devastating disease problems.¹ Three forms of calcium are in equilibrium with serum: non-diffusible calcium bound primarily to albumin; diffusible complexes of calcium with lactate, bicarbonate, phosphate, sulfate, citrate, and other anions; and diffusible ionized calcium (Ca²⁺). Ionized calcium accounts for

approximately half the total serum calcium, and non-diffusible and complexed calcium account for 45% and 5%, respectively. Ionized calcium is the physiologically active form; its concentration is regulated by the parathyroid gland. A decrease in serum ionized calcium can cause involuntary muscle contraction and related neurological symptoms, regardless of the total serum calcium concentration.²

Total calcium is more stable than ionized calcium and is currently the easiest and recommended form to analyze. Although some studies show that total calcium is reasonably associated with ionized calcium

concentrations in bovine blood, this relationship changes near calving.³

Ionized calcium (abbreviated as iCa but refers to free Ca²⁺ ion) measurement in blood is not only expensive but also requires special handling of samples. Samples must not be exposed to air. Exposure of blood sample to air changes the pH and thus the amount of ionized calcium in the sample. Also, samples must be processed as soon as possible as the stability over time is questionable. They can be submitted to veterinary diagnostic labs or veterinary clinics but the results are often inaccurate due to improper handling

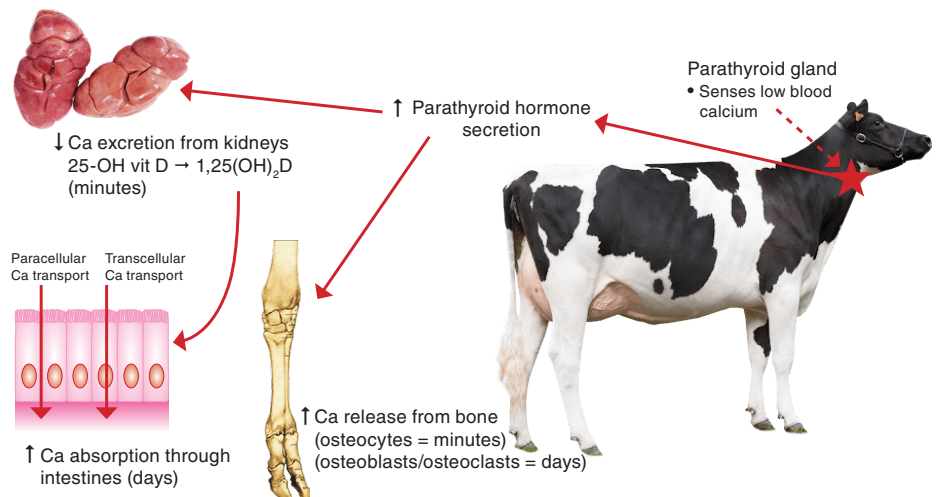


Figure 1. Schematic diagram showing the homeostatic response of a cow to low blood calcium concentration. These mechanisms act in parallel to increase blood calcium to a normal concentration. J. McArt, 2018.

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and long elapsed time from collection. There are commercially available instruments designed for farm or field use, however, their high prices in addition to per sample costs make them too expensive for regular testing of large group of animals. Currently, there is no cost-effective solution for individual animal and herd-level monitoring on farms.

After extensive research and optimization of prototype, HORIBA launched the LAQUAtwin Ca-11C bovine blood calcium meter to deliver inexpensive, smaller, and simpler yet faster alternative to commercially available instruments intended for farm or field use. It measures ionized calcium in cow's blood and can serve as screening device for detection of hypocalcemia in cows on dairy farms.

The LAQUAtwin Ca-11C has a replaceable sensor with built-in thermistor that detects sample temperature and 0.8mm thick flat calcium ion selective membrane that accepts as little as 0.3ml sample. The meter displays sample reading in mmol/L unit in just few seconds.

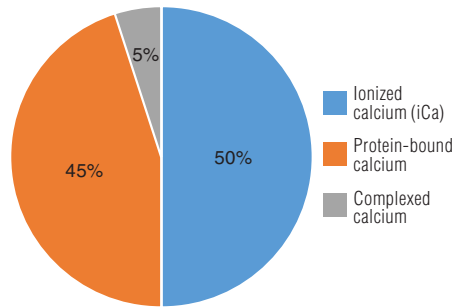


Figure 2. Three forms of calcium and their respective percentages in blood



Figure 3. Horiba Infrared thermometer and blood collection tube for iCa measurement

Method

Meter Calibration

Calibrate the LAQUAtwin Ca-11C using the 1.25 mmol/L and 2.50 mmol/L ionized calcium (Ca^{2+}) standard solutions that come with the meter according to the instruction manual. The composition of the standard solutions is similar to the electrolyte composition of blood. To minimize error in the measurement, the standard solutions and sample should be at the same temperature.

Sample Collection and Preparation

1. Collect blood sample from clean tail vein or neck jugular vein of cow into a blood collection tube that contains lithium heparin anticoagulant. Note: Blood collection tubes containing other anticoagulants may adversely affect the reading.
2. Take the temperature of the blood sample using a non-contact infrared thermometer. Wait for the sample temperature to fall in the range 20 to 25°C.
3. Place drops of blood sample onto the sensor and close the sensor cover. Wait for the reading to stabilize.
4. Record the calcium concentration.

Immediately after measuring the blood sample, wash the sensor with cleaning solution 251, which contains proteolytic enzyme that removes protein-based stains.

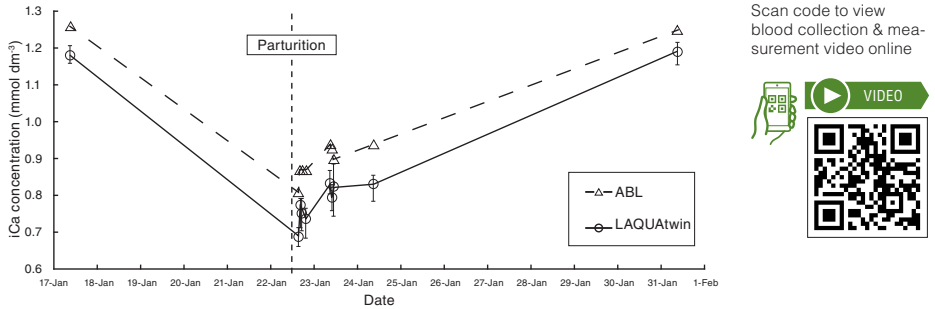


Figure 4. LAQUAtwin Ca-11C and ABL-800 FLEX measurement results of blood samples obtained from a dairy cow suffering from hypocalcemia

Results and Benefits

Subclinical hypocalcemia (cows look normal) is more common than clinical hypocalcemia (milk fever). The iCa concentration in blood of dairy cows is considered to be normal between 1.05 and 1.30 mmol dm⁻³ (mmol dm⁻³ = mmol/L), although there are some differences among studies, and hypocalcemia is diagnosed at 0.9 mmol dm⁻³ or less.⁴ Screening dairy cows for hypocalcemia by measuring ionized calcium in blood using the LAQUAtwin Ca-11C can help dairy farmers and veterinarians assess cows' risk and maximize their health by establishing monitoring and preventative strategies for individual animal or herd-level on farms.

A study was performed by HORIBA Advanced Techno, Co. Ltd. involving the LAQUAtwin Ca-11C and ABL-800 FLEX (laboratory gold standard; hereinafter referred to as ABL) to determine the optimum temperature of cow's heparinized blood sample for measurement. ABL uses an ion electrode with flow-through type cell and performs calibration and measurement at 37°C. When the samples were adjusted to 20 to 25°C using a water bath and measured by LAQUAtwin Ca-11C, the differences between the measurement values of LAQUAtwin Ca-11C and those of ABL were 0.1 mmol dm⁻³ or less. Note that the two instruments have different measurement systems and temperatures. The LAQUAtwin

Ca-11C cannot be used for diagnosis of hypocalcemia but it can help find cows possibly suffering from hypocalcemia and needing further examination and treatment.⁴

A separate study has been performed at Cornell University by Neves et al. (2017) to assess the performance of LAQUAtwin Ca-11C against the VetScan i-STAT (farm gold standard; hereinafter referred to as Vi) and ABL-800 FLEX. A linearity experiment showed no deviation from linearity over a range of iCa concentrations compared with the two instruments. A validation experiment was conducted thereafter. Ionized Ca results from LAQUAtwin Ca-11C cow-side were, on average, 0.06 mmol/L higher than the Vi. With heparin-balanced samples under laboratory conditions, the LAQUAtwin Ca-11C and Vi measured an average 0.04 mmol/L higher and lower, respectively, compared with the ABL. Results from the LAQUAtwin Ca-11C and Vi cow-side were 0.01 mmol/L higher and 0.05 mmol/L lower, respectively, compared with results from the laboratory ABL on heparinized blood. Sensitivity and specificity for the LAQUAtwin Ca-11C and Vi under farm conditions at 3 potential subclinical hypocalcemia cut points were 100 and ≥93.5%, respectively. The LAQUAtwin Ca-11C could become a rapid low-cost tool for assessing iCa cow-side while qualitatively allowing classification of subclinical hypocalcemia on farm.⁵

References and Suggested Readings

1. Christenson, D. Veterinary Medical Terminology E-Book, p. 248-250
2. Bhagavan, N.V., Chung-Eun Ha. Essentials of Medical Biochemistry, p. 488
3. McArt, J. Being a Mom is Hard: Calcium Demands of Early Lactation Dairy Cows. Retrieved from <https://bit.ly/2MoTVvu>
4. Utsumi, R. On-site Quick Measurement of Calcium Ion in Blood of Dairy Cow with Flat Ion Sensor by Rika Utsumi. Retrieved from <https://bit.ly/2LoeFwF>
5. Neves, R.C. et al. Method comparison and validation of a prototype device for measurement of ionized calcium concentrations cow-side against a point-of-care instrument and a benchtop blood-gas analyzer reference method. J. Dairy Sci. 2017, 101, p. 1334-1343

Revision 0, 1 June 2020

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