Title

Evaluation of macromineral meters to detect cation-anion difference concentration and uniformity in a total mixed ration

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Evaluation of macromineral meters to detect cation-anion difference concentration and uniformity in a total mixed ration

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Title: **Evaluation of macromineral meters to detect cation-anion difference concentration and uniformity in a total mixed ration**

Running Title: Predict macromineral concentrations within a TMR

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Declaration of Conflict of Interest
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KEYWORDS: hypocalcemia, TMR, DCAD, macromineral

Interpretive Summary

This study examined the ability of 5 commercially available mineral meters to predict mineral concentrations within a TMR to detect DCAD failure and examine TMR uniformity. The Cl and K meters were best able to predict Cl and K mineral concentrations in the TMR, could be combined to predict DCAD of a TMR and could also be used to predict TMR uniformity in the feed bunk. Use of these meters to estimate Cl and K in the TMR is cheap and more efficient method to detect DCAD failure and TMR uniformity than the current methods of urine pH and laboratory analysis used.

ABSTRACT

Some of the most common failures of dietary cation-anion difference (DCAD) TMR to prevent hypocalcemia, are a lack or dilution of acidifying minerals or inadequate mixing of the TMR. Currently, urine pH is used to monitor metabolic acidification in cows 3 wk prior to parturition. However, urine is difficult to collect and does not indicate why the close up TMR failed to acidify the cows. Mineral meters that measure solubilized mineral from the TMR could be used to approximate the amount of mineral in the TMR. The objectives of this study were to 1) evaluate how well meters predicted macromineral concentration in a TMR compared to laboratory macromineral analyses; 2) determine if the meters could estimate DCAD in TMR for close up and lactating cows; and 3) to determine if the meters could measure TMR uniformity. Meters used were Cl (Oakton SaltTestr,Oakton Instruments, Vernon Hills, IL), K (LAQUA Twin K meter, Horiba Scientific, Kyoto, Japan), S (Hanna Instruments Smithfield, RI) Ca and Mg (Hanna Instruments Ca/Mg Photometer , Smithfield, RI). The TMR samples were collected from close up and lactating cow pens at 10 commercial dairies. Ten subsamples of TMR were

collected per pen at the time of feeding and sent to Analab (Agri-King Inc., Fulton, IL) for analysis. Time length of soaking each TMR subsample for each mineral was pre-determined by identifying the time of plateau of mineral concentration (Cl for 90 min, Ca for 150 min, K for 180 min and Mg for 210 min). Data were analyzed using PROC REG in SAS (SAS Institute v. 9.4, 2021), regressing lab mineral concentration on meter mineral concentration with pen as a covariate. Prediction of Cl and K concentrations in the TMR on a DM basis were best (R^2 = 0.69 and R^2 = 0.78, respectively). The combination of both K and Cl meter concentrations were able to predict DCAD in the TMR in mEq (R^2 = 0.88), and the Cl meter was also able to predict TMR coefficient of variation (CV) (R^2 = 0.89) to evaluate TMR uniformity. All regressions had slopes not different from 1, y-intercepts not different from 0, and normally distributed residual errors. Therefore, Cl and K meters can be used to predict the macromineral concentration, DCAD of TMR, and Cl or K meters can be used to predict overall TMR uniformity of a feed drop.

Keywords: DCAD, TMR uniformity, Cl in TMR, K in TMR

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LITERATURE REVIEW

Introduction

Hypocalcemia, commonly known as milk fever (MF), can be a serious health concern in dairy cattle, increasing the risk of other diseases and affecting overall production. While MF is often not treated until it is clinical, subclinical cases are frequent with an incidence rate of 50 % in multiparous cows and 25 % in first lactation heifers (Rodriguez, 2016: Horst et al. 2003; Reinhardt, 2011). Rodriguez (2017) found that cows diagnosed with subclinical hypocalcemia increased the risk of other diseases, such as ketosis, metritis, displaced abomasum, and retained placenta. This increases the probability for the cow to not only decrease in milk production but have poor reproductive performance as well. One method of prevention for hypocalcemia is the use of a dietary cation-anion difference (DCAD) diet. This method causes a compensated metabolic acidosis by increased feeding of Cl, and S to increase the labile pool of Ca before giving birth. However, acidification may not be successful if the TMR is not properly prepared, mixed or delivered. Avoidance of operator errors and uniform mixing of feed rations plays a vital role in a cow consuming the correct proportions of DCAD macromineral. The feeder may not add the correct ingredients, including the close-up mineral, or may not mix the TMR for the correct time, causing poor uniformity. Poor uniformity will then cause cows to sort through the TMR, only eating certain ingredients from the mix, and not receive the correct macromineral proportions for the DCAD TMR to be successful (Benhke, 2005). A common method used for the detection of a negative DCAD within a TMR is to measure the urine pH of close up cows consuming the acidified TMR. If the cow has been consuming the DCAD TMR between 3 - 7 d, then the urine pH will drop from 7.8 - 8.2 to 6 - 6.7, indicating a metabolic acidification (Sanchez et al., 1999). This method is time consuming as urine can be difficult to collect from

cows, and records need to be kept, making sure the cow has been consuming the negative DCAD TMR long enough for acidification to occur. Ultimately this method will only indicate that there was a failure, but not where the failure has occurred.

The use of handheld anion and cation meters may allow dairy farmers and nutritionists to estimate the proportions of cations and anions within the TMR. By directly testing deionized water soaked TMR samples with the meters, it allows farmers and nutritionists to evaluate DCAD macromineral proportions in a fast and timely manner as well as detect where the failure is occurring since mineral concentrations are measured directly from the TMR. Some of these failures may be, no presence of DCAD mineral within the TMR, poor TMR uniformity making it difficult for cows to consume enough of the mineral, or not enough mineral added to the TMR load. This method would then give same day results and provide solutions for the dairy to implement right away. The objective of this literature review is to assess how cation and anion meters can be utilized to detect proportions of DCAD TMR macrominerals Ca, Na, Mg, K, Cl, and S a within a close-up cow TMR.

Dietary Cation Anion Difference Diet

Close up cows rely on the feeding of a DCAD TMR to mobilize Ca during parturition and early lactation. Before calving, cows require about 18 g Ca for regular body maintenance and calf development, while on the first day of lactation, they require about 55 g of Ca for milk production and body maintenance. Onset of lactation and colostrum production by the cow increase the need for Ca by about 32 g in the blood to avoid hypocalcemia (Horst et al., 1997). An acidifying TMR induces a mild metabolic acidosis. To compensate, bone releases cations, primarily Ca and to help counteract the drop-in blood pH and maintain blood pH within a normal

range. This releases approximately 4 – 5 g of Ca per day prior to parturition (Goff et al., 2003). When cows are fed TMRs higher in cations, parathyroid hormone (PTH) receptors have reduced sensitivity, compromising Ca homeostasis and increasing risk for hypocalcemia. When feeding Cl or SO_4, the PTH receptor changes conformation and is much more effective at mobilizing Ca and promoting secondary signals supporting Ca retention and mobilization. The metabolite I ,25-dihydroxyvitamin D (1,25-$(OH)_2$ D) stimulates intestine to increase intestinal Ca absorption, along with PTH activating resorption from bone to increase Ca within blood (Goff, 2006). This increases sensitivity of the target tissues responsiveness to PTH prior to parturition and lactation to create a pool of ionized Ca for mobilization and prevention of hypocalcemia (Gaynor et al., 1989).

There are several different equations that can be used to calculate DCAD. In the fullest form, the DCAD equation can be written as meq [(Na + K+ Ca + Mg) – (Cl + S + P)]/100 g of DM. The inclusion of Ca, Mg, and P within the equation poses as a problem for ruminants due to their incomplete bioavailability (Sanchez et al., 1999). This created the equation of meq (Na + K+ 0.38Ca + 0.30Mg) – (Cl + 0.60S + 0.5P)/100 g of dietary DM to account for macromineral bioavailability to ruminants (Sanchez et al., 1999). However, it has been found that most nutritionists utilize the equation $DCAD = (mEq\ Na^+ + mEq\ K^+) - (mEq\ Cl^- + mEq\ S^-)$ as many of the other macrominerals have a rather fixed amount within a TMR and it simplifies the DCAD equation (Goff et al., 2003). For example, this equation simplifies dietary mineral formulation by keeping dietary Ca steady between 1 - 1.2 %, P and Mg at 0.4 %, and S between 0.25 and 0.4 % (Goff and Horst, 2003; Gould et al., 1991). Due to the poor bioavailability of S, this macromineral is often not utilized within the TMR as the acidifying agent. Sodium is often

fed in the form of NaCl or salt, discounting its bioavailability as well since it is being directly paired with another DCAD anion. When utilizing this equation in close up cow TMRs, the overall recommended balance is -10 to -15 meq/100g DM (Sanchez et al., 1999). This provides a safe range to account for high K in forages, especially alfalfa, to have an overall acidifying effect on the metabolism, and providing an ionized pool of Ca as a buffer for utilization at the onset of parturition and lactation.

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Potassium. Extracellular K plays an important factor in osmotic equilibrium and maintaining acid base balance. Intracellular K acts as an important co-factor for many enzymes in protein synthesis, carbohydrate metabolism, and maintaining intracellular acid base equilibrium. But, the ratio of intracellular to extracellular K is one of the main determinants of resting cell membrane potentials, which controls nerve and muscle cell excitability (Goff, 2006). If K is low, causing hypokalemia, muscle weakness will occur. The clinical signs of hypokalemia are similar to that of MF in which the cow will become too weak to stand. However, this is not often seen in dairy cows as their K intake is often high due to high forage TMR containing high amounts of K.

Potassium is also one of the most abundant cations in dairy cattle TMRs and plays a large role in increasing DCAD, increasing the risk of hypocalcemia during the onset of lactation and parturition. Forages often used in dry cow TMRs can be especially high in K, such as alfalfa. However, corn silage, which is naturally low in K, containing about 11 - 15 g K/kg, works well for low DCAD TMRs. Goff (2006) recommends that forages from fields that have been fertilized with manure or K fertilizers should be not be used for grazing calving and early lactation cows.

Goff and Horst (1997) found that when comparing TMRs with K levels at 1.1, 2.1 and 3.1 %, cows fed the higher K TMRs had an increase in MF incidence rate by 40%, concluding that an increase of K in a TMR can increase the risk of MF within the herd. It has also been found that high levels of K can interfere with the ruminal absorption of Ca and Mg, increasing the likelihood of hypocalcemia as well as hypomagnesemia. Hypomagnesemia, or grass tetany is often mistaken as hypocalcemia as the clinical signs are very similar and often times anorectic cows in early lactation with a mild case of hypomagnesemia will also have a mild case of hypocalcemia (Divers et al., 2008). Rerat et al. (2009) found that dairy cows given a low K hay prepartum increased DMI during the first few days after parturition by about 2 kg/d. The low K TMR also had a positive effect on the balance of plasma Ca and phosphorus, as Ca and P levels recovered faster than that of cows on a high K TMR. They were able to demonstrate this affect by feeding two groups of Holstein and Brown Swiss dairy cows two different TMRs, one with a low K content of about 13.4 g/kg of TMR and one with a high K content of about 33 g/kg. Increased intakes and rapid plasma Ca recovery were also observed in Rerat (2014) as well as Ramos-Nieves et al. (2009). Rerat (2014) concluded that the increase in Ca urine excretion found pre partum cows with reduced dietary K prepared cows for the Ca demand at the onset of lactation.

To measure potassium ions within the TMR, the LAQUA Twin K meter which utilizes a laboratory grade ion electrode in a flat sensor style, can be used to measure the amount of K+ ions in a sample of water soaked TMR. It can read K ion levels ranging from 39 - 3,900 ppm with an accuracy range of ± 10 % or ± 10 ppm (cite Laqua manual). Kallebach (1997) found that the utilization of handheld ion electrode specific meters, also manufactured by Horiba, were able

to detect K concentrations from alfalfa stem sap taken immediately from the field and be relatively comparable ($R^2 = 0.68$) to that of laboratory oven dried flame photometry results. The meter has also been utilized to sample biological fluids such as blood and plasma. Trefz et al. (2018) found that the meter serves as a reliable cow and calf side test. However, adjustments of -5.1 % for calves and -7.3 % in cows needed to be made to the K concentration meter reading when measuring plasma K concentrations to be comparable to their gold standard indirect ion selective electrode (ISE) method.

Chloride. Chloride is the major anion within extracellular fluid, gastric secretions, and protein digestion. It is also a component in bile, pancreatic and intestinal juices, as well as being responsible for activation of intestinal amylase (Coppock, 1986). Deficiencies in Cl are manifested as pica, anorexia, decreased milk yield, constipation and cardiovascular depression (Coppock, 1986). These symptoms can occur in secondary hypokalemia but are most commonly seen as an increase in hypocalcemia in fresh cows due to the increase in metabolic alkalosis or cations within the body.

In all of Goff's experiments, chloride salts proved to have about 1.6 times the acidifying activity of sulfate (Goff et al., 2004). The absorption of chloride by ruminants is about 95 %, making it the most efficient anion for acidification within a DCAD TMR (Tucker et al.,1991; Church and Fremont, 1979). Chlorides can be highly caustic and decrease DMI severely if too much is added (Goff, 2006). Chlorides are commonly fed as magnesium chloride ($MgCl_2$), calcium chloride ($CaCl_2$), and hydrochloric acid (HCL), however HCl has the largest effect on acidification. While it has the largest affect, safety is required when handling any liquid form of it (Goff, 2006).

Chloride anions within the TMR may be measured using the Oakaton SaltTestr Pocket Meter. This meter utilizes electrical conductivity to measure chloride anions within the water soaked TMR sample. It has the ability to measure concentrations ranging in 0 - 10 ppt with an accuracy of ± 1 %. The Oakton SaltTestr has been previously used to measure TMR uniformity across mixer wagon loads. Chloride was measured in 10 subsamples of water soaked TMR from 1 mixer wagon load. The coefficient of variation (CV) of the measurements has been found to be indicative of a need for mixer wagon maintenance. When the CV was high, mixer wagon maintenance was needed, and when measured directly after maintenance, low CVs were present (H. Rossow, University of CA, Davis, Tulare, CA, personal communication).

Sulfur. Sulfur is important in rumen health. Feeding a low supplemental S additive such as sodium sulfate, within a dairy cattle TMR has been found to increase microbial growth and microbial protein supply within the rumen (McSweeney et al., 2007). But excessive sulfur levels can be harmful to dairy cattle by decreasing DMI, as well as decreasing ruminal microorganisms (Kandylis, 1984). Too much sulfur within the TMR can become toxic as it can be reduced to sulfide within the rumen. Gould et al. (1991) fed calves high levels of sulfur which increased rumen sulfide concentrations and caused sulfide toxicosis. This has also been seen in sheep fed H_2S in its anionic form, causing neuronal necrosis (Gould et al.,1991; Dirksen et al., 1982) The amount of sulfur that can be allowed within the TMR is limited to 0.4 % as recommended by the NRC in 2001.

Sulfur is less efficient than chloride salts to induce metabolic acidification, as they only acidify 60 % as well as chloride (Goff, 2006). Sulfur is excreted through the urine and bile, having a less acidifying affect than that of chloride. This has now been adjusted for within the DCAD equation as $DCAD = (mEq\ Na^{+} + mEq\ K^{+}) - (mEq\ Cl^{-} + 0.6mEq\ S^{-})$ (Goff et al., 2004). Sulfur can be added to the TMR using calcium sulfate and sulfuric acid, which has been found to be more palatable than chloride, but still has palatability issues of its own. However, sulfur is often purer than that of reagent grade HCl, therefore it would require a very low amount within the TMR than that compared to HCl. For example, there is 1 Eq of anion/ 28 mL of concentrated H2SO4 where HCL supplies 1 Eq of anion/ 83 mL (Goff et al., 2004).

Sulfate may be measured within the TMR by utilizing the Hanna Instruments Portable Sulfate Photometer to read sulfate concentrations in a water soaked TMR sample. This meter uses an adaptation of the turbidimetric method which uses barium chloride reagents and causes turbidity when mixed with the sample. Turbidity causes a loss of light due to the scattering effects of the particles suspended. The photometer is then able to read the sulfate based on the loss of light. The meter is able to measure sulfate concentrations from 0 - 150 ppm, with an accuracy of ± 1 ppm or ± 5 % of the reading. This meter has been used to detect sulfate concentration of groundwater in Ahmad et al. (2016). It has also been used in Aurica (2020), to determine the hardness level of drinking water.

Magnesium. Magnesium is an essential nutrient in a dairy cattle TMR. It acts as a cofactor for many enzymes and is involved in energy metabolism, protein synthesis, cell growth, and DNA and RNA synthesis (Schonewille, 2013). Mg is also largely involved in the gating of

ion channels, which is why a deficiency in Mg is associated with neurological symptoms due to the impairment of the channel functions in the CNS (Martens et al., 2018). This is commonly known as hypomagnesemia, or grass tetany. Because there is not a known specific regulatory system of Mg within the body, it is often common to maintain Mg in the plasma within the range of 0.9 - 1.2 mmol/l. This range is largely dependent upon the Mg absorption from the rumen, as Marten et al. (1980) found during an in vitro study when the transport capacity of the rumen epithelium greatly outweighed that of the forestomach and large intestine (Marten et al., 2018). To maintain the range, the TMR may contain up to 0.4 % of a ration in Mg on a dry matter basis (Urdaz et. al, 2003).

Mg works in conjunction with Ca ions to aid in the proper functioning of muscles and nervous system. Mg also plays a large role in the metabolism of the PTH and Vitamin D and increasing sensitivity of target tissues for Ca mobilization (Schonewille, 2013). When there is a Mg deficiency within the body, PTH production may fall. Due to adenylate cyclase and phospholipase C, both initiated by PTH, not having a Mg ion bind to their active sites for full activity. This not only can cause hypomagnesemia, but hypocalcemia as well, as it has been found that cows that consume a TMR containing less than 85 mmol per L of Mg can increase the incidence of hypocalcemia (Van De Braak et al.,1987; Sansom et al., 1983).

Hanna Instruments Ca/Mg portable photometer (Smithfield, RI) presents an opportunity to be able to measure Ca and Mg concentration available within the TMR. The photometer operates by using a combination of an LED light source with a narrow band interference filter and silicon photodetector to receive accurate photometric readings of magnesium by utilizing the calmagite

colorimetric method. The calmagite method utilizes liquid reagents with a colored dye that forms a purplish-blue color that changes to a red color when reacting with free magnesium within a solution (Hanna Instruments, 2020). With the color change, the photometer is then able to detect the amount of Mg within the solution by utilizing the light wavelength read within the photometer. It can detect up to 150mg/L with an accuracy of ± 3 mg/L or ± 3 % of reading. The meter also utilizes the oxalate method for the determination of Ca within a solution. This will also cause a color change due to the Ca concentrations and using the beer lambert law, the meter is able to determine the concentration due to the narrow band interference filter. The Ca meter ranges from 0-400ppm with an accuracy of ± 10 ppm or ± 5 % of reading. This may allow Mg and Ca concentrations contained within the TMR to be determined on farm.

Conclusion

The use of a DCAD TMR is one of the most popular and successful preventative methods used for preventing MF in dairy cattle. However, there are many failures that can occur when utilizing the DCAD method, including operator errors, lack of mineral in the TMR, or poor TMR uniformity. Detection of failures is time consuming and often inconclusive. This raises the question if portable anion and cation meters can be used to detect DCAD macrominerals within a ration. The use of these meters can provide faster and more conclusive answers to dairy producers and nutritionists utilizing DCAD TMRs and preventing MF.

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INTRODUCTION

Hypocalcemia is a major health concern during the pre and postpartum periods because it decreases milk production and increases the cow's risk of other diseases, such as ketosis, metritis, displaced abomasum, and retained placenta (Rodriguez, 2017). Metabolic acidification is often used to prevent hypocalcemia. This is achieved by using a negative DCAD TMR 3 wk prior to parturition. When cows are fed TMR high in cations, parathyroid hormone (PTH) receptors have a reduced sensitivity, compromising Ca homeostasis and increasing risk for hypocalcemia (Goff, 2006). To counteract this effect, close up (CU) cows rely on a negative DCAD TMR which utilizesCl or S to increase sensitivity of the target tissues responsiveness to PTH. This creates a pool of ionized Ca for mobilization and prevention of hypocalcemia (Gaynor et al., 1989). The most common DCAD equation used is $DCAD = (mEq\ Na^{+} + mEq\ K^{+}) - (mEq\ Cl^{-} + mEq\ S^{-})$. The equation keeps dietary Ca steady between 1-1.2 %, P and Mg at 0.4 %, and S between 0.25 and 0.4 % (Goff and Horst, 2003; Gould et al., 1991). When utilizing this equation in CU cow TMR, the overall recommended balance is -10 to -15 meq/100g DM (Sanchez et al., 1999). This provides a safe balance to account for high K in forages, while having an overall acidifying effect on the metabolism, and providing an ionized pool of Ca as a buffer for utilization at the onset of parturition and lactation.

Two common failures of metabolic acidification are related to feed management: 1) dilution of the acidification minerals in the mixer wagon or 2) the incomplete mixing of the TMR due to operator error or poor mixer wagon maintenance. In the first failure, if there is residual feed from the previous mixer wagon load mixed with the acidifying TMR, then there may be too much K and not enough Cl or S to adequately acidify the cows. This can also occur if the feeder does not

add enough of the acidification minerals to the TMR. Urinary pH has also been used to determine degree of acidification of the cows. However, this method is time consuming, prone to error if cows have not had adequate exposure to an acidification TMR and urine can be difficult to collect from cows.

In the second failure, the feeder may not mix the TMR long enough or the mixer wagon may not be mixing the TMR efficiently causing poor TMR uniformity. Poor TMR uniformity will cause cows to not receive the correct mineral proportions for acidification to be successful (Benhke, 2005). Ration uniformity can be monitored by TMR audits, which use either a particle sorter such as the Penn State Particle Separator (Pennsylvania State University, University Park, PA) or random TMR grab samples which can be analyzed for an indicator mineral such as Cl (Behnke, 2005). These audits can be time consuming, prone to error and expensive (Oelberg and Stone, 2014).

Meters that measure soluble mineral concentration within a TMR sample, however, can provide results at lower cost and within the same day that TMR samples are collected. These meters could also be used to estimate acidification within a CU TMR and monitor TMR uniformity. The objectives of this study are to 1) to evaluate the ability of the meters to estimate Cl, K, Ca, Mg, and S from water soaked TMR as representative of mineral content in the TMR from laboratory analysis, 2) to determine if the mineral meters can estimate DCAD in lactating cow and CU TMR and 3) to determine if the commercial meters can evaluate TMR uniformity.

MATERIALS AND METHODS

No approval from the University of California, Davis, Animal Care and Use Committee was needed for this study since it was conducted using only feed samples and live animals were not involved in this research.

Dairy Description and Pen Selection

The TMR samples for this study were collected from 10 commercial dairies located in Tulare County, Kings County and Glenn County (California) from December of 2020 through March of 2021. All dairies utilized a feed management program, such as EZ Feed (DHI- Provo, Provo, UT) or FeedWatch (Valley Ag Software, Tulare, CA), and backup files of each dairy were used to obtain feed management and herd data for the statistical analysis. A negative DCAD TMR was utilized in all CU pens and a positive DCAD TMR was used in all lactating cow (LC) pens. The highest milk producing pens were selected as LC pens for each dairy.(Table 1).

Sample size determination

Minimum sample for regression is 8 (Jenkins and Quintana-Ascencio, 2020). This study used 10 TMR samples per pen for TMR uniformity, 10 dairies with 2 pens per dairy for a total sample size of 20 for mineral meter prediction of laboratory mineral analysis and mineral meter prediction of DCAD.

TMR collection

The TMR samples were collected immediately after the TMR was dropped at the pen. This ensured that no alterations could be made by cows consuming or sorting through nutrients. Samples were collected using the hand grab method described by Robinson and Putnam (1998). Gallon bags were labeled 1-10 and taken in ascending order, starting at the beginning of the TMR drop and ending at the end of the TMR drop from the mixer wagon. Then the TMR was weighed into sub-samples. The first set of 10 sub-samples were soaked in deionized water and

analyzed for Cl, K, Ca, Mg, and S using the meters. The second 10 TMR sub-samples were sent to Analab (Fulton, IL) to be analyzed for mineral content.

TMR laboratory mineral analysis

The second 10 TMR sub-samples, were analyzed using wet chemistry (AOAC International, 1999; methods 935.29, respectively), and mineral analyses (Ca, P, Mg, K, S, Na, Cl, Fe, Cu, Mn, and Zn) using inductively coupled plasma-mass spectrophotometry (AOAC International, 1999; method 985.01 for Ca, P, Mg, K, Na, Fe, Cu, Mn, and Zn; method 923.01 for S; and method 915.01 for Cl).

TMR meter mineral analyses

This experiment utilized 5 mineral meters to measure Cl, K, Mg, Ca, and S concentrations in the first TMR sub-samples. Chloride concentrations were determined using the Oakton SaltTestr (Oakton Instruments, Vernon Hills, IL) which uses electrical conductivity to measure Cl anions within water. Potassium concentrations were determined using the LAQUA Twin K meter (Horiba Scientific, Kyoto, Japan) which uses a laboratory grade ion electrode in a flat sensor style. Magnesium and Ca concentrations were determined using the Hanna Instruments Ca/Mg portable photometer (Hanna Instruments, Smithfield, RI). This photometer uses the calmagnite method to detect Mg, and the oxalate method to detect Ca. Sulfate concentrations were determined using the Hanna Instruments Sulfate portable photometer (Hanna Instruments, Smithfield, RI), using an adaptation of the turbidimetric method to detect S concentrations in water. We were unable to find a sodium meter and so sodium was not included in our analyses. All meters were operated according to manufacturer's protocol and the meters could only measure mineral concentration in liquid, i.e., soluble form. Therefore, TMR samples had to be soaked in de-ionized water to estimate the mineral concentration and only the soluble content of

the mineral concentration could be estimated. Therefore we would not expect mineral levels from laboratory analysis to be the same as mineral levels using the mineral meters. Our analyses focused on which meter mineral amounts were representative or proportional to laboratory analyzed mineral amounts in the TMR.

Determination of measurement protocol for each meter

To use the meters to estimate the concentration of minerals in the TMR, the amount of time needed to soak the TMR sample to maximize the concentration of soluble mineral, needed to be determined. Peak measurement time was defined as the time at which soluble mineral concentration was highest and was the time to measure mineral concentration using the meter. At this time there would be minimal loss due to volatilization and further soaking would not leach more mineral into solution.

To estimate peak measurement time for each meter, 10 other TMR samples from a CU pen were soaked in deionized water. For the Cl and K meter, 30 g of TMR were soaked in 150 mL of room temperature deionized water. This gave the appropriate concentration range of mineral for the meter. The Ca/Mg and S meters used 7.5 g TMR soaked in 300 mL of deionized water because the photometers had a smaller concentration reading range than that of Cl and K. During soaking, each beaker was stirred to ensure water covered the entire sample. For the S meter, the TMR soaked water was filtered through qualitative Grade 613 filter paper because turbid water would interfere with the meter concentration reading. TMR samples were soaked for a total of 240 min and mineral concentrations were measured every 30 min. The time of 240 min for soaking was chosen because soaking beyond 4 h would make this method too time consuming to be used in the field and, for potentially volatile compounds involving minerals such as Cl,

mineral concentrations could begin to decrease. For example, preliminary tests examining effects of length of storage time on mineral concentrations and soaking time length indicated that TMR samples needed to be analyzed within 4 h of collection or else soluble mineral content was decreased (data not shown).

Using meters to determine mineral concentrations in TMR

To compare meter mineral concentrations to laboratory analyzed mineral concentrations in the TMR, meter mineral concentration was converted from ppm or mg/kg to a percent DM basis according to the following equations for each meter:

K mg/kg TMR solution * 0.03 kg of TMR / 0.18 kg TMR solution / lab kg TMR on a DM basis = K mg/kg TMR on a DM basis / 1,000,000 mg/kg * 100 = % K in TMR on a DM basis (% DM)

The Cl meter measures Cl concentration in ppt. A conversion of ppt to ppm was provided by the manufacturer ((Oakton Instruments, Vernon Hills, IL). This led to the following equation for Cl: Cl mg/kg TMR solution * 0.03 kg of TMR / 0.18 kg TMR solution / lab kg TMR on a DM basis = Cl mg/kg TMR on a DM basis / 1,000,000 mg/kg * 100 = % Cl in TMR on DM basis (% DM).

The Mg/Ca and S photometers required a dilution of TMR to deionized water ratio and therefore used the following equation:

Mg, Ca, or S mg/kg TMR solution * 0.0075 kg of TMR / 0.30075 kg of TMR solution / lab kg TMR on a DM basis

= Mg, Ca or S mg/kg TMR on a DM basis / 1,000,000 mg/kg *100 = % Mg, Ca or S in TMR on DM basis (% DM).

DCAD estimation

The Cumberland Valley Analytical Services DCAD calculator (CVAS, Waynesboro, PA) was used to estimate DCAD. The calculator required the % of each mineral within the TMR to be input on a DM basis and automatically converted % DM to mEq. Observed DCAD was calculated using the equation from Goff et al. (2003), (K + Na) – (Cl + S). Predicted DCAD was calculated as a reduced equation of (meter K – meter Cl). The Cl and K are the most influential cation and anion in dairy TMR because K is the most abundant cation and Cl is the most acidifying anion present in a TMR (Goff, 2006; Goff et al., 2004). Therefore, Cl and K were used predict DCAD using the reduced equation.

TMR uniformity

TMR uniformity was assessed according to Behnke (2005) with some modifications. The 10 TMR sub samples from each pen were soaked as described in the previous section and at peak measurement time, each meter was used to estimate the concentration of mineral in each TMR sub sample. Then coefficient of variation (% CV) was calculated for each pen based on mineral concentrations of the 10 samples. The % CV was also estimated for each pen using the mineral concentrations in the 10 subsamples that were analyzed by Analab. The % CV from the lab analyzed samples were then compared to the meter estimated % CV to determine which meter best estimated the variation among pen TMR samples.

Statistical Analysis

The unit of interest in this study was pen. For all analyses except TMR uniformity, % minerals were averaged for the 10 TMR samples per pen.

To determine peak measurement time, soaked mineral concentrations determined by each meter over 240 min were evaluated by a box and whisker plot. The average mineral concentration was denoted by the x, standard deviation (SD) by the box edges, and error ranges by the error bars or whiskers (Figure 1). Peak measurement time was determined to be the time when mineral concentration was highest on the graph followed by a plateau with similar SD between samples (similar box sizes), within the 240 min time period. The peak or plateau concentration times were then used as the time of measurement for each mineral meter. Since we were unable to determine a peak measurement time for the S meter, analyses of S data were not continued.

To evaluate the meters' ability to predict % Cl, K, Ca, and Mg in the TMR on a DM basis, predicted average meter estimates for each pen were regressed on observed average laboratory nutrient analyses using the PROC REG procedure of SAS (Statistical Analysis System, v.9.4). Ten TMR sub-samples per pen mineral %, were averaged for the lab analysis and for the meter analysis. The dependent variable was the average % of mineral on a DM basis from the feed analysis laboratory for each pen and the independent variable was the average % mineral in the TMR from the mineral meter for each pen. Pen TMR type (CU or LC) and dairy were also included as covariates in the regression equation, but dairy was eliminated because it did not contribute to the regression. If the confidence interval for the intercept included 0, the intercept was removed from the regression analyses. To determine if there was a pattern or bias in the residuals, the residuals were regressed on the observed average laboratory analyzed % mineral. Meter and lab analyses estimates for each mineral were also compared by partitioning of the mean square predicted error (MSPE; Bibby and Toutenburg, 1977).

To determine if the mineral meter could predict level of DCAD in LC and CU TMR, the predicted average mineral meter mEq from the reduced equation (K – Cl) per pen was regressed on the observed average DCAD mEq estimated from the TMR laboratory analyses K, Na, Cl and S concentrations per pen using the PROC REG procedure of SAS. The dependent variable was the DCAD in mEq from the TMR analysis laboratory and the independent variable was the mineral meter DCAD in mEq from each pen TMR type (CU or LC). Dairy was included as a covariate in the regression equation but was eliminated because it did not contribute to the regression. If the confidence interval for the intercept included 0, the intercept was removed from the regression analyses. To determine if there was a pattern or bias in the residuals, the residuals were regressed on the observed average laboratory analyzed DCAD from each pen. Meter and lab analyses estimates for DCAD were also compared by partitioning of the MSPE.

Determination of diagnostic sensitivity and specificity for DCAD predictability was assessed using MedCalc's diagnostic test evaluation calculator (MedCalc v. 20.027; MedCalc Software Ltd., Ostend, Belgium). Generally, 0 is used as a cutoff point to differentiate between a CU and LC DCAD, CU DCAD being less than 0 and LC DCAD being greater than 0. This held true for the laboratory observed DCAD, but not the meter predicted DCAD. The Cl meter was able to read a larger concentration than that of K meter, resulting in more negative readings of the LC DCAD after the combination occurred. Therefore, a shift from 0 to -35 was needed for the meter predicted DCAD resulting in TMR DCAD ≥ 0 mEq from the laboratory analyses and meter DCAD $\geq$ -35 mEq to be defined as a positive DCAD (LC ration). The DCAD values ≤ 0 mEq

from the laboratory analyses and meter DCAD values ≤ -35 mEq were defined as a negative DCAD (CU ration).

To determine if the mineral meters could predict TMR uniformity, meter mineral % CV for each pen was regressed on % CV for mineral concentrations from the TMR feed laboratory analyses using the PROC REG procedure of SAS. The independent variable was the TMR laboratory analyses mineral concentration CV and the dependent variable was the mineral meter concentration % CV for each pen. Pen TMR type (CU or LC) and dairy were included as covariates in the regression, but dairy was eliminated because it did not contribute to the regression. If the confidence interval for the intercept included 0, the intercept was removed from the regression analyses. To determine if there was a pattern or bias in the residuals, the residuals were regressed on the observed averaged laboratory analyzed % CV for each mineral. The % CV from meter and from lab analyses estimates for each mineral were also compared by partitioning of the MSPE.

RESULTS AND DISCUSSION

In order to test the meters ability to predict mineral concentrations, DCAD and uniformity of a TMR, 10 TMR samples per pen from CU and LC pens at 10 dairies were collected and analyzed. These dairies represented a wide range of mineral values, feed management practices, and DCAD concentrations (Table 1). The number of cows per dairy, cows per pen and pen types differed among dairies with CU pens being all dry lot pens and LC pens varying between freestall and dry lot pens. Two herds were mixed breed with both Holsteins and Jerseys in the CU and LC pens. Management factors such as milk production level, mixer load size, feeding

frequency and feeding times also varied among the dairies. To provide data representing different mineral and DCAD concentrations, TMR samples were collected from the CU and LC pens. In both CU and LC pens, % K in the TMR ranged from 1.05 - 1.86 % DM, % Cl in the TMR ranged from 0.4 – 1.2 % DM, % Ca in the TMR ranged from 0.5 – 2.12 % DM, % Mg in the TMR ranged from 0.3 – 0.7 % DM, and % S in the TMR ranged from 0.19 – 0.6 % DM according to laboratory analyses (Analab, Fulton IL). All dairies used a negative DCAD TMR for the CU pens, ranging from -1.3 to -22.1 mEq, and a positive DCAD for the LC pens ranging from 16.3 – 30.4 mEq. This data represented mineral concentration differences in both CU and LC TMR and were used to determine the predictability of mineral meters to estimate mineral concentration, DCAD concentration in CU and LC TMR and to determine if the meters can be used to evaluate TMR uniformity.

Measuring mineral levels in TMR using the meters

To use the meters to estimate amounts of minerals in the TMR, how much time was needed to soak the TMR sample to maximize the yield of soluble mineral and if the soluble mineral concentration was representative of the total mineral concentration needed to be estimated. Peak measurement time (Figure 1), the time at which soluble mineral content is highest in the TMR water solution, was determined to estimate the ideal soaking time for each meter (Table 2). The Cl meter had the fastest peak measurement time followed by Ca and then K. The Cl, Mg, and S meters had a small SD and error within the data across all time points compared to the K and Ca meters. This is not surprising because the K meter had the largest error of measurement followed by the S and Ca meters. However, the K meter was also able to determine a much larger reading range of % K compared to the ranges of the other meters. For the Mg meter, 240 min was not recorded because the meter malfunctioned due to low light in the photometer (Figure 1). For the

Ca meter, 30 min reading was not recorded as the concentration was below the meter reading range. All meters also tended to have greater SD as time of soaking increased. But, the S photometer had 50 % more variation at 210 min, and it was more difficult to identify a peak measurement time for the S meter. Therefore, we did not continue our analyses with the S meter.

To determine if the soluble mineral concentrations as measured by the meters were representative of the total mineral concentration as determined by laboratory analyses, meter and lab analyses estimates for each mineral were compared using regression analyses of predicted (meter soluble mineral %) on observed (lab analyses of total mineral %) and partitioning of the MSPE (Table 3). The partition of the MSPE is a measurement of the quality of an estimate, and splits the error associated with the estimate into 3 different categories; bias of prediction (difference contained within the model's predicted values versus the observed), slope not equal to 1(error associated with the slope not being equal to 1) and random variation (variation contained within the observed data). Ideally, all the error should be due to random variation. The meter predicted % Cl and % K were the best estimates of laboratory observed total % Cl and K in the TMR. The predicted on observed regressions for both % Cl and K had no intercept, high coefficients of determination, low MSPE and the majority of their MSPE was due to random variation in the data, not to the ability of the meters to estimate % Cl and K. The lack of intercepts indicated that the soluble % Cl and K estimated by the meters are proportional to the total % Cl and K in the TMR and the relatively high slope reflects the relationship between the meter estimates and laboratory analyses. Plots of predicted vs observed for % Cl and K, Figures 2 and 3, respectively, show the deviation of the slope from the line of unity and lack of bias in the residuals for both % Cl and % K.

The Cl meter's larger slope when compared to that of K, indicated that the Cl meter was able to detect a larger proportion of the soluble Cl than the K meter detected soluble K (Table 3). This could be due to Cl being more soluble than K, as Cl was mainly contained within the mineral portion of the TMR (Rippel et al., 1998) and K was largely contained within the stems and leaves of the hay or silage, making K less soluble than that of Cl (Goff, 2006). Kallenbach (2000), used a previous version of the Laqua Twin K meter to determine K concentration in ppm of alfalfa in the field utilizing plant sap and compared it to flame photometery. Kallenbach concluded that the meter accurately predicted K concentration in ppm contained within the alfalfa plant by regressing meter K concentration in ppm (predicted) on lab K concentration in ppm (observed) with moisture content and stem length as covariates ($R^2 = 0.89$). The study found that the K meter reading alone would underestimate the K concentration in ppm in the plant tissue but would accurately predict the concentration in ppm when used in the predictive model. These results were also in agreement with the current study, which found that the K concentration from the meter alone would slightly underestimate the concentration contained within the TMR

Both the Mg and Ca meters had slopes equal to 0 indicating that there was no relationship between predicted meter % Mg and Ca and observed total % Mg and Ca in the TMR (Table 3). This is not unexpected since it was more difficult to determine a peak measurement time for these meters even though the Mg meter was more precise (Figure 1). The use of reagents for the Mg and Ca photometer could have introduced more variation into the meter measurement because as the reagents were difficult to accurately measure with the tools and instructions given

in the kit. Therefore, the Mg and Ca photometer should not be used to predict the mineral concentrations within the TMR.

DCAD calculation and detection

There are several problems in feed management that can lead to a lack of acidification in a CU DCAD diet. Some common problems are not adding the correct (negative) mineral premix to the CU TMR or leaving some residual feed from a positive DCAD TMR (previous load) in the mixer wagon thereby diluting the negative DCAD in the CU TMR. Therefore, having a relatively simple and quick method to estimate DCAD in a TMR is valuable for a dairy to help troubleshoot or monitor negative or positive DCAD in the TMR. To determine if the Cl and K meters could predict DCAD level of a CU or LC TMR, the full DCAD equation in mEq (Goff et al., 2003) of (K + Na) – (Cl + S) was reduced to (K – Cl) in mEq (Goff, 2006; Goff et al., 2004). The minerals Cl and K are the most influential cation and anion in dairy TMR because K is the most abundant cation and Cl is the most acidifying anion present in a TMR (Goff, 2006; Goff et al., 2004). Therefore, Cl and K were combined to predict DCAD using the reduced equation. Predicted DCAD was calculated from the meter % K and Cl values from the soaked TMR converted to mEq in the reduced DCAD equation. Observed DCAD was calculated from % K, Na, Cl and S from the laboratory analyses converted to mEq using the full DCAD equation using a DCAD calculator (CVAS, Waynesboro, PA). Regression of predicted DCAD on observed DCAD had a zero intercept, a relatively high coefficient of determination and high MSPE but, most of the MSPE was due to random variation in the laboratory analyses data (Table 3). Similar to the prediction of % Cl and K, the slope indicated that the reduced DCAD predicted from the TMR soaked Cl and K was proportional to the observed full DCAD from the lab analyses. Data were in 2 distinct clusters indicating that the meters could be used to differentiate negative and

positive DCAD TMR. There was little bias in the residuals overall ($P > 0.01$) showing a general trend of increasing with increasing observed DCAD but the meter DCAD may not be able to distinguish how low or high is the DCAD within its negative or positive category (Figure 4). Predicted DCAD had a high sensitivity, specificity and accuracy (Table 4) indicating that 90 % of the time the meter predicted DCAD would correctly report a positive DCAD reading, 100% of the time a negative DCAD and 95% of the time the predicted DCAD could differentiate between and positive and negative DCAD TMR. But, further testing is needed using more TMR DCAD estimates within the negative and positive DCAD categories including TMR that have negative DCAD failure.

Using these meters and methods, a dairy could predict negative or positive DCAD of a TMR using data from the Cl and K meters. Because this method estimates DCAD in the TMR instead of using urine pH to detect failures of metabolic acidification, it has several advantages. It can relatively quickly determine if the CU TMR is adequate rather than waiting for approximately 4 d before testing cows (Sanchez, 1999). Therefore, the dairy can tell the difference between a feed management problem and a cow problem. Using urine pH to detect acidification does not directly identify the reason for failure. The meters high sensitivity, specificity and accuracy (Table 4) indicate that by testing the TMR directly using the K and Cl meter concentrations, the results could indicate if there was a lack or dilution of acidifying mineral within the CU DCAD TMR, without the need to collect urine.

Meter ability to predict TMR uniformity

Delivering a uniform TMR ensures that all cows within a pen consume the same nutrients. Poor TMR uniformity can cause sorting or bolus feeding of critical nutrients which can cause poor

performance, wasted feed, unnecessary costs, and toxicities. Using % coefficient of variation (CV) of Cl in at least 10 samples per mixer wagon load to assess TMR uniformity was suggested by Behnke (2005). He determined that a CV greater than 10% meant that the TMR was not distributed across the bunk uniformly and identified associated mixer wagon malfunctions associated with the order of concentration of Cl in the TMR samples. Other studies have also found that about 42 % of dairies have a CV of < 10 %, 46 % between 10 - 20 %, and 12 % greater than 20 %. (Behnke, 2005; Wicker and Poole, 1991; Stark et al., 1991). These CV were then used to diagnose TMR mixing problems such as insufficient mixing time, mixer overload, worn augers or paddles, and improper loading sequence (Oelberg and Stone, 2014). Rippel et al. (1998) also used Cl concentration to measure TMR uniformity and compared the Cl method to the Penn State Particle Separator. He found the Penn State Particle Separator did not work well to detect TMR uniformity because there was a large amount of variation associated with the amount of TMR that would appear on each tray for a given sample.

The reason why this method was not adopted to measure uniformity was because Behnke used Quan Tab Cl strips (Hach, Loveland, CO) to measure Cl (mg/L). The strips are expensive, about $0.50 to $2.00 a piece and so measuring uniformity for 1 pen or mixer wagon load would cost about $5-$20. The strips also require that the TMR sample be soaked in hot deionized water and that the TMR sample was strained from the water before using the strip as the strips could easily plug up form small feed particles in solution. Because the strips can get expensive, we used the Cl and K meters to measure the soluble % Cl and K content in 10 TMR samples per pen for both CU and LC pens at each dairy. The % CV of Cl and K meters and laboratory total Cl and K % CV were compared for each respective meter (Table 5). Both the Cl and K meters had good

agreement between predicted meter soaked and observed laboratory analyzed % CV for Cl and K. Both had zero intercept and low MSPE but, the Cl meter had a much higher coefficient of determination and more of the MSPE was due to random variation in the data than for the K meter. Therefore, the Cl meter was better able to predict the % CV of total Cl in the TMR samples than the K meter.

Meter performance evaluation

The Cl and K meters were both simple to use and required no extra reagents (Table 2). The Cl meter was the simplest and fastest to use, needing very little calibration. While the K meter was the most expensive meter, it could estimate a large range of % K in the TMR on a DM basis. However, it required 3 mL pipets to extract 3 mL of water to place within the meter and needed to be calibrated before each use.

CONCLUSION

This study showed that the Cl and K meters best predicted their respective macromineral concentrations within a TMR, could be used to monitor TMR uniformity and they could be used to estimate % Cl and K in a TMR. Using a reduced DCAD equation, they could also predict DCAD in CU and LC TMR. The meters not only provide a cheaper and more efficient solution for detecting DCAD failure but can also detect where the failure is occurring within the feeding process.

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APPENDIX

Table 1. Dairy description of close-up (CU) and lactating cow (LC) pens sampled by dairy

	Total Cows Milking	Breed[1]	Cows in CU Pen[2]	Average Days Carry Calf[3]	Hypocalcemia Incidence Rate[4], %	CU mineral type[5]	LC Pen Type[6]	Cows in LC Pen[7]	Average DIM[8]	305 ME, kg	LC mineral type[5]
Dairy											
1	1567	H	82	268	2%	Pdr/Plt	DL	140	129	30874	Pdr
2	5198	H	338	266		Pdr/Plt	FS	363	158	32007	Pdr
3	4754	Mix	110	286		Pdr/Plt	FS	233	181	28752	Pdr
4	2375	H	149	267		Pdr	DL	263	143	29876	Pdr
5	742	Mix	57	258	1%	L/Plt	FS	129	180	30724	Pdr
6	878	H	51	266	<1%	L/Pdr	FS	159	184	32381	Pdr
7	2030	H	92	265	1%	Pdr	FS	269	174	29574	Pdr
8	5882	H	183	262		Pdr	FS	395	108	30196	Pdr
9	10080	H	317	248		Pdr	FS	228	115	18033	Pdr
10	1653	H	69	272		Pdr	FS	194	201	29674	Pdr

[1] Breed of cows' dairy is milking being that of all Holstein (H) or mixed (40% Holstein and 60% Jersey)
[2]Total number of cows listed in pen for cows 3 weeks before parturition (CU) at time of sampling
[3]Average number of days cow has been carrying calf when moved to CU pen
[4] Hypocalcemia incidence rates as a self-reported event by the dairy
[5]Type of mineral form used for pen, being that of a powder (Pdr), pellet (Plt), or liquid form
[6] Pen type description being that of a dry lot (DL) or free stall (FS) for lactating cow pens (LC) sampled
[7] Total number of cows listed in the LC pen at time of sampling
[8] Average DIM for LC pen sampled

Table 2. Mineral meter descriptions

	K	Cl	S[1]	Mg[1]	Ca[1]
Meter Cost, $	435	88	220	235	235
Cost/Sample[2], $	0	0	0.40	0.29	0.29
Error of Measurement[3], %	10	1	5	3	5
Reading Range, mg/kg	39 – 3,900	0 – 1.0	0 – 150	0 – 150	0 – 400
TMR to Water Ratio[4], g/g	30/150	30/150	7.5/300	7.5/300	7.5/300
Peak Measurement Time, min	180	90	N/A	210	150
Calibration Frequency	Each use	Every 6 months	Each use	Each use	Each use

[1] Hanna Instruments portable photometer

[2] Reagent cost per sample was calculated by dividing the cost of the reagent by the number of samples the reagent provided for

[3] Error of measurement is how close the meter measurement is to the true value of mineral within solution as reported by manufacture

[4]Deionized water used

Table 3. Comparison of meter predicted mineral % in the TMR on a DM basis to observed laboratory mineral % in the TMR on a DM basis

Descriptive statistics	Cl	K	Mg	Ca	DCAD[1,2]
Observed mean, % in TMR DM basis	0.91	1.4	0.45	1.1	5.9
Predicted mean, % in TMR DM basis	0.91	1.4	0.45	1.1	6.2
Observed SD, % in TMR DM basis	0.44	0.23	0.091	0.37	19
Predicted SD[3], % in TMR DM basis	0.39	0.20	0.062	0.24	18
Linear regression of meter predicted mineral concentration on observed laboratory mineral concentration					
Intercept (H0: $\beta = 0$)	$P > 0.01$	$P < 0.01$	$P < 0.01$	$P < 0.01$	$P > 0.01$
Intercept	0	0.41	0.94	1.2	0
Slope	0.92	0.73	0	0	0.86
Mean Square Error	0.068	0.014	0.0046	0.084	53
Root Mean Square Error	0.26	0.12	0.068	0.29	7.3
Coefficient of Determination	0.69	0.78	0.47	0.42	0.88
Mean Bias, %	0.92	0.0077	0	0	-6.1
Mean Absolute Error	0.21	0.097	0.053	0.18	6.1
Mean Square Predicted Error	0.062	0.013	0.0042	0.076	48
Root Mean Square Predicted Error	0.25	0.11	0.065	0.28	6.9
Partitioning of the mean square predicted error, %					
Error due to bias of prediction, %	0.60	0.1	0	0	0.26
Error due to slope $\neq 1$, %	5.4	6.9	19	21	4.2
Error due to random variation, %	94	93	81	79	95

[1] For Cl, K, Mg, and Ca, 20 samples included 10 samples from a close-up pen and 10 samples from a lactating cow pen
[2] Dietary cation anion difference (DCAD) measured in mEq
[3]Standard Deviation (SD)

Table 4. The ability of Cl and K meters to predict positive or negative dietary cation-anion differences for close up and lactating cow TMR in mEq.

Meter[1]	Sensitivity[1] (%)	Specificity (%)	Accuracy (%)
DCAD[2]	90.0 (55.5 - 99.8)	100 (69.2 - 100)	95.0 (73.1 - 99.9)

[1]Sensitivity, specificity, accuracy, and 95 % confidence intervals were calculated by using the diagnostic test evaluation calculator by MedCalc (MedCalc v. 20.027; MedCalc Software Ltd., Ostend, Belgium)
[2]Dietary cation anion difference (DCAD)

Table 5. Comparison of meter predicted Cl and K % coefficient of variation to observed laboratory Cl and K % coefficient of variation from 10 TMR samples per pen to measure TMR uniformity

Descriptive statistics	Cl[1]	K[1]
Observed mean, % CV[2]	0.027	0.035
Predicted mean, % CV	0.026	0.031
Observed SD[2]	0.011	0.040
Predicted SD	0.0079	0.0085
Linear regression of meter predicted % CV on observed laboratory % CV		
Intercept (H0: $\beta = 0$)	$P > 0.01$	$P > 0.01$
Intercept	0	0
Slope	0.17	0.56
Mean Square Error	0.00010	0.0018
Root Mean Square Error	0.010	0.043
Coefficient of Determination	0.89	0.37
Mean Bias, %	2.7	11
Mean Absolute Error	0.0076	0.019
Mean Square Predicted Error	0.000089	0.0017
Root Mean Square Predicted Error	0.0094	0.041
Partitioning of the mean square predicted error, %		
Error due to bias of prediction, %	0.59	0.92
Error due to slope $\neq 1$, %	8.4	55
Error due to random variation, %	91	44

[1]For both Cl and K, 20 samples included 10 samples from a close-up pen and 10 samples from a lactating cow pen from each of 10 dairies
[2]Coefficient of variation (CV), standard deviation (SD)

Figure 1. Changes in meter mineral % in TMR on a DM basis during soaking in deionized water over time. TMR samples (10) from the close-up pens were measured every 30 min to determine peak measurement time. X represents the average meter mineral percentage, box edges represent the standard deviation, whiskers represent the standard error.

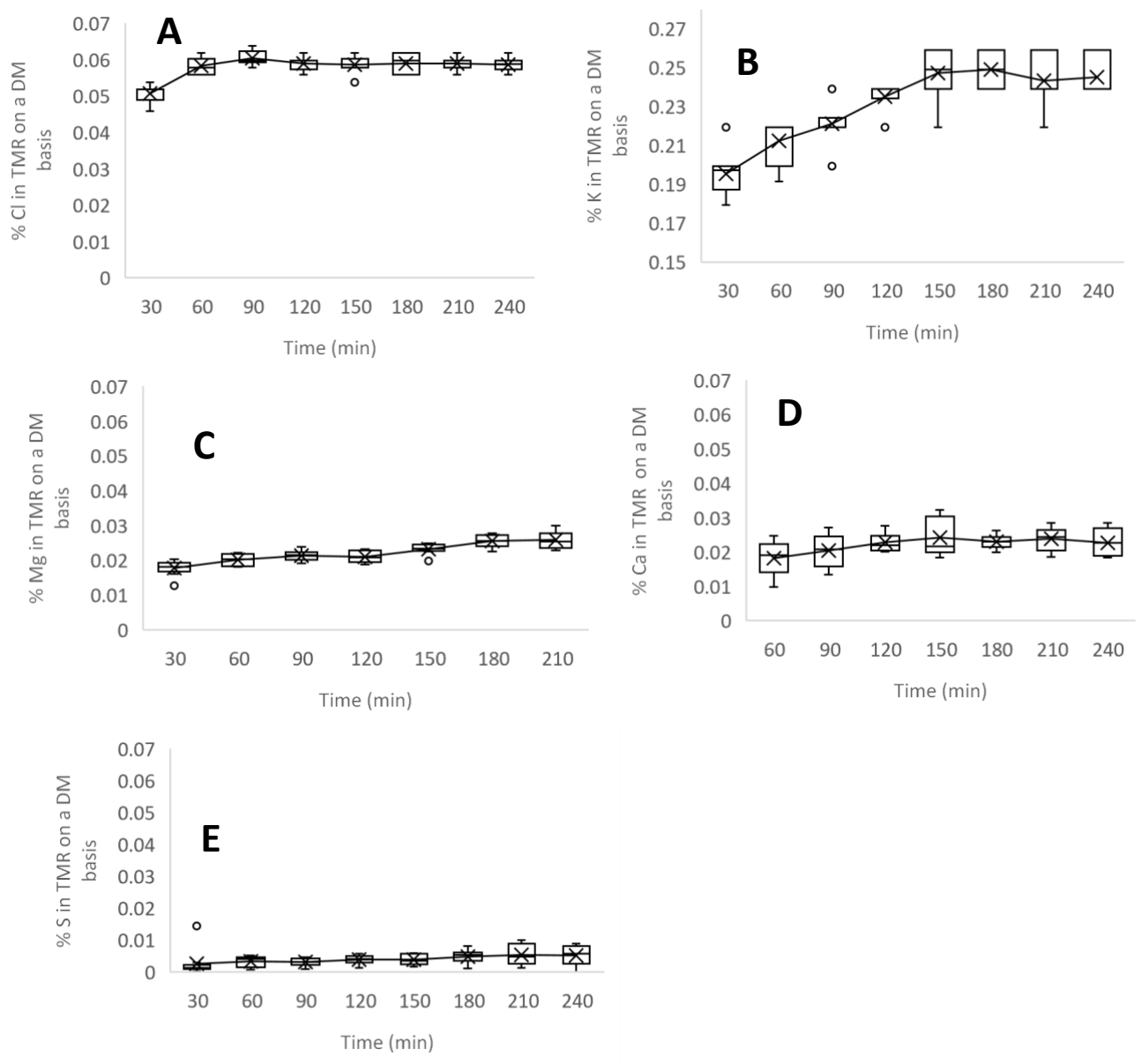

Figure 2. Plot of meter predicted % Cl in the TMR on a DM basis versus laboratory observed % Cl in the TMR on a DM basis contained within a close up and lactating cow TMR on a DM basis. The 10 TMR samples were averaged for each pen.
Cl Data (•), Cl residuals (▲), Regression (·····), Slope (——), Bias (——), Regression of residuals on observed laboratory analyzed Cl, *P* = 0.03

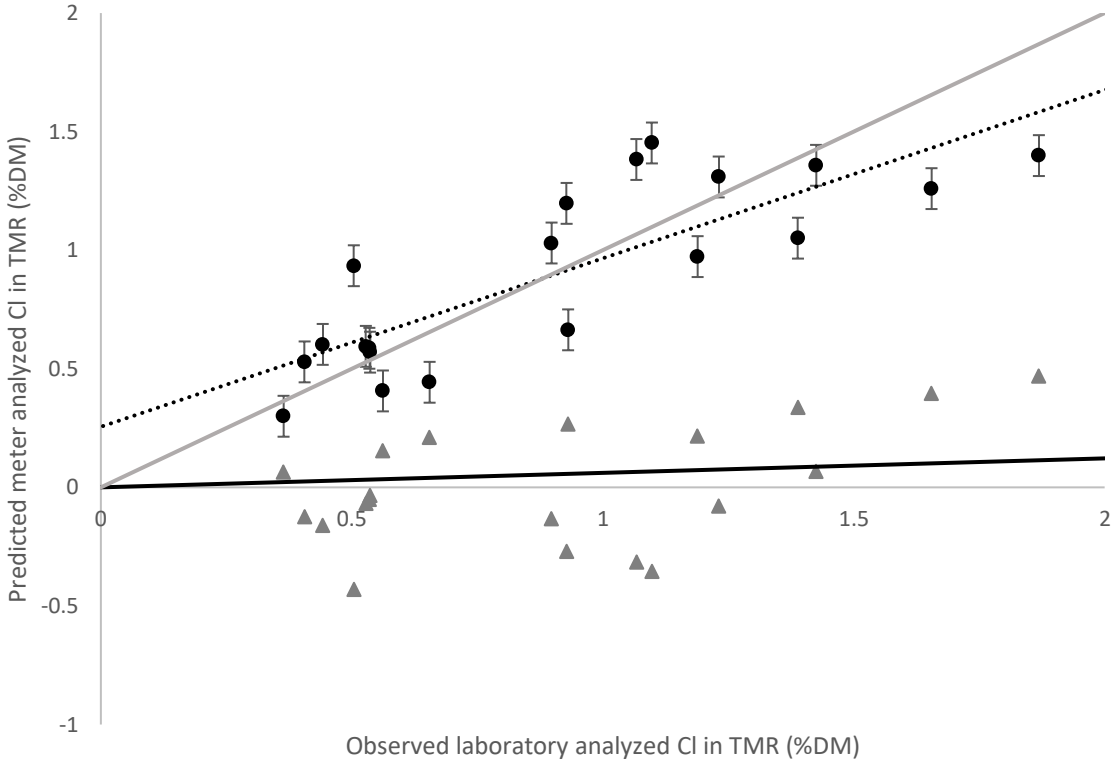

Figure 3. Plot of meter predicted % K in the TMR on a DM basis versus laboratory observed % K in the TMR on a DM basis contained within a close up and lactating cow TMR on a DM basis. The 10 TMR samples were averaged for each pen.
K Data (•), K residuals (▲), Regression (⋯⋯), Slope (——), Bias (——), Regression of residuals on observed laboratory analyzed K, *P* = 0.03

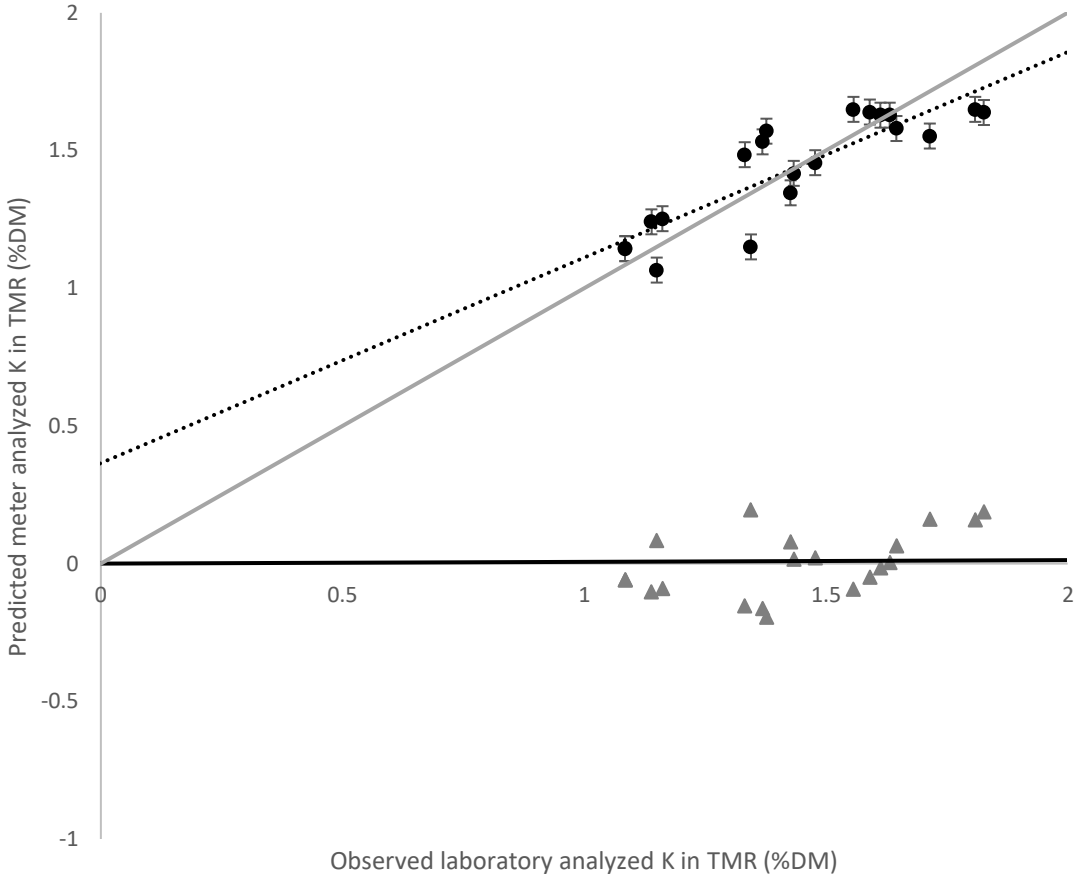

Figure 4. Plot of meter predicted dietary cation anion difference (DCAD; mEq) versus laboratory observed DCAD for close up and lactating cow TMR. Ten TMR samples were averaged by pen.
DCAD data (•), DCAD residuals (▲), Regression (⋯⋯), Slope (——), Bias (——), Regression of residuals on observed laboratory analyzed DCAD, *P* = 0.11