ASSOCIATION OF SERUM CALCIUM STATUS AT CALVING ON SURVIVAL, HEALTH, AND PERFORMANCE OF POST-PARTUM HOLSTEIN COWS AND CALVES

THESIS

Presented in Partial Fulfillment of the Requirements for the Master of Science Degree in the Graduate School of The Ohio State University

By

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The Ohio State University
2015

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Abstract

Limited evidence is available in the literature about the effect of subclinical hypocalcemia (HYPO) of dams at calving on survival and health of calves. The objective was to assess the effect of clinical and subclinical hypocalcemia (HYPO) (≤8.0 mg/dL) of the dam at calving on survival, health, and performance of lactating dairy cows and calves. Prepartum dairy cows (primiparous, n = 445; multiparous, n = 328) from one dairy herd were monitored (close-up pen) for imminent signs of parturition (appearance of amniotic sac outside the vulva) until birth. Calving ease, date and time of birth, single or multiple calves, calf sex, stillbirth (born dead or died within 24 h after birth), BCS immediately after calving, and hygiene score of the perineum were recorded. All female calves were subject to the same newborn care and colostrum management. Total postpartum serum calcium of cows was determined within 2 h after calving. The association of HYPO on survival (died or culled within 60 DIM), metritis, and pregnancy per AI (P/AI) for first services of lactating cows were analyzed using the GLIMMIX procedure of SAS. Pregnancy status up to 300 DIM was assessed using the PHREG procedure of SAS. Additionally the association of HYPO at calving between milk yield, milk components (percent fat and protein), and SCC were analyzed for the first three postpartum DHIA tests using MIXED procedure of SAS. The effect of HYPO at calving on calf survival, failure of passive transfer (FPT; serum total proteins ≤5.5 mg/dL), and diarrhea within 10 d of age were assessed using GLIMMIX. The overall prevalence of
HYPO was 15% (9% for primiparous and 30% for multiparous cows). Cows experiencing HYPO at calving had greater proportion ($P < 0.05$) of metritis (29.4%) were more likely to be culled within 60 DIM (15.9%) compared to non-HYPO cows (17.3% and 6.8%, respectively). For the first three DHIA tests, milk yield and components did not differ between HYPO and non-HYPO cows. The proportion of P/AI at first service was not different ($P > 0.05$) between HYPO (30%) and non-HYPO cows (37%) up to 300 DIM. The proportion of stillbirth and FPT did not differ ($P > 0.05$) between calves born from HYPO or non-HYPO cows. However, calves born from HYPO cows had greater (49%; $P < 0.05$) proportion of diarrhea than those calves born (33.3%) from non-HYPO cows. Dairymen, consultants, and veterinarians often trouble-shoot transition cow diseases and this process requires constant monitoring and comprehensive assessment of several events. Findings from the present study showed that HYPO at calving had significant health implications for both dams and calves.
I dedicate this thesis to Andrew Wilhelm. Thanks for pushing me the extra mile.
Acknowledgements

I would like to express my utmost gratitude to Dr. Gustavo M. Schuenemann for his constant help; genuine persistence, hard work, and guidance in making this project a success. I could not have asked for a better advisor. I would like to thank the collaborating dairy farm and their staff for providing the animals used in the present study and their assistance during the project. Thank to Drs. Juan S. Velez and Hans Bothe, from Aurora Organic Farms, for their input and assistance with this project. Additionally I would like to thank Dr. Martin G. Maquivar for all of his help evaluating calves on a daily basis, and for answering all of my questions while we were collecting data at the farm. Without their support this project would not have been possible. Also, the financial support of the OSU Veterinary Extension, Merial and T35 Fellow Scholarship, the Ohio Dairy Producers Association is greatly appreciated. Thanks Donna Wyatt for calcium assessment and all of your work in the lab. Thanks to my fellow graduate students, Drs. Adrian A. Barragan and Juan M. Piñeiro, for their contribution and assistance with data evaluation. They put in many long hours and were able to help with the project and data entry when I was not available. Also thanks to my fellow veterinary student Theresa for proofreading my work, and supporting me through the combined degree program. And thanks to my committee members, Drs. W.P. Weiss, T.A Brick, and S. Bas for the support and constant feedback throughout my pursuit of my degree. From selecting classes to interpreting results, you were always willing to help.
My family has been instrumental in getting me here. Without the constant love and support from my mom, I would not be where I am today. She has always provided the advice I need, was happy to listen, and knew what to say to keep me going through my academic program. Additionally, Andrew Wilhelm and his parents: Stan and Lisa for the free food, words of encouragement, and always allowing me to stay late at the house to keep working. I have also had many mentors along the way that have greatly influenced my education and will have a lasting impact on my veterinary career. These include: Dr. Marty Masterson, Dr. Kristy Daniels (who always wanted me to pursue an MS over veterinary school), Dr. Maurice Eastridge, and the numerous professors that took the extra time out of their day to help me with class. Lastly, these acknowledgements would not be complete without giving a huge thanks to my “adopted” family in Marysville. With their support I have had a place to live, and cows to milk. This has provided me with excellent practical experience and an acute understanding of owning and managing dairy cattle. So a big thanks to Don, Phyllis, Rob, Drew, and Lydia for putting up with me these past six years. Thank you for all of the support and the wonderful experiences that accompanied this project.
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Chapter 1

Introduction

Management of transition cows (the three weeks before and after calving) is critical for the economic success of dairy herds. It is well known that periparturient disease or conditions such as dystocia, metritis, milk fever (periparturient paresis), mastitis, displaced abomasum (DA), ketosis, and lameness have a negative impact on milk yield, reproductive performance (LeBlanc et al., 2002; Dubuc., et al., 2010), and overall animal well-being. This may be a consequence of periparturient cows being more restless 24 hours prior to calving (Proudfoot et al., 2009; Titler et al., 2013) and consequentially have reduced feeding time (Huzzey et al., 2007; Proudfoot et al., 2009) and dry matter intake (DMI; Huzzey et al., 2005). The initiation of lactation challenges the dairy cow’s metabolic capabilities at maintaining normocalcemia due to reduced DMI (Huzzey et al., 2005) and calcium (Ca) loss in colostrum and milk (Kehoe et al., 2007; Tsioulpas et al., 2007). The transition period impacts Ca homeostasis and is a risk factor for many diseases affecting the overall health and productivity of lactating dairy cows (DeGaris and Lean, 2008). A recent study has shown that cows with subclinical hypocalcemia have increased lipid metabolism, neutrophils with reduced phagocytic abilities, and an increased risk of metritis (Martinez et al., 2012). While the nadir in
blood Ca concentration most often occurs 12-24 hours after calving (Goff, 2008), the depletion of Ca stores within the cells likely begins several days before calving (Kimura et al., 2006). Cows may experience some periparturient immune suppression when their immune cells are not able to mount the necessary response to stimuli (Kimura et al., 2006). The previous study found that as the calcium demand for lactation increases, the mononuclear cells had a decline in intracellular calcium stores, and were able to recover as plasma calcium normalized (Kimura et al., 2006). Prior research suggests that when serum Ca concentrations drop below 8.5 mg/dL, cows develop subclinical hypocalcemia (Cahpinal et al., 2011; 2012), which results in an increased risk of DA, metritis, ketosis, and impaired reproductive performance (Martinez et al., 2012; Oetzel, 2013). The ability of the cow to mobilize lipids may be associated with the development of subclinical hypocalcemia (Chamberlin et al., 2013), which is supported by greater circulating non-esterified fatty acid (NEFA) levels in cows with subclinical hypocalcemia (Horst et al., 2003). Ca concentrations at calving have been shown to be associated with plasma NEFA concentrations on day -14 and 0 relative to calving (Chamberlin et al., 2013). LeBlanc et al., (2005) supported these finding when proposing that hypocalcemia may be associated with decreased DMI as a result of elevated NEFA’s before calving. While the disease consequences of clinical and subclinical hypocalcemia have been documented for lactating cows (Kimura et al., 2006; Reinhardt et al., 2011), there is very little science-based research regarding the implications on calves from affected cows. The onset of hypocalcemia (clinical or subclinical) is highly associated with parturition; furthermore, there are many risk factors contributing to its development in the days leading up to, and
after parturition. These same risk factors resulting in an immunocompromised dam with a negative energy balance may also influence the growth and health of the neonatal calf.
Chapter 2

Literature Review

1. Physiology of Calcium Balance in Dairy Cows

Initiation of lactation challenges a dairy cows’ ability to maintain calcium (Ca) homeostasis as the demand of colostrum and milk synthesis increases around parturition, and DMI transiently decreases, the result is a transient period of hypocalcemia (Reinhardt et al., 2011). Subclinical hypocalcemia has been shown to affect 25% of primiparous and 47% of multiparous cows (Reinhardt et al., 2011). While lactating dairy cows may not show the clinical signs (milk fever), they are at risk for additional postpartum diseases such as metritis (Martinez et al., 2012). Ionized Ca is necessary for vital cellular functions such as signaling, neurotransmission, muscle contraction, metabolism, growth and proliferation, and activation of immune responses (Saris and Carafoli, 2005; Parekh, 2006; Vig and Kinet, 2009). For cellular functions to occur, homeostatic mechanisms work to maintain total Ca between 8.5 and 10.0 mg/dL in lactating dairy cows (Goff et al., 1996). When approximately 50% of the circulating blood Ca is lost, a hypocalcemic event, such as milk fever, is likely to occur (DeGaris and Lean, 2008).
According to DeGaris and Lean (2008), the pre-partum demand for Ca is about 30 g per day, allowing 15 g for fecal and urinary loss, and the remaining 15 g to be available for the gestating calf. When intake does not meet the demands of calcium metabolism the pathogenesis of hypocalcemia is initiated. The available calcium pool becomes overwhelmed requiring the animal to mobilize and conserve calcium through increased absorption of intestinal calcium, resorption of calcium in the renal tubules and from bone. Calcium is eliminated from the cow to endogenous fecal Ca, clearance in glomerular filtration, placental Ca transport to the fetus, bone deposition, and Ca secretion in the mammary gland (El-Samad et al., 2002). Rigid endocrine control over metabolic processes is required to maintain Ca balance in the postpartum dairy cow particularly those with increased Ca demands. If intake and homeostatic mechanisms cannot meet the increased demands for Ca metabolism, the pathogenesis of hypocalcemia is initiated.

The regulation of serum Ca is controlled by two potent calcitropic hormones: parathyroid hormone (PTH) secreted from the parathyroid gland and 1,25-dihydroxyvitamin D [1,25-(OH)2 D], and a metabolite of vitamin D produced in the kidney (Goff, 2008). When Ca levels decrease rapidly, there is a more intense response opposed to a slower release if Ca falls gradually. PTH reduces urinary Ca excretion and mobilizing Ca from bone, which can only support the cow for a brief period of time. Increasing PTH concentration initiates a process known as osteocytic osteolysis, in which PTH causes the removal of Ca from the bone matrix (El-Samad, 2002). This process can take place in minutes to provide a rapid source of Ca to the blood. If blood levels of PTH are elevated for several hours or more, it will begin to resorb bone matrix and proceeds to enhance the final hydroxylation of 25-hydroxycholecalciferol (25-hydroxyD3) to 1,25-
Active vitamin D metabolite functions at the enterocyte level to stimulate more efficient absorption of Ca by the intestine. This occurs through increasing calcium-binding protein in the intestinal epithelial cell (Duke, 1993) and 1,25-(OH)₂ D is a potent stimulator of Ca absorption from the intestine. Calcitropic hormones facilitate the transition to lactation in order to support the 4 to 5-fold increase in the rate of Ca clearance from the blood observed at parturition (Anderson, 1970).

Conversely, in response to elevated blood Ca levels, calcitonin is released (DeGaris, and Lean, 2008). Calcitonin lowers blood Ca by promoting clearance via glomerular filtration, and preventing bone deposition (Rosol, 1995). While calcitonin plays a valuable feedback relationship with hypercalcemia, or managing blood Ca concentrations after an intravenous Ca treatment, it has a lesser impact on Ca homeostasis (El-Samad, 2002). When Ca concentration exceed 9.5 mg/dL, calcitonin is secreted (Goff, 2000) and the calcitonin feedback mechanism also decreases secretion of PTH. These Ca regulatory hormones are responsible for a self-limiting negative feedback relationship to “set” the level of ionized Ca in the blood (McDonald et al., 2001).

2. Late Gestation and Physiology of Parturition

2.1. Fetal Nutrient Requirements

Nutrient requirements of gestating dairy cattle have been outlined within the National Research Council, Nutrient Requirements of Dairy Cattle (NRC, 2001). The studies of fetal nutrient requirements were performed using Red Danish dairy cows, with a small sample size. However, they resulted in widely accepted guidelines outlining the
prediction of energy and protein requirements of dairy cattle. Additional studies (Ferrell, 1976; Eley, 1978; Prior, 1979) evaluated fetal development in the bovine using populations of animals that were half Jersey, mixed breed beef, and Hereford heifers respectively. Two of these studies (Eley, 1978; Prior, 1979) suggested that fetal growth rate peaks at about 230 days of pregnancy and declines rapidly to near 50% of the maximum by the end of term. In order to further outline the pattern of fetal and total conceptus growth throughout pregnancy, Bell et al. (1995) assessed the rates of growth and chemical composition of conceptus in Holstein cows that were slaughtered from 190 to 270 days of pregnancy. This work resulted in the publication of two studies outlining the mineral (House and Bell, 1993) and protein and energy (Bell et al., 1995) requirements of modern Holsteins during pregnancy.

Nutritional requirements of pregnancy increase markedly during the last trimester (Rattray et al., 1975). A study by House and Bell (1993) showed that 85% Ca, 84% phosphorus (P), 81% magnesium (Mg), 70% potassium (K), and 71% sodium (Na), accumulated during the final stage of pregnancy. These minerals are required for fetal growth while the cow is dry, while also maintaining maternal mineral requirements. The NRC states that the “pregnancy requirement is defined as the amount of mineral retained within the reproductive tract (fetus, uterine contents, and uterus) at each day of gestation. For most minerals, the requirement of the animal pregnant for <190 days is small and not considered in the model” (NRC, 2001). This is true for Ca, which has a minor requirement to the conceptus until after day 190 of gestation when the fetal skeleton begins to calcify. The estimated Ca accretion rate in the conceptus increased from 2.3 g/d
at 190 days of gestation to 10.3 g/d at 280 days of pregnancy; while the corresponding P accretions were 1.9 and 5.4 g/d (House, 1993).

Based on the average body weight of the Holstein cows at slaughter (714 kg), it was estimated that the cows would need to absorb roughly 11 g of Ca and 10 g of P to just meet the maintenance requirements of the dam and fetus (Jakobsen., 1957; House and Bell, 1993). The cow would need an additional 10.3 g of Ca and 5.4 g of P to meet the maximal requirements for conceptus growth (House and Bell, 1993). House and Bell (1993) concluded from their work that the current dietary Ca and P recommendations for dairy cattle appear to be adequate, and should easily accommodate maintenance and conceptus growth requirements. The exponential equation developed by House and Bell (1993) was used by the NRC to estimate the Ca requirements in the last weeks before parturition (NRC, 2001).

The Ca requirement established by the NRC for lactating dairy cattle varies based on concentration of protein in the milk by breeds. For Holstein cows, the absorbed Ca/kg of milk is 1.22 g with an additional requirement of about 2.1 g of absorbed Ca/kg of colostrum produced (NRC, 2001). As discussed previously, dairy cows have an effective mechanism for regulating the Ca in blood, but this mechanism may fail at times. At parturition, dairy cows produce on average 10 liters of colostrum containing 23 g or more of Ca, approximately 6 times as much Ca as the blood Ca pool available (Goff, 2000).

The rates of accretion of Mg, K, and Na in the conceptus in late pregnancy were about 0.2, 1.0, and 1.4 g/d, respectively, and Fe, Zn, Cu, and Mn accumulated in the conceptus at rates of 18.0, 11.7, 1.6, and .3 mg/d, respectively (House and Bell, 1993). These daily rates represent net mineral requirements for conceptus growth during late
pregnancy in mature Holstein cows. Overall, the concentration of fetal macrominerals decreased over gestation, but the total fetal content increased with gestational age. The increase in this total content is largely due to the accretion of Ca and P.

Overall, the predicted values for net energy and crude protein from Bell et al. (1995) coincided well with NRC recommendations, and are unlikely to underestimate the needs of modern Holsteins.

2.2. Maternal Nutrition, Conceptus Growth and Performance

Fetal growth and development is influenced by maternal nutrition. There has been a resurgence in studies evaluating the effect of fetal over or undernutrition during pregnancy, and how this can impact the offspring later in life (Robinson et al., 1977; Reynolds and Redmer, 1995). Within these studies, a finding that has caught the attention of animal and human scientists is the intrauterine environment as a factor associated with intrauterine growth retardation (IUGR). Interestingly, in the livestock species studied, including cattle, offspring with an above-average birth weight have a better chance of survival in comparison to those with a below-average birth weight (Funston et al., 2010). Additionally, calves from undernourished dams displayed poor growth and productivity as well as developed diseases after birth (Funston et al., 2010). Complications described in livestock included increased neonatal mortality, intestinal dysfunction, reduced postnatal growth, and reduced meat quality (Wu et al., 2006).

The programming of growth and development via maternal nutrient delivery may impact the fetus during pregnancy and later into adult life. However, it is not fully understood as to which stage during development is most impacted by maternal nutrition.
It could be assumed that the last two months of gestation may be the most sensitive, as 75% of the growth of the ruminant fetus occurs during this time (Robinson et al., 1977). For example, feeding cows 70% of their calculated energy requirements during the last 90 days of gestation resulted in calves with increased morbidity and mortality rates (Corah et al., 1975). However, the majority of placental growth, differentiation, and vascularization occur early on during fetal development (Reynolds et al., 1995). Two studies have evaluated growth restriction of the fetus to find that this is highly related to uteroplacental growth and development (Reynolds and Redmer, 1995; 2001). The growth of the placenta allows maximal exchange of nutrients between the dam and fetus, and largely relies on growth of uteroplacental vascular beds during the first half of pregnancy (Meschia et al., 1983). Knowing this, any process early in gestation that may affect placental development and vascularization may have lasting impacts on fetal growth potentially impacting the last trimester of gestation.

Much of the early work on maternal nutrition in ruminants concluded that fetal size or composition is generally not affected by nutrition, except when nutritional levels are markedly low or deficient (Ferrell et al., 1976; Prior et al., 1979; Rattray et al., 1974; 1975). As the effects of maternal nutrition gained more interest, the impact of prepartum nutrition on disease susceptibility of the neonatal calf were explored. Feeding heifers and cows a high-energy diet 100 days prepartum increased body weight before calving and calf birth weight (Corah et al., 1975). Roughly 19% more calves from mature cows fed the low-energy diet in the previous study were treated for scours, and weaning weights were greater for calves from cows consuming the high-energy diet (Corah, et al., 1975). Wittum et al. (1994) has shown that diarrhea and respiratory disease have lasting impacts
on the growth and development of the neonate. Within this study, respiratory and diarrhea events from birth to weaning resulted in 16.5 and 10.7 kg reductions in weaning weight, respectively (Wittum et al., 1994). Van Amburgh and coworkers (2014) reported that calves within the herd that experienced diarrhea had reduced average daily gains (ADG). Furthermore, calves that were recorded as having diarrhea and were also treated with antibiotics had a 50 g per day lower ADG (Van Amburgh et al., 2014).

2.3. Colostrogenesis

Colostrum is the first secretion from the mammary gland after parturition and is unique in the properties that it provides to the newborn. Colostrum nourishes the calf with immune components and nutrients; and contains more protein, immunoglobulins, nonprotein nitrogen, fat, ash, vitamins, and minerals than milk (Quigley and Drewry, 1997). Nutrients are in much higher concentration in colostrum (solids: 23.9%, protein: 14.0%, IgG: 48 mg/mL, fat: 6.7%, minerals: 1.1%, and vitamin A: 295 µg/dL), compared to whole milk (solids: 12.9%, protein: 3.1%, IgG: 0.6 mg/mL, fat: 4.0%, minerals: 0.7%, and vitamin A: 34 µg/dL; Hugh, 2009). The prepartum transfer of immunoglobulins from maternal circulation into mammary secretions defined the process of colostrogenesis. Estrogen and progesterone are necessary for initiation of IgG transfer into colostrum (Barrington et al., 2001).

Several factors can impact the quality of colostrum produced. When studying the effects of diet on colostrum quality, the concentration of crude protein in the diet does not appear to impact colostrum quality (Quigley and Drewry, 1997). The concentration of immunoglobulins (Ig) in colostrum was not significantly associated by prepartum crude
protein; high crude protein increased the non-protein fraction of colostrum, but low crude protein did not affect the IgG or crude protein within colostrum (Quigley and Drewry, 1997). In studies feeding Angus cattle 100 or 57% of NRC crude protein requirements for 90 days prepartum, the IgG concentration in colostrum or calf serum total proteins at 24 hours were not impacted (Hough et al., 1990). While it has been reported that dietary crude protein restriction does not affect IgG concentration in colostrum, the absorption of IgG1, IgG2, IgM, and IgA were reduced in calves from dams on restricted diets (Burton et al., 1984). However, there have been several studies reporting no effect of dietary crude protein restriction on colostral IgG, and the calves’ absorption of IgG (Halliday et al., 1978; and Olson et al., 1981). Additionally, it has been shown that heat stress impacts the composition of colostrum (Nardone et al, 1997). While yield is not significantly changed when Holstein heifers were heat stressed, total fat, lactose, energy, crude protein, IgG, and IgA were lower than heifers maintained within their thermoneutral zone (Nardone et al., 1997). Many other factors that are known to impact the quality of colostrum include: parity of dams, length of dry period, time of first milking in relation to calving, and vaccination strategies (Quigley and Drewry, 1997).

While many studies have focused on colostrum components for its immunological benefits to the calf, it also provides the calf with many other nutrients. For instance, the energy content of colostrum may affect the calf thermoregulation and oxidation of fatty acid processes that are necessary for gluconeogenesis (Quigley and Drewry, 1997). Without this source of energy, it has been estimated that the fat stored in the calf at calving would only support metabolism for about 15 hours and glycogen reserves would be depleted in less than three hours (Okamoto et al., 1986). The fat content of colostrum
is variable, 0.3-18.0% (Parrish et al., 1950), and manipulation of the diet fed prepartum has not successfully resulted in an adjustment in colostral fat content (Weiss et al., 1994).

The calf is also dependent on colostrum for a source of vitamin E, as it does not cross the placenta in appreciable amounts. Unless the cow is provided supplemental vitamin E, the amounts accumulated within colostrum are typically low (Weiss et al., 1990). Studies in pigs have reported that supplemental vitamin E did not affect colostral IgG absorption, but improved the development of cellular immunity (Nemec et al., 1994). In a study by Weiss et al. (1990), cows were supplemented with vitamin E pre-calving, and α-tocopherol was increased in concentration in colostrum. Interestingly, similar work in gilts did not show an increase in α-tocopherol in colostrum, but in the milk instead (Hidiroglou et al., 1993). Currently the recommendation for supplemental vitamin E in the dry period is 1000 IU/d to provide optimal immune function to neonates and their dam (Weiss et al., 1990; 1992).

According to Quigley and Drewry (1997), other vitamins such as A and D do not cross the placenta; this, the calf must rely on ingestion of colostrum for these vitamins. Colostrum serves as an important source of these vitamins, especially vitamin A, for neonates. Unlike vitamins (E, A, and D), selenium is able to cross the placenta readily, reinforcing the importance of proper selenium supplementation of the dam. In beef cattle, the supplementation of selenium via a salt-mineral mix at 120 mg/kg increased colostral IgG and the absorption of IgG by calves (Swecker et al., 1995). Cows receiving selenium supplementation from a ruminal bolus had calves with higher selenium concentrations in their blood and liver at birth and 42 days of age compared to non-supplemented cows (Abdelrahman and Kincaid, 1995). Due to the type of placentation, and reliance upon
colostrum for many nutrients and immune function, dry cow management should be a top priority for dairy producers. Energy intake, proper environmental management, and vitamin and mineral supplementation should all be managed in order to prevent nutrient deficiencies and improve the quality of colostrum.

2.4. Physiology of Parturition

When the pregnant cow approaches the end of the gestation period, the calf secretes adrenocorticotropic hormone (ACTH) from the pituitary gland and to stimulate the adrenal glands to release cortisol (Kindahl et al., 2004). ACTH triggers prostaglandin synthesis and initiates the cascade of events that leads to parturition (Kindahl et al., 2004). The increase in prostaglandins causes a drop in progesterone that coincides with rising estrogens (Goff and Horst, 1997). It is important to note that cows with hypocalcemia have circulating cortisol concentrations twice as high as cows with subclinical hypocalcemia, and three times higher than normal cows at the time of calving (Horst and Jorgensen, 1982). The high concentration of blood cortisol may compromise the immune system around calving, possibly providing an explanation for the increased amount of disease among transition cows.

3. Risk Factors for Hypocalcemia

With the onset of lactation, most lactating dairy cows enter a stage of negative Ca balance (Reinhardt et al., 2011). The cow will spend the next six to eight weeks of her lactation increasing Ca intake by increasing feed intake and improving intestinal Ca absorption (Huzzey et al., 2007). Cows are challenged to maintain normal Ca
concentration due to the increased demand in early lactation; however, many cows recover and return to normocalcemia after a period of transient hypocalcemia postpartum (Goff, 2008; Reinhardt et al., 2011). The nadir in blood Ca concentration occurs at approximately 12-24 hours after calving (Goff, 2008), and up to 48 hours after calving (Martinez et al., 2012). Cows that fail to return to normal plasma Ca concentrations may develop clinical signs of hypocalcemia known as milk fever. Hypocalcemia seems to negatively affect multiparous cows at the onset of lactation more profoundly than primiparous cows (Reinhardt et al., 2011). Clinical milk fever (hypocalcemia) results in a cow that is unable to rise (from lying to stand position) and is the most easily recognized form of hypocalcemia (Goff, 2008). Subclinical hypocalcemia results in less severe disturbances in blood Ca and does not have any outward signs of milk fever. During subclinical hypocalcemia, blood Ca concentration ranges between 5.5 and 8.0 mg/dL (Goff, 2008). While there are not clinical signs of disease early in the process, the overall performance of the cow can be significantly impacted due to a compromised immune system (Kimura et al., 2006) and the subsequent increased risk for diseases (Curtis et al., 1983).

The incidence of clinical milk fever in the United States is estimated to be 3.5% based on national surveys (DeGaris and Lean, 2008). The rate of subclinical hypocalcemia in dairy cows is 25, 41, 49, 51, and 54% for lactations 1-5, respectively (Reinhardt et al., 2011). This study showed that subclinical hypocalcemia affects many more cows within a herd than clinical hypocalcemia, making it a costly disease. For instance, the cost of clinical hypocalcemia revealed an estimated loss of $300 per clinical case, and $125 for every subclinical case (using an estimate that accounts for milk loss,
and increased costs from resulting ketosis and DA’s; Guard, 1996). For a 1,000 cow dairy, this would represent a cost of $6,000 from clinical cases, and $37,500 for subclinical disease (assuming a prevalence of 2% and 30% respectively); thus, highlighting the need as a priority to prevent hypocalcemia.

There are many consequences to subclinical hypocalcemia in postpartum cows such as reduced feed intake, decreased rumen and abomasal motility, and the increased the risk of left abomasal displacement (LDA; Goff, 2008). Due to reduced DMI, milk yield may be reduced, and there is an increase in susceptibility to infectious disease, resulting in a higher risk of removal from the herd (Curtis et al., 1983; Seifi et al., 2011). In a recent study, the risk of culling was greater for cows with subclinical hypocalcemia than cows with milk fever (Duffield et al., 2005). An early study found the risk of LDA’s to be 5 times greater in cows with subclinical hypocalcemia compared to non-hypocalcemic cows (Massey et al., 1993). However, Leblanc et al. (2005) did not find a direct relationship between Ca concentrations and LDA incidence and suggested that subclinical hypocalcemia may be a function of decreased feed intake, resulting in other diseases such as LDA’s, and subclinical ketosis.

The relationship between hypocalcemia and milk yield are inconsistent. Rajala-Schultz et al. (1999) found that cows with milk fever produced more milk, on average, than cows without hypocalcemia. However, when the cows own midlactation milk yield was used as a reference level, milk fever was associated with milk losses during the first 4-6 weeks of lactation. When Ostergaard and Larsen (2000) assessed cows with either clinical or subclinical hypocalcemia (controlling for the effect of parity and lactation stage), they did not find a relationship between low total plasma Ca and reduced milk
yield. Jawor et al. (2012) matched cows diagnosed with subclinical hypocalcemia with normocalcemic cows. The effects of parity and other diseases were controlled for to evaluate subclinical hypocalcemia and its effects on milk yield, feeding, drinking and standing behavior. In this study, dry cows that were later diagnosed with hypocalcemia had an average 1.7 kg increase in DMI over control cows, when disease and its’ confounding effects were controlled for. Additionally, cows with subclinical hypocalcemia stood an additional 2.6 hours more during the 24 hours before calving (Jawor et al., 2012). Cows with subclinical hypocalcemia had greater milk yield compared to matched control cows; however, the design of the study removed the effects of confounding disease (Jawor et al., 2012).

The harmful effects of hypocalcemia may be explained if one considers that these cows may also experience a more profound negative energy balance. Reinhardt et al. (2011) demonstrated that cows not suffering from hypocalcemia had significantly lower serum NEFA concentrations, indicating an improved energy balance over hypocalcemic animals. Similar findings of negative energy in cows with subclinical hypocalcemia were later confirmed by additional studies (Martinez et al., 2012; Chamberlin et al., 2013). Lactating dairy cows with subclinical hypocalcemia had higher concentrations of β-hydroxybutyrate (Martinez et al., 2012). Chamberlin et al. (2013) found significantly higher NEFA at calving (day 0) in hypocalcemic cows when evaluating the association of Ca status and energy balance. However, there were not differences observed in the occurrence of other diseases such as clinical mastitis, ketosis, displaced abomasum, dystocia, retained placenta, metritis, or reproductive performance in this study (Chamberlin et al., 2013).
An additional consequence of hypocalcemia is the effect of the stress at parturition on the immune system. Kimura et al. (2006) were able to measure the intracellular Ca release in response to an activation signal in mononuclear cells harvested from cows spanning a period of two weeks before and after calving. This study revealed that hypocalcemia was associated with decreased intracellular Ca stores in peripheral mononuclear cells, especially in cows that develop milk fever (Kimura et al., 2006). The decrease in intracellular Ca was apparent before parturition and the subsequent development of hypocalcemia. The authors suggested that this decrease in mononuclear intracellular Ca stores likely contributes to the immune suppression seen in periparturient animals (Kimura et al., 2006).

Cows with milk fever are at increased risk of reproductive complications including dystocia, retained placenta, and metritis (Erb et al., 1985). Erb et al. (1985) reported that in multiparous cows, milk fever increased reproductive disorders by 1.6 to 4.2 times. It was also noted that these cows had higher culling rates by 2.1 to 3.7 times due to mastitis, dystocia, or poor reproductive performance (Erb et al., 1985). In a more recent study by Martinez et al, cows with subclinical hypocalcemia (≤8.59 mg/dL serum Ca) were more likely to develop fever, metritis, and puerperal metritis compared to cows without hypocalcemia. The relative risk of developing metritis decreased by 22% with each 1.0 mg/dL increase in serum total Ca, and the population at risk of uterine disease from subclinical hypocalcemia was 66.6% for metritis and 91.3% for puerperal metritis (Martinez et al., 2012). Interestingly, neither metritis nor subclinical hypocalcemia influenced return to cyclicity, but cows with subclinical hypocalcemia had reduced
pregnancy per artificial insemination and a longer interval to pregnancy (Martinez et al., 2012).

In a recent study 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 (Chamberlin et al., 2013). However, these findings do not correlate to the increased incidence of mastitis within the first month following calving (Smith et al., 1985). It has been suggested that mammary infections may increase around parturition due to the cows’ weakened immune system (Goff, 1999). There is also a decrease in the level of lactoferrin as the gland transitions from colostrum to milk, increasing available iron for bacterial growth (Todhunter et al., 1990). Another possible explanation for increased mastitis incidence in recently fresh cows may be a reduction in smooth muscle contractions associated with hypocalcemia. The teat sphincter, a smooth constrictor muscle at the teat end, is responsible for closing the teat opening after milking, and may be negatively affected in states of low Ca balance. If this function is lost, the risk of mastitis increases substantially (Goff, 1999). A large study evaluating 33 dairy herds (2,910 cows) found that mastitis, ketosis, retained fetal membranes, and dystocia were significantly associated with hypocalcemia (Curtis et al., 1983). Additionally, there was a significant association between coliform mastitis and milk fever (Curtis et al., 1983).

3.1. Prevention of Hypocalcemia

Hypocalcemia is a gateway disease with significant economic implications for dairy herds. While some of the diseases resulting from subclinical hypocalcemia can be easily diagnosed based on clinical signs, subclinical hypocalcemia itself does not display
any evident signs (Oetzel, 2013). This makes it an especially frustrating and costly disease for farms, making prevention the top priority. Multiple strategies have been utilized to prevent hypocalcemia and mobilize Ca in dairy cows through nutritional management including: feeding anionic salts, low CA diets, low potassium forages, and vitamin D supplementation (Amaral-Phillips, 2014). Prevention of hypocalcemia through feeding strategies usually occurs within the last 21 days prepartum by adjusting the dry cow diet.

One common prevention strategy is supplementing anionic salts to reduce diet cation-anion difference (DCAD; Overton and Waldron, 2004). The goal of this type of supplementation is to reduce absorbable cations (Na, and K), while increasing available anions (Cl, and SO₄) in the diet (Goff, 2008). These ions all carry a charge and impact blood pH as a result. The difference in the charged particles that are absorbed from the diet determines the acid-base status of the blood (Goff, 2008). Due to the high potassium content of most feeds, many cows are in a state of metabolic alkalosis. This can further induce both clinical and subclinical hypocalcemia (Craigie and Stoll, 1947). Currently it is understood that the conformation of the PTH receptor is changed in the cow experiencing a metabolic alkalosis (Goff, 2008). Goff (2008) explains that this change reduces the tissues sensitivity to PTH and prevents utilization of osteoclastic bone resorption, and decreases Ca sparing mechanisms by the kidney. It has been reported by Ramberg et al., 1996 that inducing a slight metabolic acidosis, through DCAD reduction, renal production of the active metabolite of vitamin D (1, 25-(OH)₂ D₃) is enhanced. As a result, target tissues become more responsive to 1, 25– (OH)₂ D₃ and the efficiency of Ca absorption and mobilization from bone are enhanced (Ramberg et al., 1996). In order
to measure the degree of acidification, urinary pH must be monitored on cows fed anionic salts (Goff, 2008). The method of anionic salt supplementation has been successful when managed well. A meta-analysis performed in 2006 of previous studies found a fivefold reduction in the risk of clinical milk fever when feeding a typical dose of anions (Charbonneau et al., 2006). There is less known about the reduction of subclinical milk fever on farms feeding anions. In the National Animal Health Monitoring System (NAHMS) 2002 Dairy study, blood was drawn on 1,446 cows within 48 hours of calving on over 400 dairies. In a separate study, where nutritional management was evaluated, 38.7% of the animals were on a DCAD diet, and those supplemented had significantly less incidence of subclinical hypocalcemia (Horst et al., 2006).

Another method of preventing hypocalcemia includes feeding a Ca deficient diet to stimulate PTH secretion prepartum (Horst et al., 1997). With a negative Ca balance, PTH secretion is stimulated which results in osteoclastic bone resorption and renal production of 1, 25-(OH)2 D3 (Horst et al., 1997). This can prime the cow for periods of low Ca after parturition as the drain of Ca is more easily replaced. The challenge of this type of diet is feeding Ca in low enough quantities to stimulate PTH secretion. The NRC recommends absorbable Ca requirements of 14 g per day in Jersey’s and 22 g per day in Holstein cows (NRC, 2001). For instance, a low Ca diet for a 600 kg Holstein cow consuming 13 kg of DM would require <1.5 g/kg or 0.15% absorbable Ca in her diet (Goff, 2008). While there are feed supplements available to bind Ca in the diet (zeolite and vegetable oils), this method is not practical or attainable for most dairy herds (Goff, 2008).
Feeding low potassium forages in prepartum diets also changes the DCAD balance. The change may prevent clinical manifestations of hypocalcemia, but the change in the acid-base status of the blood generally is not significant enough to prevent subclinical hypocalcemia (Amaral-Phillips, 2014). Amaral-Philips (2014), advises that chlorine and sulfur may need to be fed in addition to low potassium forages.

A practice by some farms is supplementing high amounts of vitamin D to prepartum dry cows either in the feed or parenterally (Goff, 2008). Supplementation requires that up to 10 million IU of vitamin D must be injected or fed daily for 10-14 days before calving (Goff, 2008). While these doses are effective in preventing pariparturient paresis, if administration is stopped more than four days before calving, the cow becomes predisposed to hypocalcemia (Horst et al., 1997). The feeding practice cannot simply be continued as toxicity levels are approached and the effective dose of vitamin D for injection (10 million units) may cause irreversible metastatic calcification of soft tissues (Goff, 2008). Other vitamin D metabolites are available and can be more effective and safer than using vitamin D for injection. However, there are issues associated with the timing of these in relation to parturition, as production of renal 1, 25-(OH)2 D3 can be suppressed resulting in milk fever further into lactation (Littledeike and Horst, 1980). When utilizing these methods, providing an oral source of Ca at calving and a diet high in Ca is prudent.

4. Colostrum and Immunity of the Calf

Although fully developed at birth, he calf immune system is immature, making the newborn calf relatively vulnerable and unprotected to pathogens at birth. The immune
system matures very slowly in mammals, continually developing until the animal reaches sexual maturity and begins to cycle. The immune system of the calf begins to develop early in gestation, and it changes and specializes as the fetus grows in small steps from conception to approximately six months after birth (Chase et al., 2008). In cattle, most of the immune system maturity is seen by five to eight months of age (Cortese, 2009). According to Cortese, 2009, the newborn calf is not especially susceptible to pathogens or unable to mount an immune response at birth, the immune system is simply unprimed. This results in a slower and weaker response to antigens by the innate immune system of the calf. The neonatal calf actually has a greater number of phagocytic cells; however these cells have decreased function, which can be appreciated up to four months of age (Hawser, et al, 1986).

4.1. Neonatal Immunologic Development

The ontogeny of the neonatal immune system begins early in fetal development and continues to diversify until the animal reaches puberty. In fetal lambs and calves the embryonic thymus can be seen as early as 27 and 30 days of gestation (Casaro et al., 1971; Jordan et al., 1976). During gestation there is differentiation of thymocytes into specific T cells lines (Cortese, 2009). The B cells develop and differentiate in fetal bone marrow and are present in much lower numbers in the periphery of the fetus (1-2%) compared to the mature calf (10-20%; Kampen et al., 2006); however, most of the circulating fetal lymphocytes are T cells. Peripheral blood T cells begin populating lymphoid tissues about one month before birth, decreasing circulating levels from 60 to 30% (Chase et al., 2008). While the lymphoid system is developed at birth, calves are not
born with any memory T or B-cells (Chase et al., 2008). As a result, when a ruminant leaves the sterile uterus and is exposed to its environment, the ability of the neonate to generate a protective immune response is hindered by the immaturity of the immune system and the delay between initiation of response and effective protection (Barrington, 2015). While all of the immune components are present at birth, many of these are not functional. The active immunity of the calf continues to develop until puberty, and many of the mechanisms are not functional until the calf is two to four weeks of age (Reber et al., 2006). This is a result of lingering immunosuppressive effects of the hormonal and interaction of the placenta, near-term fetus, and dam. The placenta produces progesterone, prostaglandin E2, and cytokines that suppress cell-mediated and memory (TH1) responses in the fetus and dam, while promoting antibody production by the dam (Chase et al., 2008). The overall effect on the acquired immune mechanisms by the hormones results in decreased lymphocyte responsiveness, reduced antigen presentation by MHC II complex, and reduced antibody production due to a lack of B-cell differentiation (Chase et al., 2008).

Fetal calves are protected mostly by the innate immune system. Phagocytic cells such as: neutrophils and macrophages comprise the innate immune response, and do not fully develop until late in gestation (Barrington, 2001). There is a decline in the functional capacity of these cells as the fetus reaches term and fetal cortisol levels increase as part of the parturition process. The release of corticosteroids and the presence of an increased amount of suppressor T cells can dramatically impact systemic immune responses of the calf for the first week of life. In fact, immune responses continue to decline from birth until day three when they reach a nadir (Rajaraman et al., 1997).
Diminished cytokine and blastogenic responses are seen in the first few days after birth, and take five days to return to the functional level comparable to birth (Rajaraman et al., 1997). By one week of age, the calf’s neutrophils are functional and have the ability to mount an immune response (Kampen et al., 2006). Interestingly, the number of neutrophils circulating in the newborn calf is approximately four times higher than in 3-week-old calves. However, the ability of the neutrophils and macrophages of the calf to phagocytose are reduced, and the ingestion and absorption of colostrum potentiates this ability (Menge et al., 1998). Additional compromised native immune mechanisms include: decreased complement activity along with reduced interferon production, and reduced function of both natural killer and dendritic cells (Chase et al, 2008). Ultimately, the neonatal calf has a decreased native and acquired immune defense mechanism.

4.2. Passive Immunity

The bovid has an epitheliochorial placentation resulting in no maternal transfer of antibodies or white blood cells in utero. As a result, bovine fetuses are born agammaglobulinemic, having almost no antibodies unless they were infected in utero (Barrington, 2001). The protective environment of the uterus prevents the adaptive immune system of the calf from developing any memory. This makes the ingestion of colostrum essential in order for neonates to have a source of immunoglobulin for the first few weeks of life (Cortese, 2009).

Colostrum provides the best and most important example of passive immunity as it has numerous short and long term effects on calves. Colostrum is the first milk from the mammary gland after calving and is composed of concentrated levels of antibodies.
and immune cells (B cells, CD cells, macrophages, and neutrophils) that are fully functional after absorption by the calf (Riedel-Caspari et al., 1991). When a calf is born, the digestive tract absorbs these colostral proteins via pinocytosis across the epithelial cells. As soon as the digestive tract is stimulated by ingestion of any material, this population of cells within the gut begins to change to no longer permit absorption (Cortese, 2009). Six hours after birth approximately 50% of the absorptive capacity remains; followed by 33% at 8 hours, and virtually no absorption is observed by 24 hours (Rischen et al., 1981). To achieve maximal benefit from colostrum, the quality, quantity, and timing of feeding must all be taken into consideration. Calves that are deprived of colostrum do not have endogenous production of IgM until 4 days after birth, and functional levels are not achieved until eight days of age (Husband et al., 1975). Additionally, circulating IgA, IgG₁, and IgG₂ are not appreciable until 16-32 days of age independent of colostrum status of the calf (Husband et al., 1975).

Colostrum is also rich in cytokines that aid in the development of the fetal immune response (Hagiwara et al., 2000). These cytokines are associated with a pro-inflammatory response that can recruit neonatal lymphocytes to the gut, promoting immune development. Cytokines are also responsible for improving the ability of neutrophils to phagocytize bacteria (Menge et al., 1998). Another important component of colostrum is cells (Riedel-Caspari et al., 1991). The leukocytes in colostrum are viable and are present in percentages similar to blood. Maternal cells enter circulation and reach peak levels at one day of age (Reber et al., 2006). The neonate then gains cell-mediated immunity from the transfer of leukocytes and is provided local bactericidal and

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phagocytic activity within the gastrointestinal tract, and enhanced passive transfer of immunoglobulins (Rischen et al., 1981).

While all of the components of colostrum are essential to development of the neonate’s immune system, the concept of Failure of Passive Transfer (FPT; serum total proteins ≤ 5.5 mg/dL) is used to describe a neonate that does not absorb adequate levels of immunoglobulin. Many factors can account for the incidence of FPT in calves. These include: colostrum with an inadequate immunoglobulin concentration, ingestion of an appropriate mass of immunoglobulin, and absorption of immunoglobulins in a timely manner (Barrington, 2015).

5. Preweaning Calf Morbidity and Mortality

There are several factors associated with the morbidity, mortality, and growth of dairy calves from birth to weaning. According to McGuirk and Ruegg (2009), the highest morbidity and mortality rates of any dairy operation are for replacement heifers, primarily due to diarrhea prior to weaning. The most common pre-weaning diseases include: neonatal calf diarrhea, septicemia, and pneumonia, with diarrhea being the most common cause of death or reduced growth rate (McGuirk and Ruegg, 2009, van Amburgh et al., 2014). According to the National Animal Health Monitoring System, 7.8% of preweaned heifers died due to diarrhea, pneumonia, or other digestive problems (USDA, 2010); similar findings on calf mortality were observed by Walker et al. (2012). The overall preweaned diarrhea morbidity at the herd level was 20% with a range of 4.1% to 43% while respiratory morbidity was 5.3% (Walker et al., 2012).
The most efficient and effective management of the newborn may be achieved via the implementation and use of protocols for calving and also for calf management at the calf raiser (Mee, 2008). Calving protocols allow for early disease detection, implementation of treatments, and herd level corrective strategies to be performed. Management of the peripartum cow, prepartum facilities, maternity facilities, and attention to the cow and calf at calving is necessary to best support the cow and calf at calving (Schuenemann et al., 2013). Immediately after birth, the calf requires proper and timely administration of colostrum, navel disinfection, and movement of the calf to clean and dry environment (Mee, 2008). Once the calves reach the calf raiser facility, nutrition and environment are perhaps the top priorities in order to prevent diseases. Additionally, early identification of sick calves with appropriate interventions, are key factors to a successful calf rearing program (McGuirk and Ruegg, 2009). The five C’s of raising replacement heifers from birth to weaning include: colostrum, cleanliness, comfort, calories, and consistency (McGuirk anfd Ruegg, 2009) to ensure best welfare and productivity of future replacements of the herd (Mee, 2008).

6. Statement of the Problem and Rationale

Prevention of disease at the herd level requires a constant effort with effective coordination of feed management, environment, and personnel. Management of transition dairy cows (the weeks prior to- and after calving) is paramount for postpartum cow health and survival with profound implications on profitability of dairy herds. Calving-related losses such as metritis significantly reduced herd profitability through decreased milk
yield, extended days open, and increased replacement costs (Rajala-Schultz and Gröhn, 1999; Groenendaal et al., 2004; Meadows et al., 2005). Transition cow diseases have been associated with blood Ca status (clinical or subclinical) of postpartum cows (Reinhardt et al., 2011). Hypocalcemic cows are more likely to experience dystocia, retain fetal membranes, and develop metritis or DA (Curtis et al., 1983), which in turn reduce milk yield and reproductive performance of lactating cows (LeBlanc et al., 2002; Dubuc., et al., 2010) and increase the risk for culling (Sheldon et al., 2006; de Vries et al., 2010). However, the literature contains limited science based evidence concerning how the dam’s Ca status (clinical or subclinical) at calving effects the calf’s health and survival.

Therefore, the overall objective of the present study was to assess the effect of hypocalcemia (≤8.0 mg/dL) at calving on survival, health, and performance (reproduction, milk yield and components) of lactating dairy cows and calves.
Chapter 3

Association of Serum Calcium Status at Calving on Survival, Health, and Performance of Post-Partum Holstein Cows and Calves

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3.1. Abstract

The objective was to assess the effect of clinical and subclinical hypocalcemia (HYPO) (≤8.0 mg/dL) of the dam at calving on survival, health, and performance of lactating dairy cows and calves. Prepartum dairy cows (primiparous, n = 445; multiparous, n = 328) from one dairy herd were monitored (close-up pen) for imminent signs of parturition (appearance of amniotic sac outside the vulva) until birth. Calving ease, date and time of birth, single or multiple calves, calf sex, stillbirth (born dead or died within 24 h after birth), BCS immediately after calving, and hygiene score of the perineum were recorded. All female calves were subject to the same newborn care and colostrum management. Total post-partum serum calcium of cows was determined within 2 h after calving. The association of HYPO on survival (died or culled within 60 DIM), metritis, and pregnancy per AI (P/Al) for first services of lactating cows were analyzed using the GLIMMIX procedure of SAS. Pregnancy status up to 300 DIM was assessed using the PHREG procedure of SAS. Additionally the association of HYPO at calving between milk yield, milk components (percent fat and protein), and SCC were analyzed for the first three postpartum DHIA tests using MIXED procedure of SAS. The effect of HYPO at calving on calf survival, failure of passive transfer (FPT; serum total proteins ≤5.5 mg/dL), and diarrhea within 10 d of age were assessed using GLIMMIX. The overall prevalence of HYPO was 15% (9% for promiparous and 30% for multiparous
cows). Cows experiencing HYPO at calving had greater proportion \((P < 0.05)\) of metritis (29.4%) were more likely to be culled within 60 DIM (15.9%) compared to non-HYPO cows (17.3% and 6.8%, respectively). For the first three DHIA tests, milk yield and components did not differ between HYPO and non-HYPO cows. The proportion of P/AI at first service was not different \((P > 0.05)\) between HYPO (30%) and non-HYPO cows (37%) up to 300 DIM. The proportion of stillbirth and FPT did not differ \((P > 0.05)\) between calves born from HYPO or non-HYPO cows. However, calves born from HYPO cows had greater \((49%; P < 0.05)\) proportion of diarrhea than those calves born (33.3%) from non-HYPO cows. Dairymen, consultants, and veterinarians often trouble-shoot transition cow diseases and this process requires constant monitoring and comprehensive assessment of several events. Findings from the present study showed that HYPO at calving had significant health implications for both dams and calves.

3.2. Introduction

Clinical hypocalcemia (HYPO), also known as milk fever in postpartum dairy cows, significantly increases the risk for dystocia, ketosis, retained fetal membranes, displaced abomasum, metritis, and mastitis (Curtis et al., 1983). For instance, postpartum cows that develop hypocalcemia had a leukocytes with a compromised ability to respond to stimuli (Kimura et al., 2006); which in turn leads to uterine diseases (Dubuc et al., 2010) and ovarian dysfunction (e.g., low plasma estradiol, prolonged luteal phase, and cysts; Sheldon et al., 2009) and the subsequent reduced reproductive performance (Dubuc et al., 2010; Brick et al., 2012), increased risk of culling due to reproductive failure (LeBlanc et al., 2002) and reduced milk yield (Dubuc et al., 2011). According to the
National Animal Health Monitoring System (NAHMS), the average incidence of milk fever in US dairy herds was 5.2% (NAHMS, 2010). Recent field studies reported incidences of milk fever for all lactations of 4.5% (Reinhardt et al., 2011) and up to 7% (DeGaris and Lean, 2008; Goff, 2008; Mulligan and Doherty, 2008). According to one study, the prevalence of clinical HYPO for first-lactation is 1% and 4%, 7%, and 10% for second, third and fourth lactation Holstein cows (Reinhardt et al., 2011). However, the incidence of subclinical HYPO in postpartum cows increased with age, and was present in 25% of first lactation cows and 41-54% of second or greater lactation cows (Reinhardt et al., 2011). The immune system of lactating dairy cows experiencing subclinical HYPO may be compromised severely enough to alter physiological and immune functions with the subsequent risk to develop postpartum diseases (Kimura et al., 2006).

There is limited science based evidence available in the literature about the effect of clinical or subclinical hypocalcemia of dams at calving on survival, health and performance of calves. It was reported that calves born from HYPO cows were at greater risk of developing diarrhea and respiratory events prior to weaning compared to calves born from non-HYPO cows (Planski and Abrashev, 1987). However, the information on pre- and postpartum nutrition (i.e., diets) and colostrum management as well as diagnosis of calf diseases were not available. Therefore, the objective was to assess the effect of clinical and subclinical HYPO (≤8.0 mg/dL) at calving on survival, health, and performance of lactating dairy cows and their calves. The hypothesis was that dairy cows experiencing HYPO at calving would have increased risk for postpartum diseases and reduced performance; in addition, it was hypothesized that the risk for disease would be
increased in calves born from cows with HYPO as opposed to calves born from non-
HYPO cows.

3.3. Materials and Methods

3.3.1. Animals, Feeding, and Facilities

In total, 773 lactating Holstein cows (445 primiparous and 328 multiparous) from
one certified organic dairy herd were used in the present study. Briefly, cows and heifers
were housed in free-stall barns and milked thrice daily at approximately 8-h intervals.
Cows were fed twice daily, in the morning and afternoon, with a TMR formulated to
meet or exceed dietary nutritional requirements for lactating and dry dairy cows (NRC,
2001). Additionally, all cows had access to pasture (mixture of alfalfa, ryegrass, and
orchardgrass, as well as triticale, wheat, and sorghum). At one day of age, female calves
were moved to a calf raising facility and were housed individually in calf hutches. The
calves were fed pasturized waste milk twice daily. This study was conducted from May
2013 through August 2013. The procedures described below were reviewed and approved
by the Institutional Animal Care Use Committee, The Ohio State University.

3.3.2. Management of Dry Cows, Calving and Colostrum

Daily, a list of cows was obtained based on their calving dates using on-farm
computer records (PCDART, Raleigh, NC). Pregnant cows were dried-off 60 ± 3 d prior
to the expected calving date and moved into a far-off dry pen immediately after last
milking. All cows were moved to close-up prepartum pens 21 d prior to the expected
calving date and were closely monitored by on-farm personnel for signs of parturition
(appearance of amniotic sac or feet of the calf outside the vulva) every 1 h (Schuenemann et al., 2013) and calved within their pen. Calving ease (CE) of cows (CE; assistance provided at birth) was recorded using a 4-point scale (1 = no assistance provided; 2 = light assistance by one person without the use of mechanical traction; 3 = mechanical extraction of the calf with an obstetric calf-puller; and 4 = severe dystocia: surgery or fetotomy needed; Schuenemann et al., 2011). Furthermore, calving date and time, and stillbirth were recorded. Additionally, cows had their body condition scores (BCS) using a 5-point scale with 0.25 unit increments (Ferguson et al., 1994) performed by the same person from the research team. The manure hygiene score (MHS) was recorded using a 3-point scale with 1.0 unit increments recorded at calving (Schuenemann et al., 2011b). Stillbirth was defined as a calf born dead or died (normal gestation length) within 24 h after birth (Schuenemann et al., 2011a). After birth, all calves received 3.7 L of quality colostrum, not necessarily from their dam, (colostrometer; >50 IgG mg/dL; Bartier et al., 2015) within 3 hours after birth. Two days after colostrum administration, blood samples were collected from jugular veinipuncture from females calves to assess failure of passive transfer (FPT; ≤5.5 mg/dL).

3.3.3. Calcium Status of Dams

Blood samples (8 mL) for determination of serum calcium status were collected from cows within 2 hours after parturition by coccygeal venipuncture (BD Vacutainer, Franklin Lakes, NJ). Immediately after collection, blood samples were centrifuged at 2,785 × g for 20 min immediately after collection, and serum samples were stored at −20 °C until assayed for total calcium. Total serum concentration of calcium were determined
in duplicates using a commercially available kit (Calcium Liquicolor No. 0150, Stanbio Laboratory, Boerne, TX) according to manufacturer’s instructions. Cows were classified as hypocalcemic when the concentration of calcium from the blood sample was ≤8.0 mg/dL (Reinhardt et al., 2011).

3.3.4. Diagnosis of Health Events and Milk Weights and Components

Weekly, a list of cows to screen was obtained based on their calving dates using on-farm computer records (PCDART, Dairy Record Management Systems, Raleigh, NC). Briefly, postpartum cows were screened for retained fetal membranes (RFM), metritis and mastitis. RFM was defined as a failure to expel the fetal membranes by 24 hours after parturition. Metritis was defined as a foul-smelling, brown-red, watery vaginal discharge within the first 20 DIM and fever (≥39.5 °C; Dubuc et al., 2010). Clinical mastitis was defined as an inflammation of the udder with visible changes in milk (presence of clots or blood at fore stripping) within 20 DIM. The incidence of diarrhea in female calves was assessed within 10 days of age. Diarrhea was defined as the presence of fluid or bloody feces (score 2-3; 0-3 scale) and either >5% dehydration (score 3; 1-3 scale) or fever (≥39.5 °C) within 10 days of age (Goodell et al., 2012). The proportion (%) of weaned calves was assessed at approximately 8 weeks of age. Milk weights, components (percent fat and protein), and SCC for the first three postpartum DHIA tests were collected to assess its association with HYPO cows.
3.3.5. Breeding Management

For all lactating dairy cows, the reported voluntary waiting period was 45 d. The breeding program was based on estrus detection (using tail chalk plus visual observation) twice daily and all animals presenting signs of standing estrous behavior received AI. Animals that did not display estrous behavior or were not pregnant after 5 consecutive AI were turned into the bull pen. Additionally, open cows at the time of pregnancy diagnosis were re-enrolled in the estrus detection program as described previously. All bulls were subjected to the Breeding Soundness Examination once per year and only bulls with a satisfactory score were used. Pregnancy diagnosis (PD) was performed at 40±3 d post-AI via transrectal palpation. The survival of lactating dairy cows (died or were culled within 60 DIM) and the subsequent reproductive performance up to 300 DIM were assessed.

3.3.6. Statistical Analyses

Data from individual lactating dairy cows (e.g., parity, dystocia, and stillbirth) were exported from PCDART into an Excel spreadsheet (Microsoft Corp., Redmond, WA). Distribution of Holstein dairy cows by parity (primiparous vs multiparous) with respect to MHS at calving, BCS immediately after calving, dystocia, stillbirth, and FPT were reported (Table 2). The association of cows experiencing HYPO at calving on metritis, mastitis, risk of culling within 60 DIM, and pregnancy per AI (PAI) at first service were analyzed using the GLIMMIX procedures of SAS (Table 3; SAS, 2009). The association of cows experiencing HYPO at calving on milk weights (kg/d) and components (SCC and percent fat and protein) were analyzed using MIXED procedure of SAS (SAS, 2009). A Cox proportional hazard model was used to assess the effect of
HYPO and non-HYPO cows on the time to pregnancy up to 300 DIM using the PHREG procedure of SAS (SAS, 2009), controlling for the effect of parity. Additionally, the effect of dam’s HYPO status at calving on stillbirth, diarrhea within 10 days of age and the proportion of weaned calves were analyzed using the GLIMMIX procedures of SAS (Tables 5; SAS, 2009). A $P < 0.05$ was considered statistically significant.

3.4. Results

The ingredient and nutrient composition of pre- and postpartum diets (DM basis) is provided in Table 1. The distribution of Holstein cows by parity (primiparous vs multiparous) with respect to MHS at calving, BCS immediately after calving, dystocia, stillbirth and FPT are provided in Table 2.

3.4.1. Association of HYPO at calving on health, culling and reproductive performance of cows

A total of 773 Holstein cows (with and without HYPO at calving) were included in the analyses (Table 3). The overall prevalence of HYPO ($\leq 8.0$ mg/dL) was 9% for first lactation and 30% for second or greater lactations (Figure 1). Lactating dairy cows with HYPO had increased risk for metritis and culling within 30 DIM (Table 3). The proportion of cows with mastitis and pregnancies per AI at first service was not different (Table 3).
3.4.2. Association of HYPO at calving on milk yield and milk components

Milk yield, components (percent fat and protein) and SCC were not different in HYPO and non-HYPO cows for the first three DHIA tests post-partum. (Table 4).

3.4.3. Association of HYPO at calving on survival and health of calves

All calves (male and female) were considered when assessing the association of dam’s HYPO status at calving on stillbirth. Only female calves were used in the present study to assess the association of HYPO at calving on FPT, diarrhea within 10 days of age and the proportion of weaned calves (Table 5). The proportion of stillbirth, FPT, and weaned calves did not differ between HYPO and non-HYPO cows (Table 5). However, calves born from HYPO cows had significantly higher proportion of diarrhea compared to non-HYPO cows (Table 5).

3.5. Discussion

The objectives of the present study were to assess the effect of HYPO at calving on health and performance of calves and cows. The study showed that (1) the overall prevalence of HYPO (clinical and subclinical combined) was 9% for primiparous and 30% for multiparous cows; (2) cows experiencing HYPO at calving had increased risk for metritis and early removal from the herd (culling within 30 DIM) compared to non-HYPO cows; (3) milk yield and components (percent fat and protein as well as SCC) were not different between HYPO and non-HYPO cows; and (4) female calves born from HYPO cows had greater proportion of diarrhea within 10 days of age than those calves born from non-HYPO.
In Holstein lactating cows, the prevalence of clinical HYPO for first-lactation is 1% and 4%, 7%, 10% for second, third and fourth lactations, respectively (Reinhardt et al., 2011). Similarly, subclinical HYPO in postpartum cows increased with age and was present in 25% of first lactation cows and 41-54% of second or greater lactation cows (Reinhardt et al., 2011). In the present study, blood samples to assess total calcium concentration were collected from cows within 2 hours of calving. Previous work assessed HYPO within 48 hours after calving to determine the prevalence of clinical and subclinical HYPO within herd (Reinhardt et al., 2011). Martinez et al. (2012) assessed total serum calcium concentrations at 0, 1, 2, 3, 4, 7, and 12 DIM and observed the nadir in blood calcium concentrations at 2 days after calving. It is important to note that in our study calcium status was assessed within 2 hours of calving. Calcium concentrations are reported to reach a nadir within 24 to 48 hours of calving (Goff, 2008; Martinez et al., 2012) thus, the prevalence of HYPO in cows by 48 hours after calving may be higher than reported in our study. Regardless of parity, the HYPO prevalence (either clinical or subclinical) from the present study is in agreement with previous reports (Reinhardt et al., 2011; Martinez et al., 2012).

Ionized calcium is necessary for vital bodily functions such as cellular signaling, muscle contraction, and activation of immune cells among others (Saris and Carafoli, 2005; Kimura et al., 2006; Parekh, 2006). Calcium concentrations in cows at low risk for developing metritis did not differ from high risk cows (experiencing one or more calving disorders such as dystocia, birth of twins, stillbirth, and retained fetal membranes) on the day of calving; however, a significant reduction in calcium concentration was observed one day after calving for high risk cows (Martinez et al., 2012). Cows with subclinical
HYPO (total serum calcium \( \leq 8.59 \text{ mg/dL} \)) for at least one day, between 0 and 3 DIM, had less neutrophils in their blood with reduced functions, and increased risk for metritis (Martinez et al., 2012). In the present study, HYPO cows had significantly increased risk for metritis and culling within 30 DIM as opposed to non-HYPO cows. Furthermore, PAI for first services and clinical cases mastitis within 20 DIM were not different between HYPO and non-HYPO cows. Although DMI was not assessed in the present study, it has been reported that cows with metritis have decreased feed intake and reduced feeding time prior to and after calving (Huzzey et al., 2007). It has been proposed that decreased DMI prepartum may be associated with HYPO (LeBlanc et al., 2005), which in turn decreased DMI leads to elevated NEFA and \( \beta \)-hydroxybutyrate (BHBA) concentrations and development of ketosis (LeBlanc et al., 2005; Chapinal et al., 2011; Martinez et al., 2012). Additionally, the increased concentration of energy metabolites (NEFA and BHBA) within 12 DIM were interrelated; thus, influencing the risk for postpartum diseases in lactating dairy cows (Martinez et al., 2012).

HYPO cows had increased risk of culling within 30 DIM compared to non-HYPO cows. A retrospective study assessed the relationships of energy (serum NEFA, BHBA, and glucose) and calcium status of cows on postpartum diseases and culling (Seifi et al., 2010). Postpartum cows with serum calcium concentrations \( \leq 2.2 \) or \( \leq 2.3 \text{ mmol/L} \) had 2.4 and 5.3 increased odds of culling, respectively (Seifi et al., 2010). Similarly, cows with elevated serum NEFA and BHBA concentrations and low serum calcium (\( \leq 2.3 \text{ mmol/L} \)) within 1 week prepartum through 2 weeks postpartum had increased risk of culling (Roberts et al., 2012). Different serum calcium ranges have been published as normal reference values in cattle around parturition such as 8.5 to 10 mg/dL (Goff, 2008), \( >8.59 \)
mg/dL (Martinez et al., 2012), or >8.8 mg/dL (Chapinal et al., 2011). A cut-off value of 
≤8.0 mg/dL (or ≤ 2.0 mmol/L) was used in the present study to assess the effect of HYPO
at calving on survival, health and performance of dairy cattle; thus, this cut-off value may
have resulted in the inclusion of cows with more severe HYPO.

Studies have reported that cows with HYPO (<7.2 mg/dL; Jawor et al., 2012)
produced 5.7 k/d more milk or that milk yield did not differ (HYPO ≤8.59 mg/dL;
Martinez et al., 2012) in the first month of lactation when compared to non-HYPO cows.
It could be hypothesized that HYPO may compromise DMI postpartum, as reported
elsewhere (Jawor et al., 2012), which in turn could lead to higher blood concentrations of
NEFA and BHBA (Martinez et al., 2012) and increased risk for metritis with reduced
milk yield (Dubuc et al., 2011). In the present study, milk yield and components (percent
fat and protein as well as SCC) for the first three DHIA tests were not different in HYPO
and non-HYPO cows. In the present study, only 4 cows had Ca concentration <6.0 mg/dL
and most of the HYPO cows had Ca concentrations of 6.0 to 8.0 mg/dL. This low
prevalence of clinical HYPO could explain, at least in part, the differences observed
between studies on milk yield in HYPO and non-HYPO cows during the first month of
lactation.

An interesting finding was that female heifer calves born from HYPO cows had
greater proportion of diarrhea within 10 days of age compared to those born from non-
HYPO cows. Prewening calf diarrhea, defined as a calf presenting fluid or bloody feces
and >5% dehydration or fever (≥39.5 °C) within 10 days of age, is a common health and
welfare problem on dairy herds (Walker et al., 2012; Al Mawly et al., 2015). Infectious
agents such as Cryptosporidium parvum, bovine rotavirus, and co-infection with more
than one agent (*E. coli, Salmonella*) as well as housing (environment) were associated with increased odds of liquid feces in calves within 9 to 21 days old (Al Mawly et al., 2015). There are several factors associated with the morbidity and mortality of dairy calves from birth to weaning. According to McGuirk and Ruegg (2009), the highest morbidity and mortality rates of any dairy operation are for replacement heifers, primarily due to diarrhea prior to weaning (Walker et al., 2012). The most common pre-weaning diseases include: neonatal calf diarrhea, septicemia, and pneumonia, with diarrhea being the most common cause of death or reduced growth rate (McGuirk and Ruegg, 2009, van Amburgh et al., 2014). A study employing a cross-sectional survey of heifer rearing facilities reported that overall neonatal calf diarrhea morbidity at the herd level was 20% with a range of 4.1% to 43% (Walker et al., 2012).

The most efficient and effective management of the newborn health is achieved by feeding adequate quantity (at least 4 L) of quality colostrum (>50 g/L) with minimal bacterial contamination within the first 3 hours after birth (Stewart et al., 2005; Mee, 2008; Godden et al., 2009). Immediately after birth the calf requires proper navel disinfection, removal from the dam, and movement to a clean and dry environment (Mee, 2008). Once calves (ranging anywhere from one to seven days of age) reach the calf raiser facility, nutrition and housing environment are perhaps the top priorities to prevent disease and identify sick calves early (McGuirk and Ruegg, 2009; Walker et al., 2012). Feeding heifers and cows a high-energy diet (100% of recommended prepartum energy) 100 days prepartum increased body weight of dams before calving as well as calf birth and weaning weight (Corah et al., 1975). When mature cows were fed a low-energy diet (65% of NRC [1970] energy level), more calves (roughly 19%) were treated for scours,
and weaning weights were lower (Corah, et al., 1975). An increased growth rate or plane of nutrition during the preweaning period is positively associated with future milk production (Bach, 2012). While pre-weaning growth rate was not assessed in this study, previous work has shown that average daily gains in neonatal calves experiencing diarrhea that were treated with antibiotics had reduced average daily gains of 50 g per day (Van Amburgh et al., 2014). In the present study, all calves received the same quantity (3.7 L) and quality (colostrometer; >50 IgG mg/dL) of colostrum within 3 hours after birth. However, heifers did not commonly receive colostrum from their dams. This highlights the importance of maternal factors on the development of diarrhea within calves. Furthermore, the proportion of dystocia, stillbirth, and FPT (≤5.5 mg/dL) were not different between calves born from HYPO and non-HYPO cows.

It has been proposed that the neonate can be programmed maternally (in utero) and post-natally, through milk or colostrum related factors (Bartol et al., 2008). It is well known that colostrum plays a major role on calf health, primarily through passive absorption of IgG (Godden et al., 2009). However, colostrum is also rich in many vitamins, minerals, and molecules such as lactoferrin, relaxin, prolactin, insulin, IGF-1 among others (Odle et al., 1996; Blum and Hammon, 2000; Kehoe et al., 2007; Bartol et al., 2008). Although IgG concentration was the only colostrum component measured in the present study, colostral vitamins and nutrients in HYPO and non-HYPO cows were not assessed. Since the concentration of IgG were relatively the same for all calves, it could be hypothesized that HYPO cows could yield colostrum of inferior quality as opposed to non-HYPO cows, which in turn could compromise neonatal calf health prior to weaning. Further studies are needed to assess the association between prepartum cow management
(i.e., Ca and energy status at calving) and neonatal calf health, accounting for the effect of colostrum components.

In conclusion, the overall prevalence of HYPO was 30% (9% for primiparous and 30% for multiparous cows). Cows experiencing HYPO at calving had increased risk for metritis and culling within 30 DIM compared to non-HYPO cows; however, milk yield and components as well as the proportion of P/AI at first service was not different between groups. Although the proportion of stillbirth and FPT was not different, calves born from HYPO cows had greater proportion of diarrhea than those calves born from non-HYPO cows. Dairymen, consultants, and veterinarians often trouble-shoot neonatal calf diseases and this process requires constant monitoring and comprehensive assessment of several events. Findings from the present study showed that HYPO at calving had significant health implications for both dams and calves.
### 3.6 Tables and Figures

Table 1. Ingredients and nutrient composition of pre- and postpartum diets (DM basis).

<table>
<thead>
<tr>
<th>Ingredients, %</th>
<th>Prepartum</th>
<th>Early postpartum</th>
</tr>
</thead>
<tbody>
<tr>
<td>Grass hay</td>
<td>45.12</td>
<td>5.51</td>
</tr>
<tr>
<td>Wheat straw</td>
<td>-</td>
<td>0.95</td>
</tr>
<tr>
<td>Alfalfa hay</td>
<td>25.72</td>
<td>28.73</td>
</tr>
<tr>
<td>Corn silage</td>
<td>10</td>
<td>16.07</td>
</tr>
<tr>
<td>Grain mix</td>
<td>15.93</td>
<td>46.14</td>
</tr>
<tr>
<td>Mineral mix</td>
<td>3.19</td>
<td>2.49</td>
</tr>
</tbody>
</table>

**Nutrient profile**

<table>
<thead>
<tr>
<th>Nutrient</th>
<th>Prepartum</th>
<th>Early postpartum</th>
</tr>
</thead>
<tbody>
<tr>
<td>NE&lt;sub&gt;L&lt;/sub&gt;, Mcal/kg</td>
<td>1.30</td>
<td>1.62</td>
</tr>
<tr>
<td>CP, %</td>
<td>11.78</td>
<td>18.8</td>
</tr>
<tr>
<td>NDF, %</td>
<td>45.79</td>
<td>28.39</td>
</tr>
<tr>
<td>ADF, %</td>
<td>30.78</td>
<td>21.23</td>
</tr>
<tr>
<td>Starch, %</td>
<td>13.79</td>
<td>26.69</td>
</tr>
<tr>
<td>Ca, %</td>
<td>1.43</td>
<td>1.33</td>
</tr>
<tr>
<td>P, %</td>
<td>0.34</td>
<td>0.39</td>
</tr>
<tr>
<td>Mg, %</td>
<td>0.42</td>
<td>0.36</td>
</tr>
<tr>
<td>K, %</td>
<td>1.47</td>
<td>1.28</td>
</tr>
<tr>
<td>Na, %</td>
<td>0.25</td>
<td>0.42</td>
</tr>
<tr>
<td>Cl, %</td>
<td>1.10</td>
<td>0.38</td>
</tr>
<tr>
<td>S, %</td>
<td>0.36</td>
<td>0.23</td>
</tr>
<tr>
<td>DACD, mEq/100g DM</td>
<td>-14</td>
<td>15.8</td>
</tr>
</tbody>
</table>

Dietary cation-anion difference calculated as follows: DCAD = (mEq of Na + mEq of K) – (mEq of S + mEq of Cl).
Table 2. Distribution of Holstein dairy cows by parity with respect to MHS, BCS, dystocia, stillbirth, and FPT*

<table>
<thead>
<tr>
<th>Items</th>
<th>Primiparous (n = 445)</th>
<th>Multiparous (n = 328)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MHS at calving (n)</td>
<td>1.8</td>
<td>1.9</td>
</tr>
<tr>
<td>BCS†</td>
<td>3.32</td>
<td>3.58</td>
</tr>
<tr>
<td>Dystocia, %</td>
<td>6.6</td>
<td>7.4</td>
</tr>
<tr>
<td>Stillbirth‡, %</td>
<td>2.8</td>
<td>2.1</td>
</tr>
<tr>
<td>FPT (≤5.5 mg/dL)§, %</td>
<td>1.2</td>
<td>3.3</td>
</tr>
</tbody>
</table>

*Distribution (mean) of lactating Holstein dairy by parity (primiparous vs multiparous) with respect to manure hygiene score (MHS), BCS immediately after calving, dystocia, stillbirth, and failure of passive transfer (FPT).

MHS = manure hygiene score using a 3-point scale was recorded at calving (Schuenemann et al., 2011b).

†Body condition score (BCS) was assessed immediately after calving using a 5-point scoring system (Ferguson et al., 1994).

Dystocia was recorded at calving using a 4-point scale (Schuenemann et al., 2011a) and cows were classified as assisted or unassisted.

‡Stillbirth was defined as a calf born dead or died within 24 h after birth, and with a normal gestation length.

§FPT = failure of passive transfer. Blood samples were collected from female calves 2 days after colostrum administration to assess the concentration of total serum proteins.
Table 3. Association of calcium status of Holstein dairy cows at calving on metritis, mastitis, risk of culling within 30 DIM and pregnancy to first service*

<table>
<thead>
<tr>
<th>Items</th>
<th>HYPO (≤8.0 mg/dL; n=113)</th>
<th>non-HYPO (&gt;8.0 mg/dL; n = 660)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Metritis†, %</td>
<td>29.4</td>
<td>17.3</td>
<td>0.02</td>
</tr>
<tr>
<td>Mastitis†, %</td>
<td>7.8</td>
<td>4.4</td>
<td>0.11</td>
</tr>
<tr>
<td>Culled within 60 DIM‡, %</td>
<td>15.9</td>
<td>6.8</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Pregnancies per AI for FS§, %</td>
<td>30</td>
<td>37</td>
<td>0.21</td>
</tr>
</tbody>
</table>

*Blood samples from lactating Holstein dairy cows were collected within 2 hours after calving to assess the calcium status. The proportion (%) of animals with HYPO (≤8.0 mg/dL) or non-HYPO (>8.0 mg/dL) was assessed using total serum calcium concentration (Reinhardt et al., 2011).

†Metritis was defined as a foul-smelling, brown-red, watery vaginal discharge within the first 20 DIM and fever (≥39.5 °C).

‡Mastitis was defined as an inflammation of the udder with visible changes in milk (presence of clots or blood at fore stripping) within 20 DIM.

§The proportion (%) of cows that were culled within 60 DIM was assessed.

§Pregnancies per AI for first service (FS). The breeding program was based only on estrus detection (using tail chalk plus visual observation) twice daily and all animals presenting signs of standing estrous behavior received AI.
Table 4. Association of calcium status at calving of Holstein dairy cows on milk yield and components (percent fat and protein, and SCC) of the first three DHIA tests*

<table>
<thead>
<tr>
<th>DHIA tests \n(≤8.0 mg/dL; n=113)</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Milk yield ‡, kg</td>
<td>33.42</td>
<td>30.84</td>
<td>28.64</td>
<td>35.55</td>
<td>31.63</td>
<td>29.34</td>
<td>0.40</td>
</tr>
<tr>
<td>Fat ‡, %</td>
<td>2.98</td>
<td>2.79</td>
<td>3.03</td>
<td>3.03</td>
<td>2.87</td>
<td>3.07</td>
<td>0.60</td>
</tr>
<tr>
<td>Protein &amp; , %</td>
<td>2.79</td>
<td>2.84</td>
<td>2.88</td>
<td>2.79</td>
<td>2.83</td>
<td>2.90</td>
<td>0.37</td>
</tr>
<tr>
<td>SCC (x10^3 cells/mL)</td>
<td>243</td>
<td>186</td>
<td>189</td>
<td>262</td>
<td>148</td>
<td>166</td>
<td>0.73</td>
</tr>
</tbody>
</table>

*Least squares means are presented. Blood samples from lactating Holstein dairy cows were collected within 2 hours after calving to assess the calcium status. The proportion (%) of animals with HYPO (≤8.0 mg/dL) or non-HYPO (>8.0 mg/dL) was assessed using total serum calcium concentration (Reinhardt et al., 2011).

†Milk yield (kg) and components (percent fat and protein, and SCC) were recorded for the first three DHIA tests postpartum.
Table 5. Association of calcium status of Holstein dairy cows at calving on stillbirth, FPT, diarrhea within 10 days of age and proportion of weaned calves*

<table>
<thead>
<tr>
<th>Items</th>
<th>HYPO (≤8.0 mg/dL; n=113)</th>
<th>non-HYPO (&gt;8.0 mg/dL; n = 660)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stillbirth†, %</td>
<td>2.0</td>
<td>2.5</td>
<td>0.32</td>
</tr>
<tr>
<td>FPT (≤5.5 mg/dL)‡, %</td>
<td>2.1</td>
<td>2.5</td>
<td>0.87</td>
</tr>
<tr>
<td>Diarrhea&amp;, %</td>
<td>49.1</td>
<td>33.3</td>
<td>0.04</td>
</tr>
<tr>
<td>Calf weaned§, %</td>
<td>89</td>
<td>92</td>
<td>0.41</td>
</tr>
</tbody>
</table>

*Blood samples from lactating Holstein dairy cows were collected within 2 hours after calving to assess the calcium status. The proportion (%) of animals with HYPO (≤8.0 mg/dL) or non-HYPO (>8.0 mg/dL) was assessed using total serum calcium concentration (Reinhardt et al., 2011).

†Stillbirth was defined as a calf born dead or died within 24 h after birth, and with a normal gestation length.

‡FPT = failure of passive transfer (≤5.5 mg/dL). Blood samples were collected from female calves 2 days after colostrum administration to assess the concentration of total serum proteins.

&The incidence of diarrhea in calves was assessed within 10 days of age. Diarrhea was defined as a calf presenting fluid or bloody feces (score 2-3; 0-3 scale) and >5% dehydration (score 3; 1-3 scale) or fever (≥39.5 °C) within 10 days of age.

§The proportion (%) of weaned calves was assessed (based on the total of female calf alive >24 hours) at 8 weeks of age.
Figure 1. Total serum calcium of Holstein dairy cows within 2 hours after parturition by lactation number.

Total serum calcium concentrations from 773 Holstein dairy cows. Blood samples were collected within 2 hours after parturition to assess the calcium status by lactation number: Lact 1 (n=445), Lact 2 (n=206), Lact 3 (n=65), Lact 4 (n=25), Lact 5 (n=8), Lact 6 (n=18), Lact 7 (n=5), and Lact 8 (n=1). The proportion (%) of animals with HYPO (≤8.0 mg/dL) or non-HYPO (>8.0 mg/dL) was assessed using total serum calcium concentration (Reinhardt et al., 2011). Cows with clinical HYPO had a total serum Ca of ≤6.0 mg/dL.
Figure 2. Survival curves for time to pregnancy after the voluntary waiting period of dairy cows with HYPO and non-HYPO. The calcium status of lactating dairy cows (n=773) cows was assessed within 2 hours after calving. Adjusted hazard ratios (AHR; 95% CI) for pregnancy ($P > 0.05$) were 0.93 (0.70-1.22) for HYPO cows (non-HYPO = Referent). Mean time to pregnancy (95% CI) was 140 d (134-146) and 147 d (129-165) for non-HYPO and HYPO cows, respectively.
Chapter 4

Summary and Conclusions

While transition dairy cows will likely experience some degree of hypocalcemia, clinical or subclinical, it is essential that we continue to improve our understanding of the underlying mechanisms associated with this costly disease. Different serum Ca ranges have been published as normal reference values in dairy cattle around parturition such as 8.5 to 10 mg/dL (Goff, 2008), >8.0 mg/dL (Reinhardt et al., 2011), >8.59 mg/dL (Martinez et al., 2012), or >8.8 mg/dL (Chapinal et al., 2011). A cut-off value of ≤8.0 mg/dL (or ≤ 2.0 mmol/L), as described by Reinhardt et al. (2001) was used in the present study to assess the association of HYPO at calving on survival, health and performance of dairy cows and calves. Previous work has shown that hypocalcemia decreased feed intake, increased risk for developing infectious disease (e.g., metritis) and culling early in lactation, and reduced milk yield (Curtis et al., 1983; Goff, 2008; Seifi et al., 2011; Martinez et al., 2012). Although the negative association of hypocalcemia on survival and health has been extensively documented, limited science based evidence is available in the literature on the relationship of maternal Ca status at calving on survival, health and performance of calves. Findings from the present study showed that hypocalcemia (≤8.0 mg/dL) at calving had significant health implications for both dams and calves.
Calves born from hypocalcemic cows had greater proportion of diarrhea than those calves born from non-hypocalcemic cows. In future studies, the focus should be placed on the association between prepartum cow management (i.e., Ca and energy status of dams at calving) and neonatal calf health, accounting for the effect of colostrum components. The association of hypocalcemia at calving and neonatal calf diarrhea provided useful information for the development of new hypotheses for future studies to elucidate the underlying mechanisms by which infection is controlled and its association with clinical recovery and calf performance.

4.1. Acknowledgements

The authors thank the collaborating dairy farm and their staff for providing the animals used in the present study and their assistance during the project. This project was partially supported by the Ohio Dairy Producer Association, Merial and T35 Fellow Scholarship, and The Ohio State University Veterinary Extension.
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