ORAL NUTRIENT SUPPLEMENTATION OF NEONATAL CALVES TO MITIGATE VITAMIN AND MINERAL DEFICIENCIES

By

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ABSTRACT

Nutritional supplementation of cows is a common management strategy that assumes calves receive adequate quantities of nutrients from dams in utero, and through consumption of colostrum and milk. This study evaluated an easy to administer, efficacious and economical oral nutritional supplement for neonatal calves as an additional approach. The primary objective of this study was to evaluate the effect of oral nutrient supplementation in neonatal beef and dairy calves by examining plasma vitamin and serum mineral concentrations. Other objectives were: 1) to assess prevalence and severity of nutrient deficiencies in newborn calves, along with intra- and inter-herd variability; 2) determine the relationship between dam and calf blood nutrient concentrations; and 3) investigate the relationship between neonatal vitamin-mineral concentrations and morbidity and mortality occurrence. Baseline blood plasma and serum concentrations of vitamin A, vitamin E, iron, and selenium were determined in newborn calves and their dams. Calves were randomly assigned to either treatment or control groups which received 10 mL of *VitaFerst-Care* or saline solution, respectively. Following neonatal supplementation, calf blood nutrient profiles were assessed at day 14 (year 1), and day 3 (year 2) for control and treatment groups. Four hundred and sixty-five beef and dairy animals (277 calves and 188 cows) were enrolled in this two-year study at Lakeland College. Blood samples were collected by jugular venipuncture for calves and coccygeal venipuncture for cows. Blood serum/plasma concentrations of vitamin A, E, selenium, and iron were determined by high-performance liquid chromatography (HPLC) and inductively coupled plasma mass spectrometry (ICP-MS). Statistical analysis was performed using a two-way ANOVA. There was no significant increase in vitamin A and selenium concentrations in response to oral nutrient supplementation. There was a statistically significant effect of supplementation on vitamin E and iron concentrations in beef calves at day 3. Pre-ruminant calves were observed to have marginal to low vitamin A and iron concentrations, low to deficient selenium concentrations, yet adequate to high vitamin E concentrations under the variable management systems deployed. Intra- and inter- herd variability was limited with respect to all nutrients except for iron. Ultimately, this study emphasizes the need for implementing a vitamin and mineral supplementation protocol to address deficiencies in pre-ruminant calves in Alberta.

keywords: vitamin A, vitamin E, iron, selenium, beef, dairy, neonatal calves, oral vitamin and mineral supplementation, nutrient deficiencies

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GLOSSARY OF TERMS

ADG: Average Daily Gain
AMR: Antimicrobial Resistance
ANOVA: Analysis of Variance
BMP: Best Management Practices
EDTA: Ethylenediaminetetraacetic Acid
Fe: Iron
FTT: Failure to Thrive
Hb: Hemoglobin
HPLC: High Performance Liquid Chromatography
ICP-MS: Inductively Coupled Plasma Mass Spectrometry
Se: Selenium
TIBC: Total Iron-Binding Capacity
WMD: White Muscle Disease

CHAPTER ONE: GENERAL INTRODUCTION

Mineral and vitamin supplementation is a common management strategy in the cattle industry to compensate for limitations and address nutrient deficiencies. As livestock production systems intensified and herds of animals were being housed indoors or in confined spaces, it altered their exposure to different environmental factors. The pervasiveness of nutrient deficiencies has since increased in Alberta cattle herds compared to previous years (Campbell et al., 1995; Waldner and Van De Weyer, 2011). Furthermore, in some countries, the trace element content of soil and roughage has decreased over the last decade, due to the exhaustion of soils and new agricultural practices involving fertilizers containing no or few trace elements (Guyot et al., 2009). In western Canada, extended periods of winter feeding, synchronous with stored feed, have negative ramifications on nutritive value and the current climate change phenomenon represents an additional challenge for ruminant production. In general, climate change reduces the availability of rangeland pastures and forages causing livestock production to depend on alternate feed sources of varying nutritional quality (McGrath et al., 2018). With the transition to a greater percentage of livestock herds maintained within confined areas, producers began to rely on preventative medicines and antimicrobial drugs to ensure the health of their livestock (Carroll and Forsberg, 2007). This has led to a growing global recognition of the importance of antimicrobial resistance (AMR) and the need for antimicrobial stewardship in veterinary medicine (Waldner et al., 2019; WHO, 2017). Scientists have since investigated the use of various nutritional strategies to enhance the immune systems of livestock throughout various stages of production. The research has shown the influence that numerous dietary factors have on performance and health of cattle. Countless studies have documented the immunomodulatory properties of various vitamins and minerals, including, but not limited to vitamin A, vitamin D, vitamin E, iron, and selenium (Carroll and Forsberg, 2007; Graham, 1991; Hodnik et al., 2020; Jenkins and Hidiroglou, 1987; Krueger et al., 2014; Spears, 2000).

Micronutrients, including vitamins and minerals, are essential dietary elements required for normal growth, development, and disease prevention. The concentrations of micronutrients must be maintained within quite narrow limits, and to prevent deficiencies,

toxicities and imbalances, animals must be supplied with a diet that is non-toxic and palatable, and which contains appropriate quantities of required nutrients (Engle, 2001). It is important to note that most micronutrients cannot be produced in the body and must be derived from the diet - Vitamin D is an exception, with exposure to sunlight or consumption of sun-cured forages (Hodnik et al., 2020). As a result, domesticated cow-calf herds in western Canada generally require supplemental minerals, vitamins, and salt. Respectively, maternal subnutrition has intensified over the years (Noya et al., 2019a; Noya et al., 2019b), and trace mineral supplementation is a common practice for animals under confined management schemes (Hulbert et al., 2023; Van Emon et al., 2020). This management strategy presumes that calves acquire adequate essential nutrients through placental transfer and suckling colostrum from their dam (Quigley and Drewry, 1998). However, calves born to nutrient deficient cows are at serious risk of nutritional deficiencies and secondary infectious diseases (Homerosky et al., 2019; Radostits and Bell, 1970). Therefore, neonatal vitamin-mineral supplementation requires further investigation as it has not been a major focus in the past. Rather, the periparturient and weaning periods have previously been thought to be the most critical periods for nutrient supplementation because decreased feed intake is often observed, which decreases nutrient intake and can yield immunosuppression (Kafilzadeh et al., 2014; Weiss et al., 1990; Galyean et al., 2022). Yet, the lack of evidence for early pre-weaning supplementation, compared to post-weaning supplementation (Galyean et al., 2022), in beef calves leads to curiosity about the potential return on investment that could be established through vitamin-mineral supplementation in young calves.

Most often, minerals and vitamins are offered free choice in either loose or block form to cows. A recent survey of western Canadian beef producers found that 97% of participating producers reported providing free-choice supplementation to their herd at some point during the year (Waldner et al., 2023). Several factors impact intake, such as: animal requirements, soil characteristics, season, forage type, forage availability, water quality, protein supplements, energy supplements, vitamin-mineral supplement palatability, source and physical form of the mineral supplement, bioavailability, freshness of the mineral, salt concentration, and mineral access (Arthington and Ranches, 2021; Patterson et al., 2013; Tait and Fisher, 1996; McDowell, 1996); as a result, intake with

these supplements is highly variable. Additionally, supplementation practices in many herds are often less consistent in the latter part of the summer and only optimized during the breeding period (Chládek and Zapletal, 2007; Waldner and Van De Weyer, 2011). Moreover, trace minerals and vitamins are typically easier to effectively supplement during the winter-feeding period, due to the accessibility of the animals and the opportunity to incorporate oral supplements with feed. Finally, a total mixed ration (TMR) may be preferred because it incorporates the mineral supplement with the other dietary components to yield a more complete diet. Although a TMR is most commonly used in dairy operations or feedlot systems, TMRs possess their own limitations as well: thoroughly mixing minerals in mixed rations is difficult because only a small quantity of mineral is required, and it separates easily from the larger particle sizes of grain and forages (Schingoethe, 2017). Current mineral and vitamin supplementation standards for cattle herds could be improved, particularly in areas where micronutrients in forage are less than adequate to achieve health and performance targets (Waldner et al., 2023).

Despite rudimentary nutritional supplementation practices, industry has demonstrated that nutritional management of cows during the dry period may have a profound effect on the performance of newborn calves because of the direct impact that cow nutrition status has on colostrum quality and quantity (Quigley and Drewry, 1998; Van Emon et al., 2020). It has been suggested that nutritional status in ewes during the periconceptual period is highly important to fertility and that some fetal programming may actually begin in the oocyte before mating occurs (Nordby et al., 1987). For cows, the effects of periconceptual nutrition on offspring is less clear, but there is some evidence that severe, chronic growth retardation in utero can result in smaller animals at any given age, with effects being seen up to 30 months after birth (Greenwood and Cafe, 2007). Clearly, the importance of adequate nutrition is critical in both early and late gestation (Van Emon et al., 2020). Blood nutrients present at birth may be linked to health status and contribute to neonatal performance in later life. However, it needs to be emphasised that during specific stages of embryonic, foetal, and neonatal calf development, long-term consequences of more specific, acute environmental influences remain to be determined (Greenwood and Cafe, 2007).

Calves up to 28 days of age are considered neonates. During the neonatal period, the rumen is underdeveloped, and calves are known as pre-ruminants (Diao et al., 2019). At this point, the immature digestive metabolic system functions similarly to that of a young monogastric animal, and the calf depends solely on milk for nutriment. Nutrients are transferred from mother to the calf via placental transfer and the umbilicus, prior to birth, and colostrum ingestion immediately post parturition. Colostrum is the first milk produced after birth, and then subsequently is called transition milk for the next several days postpartum, during the transition from colostrum to mature milk (Fahey et al., 2020; Gopal and Gill, 2000). However, time constitutes a critical element for feeding calves colostrum because the absorption of colostrum decreases significantly after several hours have elapsed due to reduced intestinal permeability (Staley and Bush, 1985; Puppel et al., 2019). For example, absorption decreases by 1/3 within 6 hours after birth and 2/3 just 12 hours after birth (Puppel et al., 2019). Colostrum contains many essential nutrients and antibodies that support postnatal growth and development, stimulates digestive activity, and ultimately promotes production of robust, young animals by assisting the immune system to target microbial threats (Hammon et al., 2020; Playford and Weiser, 2021). Most importantly, the calf receives immunoglobulins (Ig) in colostrum, which are indicative of the success of transfer of passive immunity (Quigley and Drewry, 1998; Playford and Weiser, 2021). Passive immunity provides short-term protection from infection and helps to protect the calf until its own immune system becomes fully functional. Failure of transfer of passive immunity can occur when a calf does not obtain adequate amounts of colostrum within a short-time and can lead to disease susceptibility and failure to thrive (FTT) (Dewell et al., 2006; Waldner and Rosengren, 2009). According to Puppel et al. (2019), in comparison to milk, colostrum contains two times more dry matter, three times more minerals, five times more proteins, and more fat-soluble vitamins. Thus, only small amounts of trace minerals and vitamins pass through mature milk to nursing calves, making pre-ruminating calves reliant on any stores they have in their liver until they begin to ingest forage and supplements as they grow (Van Emon et al., 2020). A lack of vitamins will persist until calves start consuming substantial fresh forage at three to four months of age (Waldner and Uehlinger, 2017). As a result, calves

are at greater risk of nutrient deficiencies during the first few weeks of their life when they are exclusively fed milk (Joerling and Doll, 2019; NRC, 1996).

Therefore, injectable vitamins and minerals have been utilized and can alter the micronutrient status of deficient or marginally deficient cattle faster than dietary supplementation (Galyean et al., 2022). Unlike traditional injectable options (subcutaneous or intramuscular), oral supplements should be advantageous as they do not leave lesions or injection-site damage (Galyean et al., 1991) and are easy to administer for unskilled users. A previous study by Ede et al., (2018) demonstrated that dairy calves find intramuscular injections in the rump aversive, whereas intranasal routes are a refinement. In fact, Kafilzadeh et al. (2014) found no significant difference between the effectiveness of injections and oral supplementation of vitamin E and/or selenium. Oral products exist for vaccines, antimicrobials, and supplements and can minimize pain and stress in animals (Jiminez et al., 2019; Kafilzadeh et al., 2014; Ohlheiser et al., 2020). Consequently, oral vitamin and mineral supplementation for neonates during this critical period is the focus of this research.

The goal of this study was to investigate the efficacy of an oral vitamin and mineral supplement targeted at newborn beef and dairy calves. First, the prevalence and severity of nutrient deficiencies, along with intra- and inter-herd variability, in cattle herds were explored. Next, the relationship between dam and calf blood nutrients after parturition were investigated. Finally, the relationship between blood serum/plasma vitamin and mineral concentrations, the prevalence of infectious disease occurrence (morbidity), and the need for treatment as well as subsequent mortality in neonatal calves was investigated.

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CHAPTER TWO: PREVALENCE AND SEVERITY OF NUTRIENT DEFICIENCIES IN CATTLE HERDS

2.1 Introduction

In western Canada, Alberta has the largest cattle inventories, contributing to 42.7% of the national herd (The Daily, 2023); however, nutrient deficiencies can severely impact livestock production efficiencies, which would be important to the economic viability of the provincial industry. Nutrient deficiencies may be responsible for reduced growth potential, a lack of disease resistance, impaired vaccine response, inferior reproductive ability, and overall poor performance in livestock such as cattle, sheep, and pigs (Perdrizet et al., 2020; Krueger et al., 2014; Spears, 2000; Vlasova and Saif, 2021). In young ruminants, macro and micro element deficiencies contribute to reduced growth as well as increased susceptibility to diarrhea and respiratory disorders because of flawed immune responses (Radostits et al., 1991; Radwińska and Żarczyńska, 2014; Waldner et al., 2013). The adoption of intensive livestock production systems may challenge cattle health and welfare status and consequently increases the incidence of metabolic and infectious diseases (McGrath et al., 2018; Waldner et al., 2023). Furthermore, drought, poor-quality forage, and extended periods of winter feeding in Canadian beef production can impact vitamin and mineral levels, resulting in deficiencies in cattle rations (Waldner and Uehlinger, 2017).

Nutrient deficiencies manifest differently depending on the age, geographical location, supplementation history, water quality, and physiological status of the animal (Waldner and Blakley, 2014; Van Emon et al., 2020). Animals can have subclinical deficiencies without exhibiting outward clinical symptoms which may still impact their immunocompetency (Kegley et al., 2016). Poor cow nutrient status and subclinical deficiencies can directly impact the nutritional status of their offspring. Corah et al. (1975) observed that progeny born to energy restricted cows were slower to suckle, had increased morbidity and mortality rates, lower weaning weights; and consequently, heifer progeny were slower to reach puberty. Recently published literature and preliminary work has shown that iron and selenium are major mineral deficiencies in calves; while vitamins of concern in cattle nutrition continue to be vitamin A, D, and E (Arthington and Ranches,

2021; Hidiroglou, 1979; Maas et al., 2008; Mehdi and Dufrasne, 2016; Nelson et al., 2016; Waldner and Uehlinger, 2017; Waldner and Van De Weyer, 2011).

If blood nutrient levels are assessed soon after birth it may provide insight regarding the ubiquity of nutrient deficiencies in Alberta cattle herds and provide for evaluation of the effectiveness of current management practices being utilized provincially. Furthermore, by comparing calf nutrient profiles to that of the dam, a better understanding can be developed of the relationship between calf and dam nutrient profiles. This could aid in the revision of best management practices (BMPs), and supplementation protocols could be developed based on inclusion of either cow, calf, or both. Therefore, we assessed the prevalence and severity of nutrient deficiencies, along with intra- and inter-herd variability, in central Alberta herds by measuring blood nutrient concentrations of vitamin A, E, selenium, and iron. Further, blood plasma vitamin and serum mineral concentrations of calves and their dam were evaluated within 72 hours of birth to determine if a relationship exists. It was expected that cattle under variable management strategies will display inter-herd variability, whereas intra-herd variability will be limited, particularly for cattle receiving a TMR compared to those offered free choice mineral. Furthermore, we expected that the relationship between dam and calf vitamin and mineral concentrations should be linearly sequential.

2.2 Materials and Methods

All protocols were consistent with the Canadian Council on Animal Care guidelines and were approved by the Lakeland College (Neonatal-12-21) and Thompson Rivers University (AUP#103169) Animal Care Committees.

2.2.1 Study farms

Herds recruited for this study consisted of both beef and dairy animals. The study utilized three primary herds at Lakeland College, in Vermilion, Alberta. A single dairy herd, under the operation of the Lakeland College Student Managed Farms was included, with a total herd size of 168 Holstein cows. Beef herds utilized in the study included the Lakeland

College Student Managed Farm's purebred black Angus herd and the Simmental-Angus commercial herd of 50 and 97 cows, respectively. In addition, inclusion of one producer commercial beef herd in 2022, as well as five producer commercial beef herds in 2023, was voluntary and contributed to an increased portion of the sample size during the two-year study. The inclusion of these producer herds was primarily used to determine the severity and prevalence of mineral and vitamin deficiencies between-farms in Alberta.

The study took place during two consecutive calving seasons: 2022 and 2023. A total of 196 beef calves and 191 beef cows were on trial with 68 calves and 67 cows sampled in year 1 and 128 calves and 124 cows sampled in year 2. A total of 106 dairy calves and 103 dairy cows were in the study, with 53 calves and 51 cows sampled in year 1, and 53 calves and 52 cows sampled in year 2. Additionally, 50 producer calves and 5 cows were included. A subsample of these animals was submitted for blood nutrient profiling (n = 465). The number of samples analyzed for vitamin and mineral testing was ultimately determined by cost/budget.

2.2.2 Herd management

The animals in the main study herds were managed by Lakeland College staff and the student members of the Student Managed Farms (SMF) team. Beef cows were provided free-choice trace mineral supplements for only a portion of the year - May to November. Prior to, and following calving, beef cows received a TMR with ground hay, corn silage, barley silage, barley grain, barley straw, and a 36:20 mineral supplement. The dairy herd received a TMR throughout the entire year.

Producer herds were under variable management. Producers provided free-choice trace mineral supplements to 50% (3/6) of the herds. The other 50% (3/6) of the producers utilized a TMR in their feeding program. Injectable vitamin A/D was given to 16% (1/6) of the herds at least once throughout the winter before calving.

2.2.3 Blood sampling

Samples were collected between January 2022 and June 2023. Beef herd sampling occurred throughout their respective calving seasons between January and March each year. Precisely, the calving season for beef cows was January 27th to March 24th, 2022, and January 9th to March 30th, 2023. Dairy samples were collected throughout the entirety of the year, whenever calves were born, starting on February 8, 2022, and ending on May 19, 2023.

Blood was collected on calves by jugular venipuncture using a 20-gauge, 1-inch hypodermic needle and a 12 mL syringe. Blood was then transferred into 10 mL draw Vacutainer® tubes: 9-10 mL of blood into a red top blood tube without anticoagulant and 2-3 mL of blood into a purple top blood tube with ethylenediaminetetraacetic acid (EDTA). Blood collection was completed within 72 hours of calving. Calves born to cows that experienced dystocia, pluriparous cows (twins), and cows that were aggressive were excluded from the study.

Blood was collected from cows by coccygeal venipuncture using a 20-gauge, 1-inch hypodermic needle, into a 10 mL draw Vacutainer® tube, after parturition, at the same time as initial blood collection was completed on their neonatal calf. Private veterinarians in central Alberta trained research students and staff to perform jugular venipuncture and coccygeal venipuncture, for the purpose of this study.

Following blood collection on farm, blood was allowed to clot and then centrifuged (80-2 Laboratory Desktop Low Speed Centrifuge) at 2,500 rpm for 7 minutes at approximately 15°C. The serum and plasma were separated into labelled 1 mL microcentrifuge tubes. All serum/plasma samples were immediately placed into a -20°C freezer and transported on ice to Chinook Contract Research, Airdrie, Alberta, at the earliest convenience. Samples were then stored at -20°C until sent for analysis.

2.2.4 Plasma vitamin A and E analysis

Vitamin analysis was conducted by a commercial laboratory (Prairie Diagnostic Services Inc, Saskatoon, SK). Vitamin A (retinol) and E (α-tocopherol) concentrations were

determined in plasma by High Performance Liquid Chromatography (HPLC), using fluorescence detection at 325 nm or 285 nm, for vitamins A and E, respectively (Catignani and Bieri, 1983; Nierenberg and Lester, 1985). Although extraction and analysis of individual vitamins were conducted separately, the procedure was identical. Samples and standards were protected from light at all times. Results for vitamins A and E were reported as ug mL^{-1} (ppm).

2.2.5 Serum trace mineral analysis

Trace mineral analysis was conducted by two commercial laboratories: Quality Analytical Services, Okotoks, AB and Prairie Diagnostic Services Inc, Saskatoon, SK in year one and two, respectively. Elemental iron (Laur et al., 2020) and selenium (Forrer et al., 1999) concentrations were determined in blood serum by Inductively Coupled Plasma Mass Spectrometry (ICP-MS) following microwave digestion (Wilschefski and Baxter, 2019). Results for selenium (Se) and iron (Fe) were reported as ug mL^{-1} (ppm).

2.2.6 Reference concentrations for vitamin and trace mineral analysis

The reference concentrations for vitamins and trace minerals were from Puls (1994a and 1994b). For newborn calves, 0.18 µg mL^{-1} to 0.23 µg mL^{-1} is considered the adequate concentration for vitamin A in blood serum, while for cows, it is only slightly higher at 0.3 µg mL^{-1} to 0.7 µg mL^{-1} (Puls, 1994a).

For vitamin E in young calves, 0.8 µg mL^{-1} to 1.2 µg mL^{-1} is the appropriate reference range (Puls, 1994a). The expected concentrations in adults are higher for vitamin E, where 3.0 µg mL^{-1} is considered the minimum adequate concentration in blood serum, up to 10.0 µg mL^{-1} (Puls, 1994a). These reference concentrations are comparable to the reference concentrations, for vitamins A and E, that were used by Waldner and Uehlinger (2017).

The expected serum selenium concentrations for calves are said to range between 0.13 µg mL^{-1} to 0.16 µg mL^{-1} (Puls, 1994b). For adult cattle, the reference range for selenium is much broader, between 0.08 µg mL^{-1} to 0.30 µg mL^{-1} (Puls, 1994b). These

values are similar to suggested values from Dargatz and Ross (1996), Leslie et al. (2019), and Van De Weyer et al. (2010).

The rate of adequate iron in the blood serum of cattle, regardless of age, is 1.3 µg mL^{-1} to 2.5 µg mL^{-1} (Puls, 1994b); although the iron requirements of ruminants are not well established, and most recommendations are an estimate. For example, many sources have utilized serum ferritin and total iron-binding capacity (TBIC) values instead (Heidarpour Bami et al., 2008).

Table 2.1. Reference ranges for expected concentrations of vitamins and trace minerals in blood serum and blood plasma of cattle

Animal	Micronutrient	**Lowest concentration considered adequate (ug mL^{-1})**	**Highest concentration considered adequate (ug mL^{-1})**
Calves	Vitamin A	0.18	0.23
	Vitamin E	0.8	1.2
	Iron	1.3	2.5
	Selenium	0.13	0.16
Cows	Vitamin A	0.3	0.7
	Vitamin E	3	10
	Iron	1.3	2.5
	Selenium	0.08	0.3

Source: Puls, 1994a and Puls, 1994b

2.2.7 Data comparisons and statistical analysis

Data were entered and analyzed in Excel (Microsoft, Microsoft Office Windows 365). Measures of central tendency and spread (mean, standard deviation, minimum, and maximum) were calculated separately for vitamin and mineral concentrations of cows and calves. Individual serum vitamin A, vitamin E, selenium, and iron concentrations were classified as deficient, adequate, or more than adequate based on the reference range criteria, and the proportion of animals considered deficient in each herd was summarized. Percentiles (k=0.05, 0.25, 0.5, 0.75, 0.9) were determined to describe the distribution of plasma/serum micronutrient concentrations in cows and calves. The Pearson correlation

coefficient between cow and calf serum/plasma nutrient concentrations were calculated using Excel.

2.3 Results

Of the 302 calves (196 beef, 106 dairy) and 294 cows (191 beef, 103 dairy) for which blood samples were collected, a total of 465 samples (277 calves and 188 cows) were analyzed for blood nutrient concentrations. The laboratory reported vitamin A and E concentrations for 195 animals (75 cows and 120 calves) and iron and selenium concentrations for 270 study animals (113 cows and 157 calves). This included animals from eight herds that were under variable management regimes.

Nutrient deficiencies were common for animals in this study (Table 2.2), with greater severity observed in calves compared to cows. Numerous calves and several cows from Alberta herds were classified as deficient based on the reference ranges used (Table 2.1).

Table 2.2. Proportion of cows and calves with lower-than-adequate vitamin A, vitamin E, iron, and selenium concentrations and the distribution of the plasma/serum micronutrient concentrations

Animal	Micronutrient	Lowest concentration considered adequate	No. of animals (%) below adequate concentration	Percentiles of plasma/serum micronutrient concentration				
				5th	25th	50th	75th	95th
Calves	Vitamin A (ug mL^{-1})	0.18	85/120 (71)	0.11	0.13	0.16	0.21	0.25
	Vitamin E (ug mL^{-1})	0.8	12/120 (10)	0.68	1.03	1.58	2.02	2.93
	Iron (ug mL^{-1})	1.3	50/157 (32)	0.56	1.16	1.6	2.39	3.42
	Selenium (ug mL^{-1})	0.13	148/157 (94)	0.04	0.06	0.08	0.1	0.12
Cows	Vitamin A (ug mL^{-1})	0.3	24/75 (32)	0.19	0.26	0.33	0.4	0.46
	Vitamin E (ug mL^{-1})	3	8/75 (11)	2.22	3.35	4.23	5.06	5.73
	Iron (ug mL^{-1})	1.3	14/113 (12)	1.05	1.69	2.52	3.7	5.61
	Selenium (ug mL^{-1})	0.08	23/113 (20)	0.06	0.09	0.1	0.13	0.16

The relationship between blood nutrient concentrations in calves and their dams, after calving, were often negligible, with few correlations of particular interest (Table 2.3). The correlation between vitamin A levels in beef calves and their dams was fairly strong

(r > 0.6). No other notable relationship exists between post-calving dam and calf nutrient levels as the remaining correlations are close to zero and therefore considered weak or negligible.

Table 2.3. Correlations between dam and neonatal calf blood micronutrient levels at parturition

	Micronutrient	Year 1 r	Year 2 r
Beef	Vitamin A	0.623	0.619
	Vitamin E	-0.026	-0.477
	Iron	-0.418	-0.112
	Selenium	-0.128	-0.258
Dairy			
	Vitamin A	-0.010	-0.139
	Vitamin E	-0.352	-0.310
	Iron	0.594	0.169
	Selenium	-0.046	-0.128

Limited intra- and inter- herd variability was observed for concentrations of vitamin A, vitamin E, and selenium in cows and calves under variable management. Average calf vitamin A concentrations for each herd were marginal to low in all study animals, with only 40% of calves displaying adequate values. All (100%) study calves were deficient in selenium. Contrarily, calf vitamin E concentrations were adequate (40%) to more than adequate (60%). Lastly, iron concentrations were inconsistent as some herds were found to be deficient (20%), some adequate (40%), and others more than adequate (40%). Within a given herd, the same trend was found. Therefore, iron concentrations were variable within- and between-herds in this study (Table 2.4).

The producer who administered injectable vitamin A/D to cows prior to calving, had higher vitamin A levels in calves at birth (0.2022 ug mL^{-1}), compared to producers who did not administer a vitamin supplement to cows prior to calving ($\leq$ 0.1729 ug mL^{-1}) (P = .0132). In addition, producers who did not administer an injection of fat-soluble vitamins had neonatal calves that showed, on average, vitamin A concentrations below the recommended adequate concentration (0.18 – 0.23 ug mL^{-1}).

Table 2.4. Minimum and maximum values for vitamin A, E, iron, and selenium concentrations of study herds under various management strategies

			Micronutrient							
			Vitamin A		**Vitamin E**		**Iron**		**Selenium**	
Animal	**Herd ID/year**	**Management**	Min.	Max.	Min.	Max.	Min.	Max.	Min.	Max.
Calves	Dairy 1	TMR	0.11	0.27	0.61	4.32	0.24	7.95	0.03	0.15
	Dairy 2	TMR	0.15	0.35	0.98	3.10	0.74	2.21	0.05	0.13
	Beef 1	TMR	0.10	0.19	1.28	3.25	0.46	2.77	0.03	0.08
	Beef 2	TMR	0.10	0.38	0.83	7.54	0.38	3.40	0.04	0.11
	Prod. 1	Free choice	0.12	0.14	0.39	1.72	0.96	2.85	0.02	0.31
	Prod. A2	Free choice	0.11	0.23	1.10	2.00	1.62	5.01	0.08	0.21
	Prod. B2	Free choice	0.12	0.25	0.82	1.32	1.77	10.4	0.09	0.15
	Prod. C2	TMR with A/D inj.	0.14	0.30	0.63	1.41	0.89	2.98	0.07	0.12
	Prod. D2	TMR	0.10	0.24	0.69	3.72	1.21	5.41	0.08	0.16
	Prod. E2	TMR	0.12	0.22	0.60	1.88	1.38	6.06	0.08	0.15
Cows	Dairy 1	TMR	0.12	0.40	2.07	7.78	0.97	8.75	0.04	0.27
	Dairy 2	TMR	0.20	0.33	2.09	7.48	1.91	4.73	0.10	0.15
	Beef 1	TMR	0.18	0.39	3.00	5.65	1.05	5.16	0.06	0.15
	Beef 2	TMR	0.18	0.51	1.81	5.81	0.86	13.7	0.06	0.22
	Prod. B2	Free choice	0.32	0.52	2.26	4.46	2.27	18.0	0.09	0.17

2.4 Discussion

The objective of this investigation was to evaluate the prevalence and severity of nutrient deficiencies in a number of beef and dairy herds in Alberta, and to determine intra- and inter-herd variability. For several years, below-adequate blood serum micronutrient concentrations have been reported in beef cattle throughout North America (Campbell et al. 1995; Dargatz and Ross 1996; Dargatz et al. 1999; Hoff et al. 2001). This study showed that nutrient deficiencies were prevalent in both beef and dairy herds; however, the severity of the deficiency differed for each nutrient in question. Nutrient deficiencies were observed in cows for vitamin A (32%), vitamin E (11%), selenium (12%), and iron (20%). Neonatal nutrient deficiencies were common for vitamin A (71%), vitamin E (10%), selenium (94%), and iron (32%). Another study examining liver biopsies and blood samples from young calves in western Canada found that micronutrient deficiencies were common for vitamin E (56%), vitamin A (84%), copper (83%), and selenium (20%) (Waldner and Kennedy, 2008). Contrarily, calves in this study were most deficient in selenium and least deficient in vitamin E, yet the percentage of calves deficient in vitamin

A was similar between these studies. The prevalence of inadequate nutrient levels in cattle warrants future evaluation of the effectiveness of current management practices.

Assessment of trace element status is crucial to identify whether current mineral supplementation of livestock is adequate; and whether improved productivity is likely to occur with changes in supplementation (Kincaid, 1999). Based on the prevalence of nutrient deficiencies observed, subsequent research should complete an in-depth analysis to determine the downfalls of current management strategies and search for alternative options that could be implemented to effectively supplement vitamins and minerals. Free-choice supplementation does not ensure adequate nutritional status in cows or their offspring (Waldner et al., 2010; Waldner et al., 2023). While it has previously been thought that feeding a TMR is superior to free-choice mineral programs, as consumption is more uniform and nutritionally complete (Schingoethe, 2017), limited difference was observed in blood nutrient levels of cattle fed a TMR versus free-choice. There was minimal variability of vitamin A and selenium concentrations in both cows and calves from different herds and given that the acceptable range of vitamin E concentrations for cows is broad (3 – 10 ug mL^{-1}), there was also minimal inter-herd variability. Similarly, the variability of vitamin E concentrations for calves in all herds was minimal, with the exception of a couple outliers. On the other hand, iron concentrations seemed to fluctuate considerably within a given herd. For example, the minimum (0.861 ug mL^{-1}) and the maximum (13.71 ug mL^{-1}) in one study herd were substantially different. The same trend was found amongst all herds in regard to iron concentrations, thus iron concentrations were variable both within- and between herds.

Ruminants are sometimes exposed to high iron intakes through ingestion of water, soil or feedstuffs that are high in iron (Spears, 2003) which could explain the variability. Due to the influence that the green colour of forage has on vitamin A (and vitamin E) content (Frye et al., 1991; Maas et al., 2008; Van De Weyer et al., 2010), it would have been logical to find greater variation between herd vitamin levels as well. Finally, considering the close proximity of study herds, and that the area is generally considered selenium deficient (Khanal and Knight, 2010; Mehdi and Dufrasne, 2016), there is little cause for fluctuation in baseline serum selenium concentrations of animals in this study. Nevertheless, detailed information regarding feed and water type/quality were not

collected in this study. Thorough information on supplementation history, feed, and management are important for optimal interpretation of results. Kegley et al. (2016) also proposed that determining the micronutrient status of a herd requires a greater understanding of the bioavailability of nutrients from forages, feedstuffs, and different supplemental sources, the impact of dietary antagonists, along with requirements for different genetic and environmental conditions. In addition, the evaluation of blood concentrations provide a less than ideal metric of an animal's micronutrient status when compared to liver biopsies; however, when screening large numbers of animals for deficiencies, serum/plasma samples can provide a practical surveillance option (Waldner and Van De Weyer, 2011).

Maternal nutritional status and the metabolic environment is critical in determining viability of off-spring, long-term health, and reproduction (Symonds et al., 2010; Van Emon et al., 2020). Previous studies have assessed the relationship between cow nutrient status prior to calving and subsequent calf nutrient status (Prom et al., 2022; Reece et al., 1985). Previous studies have found that the selenium status of the cow before calving is an important determinant in the selenium status of calves (Campbell et al., 1990; Koller et al., 1984). To our knowledge, this is the first study to assess the blood nutrient profiles of calves and their dam immediately following calving. Unfortunately, to adequately assess the vitamin and mineral status of a cow herd, the periparturient period may not be the preferred time because of the inevitable physiological changes that occur. For example, it has been previously found that vitamin A and E plasma concentrations decrease in cows around calving (Goff and Horst, 1997). Low blood vitamin A and E levels in the cow after calving may be indicative of post-partum nutrient transfer through colostrum and/or low cow vitamin levels may partially reflect feeding and management strategies, which may be compounded further by reduced body vitamin A stores resulting from winter feeding and poor-quality forage (Waldner and Uehlinger, 2017). Therefore, it is impossible to determine the reason that cows in this study were most deficient in vitamin A after parturition. Likewise, physiological factors like pregnancy and inflammation affect plasma iron, total iron binding capacity (TIBC), and ferritin concentrations (Miyata et al., 1984; Joerling and Doll, 2019). Plasma iron concentrations initially increase and rapidly decrease in the acute phase of inflammation after calving (Graham, 1991). Therefore, it

may be more suitable to evaluate colostral nutrient levels post-calving. For instance, Weiss at al. (1990) found that the vitamin E (α-tocopherol) content of colostrum is usually low unless the cow is provided supplemental dietary vitamin E. While we were unable to directly compare the impact that cow nutritional status during late gestation has on the micronutrient concentrations of progeny, it was found that there were no strong correlations between dam and calf blood nutrient concentrations after parturition. This is an area that needs to be researched in more detail to establish future good management practices.

Finally, it was observed that administrating an injection of fat-soluble vitamins to cows in this study prior to calving, improved plasma vitamin A concentrations in newborn calves. Increased calf serum levels were found in a similar study evaluating the effects of prepartum vitamin A supplementation (Prom et al., 2022). Injectable vitamins and minerals have been shown to be readily available to cattle and result in benefits like improved immune system function and improved overall health (Cipriano et al., 1982; Kegley et al., 2016). Although to ensure the newborn receives adequate nutriment early in life and considering the severity of nutrient deficiencies still found in neonatal calves, vitamins and minerals should be provided directly to the calf. Some producers routinely administer vitamin A, D, E, and selenium injections in young calves, as well as cows, as a proactive preventative measure and a common treatment of nutrient deficiencies (Nelson et al., 2016; Waldner and Uehlinger, 2017); however, there are recent animal welfare concerns associated with administering injections (Nielsen et al., 2023). New research supports oral supplementation to dairy calves as being more sufficient than a parenteral administration of selenium (Joerling and Doll, 2019). Still, additional work is required in neonatal nutrition to determine specific minerals and vitamins of utmost importance and refine the optimal method of administration.

This research provides additional data regarding the prevalence and severity of nutrient deficiencies in Alberta cattle herds. It was found that neonatal calves can have marginal to low vitamin A and iron concentrations, low to deficient selenium concentrations, yet adequate to high vitamin E concentrations under variable management systems. However, high plasma vitamin E concentrations may counteract low serum selenium concentrations (Graham 1991). Our results show that plasma vitamin

E concentrations are greater than 0.8 – 1.2 ug ml^{-1} on average, while average selenium concentrations in newborn calves are below the expected range of 0.13 and 0.16 ug ml^{-1}. Complex nutrient interactions, environmental influences and a changing physiological status may not be fully accounted for by published nutrient requirements (Herdt et al., 2000; NRC, 1996; Radostits and Bell, 1970). No clear relationship was found between dam and calf blood nutrient profiles following calving; however, it was observed that an injection of vitamin A/D in cows during late gestation led to increased serum vitamin A levels in their offspring. Finally, minimal intra- and inter- herd variability was observed for all nutrients analyzed, except iron, in both beef and dairy cows and calves.

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CHAPTER THREE: THE EFFECT OF ORAL VITAMIN AND MINERAL SUPPLEMENTATION ON NEONATAL BEEF AND DAIRY CALVES

3.1 Introduction

Mineral and vitamin deficiencies are a pervasive challenge for livestock producers in western Canada and have economically important effects (Perdrizet et al., 2020). Insufficient nutrients can result in immunosuppression and reduced growth rates, yielding disease or disorders and lessening the performance of young calves (Carroll and Forsberg, 2007). Extensive rangeland pasture systems may benefit from nutritional intervention to optimize tissue reserves of newborn calves (Palomares, 2022). Thus, neonate nutritional supplementation could mitigate mineral and vitamin deficiencies in livestock and address production challenges of current and future production systems. The National Beef Strategy 2020-2024 Productivity Pillar identifies a goal to reduce average calf death losses to 5% in each region, and a research and development priority was established on improved prevention of animal disease and welfare issues through the identification, development, and promotion of cost-effective treatment and management strategies that can be widely adopted to improve performance outcomes (Beef Cattle Research Council, 2019). Early nutritional intervention to develop healthier and more robust young animals may provide a significant return on investment regarding herd health and industry sustainability (VandeHaar and St-Pierre, 2006). In addition, it may support animal welfare (Lorenz, 2021; Murray et al., 2016), antimicrobial stewardship (AMR) (Waldner et al., 2019), and producer profitability (Mulligan et al., 2006).

The importance of optimal mineral and vitamin nutrition on improving immune function and health has been recognized in the research literature in preceding decades (Carroll and Forsberg, 2007). However, limited research is currently available regarding oral vitamin-mineral supplementation of newborns. Rather, routine mineral supplementation of cows has been the primary focus, assuming calves will receive adequate quantities of nutrients from dams in utero and through consumption of colostrum and milk (Leslie et al., 2019). Supplementation during gestation is thought to provide the most direct benefit (Quigley and Drewry, 1998). Minerals and vitamins are

often delivered free-choice in a loose or block form to cows, but intake of these supplements is often not monitored and can be highly variable (McCarthy et al., 2021). Despite the assumption that calves receive adequate nutrients from bovine colostrum, it is well known that colostrum supply is quite variable and that the quality, quantity, and timing of colostrum feeding are all major factors that may contribute to calf performance in later life (Hammon et al., 2020; Puppel et al., 2019). In fact, good colostrum management is considered the single most important factor in preventing calf morbidity and mortality (Galyean et al., 1999; Godden et al., 2019; Playford and Weiser, 2021); however, limited data suggests that inadequate transfer occurs in approximately 33% of newborn beef calves in western Canada (Gamsjäger et al., 2020) and up to 40% of dairy calves (Waldner and Rosengren, 2009). Consequently, there is increased susceptibility to calfhood diseases due to naïve immune responses, which merits emphasis on disease prevention, limiting the economic impacts and the need for subsequent intervention (Lorenz et al., 2011). Beef and dairy calf management is considerably different, as beef calves generally remain with the cow post-calving and nurse ad libitum, while dairy producers often separate calves from their dams and then provide the colostrum. In addition, it is well established that cow's milk is often not a good source of iron for neonatal calves (Mohri et al., 2006; Atyabi et al., 2006) and it may not fully meet the nutritional requirements of young, pre-ruminating animals. As a result, many calves are often reliant on any stores they may have in their liver until they begin to ingest forage and supplements as they grow (Van Emon et al., 2020). Calves are at greater risk of nutrient deficiencies when they are exclusively fed a whole milk diet for the first few weeks of their life (Joerling and Doll, 2019; NRC, 1996).

By determining the severity of nutrient deficiencies in neonatal calves and assessing how young calves respond to nutritional intervention early in life, we can increase our knowledge and improve our management of livestock in western Canada. Furthermore, BMPs may be updated to improve future calf production efficiencies. Management strategies that can be easily implemented may be especially valuable as they are often more readily adopted by producers (Liu et al., 2018). Therefore, the goal of this research was to investigate the efficacy of an oral vitamin and mineral supplement targeted at newborn calves. Unlike traditional injectable options (subcutaneous or

intramuscular), oral supplements could be advantageous as they do not leave lesions or injection-site damage (Galyean et al., 2022), minimize pain and stress in animals, and are easy to administer for unskilled users. The primary objective of this study was to determine plasma vitamin and serum mineral concentrations in neonatal beef and dairy calves in central Alberta. Specifically, our goal was to determine the blood plasma/serum concentrations of vitamin A, vitamin E, iron, and selenium before and after administration of an oral nutrient supplement, *VitaFerst-Care.* The second objective was to investigate the relationship of blood serum vitamin and mineral concentrations with morbidity occurrence and mortality in neonatal calves in the study. Our hypothesis was that orally supplemented calves would show increased nutrient blood plasma/serum concentrations compared to calves that receive the oral saline control. Moreover, if effective, supplemented calves would be expected to have increased production parameters, such as weight per day of age, and be more immunocompetent and resistant to disease, as measured through mortality and morbidity percentages.

3.2 Materials and Methods

All protocols were consistent with the Canadian Council on Animal Care guidelines and were approved by the Lakeland College (Neonatal-12-21) and Thompson Rivers University (AUP#103169) Animal Care Committees.

3.2.1 Study animals

This study included both beef and dairy animals. The study utilized three primary herds at Lakeland College in Vermilion, Alberta. Beef herds utilized in the study included the Lakeland College Student Managed Farms purebred black Angus herd and the Simmental-Angus commercial herd of 50 and 97 cows, respectively. A single dairy herd, under the operation of the Lakeland College Student Managed Farms was included, with a total herd size of 168 Holstein cows. In year 1, only cows that had a heifer calf were used in the study. Male dairy calves were excluded because they were sold before one week of age and unavailable for a second sample collection. In year 2, bull calves were

included in the study as the second sampling date for calves was reduced from fourteen days to three days. Bull calves were on site for that duration.

The study took place during two consecutive calving seasons: 2022 and 2023. A total of 196 beef calves were on trial with 68 calves sampled in year 1 and 128 calves sampled in year 2. A total of 106 dairy calves were used in the study, with 53 calves sampled each year. A subsample of these animals was submitted for blood nutrient profiling (n = 157).

3.2.2 Herd management and animal selection

The animals in the main study herds were managed by Lakeland College staff and the student members of the Student Managed Farms (SMF) team. Study animals were enrolled based on a selection criterion: unassisted calving, single calf, and docile cow. Calves born to cows that experienced dystocia, pluriparous cows (twins), and cows that were aggressive were excluded from the study. Researchers ensured, through observation, that each calf enrolled in the study had suckled colostrum prior to initial blood sampling and treatment administration. The number of blood samples collected per herd was primarily determined by the number of accessible animals and limited by the ability to collect a complete sample set.

3.2.3 Housing

The beef herd was housed outside in a corral until the time of calving. Upon calving, cow-calf pairs were brought into the calving barn, allowing the calf to warm up and dry off. The heated barn had 10 pens (3.66 x 4.87 m) that could each accommodate a single cow-calf pair. The time each pair remained in the barn was dependent on calf vigour, the outside temperature, and the demand for pen space due to the number of new calves needing to be brought into the barn. Thereafter, the cow-calf pairs were transferred to a small pen (12.75 x 17.15 m) outside where farm staff and management could continue to monitor them closely. After 3-5 days here, the cows and calves were moved to a larger pen (27.97 x 53.19 m) with other cows that previously calved.

Dry dairy cows were moved to a pen (10.24 x 16.59 m) in the dairy barn, two to three weeks prior to calving. Following calving, the calf was removed from its dam, dried off, and bottle-fed 3L of colostrum within 1 hour of birth. The colostrum from their dam was only administered if it is high enough quality (Brix > 22%), otherwise frozen donor colostrum was provided. Calves were then individually housed in a calf hutch (Agri-Plastics Flex Pen, 1.17 x 1.85 m) for 4 to 7 days. Bull calves were sold within a 4-to 7-day period. In the hutch, calves received 3L of cow's milk twice a day for four days. Afterwards, calves are offered starter ration in addition to milk replacer (3L milk replacer and ad lib access to a calf starter ration: NSI XP 19% Calf Starter Complete M56, Nutri-Source). After one week, the heifer calves were transferred to a calf room (5.23 x 6.07 m) with 8-10 other calves of similar size and age. They remained in that group until weaning time.

3.2.4 Treatment and Randomization

Calves were randomly assigned to receive oral supplementation with either 10 mL of oral nutrient supplement *VitaFerst-Care* (ferrous fumarate 300mg/mL, vitamin A acetate 50mg/mL, vitamin E acetate 30mg/mL, sodium selenite 0.5mg/mL, vitamin B12 0.5mg/mL, and vitamin D3 0.08mg/mL) or 10 mL of an oral saline control solution. Treatment allocation was predetermined by a random computer generator.

It was not possible to blind the staff, students, and researchers to the calves' treatment due to statutory farm medicine records and the different physical appearance of the oral supplement compared to the saline solution. Personnel analyzing blood samples were blinded to treatments.

3.2.5 Calf data and health records

Detailed information was recorded for each calf at the time of enrollment into the study, including calf identification, date of birth, breed, birth weight, blood collection date and time, and treatment received. The date and time of the second blood collection were also

recorded at the appropriate time for each calf. Beef and dairy herd data record sheets consisted of the same information but were kept separately.

Records were kept for all occurrences of health problems and treatments administered up to five weeks of age. The health outcomes examined included calf death, calf treatment for any reason, treatment for diarrhea, and treatment for pneumonia. Treatments included, but were not restricted to, antibiotics and other veterinary drugs. Administration of electrolytes and antibiotic tablets for neonatal calf diarrhea were considered treatments. In the case of calf mortality, it was also recorded.

Weighing of beef calves was completed periodically throughout the first year of the study. Calf weights were determined using a portable calf weighing crate, *Ritchie* (345GE, Chippenham, Wiltshire, United Kingdom) with a Tru-Test system (EziWeigh7i, Mount Wellington, Auckland, New Zealand). Birth weights and weaning weights were recorded for beef calves in year one. The average daily gain (ADG) was calculated for the study period based on weight measurements obtained at the first and final calf visit. In the second year, only birth weight was obtained, and weight data was no longer analyzed.

3.2.6 Blood sampling

Samples were collected between January 2022 and June 2023. Beef herd sampling occurred throughout the respective calving seasons between January and March each year. Precisely, the calving season for beef cows was January 27th to March 24th, 2022, and January 9th to March 30th, 2023. Dairy samples were collected throughout the entirety of the year, whenever calves were born, starting on February 8, 2022, and ending on May 19, 2023.

Blood was collected on calves by jugular venipuncture using a 20-gauge, 1-inch hypodermic needle and a 12 mL syringe. Blood was then transferred into 10 mL draw Vacutainer® tubes: 9-10 mL of blood into a red top blood tube without anticoagulant and 2-3 mL of blood into a purple top blood tube with ethylenediaminetetraacetic acid (EDTA). Additionally, fresh blood from beef calves in year 2 was immediately analyzed with a hemoglobinometer (HemoCue) to assess hemoglobin levels (g/L) as another indicator of iron status. Blood collection was completed prior to supplementation at 1-3 days of age

and again following supplementation. The second blood collection was completed between 12 and 20 days in year one and between 2 and 5 days in year two. The calves were approximately 14 days old (16.43 +/- 2.16 d) and 3 days old (3.50 +/- 0.87 d) at the time of the second blood collection each year.

Following blood collection on-farm, blood was allowed to clot and then centrifuged (80-2 Laboratory Desktop Low Speed Centrifuge) at 2,500 rpm for 7 minutes at approximately 15°C. The serum and plasma were separated into labelled 1 mL microcentrifuge tubes.

All serum/plasma samples were immediately placed into a -20°C freezer and later transported on ice to Chinook Contract Research, Airdrie, Alberta, at the earliest convenience. Samples were then stored at -20°C until sent for analysis. All samples were analyzed during the winter and spring of 2023.

Only complete sample sets with calf baseline (d0) and calf post-treatment (d14/d3) were included in this investigation. A maximum of 22 beef samples and 36 dairy samples were analyzed in year one. In year two, a total of 40 beef samples and 10 dairy samples were analyzed. The number of samples analyzed for vitamin and mineral testing was ultimately determined by cost/budget and completeness.

3.2.7 Plasma vitamin A and E analysis

Vitamin analysis was conducted by a commercial laboratory (Prairie Diagnostic Services Inc, Saskatoon, SK). Vitamin A (retinol) and E (α-tocopherol) concentrations were determined in plasma by High Performance Liquid Chromatography (HPLC), using fluorescence detection at 325 nm or 285 nm for vitamins A and E, respectively (Catignani and Bieri, 1983; Nierenberg and Lester, 1985). Although extraction and analysis of individual vitamins were conducted separately, the procedure was identical. Samples and standards were protected from light at all times. Results for vitamins A and E were reported as ug mL^{-1} (ppm).

3.2.8 Serum trace mineral analysis

Trace mineral analysis was conducted by two commercial laboratories: Quality Analytical Services, Okotoks, AB and Prairie Diagnostic Services Inc, Saskatoon, SK in year one and two, respectively. Elemental iron (Laur et al., 2020) and selenium (Forrer et al., 1999) concentrations were determined in blood serum by Inductively Coupled Plasma Mass Spectrometry (ICP-MS) following microwave digestion (Wilschefski and Baxter, 2019). Results for selenium (Se) and iron (Fe) were reported as ug mL^{-1} (ppm).

3.2.9 Reference concentrations for vitamin and trace mineral analysis

The reference concentrations for vitamins and trace minerals were adapted from Puls (1994a and 1994b). For newborn calves, 0.18 µg mL^{-1} to 0.23 µg mL^{-1} is considered the adequate concentration for vitamin A in blood serum (Puls, 1994a). For vitamin E in young calves, 0.8 µg mL^{-1} to 1.2 µg mL^{-1} is suggested to be the appropriate reference range (Puls, 1994a; Waldner and Uehlinger, 2017).

The expected serum selenium concentrations for calves are said to range between 0.13 µg mL^{-1} to 0.16 µg mL^{-1} (Puls, 1994b). These values are similar to suggested values from Dargatz and Ross (1996), Leslie et al. (2019), and Van De Weyer et al. (2010). The rate of adequate iron in the blood serum of cattle is 1.3 µg mL^{-1} to 2.5 µg mL^{-1} (Puls, 1994b); although the iron requirements of ruminants are not well established, and most recommendations are an estimate. Many sources have utilized serum ferritin and total iron-binding capacity (TBIC) values instead (Heidarpour Bami et al., 2008).

Hemoglobin (Hb) values in fresh blood of veal calves are expected to be approximately 74 to 120 g L^{-1} (Panousis et al., 2018), however, a threshold of 90 g L^{-1} should be used to classify calves as low (Allan et al., 2020; Joerling and Doll, 2019).

3.2.10 Statistical analysis

Statistical analysis was completed with Prism GraphPad Software version 9.5.1. Data were analyzed using a two-way analysis of variance (ANOVA) to assess the significant systematic effects of time and treatment on calf blood nutrient levels. Average daily gain (ADG), morbidity, and mortality percentages were calculated using Excel. Pearson's

correlation coefficient was calculated to assess the degree of linear association between variables. Results were considered statistically significant if the *P* value was less than 0.05 ($P < 0.05$).

3.3 Results

A total of 302 calves were enrolled in this trial, with a maximum of 157 calves subjected to blood nutrient profiling. Calves were born between January 2022 and June 2023. Beef calves chosen for blood nutrient profiling were born in January and February each year, while dairy calves chosen were born January through April. At sampling, calves were between 1 to 20 d old: baseline blood sampling was done within 72 hours of birth (1-3 days of age), followed by a second sample collection completed at approximately 14 d (median = 16; interquartile range = 12-20) in year one and at 3 d (median = 3; interquartile range = 2-6) in year two.

3.3.1 Plasma vitamin A and E concentrations

There was an increase in blood plasma vitamin A concentrations over time (year 1: $P= 0.0006$; year 2: $P<0.0001$), regardless of experimental group (year 1: $P= 0.3523$; year 2: $P= 0.8798$) (Table 3.1 and 3.2). The mean concentration of vitamin A (0.141 ug mL^{-1}) in beef calves, at birth, were below the recommended minimum adequate concentrations for newborn calves; however, the mean concentrations of vitamin A (0.287 ug mL^{-1}) at d14 were above the recommended maximum adequate concentration (Figure 1a). In year two, the mean concentration of vitamin A in beef calves at birth (0.147 ug mL^{-1}) were below the recommended minimum adequate concentrations; yet, at d3 vitamin A concentrations (0.221 ug mL^{-1}) were at a level considered adequate for young calves (Figure 1b).

In dairy calves, the effect of treatment was not significant, however the effect of time was significant in the second year of the study ($P = 0.0134$) (Table 3.2). The mean concentrations of vitamin A (0.188 ug mL^{-1}) were considered adequate at birth, and at d14, the average vitamin A concentration (0.322 ug mL^{-1}) exceeded the recommended

maximum adequate concentration for calves (Figure 1c). The increase over time was not significant (*P*= 0.0922), nor was the effect of treatment (*P*= 0.8546) (Table 3.1) on vitamin A concentrations in year one of treatment and control (0.264 ug mL^{-1}; 0.246 ug mL^{-1}) groups, respectively. In the following year, the mean concentration of vitamin A (0.201 ug mL^{-1}) in dairy calves were also considered adequate immediately following calving and increased at d3 (0.273 ug mL^{-1}) to above the recommended maximum adequate concentration for calves (Figure 1d). The difference between means in the treatment (0.226 ug mL^{-1}) and control (0.248 ug mL^{-1}) groups was insignificant (*P*= 0.5652).

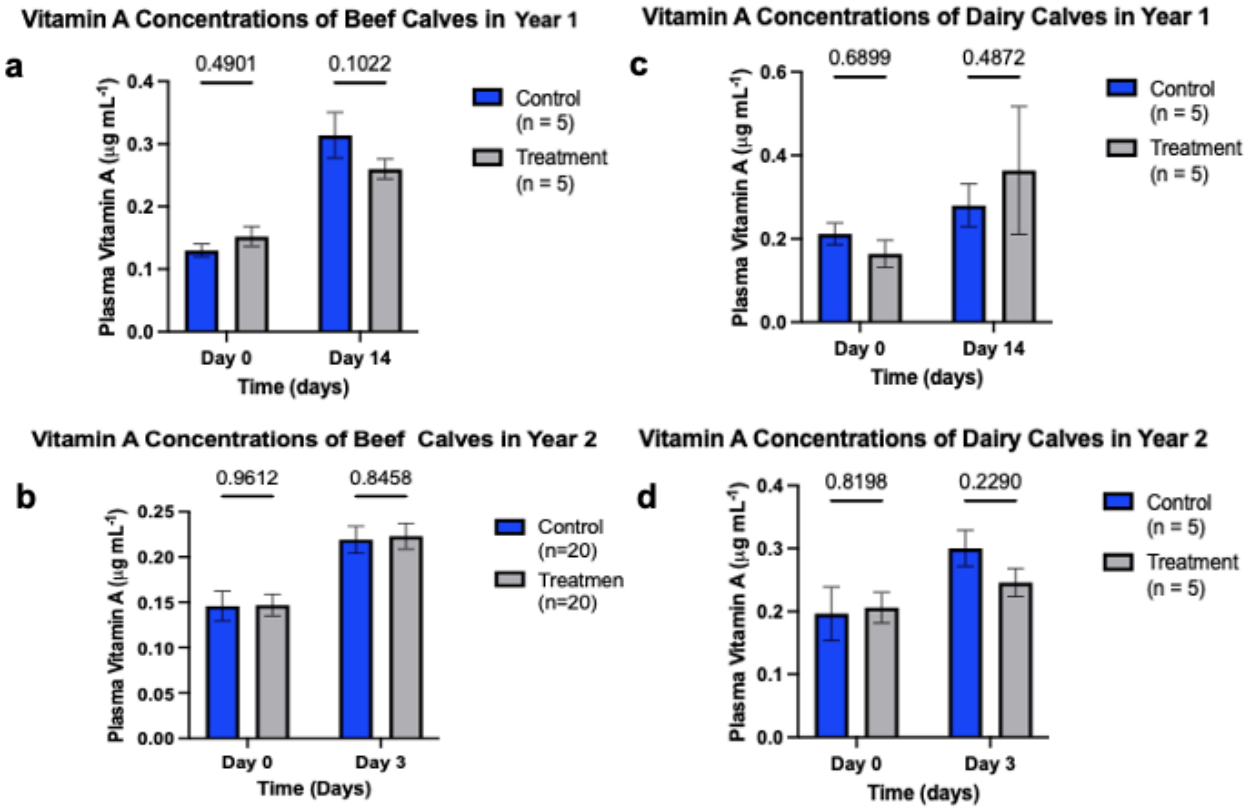

Figure 1. Plasma vitamin A blood nutrient concentrations in newborn calves before (day 0) and after (day 14, day 3) treatment. Average with standard error of the mean (SEM) are displayed in control and treatment groups for (a) beef calves in year 1 and (b) year 2 and (c) dairy calves in year 1 and (d) year 2.

Baseline plasma vitamin E concentrations in all beef and essentially all dairy calves were greater than the recommended maximum adequate concentration (1.2 ug mL^{-1}) for neonatal calves. Further, after a few days (3 to 14 days) the plasma vitamin E

concentrations increased considerably. Thus, the effect of time on mean concentrations of vitamin E is significant ($p < 0.05$) for (year 1: $P = 0.0029$; $P = 0.0001$ / year 2: $P<0.0001$; $P = 0.0392$) for beef and dairy calves in this study (Table 3.1 and 3.2). Treatment was not statistically significant for mean concentrations of vitamin E in control (6.124 ug mL^{-1}; 2.552 ug mL^{-1}) and treatment (8.408 ug mL^{-1}; 3.556 ug mL^{-1}) groups of dairy calves, in both years one (Figure 2c) and two (Figure 2d), Likewise, for beef calves the mean vitamin E concentration of the treatment group (year 1: 4.086 ug mL^{-1}; year 2: 4.080 ug mL^{-1}) is greater than the mean vitamin E concentration of the control group (year 1: 3.934 ug mL^{-1}; year 2: 2.785 ug mL^{-1}) for each year (Figure 2a and 2b). The effect of treatment on beef calf vitamin E concentrations trends toward significant ($P = 0.0515$) and the treatment x day interaction was significant in year two of this study. ($P = 0.0119$) (Table 3.2).

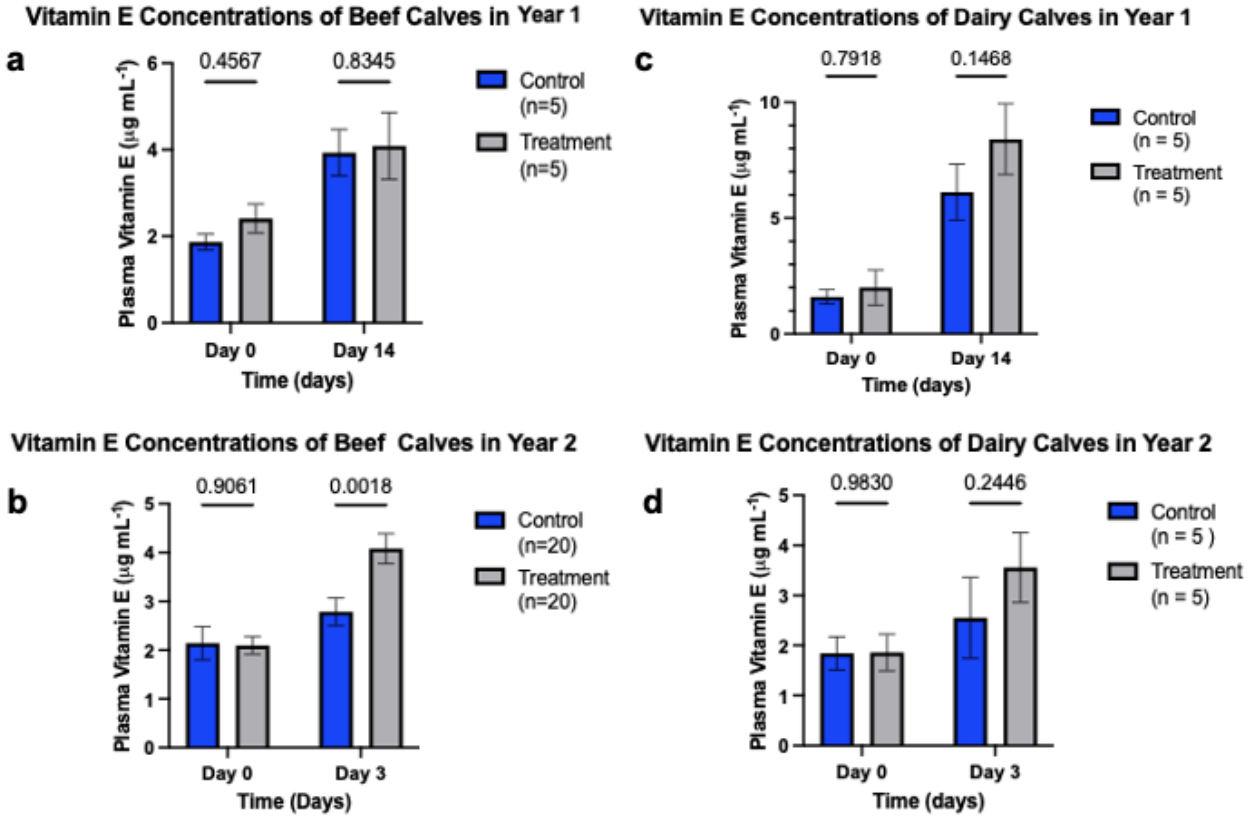

Figure 2. Plasma vitamin E blood nutrient concentrations in newborn calves before (day 0) and after (day 14, day 3) treatment. Average with standard error of the mean (SEM) are displayed in control and treatment groups for (a) beef calves in year 1 and (b) year 2 and (c) dairy calves in year 1 and (d) year 2.

3.3.2 Serum selenium concentrations

Selenium concentrations for all young calves in this study were below the recommended minimum adequate concentration (0.13 ug mL^{-1}); the average was 0.06 ug mL^{-1} in year one (Figure 3a and 3c) and 0.07 ug mL^{-1} in year two (Figure 3b and 3d). Calves in this study were considered deficient with no statistically significant effect displayed for treatment ($P > 0.05$). Time was significant for dairy calves in year one ($P < 0.0001$) and beef calves in year two ($P = 0.0122$) (Table 3.1 and 3.2).

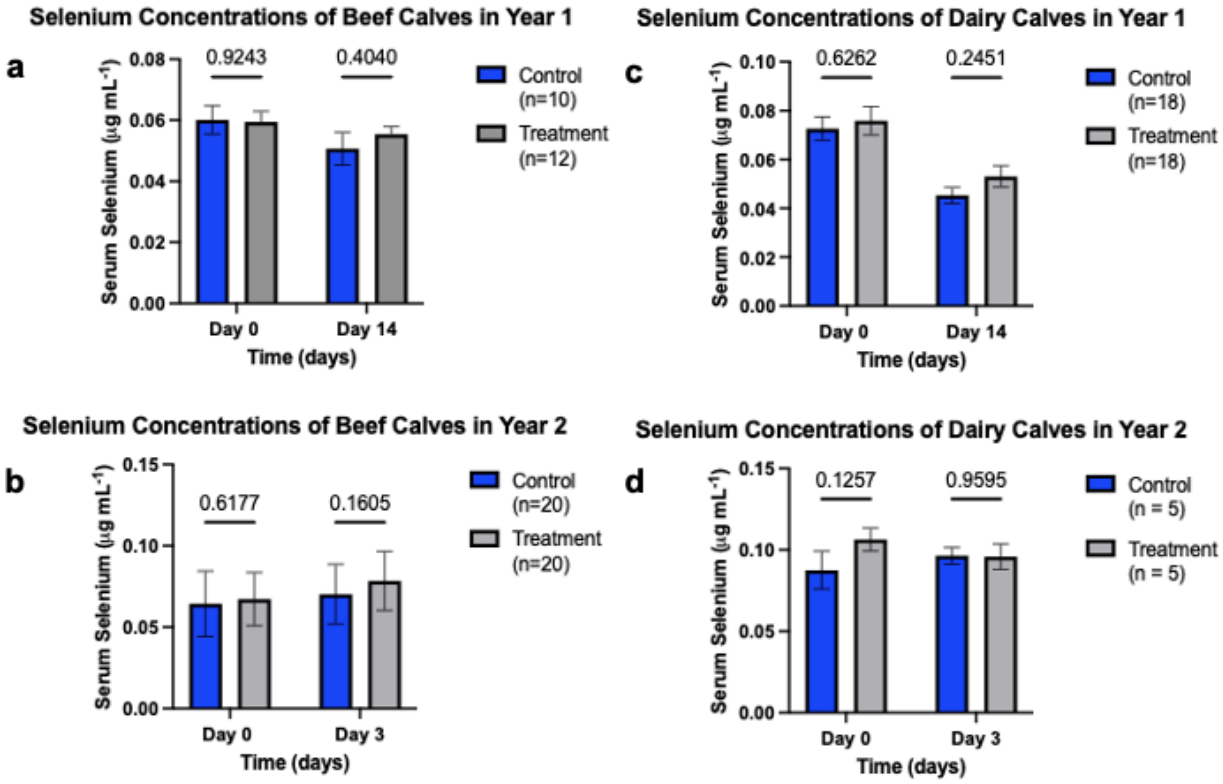

Figure 3. Serum selenium blood nutrient concentrations in newborn calves before (day 0) and after (day 14, day 3) treatment. Average with standard error of the mean (SEM) are displayed in control and treatment groups for (a) beef calves in year 1 and (b) year 2 and (c) dairy calves in year 1 and (d) year 2.

3.3.3 Serum iron and hemoglobin concentrations

The mean concentrations of iron in beef calves (year 1: 1.259 ug mL^{-1}; year 2: 1.815 ug mL^{-1}) at birth were at or above the recommended minimum adequate concentrations for young calves. The mean concentrations of iron (1.377 ug mL^{-1}; 2.226 ug mL^{-1}) at d14 and at d3 were within the recommended range of 1.3 to 2.5 ug mL^{-1} (Figure 4a and 4b). The effect of treatment in year two was statistically significant (*P*= 0.0018) (Table 3.2). Hemoglobin (Hb) was used as an additional measure of iron levels during year two of this study, where it was found that all beef calves shortly after birth averaged 98.24 g L^{-1} Hb and decreased to 94.57 g L^{-1} Hb 3 days later. Calves drenched with an oral vitamin-mineral supplement containing iron (ferrous fumarate) shortly after birth had increased levels of hemoglobin (mean = 99 g L^{-1}) compared to calves that were not provided with the oral nutrient supplement (mean = 90.8 g L^{-1}). Thus, the effect of iron supplementation on hemoglobin levels in treatment versus control calves was statistically significant (*P* = 0.0217).

The mean concentrations of iron in dairy calves at birth (1.581 ug mL^{-1}), and at d14 (2.181 ug mL^{-1}) were considered adequate (Figure 4c). Similarly, in year two (Figure 4d), the mean concentrations of iron were considered adequate at birth (1.393 ug mL^{-1}) and three days later (1.976 ug mL^{-1}). Time had a significant effect (*P*= 0.0015; *P*= 0.0154) each year, whereas treatment had no effect (*P*= 0.0761; *P*= 0.3779) (Table 3.1 and 3.2).

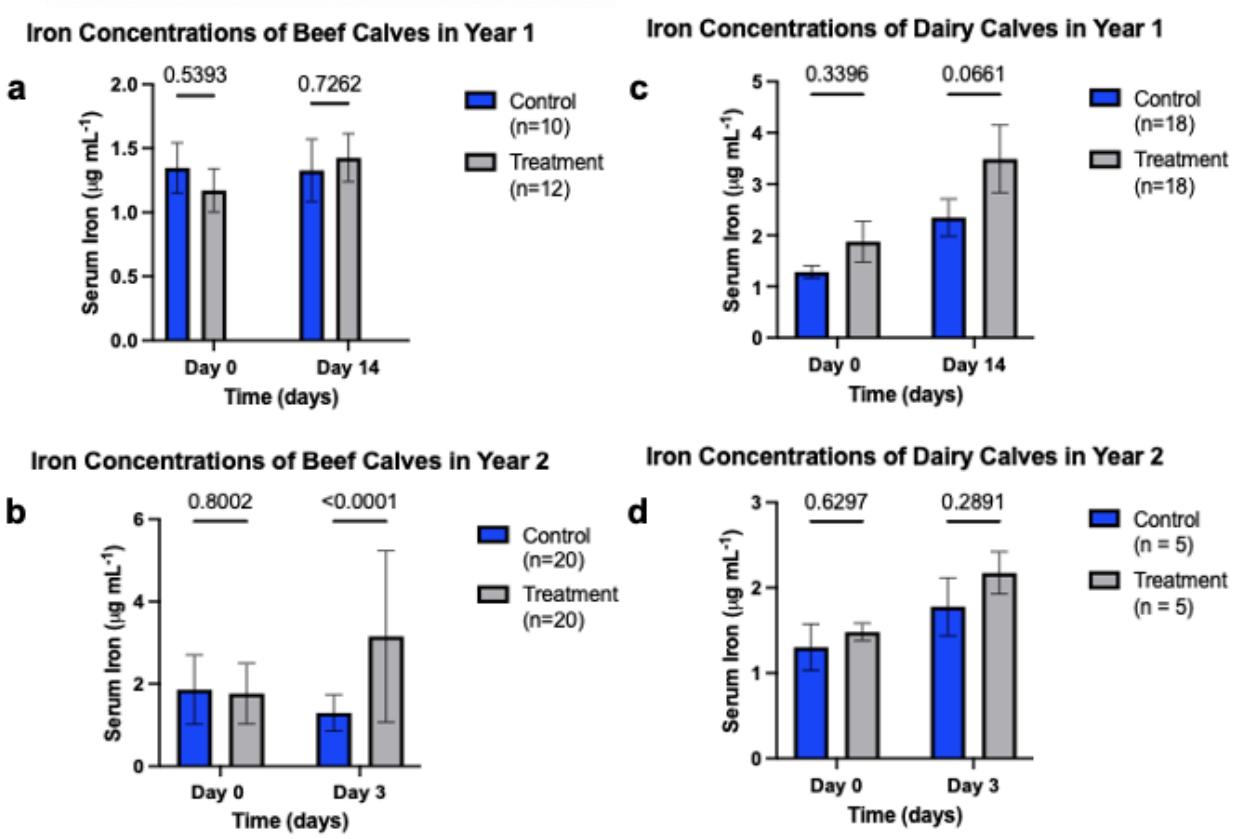

Figure 4. Serum iron blood nutrient concentrations in newborn calves before (day 0) and after (day 14, day 3) treatment. Average with standard error of the mean (SEM) are displayed in control and treatment groups for (a) beef calves in year 1 and (b) year 2 and (c) dairy calves in year 1 and (d) year 2.

Table 3.1. Effect of oral supplementation on calf blood nutrient concentrations in year one

			Treatment		P-values		
	Nutrient	Baseline	Control	*VitaFerst-Care*	Treatment	Days	Animal	Treatment*Days
Beef	Vitamin A	0.1410	0.2220	0.2060	0.3523	0.0006*	0.9088	0.1910
	Vitamin E	2.1390	2.9000	3.2490	0.5517	0.0029*	0.2595	0.6687
	Iron	1.2590	1.3370	1.2980	0.8460	0.5555	-	0.4948
	Selenium	0.0598	0.0554	0.0575	0.6377	0.0743	-	0.4719
Dairy	Vitamin A	0.1880	0.2460	0.2640	0.8546	0.0922	0.2028	0.3737
	Vitamin E	1.8030	3.8630	5.2060	0.3270	0.0001*	0.0827	0.2551
	Iron	1.5810	1.8170	2.6840	0.0761	0.0015*	0.1260	0.4799
	Selenium	0.0743	0.0590	0.0645	0.2726	<0.0001*	0.2603	0.6117

Table 3.2. Effect of oral supplementation on calf blood nutrient concentrations in year two

			Treatment		P-values		
	Nutrient	Baseline	Control	*VitaFerst-Care*	Treatment	Days	Animal	Treatment*Days
Beef	Vitamin A	0.1465	0.1825	0.1850	0.8798	<0.0001*	0.0381*	0.9034
	Vitamin E	2.1170	2.4630	3.0870	0.0515	<0.0001*	0.1114	0.0119*
	Iron	1.8150	1.5810	2.4610	0.0018*	0.1440	0.6105	0.0010*
	Selenium	0.0659	0.0674	0.0729	0.2535	0.0122*	0.0095*	0.4198
Dairy	Vitamin A	0.2010	0.2480	0.2260	0.5652	0.0134*	0.0998	0.1977
	Vitamin E	1.8530	2.1980	2.7090	0.4693	0.0392*	0.1910	0.3418
	Iron	1.3930	1.5410	1.8280	0.3779	0.0154*	0.0977	0.5810
	Selenium	0.0970	0.0920	0.1011	0.3898	0.8832	0.0803	0.1408

3.3.4 Morbidity, mortality, and weight gain

The average calf death loss for the 302 (196 beef, 106 dairy) calves in this study was 3.97% (12/302) for both the beef (4.59%, 9/196) and dairy (2.83%, 3/106) herds. This included a 7.35% (5/68) and 3.13% (4/128) mortality for beef calves and a 3.77% (2/53) and 1.89% (1/53) mortality for dairy calves in years one and two, respectively.

The majority of calves were healthy throughout the duration of this study, with an overall morbidity rate of 36.75% (111/302). This included treatment of 60 out of 196 (30.61%) beef calves and 51 out of 106 (48.11%) dairy calves throughout the five-week study period. However, the dairy herd (n = 53) treated a significantly larger number of calves in year 1 (42/53) compared to year 2 (9/53), while the beef herd treated approximately 30% in each year; year 1 (20/68) and year 2 (40/128). There were typically no differences in the number of sick calves that belonged to the treatment and control groups; however, for beef calves in year 2, there were slightly more sick calves from the treatment (25/40) group than the control (15/40) group. Calves were treated primarily with Resflor or Metacam, for reasons such as pneumonia, enteritis, lack of appetite, or navel infection.

Average daily gain (ADG) of control calves was 1.38 $\pm$ 0.09 kg/d compared to 1.41 $\pm$ 0.16 kg/d in orally supplemented calves (P = 0.3261). A positive correlation was found (r = 0.417) between ADG and serum iron concentrations in beef calves. There was a significant relationship between plasma vitamin E concentrations at birth and weaning weight in beef calves in this study (P = 0.0380).

3.4 Discussion

This study described differences in serum and plasma concentrations of several micronutrients measured before and after administration of an oral nutrient supplement to neonatal beef and dairy calves. To our knowledge, this is the first study to examine the effects of an oral multi-vitamin and mineral supplement provided to neonatal calves in Canada. No significant increase was found for vitamin or mineral concentrations in control and treatment groups in the first year, when blood nutrient profiles were assessed

approximately 14 days after supplementation. During the second year, blood nutrients were evaluated 3 days after supplementation, with no treatment effect on vitamin A and selenium concentrations; however, the effect of treatment on vitamin E and iron concentrations in treatment versus control beef calves was statistically significant. Additionally, time had a significant effect on blood concentrations of certain nutrients for both treatment and control calves in this study, namely, vitamin E, usually vitamin A and selenium, as well as iron in dairy calves.

Some nutrients, like selenium and iron, are readily transferred via placental transfer, whereas vitamins A and E do not cross the placenta in significant amounts, so the calf must rely on ingestion of colostrum for these vitamins (Quigley and Drewry, 1998). Therefore, it is plausible that the concentrations of fat-soluble vitamins in newborn calves would rise naturally after birth. For dairy calves, there was a significant time effect on blood serum iron concentrations as well, which may be related to a higher content of iron present in milk replacer compared to of that in whole milk (Allan et al., 2020; Budny-Walczak et al., 2023; Jenkins and Hidiroglou, 1987). It is well known that the mother's milk is not a good source of iron (Mohri et al., 2006; Atyabi et al., 2006), thus we see no noticeable change in iron levels of beef calves over time.

The stability of fat-soluble vitamins was an important consideration when evaluating the effectiveness of the oral vitamin-mineral supplement. Loss of vitamin activity in stored feed and premixes may lead to subclinical vitamin deficiencies and account for hidden depressions in growth, feed efficiency, and disease resistance (Shurson et al., 2011). Vitamin A, and vitamin E to a lesser extent, are sensitive to heat, light, moisture, and trace minerals and thus, tend to be susceptible to destruction in stored feed and premixes (Frye et al. 1991; Shurson et al. 2011). Inorganic trace minerals and mineral salts are oxidizing agents that hasten the rate of vitamin destruction; while organic trace mineral forms may have the ability to protect vitamins from the destructive ionic charges associated with inorganic trace minerals (Shurson et al., 2011). The stability of vitamin A and E in blood samples may also affect the results, particularly when samples collected in the field are exposed to a range of temperatures and for various time periods before being received by the laboratory (Mitsioulis and Judson, 2000). Further, hemolysis of blood by repeated freeze-thaw leads to breakdown of vitamins in blood samples prior

to analysis (Hooser et al., 2000). Storage temperature and time are important considerations for vitamin analysis as well: vitamin E (α-tocopherol) content of blood plasma from cattle decreased in just 6 days at -20°C, suggesting that extended periods of time would lead to further degradation (Shurson et al., 2011). In this study, year one blood samples were stored for a one-year period before being analyzed, whereas year two samples were analyzed only several weeks after collection. For this reason, proper care and handling of blood samples and of the oral supplement prior to administration, in a study like this, are imperative to the efficacy and could have significantly impacted the final results.

Previous studies have found that while correcting selenium deficiencies, administration of organic selenium such as selenium methionine was found to result in higher tissue, serum, and whole blood selenium concentrations than by the administration of equivalent doses of inorganic selenite (Tiwary et al., 2006; Khanal and Knight, 2010). Moreover, feeding organic selenium from selenomethionine or selenized yeast results in much higher tissue and milk selenium concentrations than are obtained with selenite (Spears, 2003). Organic forms of selenium are apparently better absorbed than are inorganic forms. The multi-vitamin and mineral drench, *VitaFerst-Care*, tested in this study contained inorganic sodium selenite. Though, it has been said that the rumen can convert calcium and sodium selenite to biologically available forms of selenium (Henry et al., 1988), during the neonatal period, the rumen is underdeveloped (Radostits and Bell, 1970), and therefore, study calves may have been unable to absorb the selenium. Although, another study found that changes in blood selenium concentrations were prolonged in young calves: blood selenium concentrations did not change for the at least the first 3 weeks and were most often observed around 10 weeks, when calves received a selenium injection at birth (Kincaid and Hodgson, 1989). In this study, no changes in blood serum concentrations were observed in calves less than two weeks of age, when they were drenched with inorganic selenium at birth. Consequently, the duration of this research trial may not have been sufficient to determine the treatment effect of selenium supplementation on young calves. Another important observation was that there was a statistically significant effect over time on selenium concentrations when the level of significance for vitamin E concentrations over time was $p \leq 0.0001$. An interrelatedness

and synergistic interaction exist between vitamin E and selenium (McGrath et al., 2018). Thus, since all calves on trial were clinically normal, the low concentrations of selenium may have been compensated for by the more than adequate vitamin E concentrations. Contrarily, deficiencies of other antioxidants (vitamin A and E) may increase expression of a selenium deficiency (Graham, 1991).

On the other hand, young calves are thought to be very efficient at absorbing iron from their diet (Allan et al., 2020; Budny-Walczak et al., 2023). One study by Kume and Tanabe (1996) found that plasma iron of calves treated with iron increased temporarily at 2 days of age, and blood hematocrit and hemoglobin of calves treated with iron increased from 2 to 10 d of age. Likewise, a pharmacokinetics study which assessed the effect of *VitaFerst-Care* on calves from one to four days after treatment found that serum iron levels increased substantially between one and three days in calves that received the oral supplement, compared to those in the control group (unpublished data). Our data indicates that at approximately 14 days the effect of iron supplementation is not detected in circulating blood serum levels, and that iron was most likely utilized previously, or excess levels have entered storage sites in the liver. As such, when concentrations of iron were examined at 3 days, there was a statistically significant effect of iron supplementation on serum iron concentrations and hemoglobin levels in beef calves; however, the insignificance observed for dairy calves is most likely explained by the much smaller sample size. There have been trials conducted on farms where supplementing calves with iron at birth either orally or by injection has increased hemoglobin levels and growth rates over the first eight weeks of life (Mohri et al., 2010; Völker and Rotermund, 2000). In the present study, the ADG of the treatment group was greater than that of the control group. There was also a positive correlation with ADG and serum iron concentrations in beef calves which is similar to another study's finding: calf weight in the first and subsequent weeks of life was positively correlated to serum iron during the first week of life (Reece et al., 1985). Furthermore, beef calves that received vitamin-mineral supplementation had a significantly greater average hemoglobin concentration compared to that of the control group. Seemingly, when morbidity and mortality rates were the greatest in this trial, so were the percentages of iron deficient animals; however, this

primarily applied to dairy calves because beef calves displayed consistently deficient iron concentrations and had comparable morbidity rates year over year.

The neonatal period exhibits a very critical time for young calves because of the high disease incidence accompanied with an underdeveloped immune system (Chase et al., 2008; Przybylska et al., 2007). Antimicrobial treatment for calf scours and pneumonia were common for neonates in this study. From birth until roughly one month of age calves are at highest risk of enteritis and pneumonia (Waldner et al., 2013). Further, a 2013 survey of western Canadian beef producers found that average calf death loss was highest between birth and one month of age and was 3.7% (Waldner et al., 2013). Other studies found that beef calf mortality was 4.5%, 3.2%, 4.8%, and 3.0%, respectively (Pearson et al., 2019; Tang and Lhermie, 2023; Waldner and Van De Weyer, 2011; Waldner et al., 2022). In the present study, average calf death loss was 4.6%. The incidence for mortality in dairy herds worldwide ranges between 3% and 9% during the perinatal period (Compton et al., 2017): this research indicates that average dairy calf death loss was only 2.8%.

Dairy calves generally experience higher on farm morbidity and mortality rates in the first few months of life compared to beef calves (Hyde et al., 2020). Regardless, average calf morbidity in the present study was remarkably high (37%), although, dairy calf morbidity was about 17% higher than beef calf morbidity. According to other studies, average herd-level incidence of preweaning treatment for disease was only 7.9% (Murray et al., 2016) and 9.4% (Pearson et al. 2019).

Production parameters such as growth and average daily gain (ADG) in calves have significant economic implications for the industry as well. The major nutritional factors affecting pre-weaning calf growth and composition at weaning are the lactational performance of the dam and the quality and availability of nutrients from pasture and/or supplementation prior to and following parturition (Greenwood and Café, 2007). According to Krueger et al. (2014), calf growth rate is the best indicator of limiting nutrients. This study provides evidence that vitamin and mineral supplementation did not significantly increase weight gain in beef calves from birth to weaning, but nutrient concentrations, like vitamin E, may be important indicators of weight gain in pre-ruminant calves.

Limitations of this study included the short duration that calves were on trial as it restricted the ability of researchers to determine the long-term impact that oral neonatal nutrient supplementation has on weight gain, morbidity, and mortality rates. There seemed to be no relationship between calf blood nutrient levels and occurrence of treatment as there was usually an even division of control and treatment calves that were administered antibiotics or other veterinary drugs during this trial. The same was found in another study where a prenatal injection of trace minerals and vitamin E had no effect on passive immune status and survival rate of calves (Daugherty et al., 2002). Though, due to the multi-vitamin and mineral complex that was offered in this study, researchers were unable to correlate morbidity and/or mortality with specific calf vitamin or mineral concentrations and determine their individual effects on immunocompetence. Linkages need to be established between early micronutrient deficiencies and long-term daily gain in calves as well as investigating the link to pre-weaning disease incidence (Allan et al., 2020).

In summary, this research found that oral vitamin and mineral supplementation with *VitaFerst-Care* was largely ineffective in terms of increasing blood nutrient concentrations of beef and dairy calves at 14 days. However, when nutrient concentrations were measured at 3 days, supplementation had a significant effect on vitamin E and iron concentrations in beef calves. Researchers should re-evaluate the appropriate time(s) to analyze vitamin-mineral concentrations following supplementation. Young calves are very efficient at absorbing iron from their diets, while selenium metabolism may be quite lengthy. It was observed that nutrients that are largely transferred through colostrum (vitamins A and E) increase naturally over-time in newborn calves. There seems to be no relationship between calf blood serum/plasma vitamin and mineral concentrations and morbidity occurrence, nor mortality, in the present study. The effect of blood nutrient profiles on growth of young calves appears minimal and should be more closely followed in future studies.

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CHAPTER FOUR: GENERAL DISCUSSION

4.1 General Discussion of Chapter 2

The second chapter of this thesis evaluated the severity and prevalence of vitamin and mineral deficiencies in several beef and dairy herds by determining blood serum and plasma concentrations of specific nutrients. This study demonstrated that nutrient deficiencies were prevalent in study herds, although the extent of severity is dependent on the micronutrient in question. For example, vitamin A and iron were marginal to low, and selenium concentrations were found in negligible amounts, while conversely vitamin E levels were more than adequate in our study calves. Deficient levels of vitamin A, vitamin E, iron, and selenium were also observed in up to one-third of cows. There was little fluctuation in herd averages for all nutrients examined, except iron. Serum iron concentrations were highly variable within a given herd, as well as when comparing herd to herd. Furthermore, the results from this study show that no strong relationships exist between cow and calf vitamin and mineral levels postpartum.

The liver is the organ that often best represents the status of several trace elements in animals as other tissues do not consistently reflect trace mineral status (Kincaid, 1999). While blood samples can be useful means of evaluating nutritional status, some limitations do exist, such as varying accuracy with specific minerals, the chosen protocol for sample collection and care while processing, and the method of analysis (Herdt et al., 2000). Analyses of minerals in milk are seldom useful in mineral assessment because most cations in milk are actively transported into the mammary gland and concentrations are regulated (Kincaid, 1999). Likewise, values for minerals in hair, wool, and hooves lack reference standards, are too slowly responsive to intakes, and can be easily contaminated (Kincaid, 1999). Therefore, a liver biopsy is described to be the most reliable in order to assess nutrient concentrations (Guyot et al., 2009). However, under ideal circumstances, biopsy samples are taken before and after treatments are applied, and such sampling methods are invasive, expensive, and technically demanding in a large population of animals in the field. Rather blood measures are frequently used for

assessment because they are significantly correlated to nutritional status of some trace elements (Claypool et al., 1975; Mills, 1987) and sampling is much less invasive.

Blood sampling can be further divided into the use of serum, plasma, or whole blood. Determining which biological fluid is the most accurate depends on the exact research goals and which vitamins or minerals are of particular interest. Common measures for estimating the selenium status of livestock include concentrations of selenium in liver, serum, and whole blood (Kincaid, 1995). It has been suggested that selenium concentrations in serum or plasma are highly correlated with rate of oral or parenteral administration and respond quickly to changes in selenium intake as compared to whole blood selenium (Stowe and Herdt, 1992; Walburger et al., 2007). Furthermore, physiological factors as well as the state of deficiency may influence the choice for best possible sampling method. For example, iron can be stored in the spleen and liver and found in red blood cells. Iron depletion occurs in three stages: stage 1 is depletion of tissue iron reserves; stage 2 is characterized by reduced serum iron and increased total iron binding capacity; and stage 3 is characterized by anemia (Johnson, 1990). Thus, assessing blood serum iron and selenium concentrations were appropriate for this study. Likewise, plasma concentrations of retinol are indicators to assess the vitamin A (retinol) status in cattle, as is the determination of plasma vitamin E (α-tocopherol) (Raila et al., 2017). Therefore, while blood sampling techniques were sufficient for the purpose of this study, cost-effective, and relatively non-invasive, liver biopsies would have been favoured.

Barriers to a diagnosis are related not only to the cost and invasiveness of sampling but also to the subtle nature of clinical signs for certain minerals (Perdrizet et al., 2020). Additionally, many nutrient deficiencies go undiagnosed and unrecorded due to poor access and vague reference standards. Published reference ranges are scarce, or often inconsistent, and frequently have overlapping values for what is considered deficient, marginal, normal, or toxic (Herdt and Hoff, 2011; Puls, 1994a; Puls, 1994b; Radostits and Bell, 1970). The National Research Council (NRC) publications for dairy cattle (NRC, 2001), beef cattle (NRC, 2000) and small ruminants (NRC, 2007), possess some limitations as they only define vitamin requirements for vitamins A, D, and E, while they underestimate the requirements of some minerals (McGrath et al., 2018). This may

be partly due to the fact that researchers have suggested that dietary requirements for minerals and vitamins for optimal immune function may be greater than those needed for maximal growth or reproductive performance (Kegley et al., 2016). Finally, vitamin and mineral concentrations suitable for optimal immune function, reproductive performance, or maximum growth have been found to be insufficient at times of physiological stress (weaning, transport, comingling, etc.), when feed intake is reduced (Kegley et al., 2016). Many nutrient deficiencies are interrelated, making this a complex field of study and to provide a comprehensive overview of all nutrition–immune system interactions is well beyond the scope of a single trial. Still, this study found that below-adequate blood serum/plasma micronutrient concentrations continue to be observed in Alberta cattle herds. Farmers and ranchers should perform feed and animal testing to best gauge herd levels and ensure adequate supplementation is provided and consumed.

4.2 General Discussion of Chapter 3

In the third chapter of this thesis, the efficacy of an oral vitamin and mineral supplement targeted at newborn beef and dairy calves was evaluated by measuring blood serum/plasma concentrations of vitamin A, E, selenium, and iron before and after supplementation. There was no change in blood nutrient profiles following supplementation for vitamins A and selenium; however, there was a significant increase in beef calf serum iron and plasma vitamin E concentrations at day three. There was also no effect of supplementation on morbidity or mortality yet there was a small difference in ADG of control and treatment groups.

Vitamin A is the most important vitamin in cattle nutrition although it can be stored in the liver for use over a two- to three-month period (“Nutrients for Cattle – Agri-Facts”, 1986). Vitamin A is essential for normal growth and development, maintenance of healthy epithelial tissues (i.e., lining of digestive and reproductive tracts), reproduction, and adequate immune function (Frye et al., 1991). A lack of vitamin A, hypovitaminosis A, particularly in growing animals, can result in decreased disease resistance and thus, increased morbidity and mortality (Waldner and Uehlinger, 2017).

Newborn calves obtain most of their vitamin A from colostrum and milk because vitamin A does not cross the placenta in significant amounts (Quigley and Drewry, 1998). According to Playford and Weiser (2021), the concentration of vitamin A in mature milk is greater than of that found in colostrum, which could explain the gradual increase observed in calf vitamin A concentrations throughout this study. However, it has been said that the vitamin A content in cows' colostrum and milk depends on their intake during late gestation (Waldner and Uehlinger, 2017). Particularly for beef cattle, this late gestational period often occurs during winter months when hays, silages, dry grasses, and straw, in addition to, damaged crops salvaged from drought or hail situations, are utilized in a feeding program. These types of feed can make up the entire diet of beef and dairy cows before and after calving (Waldner and Uehlinger, 2017). Although, dairy cattle are generally fed a TMR utilizing silages, rather than dry, stored feeds, with or without free-choice mineral and a vitamin premix, that the average cow-calf herd receives. While young, green, actively growing forages and legume blends have the potential to meet the nutritional requirements of a herd for normal growth and maintenance, they are only available for a short duration of the year. Substantially lower concentrations of vitamins A and E are found in stored feeds compared to fresh forage rations (Maas et al., 2008; Van De Weyer et al., 2010). Thus, extended periods of winter feeding, synchronous with stored feed, often of poorer quality, may significantly impact vitamin and mineral levels, resulting in deficient cattle rations. These impacts can be passed onto their offspring: there was a fairly strong correlation found between vitamins A concentrations in beef cow-calf pairs after calving.

Like vitamin A, vitamin E crosses the placenta in limited amounts, and colostrum is the primary source of vitamin E in neonatal calves (Hidiroglou, 1989). Therefore, it would be expected that calves are born with relatively low stores of vitamin E, and concentrations increase drastically from birth to roughly 3 days of age, which is consistent with our findings, where time usually had a significant effect on vitamin E concentrations, regardless of treatment group. Furthermore, there was a statistically significant interaction present between the effects of treatment and time for beef calves in year two of this study, which suggests that the success of oral supplementation may depend on colostral vitamin E levels available.

Typically, vitamin E can only be stored in the body (liver, adipose tissue, and muscle) for a limited time (Frye et al., 1991; Waldner and Uehlinger, 2017). Vitamin E plays an important role in improving disease resistance by stabilizing the biological membrane, removing active oxygen species in the body, and providing an antioxidative effect (Otomaru et al., 2022). Vitamin E has a major impact on immunity, which appears to relate to enhanced neutrophil function. Rapid recruitment of neutrophils is critical for maximizing host defense mechanisms (Spears and Weiss, 2008). Thus, neutrophils in selenium or vitamin E-supplemented calves may have an increased ability to phagocytose and kill rotavirus upon exposure, preventing the virus from establishing infection and replicating in the small intestine (Leslie et al., 2019; Teixeira et al., 2014). As such, some studies suggest that calves with enteritis had lower vitamin E concentrations or were not supplemented with vitamin E compared with calves that did not experience enteritis (Radostits et al., 1991; Krueger et al., 2014). Another study revealed that there was almost no effect in response to vitamin E supplementation, other than serum vitamin E concentration (Otomaru et al., 2022). It has previously been reported that calves that received selenium and vitamin E at birth had, on average, 28% higher concentrations of vitamin E compared with calves that did not receive such supplementation (Waldner and Uehlinger, 2017). Likewise, calves in this study had, on average, 38% higher concentrations of vitamin E compared with calves that did not receive vitamin supplementation. Yet, in the described study, there was no clear association made between the incidence of enteritis and lower vitamin E concentrations, though all calves displayed more than adequate vitamin E concentrations. The vitamin E requirement for optimal immunity and health in cattle continues to be an area of interest, with responses to supplementation varying (Spears and Weiss, 2014). Previous work has found that supplemental vitamin E in receiving diets (post-weaning) seemed to be beneficial for decreasing morbidity, increasing daily gains, and improving overall performance (Duff and Galyean, 2007; Galyean et al., 2022), all findings that were not observed in this study. Future research should assess the influence that oral vitamin E supplementation has on neonatal health and production parameters in both short- and long-term.

Vitamin E and selenium work synergistically and represent ideal antioxidant supplements to improve ruminant health, production, and reproduction (McGrath et al., 2018). When antioxidant capacities are compromised, this can lead to immunosuppression and potentially increase pre-weaning disease incidence (Przybylska et al., 2007). Selenium is involved in the antioxidant system via its role in the enzyme glutathione peroxidase and prevents oxidative damage to the body tissues (Khanal and Knight, 2010). Selenium is known to readily cross the placenta to the calf, and accumulates as stores in the liver, however, it is not transferred well through colostrum or milk (Campbell et al., 1990; Koller et al., 1984; Quigley and Drewry, 1998). As a result, there was little change in blood serum selenium levels in pre-ruminating calves across the study period. In fact, over the long-term, it is thought that the mineral transfer in the milk is not efficient in maintaining adequate selenium status in calves (Mehdi and Dufrasne, 2016). Therefore, the decline in selenium stores of the beef calf from parturition onward is an important consideration (Maas et al., 2008) which was observed in year one of this study.

Selenium has a very narrow range of acceptability and excess can lead to toxicity. Selenium deficiency is more of a problem geographically than is selenium toxicity (Khanal and Knight, 2010; Mehdi and Dufrasne, 2016). Low selenium in soil and forage is recognized in many areas of western Canada (Owen et al., 1977). Moreover, selenium deficiency has become more common in more recent years for beef cows in Alberta (Waldner and Van De Weyer, 2011) than had been reported in earlier surveys (Campbell et al., 1995). Further, selenium deficiency is often listed as a differential diagnosis when investigating excessive calf losses in cow-calf herds (Waldner and Van De Weyer, 2011). A vitamin E or selenium deficiency in young calves is characterized by skeletal and cardiac muscle tissue degeneration, called nutritional myodegeneration (NMD), or more commonly known as white muscle disease (WMD). Fast-growing calves up to 4 months of age are often highly susceptible to WMD (Maas and Valberg, 2015). In general, selenium deficiencies in animals are corrected by giving injections, dietary supplements, salt licks, and drenches.

Iron is an important trace mineral in mammals that plays a critical role in normal development and performs several vital functions, such as binding and transporting

oxygen as a key component of hemoglobin (Joerling and Doll, 2019). Iron deficiency anemia is a common disease in calves, predominately caused by an undersupply of iron during exclusive feeding of whole milk, without the additional administration of dietary supplements (Matrone et al., 1957; Andrews, 2004). In calves, first symptoms can appear at approximately 2 months of age as loss of appetite, fatigue, apathy, and increased infection rates (Radwińska and Żarczyńska, 2014). Mature milk is a poor source of iron (Atyabi et al., 2006; Mohri et al., 2006; Mohri et al., 2010), resulting in depletion of calf iron stores, primarily in the liver, but also in the spleen and bone marrow, in the first 3–4 weeks of life, without nutritional intervention (Joerling and Doll, 2019). It has been proposed that iron supplementation to alleviate neonatal anemia should start on the first day of life (Atyabi et al., 2006). Previous work suggests that iron supplementation may be advantageous: Bunger et al. (1986) reported that the prevalence of pneumonia and diarrhea and the frequency of treatments for these diseases were higher in a group of calves that were not supplemented with iron than the calves that were given oral iron supplementation. Three key studies also found that average daily gain (ADG) and total weight gain differed significantly in treatment groups who received iron-supplementation compared to those in the control groups (Atyabi et al., 2006; Mohri et al., 2006; Mohri et al., 2010). Iron deficiency in calf herds can adversely affect growth, immunity, and feed conversion leading to negative economic implications (Graham, 1991). Maintaining adequate iron levels in the pre-weaning stages is crucial (Allan et al., 2020).

Iron deficiency in neonatal calves suckling milk appears to be relatively common but can be quickly corrected if the animals are raised with access to soil or forage or are given iron supplements (Reece et al., 1985). Soil ingestion often represents a major dietary source of iron (Hansen and Spears, 2009). However, snow- and ice-covered grounds restrict ruminant exposure to soil, and hence iron acquisition for a large portion of the year (Hansen and Spears, 2009). Further, dairy animals raised indoors, without access to soil, are particularly susceptible to iron deficiency, nonetheless, the percentage of beef versus dairy calves deficient in iron was the same (32%) for this study.

Weather events, including wet and cold, can have negative impacts on health as well. Subsequently, typical western Canadian calving seasons occur in the winter and spring months, often between January and June, which can introduce stressors due to

extreme climate. A survey done by Waldner et al. (2013) reported that later calving herds treated fewer sick calves. One interpretation is that these herds had less intensive exposure to infectious agents because they were no longer calving in small, confined areas. A later calving season might also be more likely to avoid the inclement weather in western Canada. Stress factors such as cold ambient temperatures increase the risk of morbidity and mortality during early calf rearing (Quinn et al., 2011). Calfhood diseases have a major impact on the economic viability of cattle operations, due to the direct costs associated with treatment or loss and the long-term effects on performance (Lorenz, 2021). It was suggested that effective nutrient supplementation cannot prevent all disease, although it may reduce the severity of morbidity, and incidences of mortality in young calves (Kegley et al., 2016). This study found that intervening with oral nutrient supplementation was not effective overall; and as a result, it did not reduce the severity of morbidity, nor incidences of mortality in young calves. As such, its use in disease prevention for neonatal calves cannot be recommended at this time until formulation effectiveness can be improved.

4.3 Conclusions

This research provides insight regarding the severity and prevalence of nutrient deficiencies in newborn beef and dairy calves. Pre-ruminant calves can have marginal to low vitamin A and iron concentrations, low to deficient selenium concentrations, yet adequate to high vitamin E concentrations under variable management systems. Serum iron concentrations were highly variable, both within- and between farms. However, the blood nutrient concentrations of vitamin A, vitamin E, and selenium were relatively consistent, with limited intra- and inter-herd variation.

This study indicated that there was almost no effect on blood nutrient concentrations in response to oral vitamin and mineral supplementation other than vitamin E and iron concentrations at 3 days, exclusively in beef calves. Nutrient levels naturally rise over time, particularly so for the nutrients that are transferred well through colostrum (vitamin A and E). Yet, no clear relationships exist between cow and calf blood nutrient levels.

The results from this study supply additional data relative to the expected plasma vitamin A and E and serum iron and selenium concentrations in newborn beef and dairy calves, to help address the inadequacy of consistent guidelines for what constitutes a deficient versus normal blood nutrient concentration. The data suggest that the reference ranges currently used to gauge nutrient status do not fully account for complex nutrient interactions.

The interactions between nutritional status, immunology, and disease resistance are extremely complex. No clear association was found between blood nutrient concentrations and the probability of treatment for illness during the early pre-weaning period for calves in this study. Further research should be done on timing of sickness of young calves to determine if supplementation during this critical period can defer or prevent disease incidence.

Livestock rearing in a typical Canadian winter has several challenges including inclement weather, previous drought, and extended periods of winter feeding that impact vitamin and mineral deficiencies in a calf crop. Furthermore, thorough information on supplementation history, management systems, and climate conditions should be provided as part of the herd details to aid in the interpretation of diagnostic data.

Ultimately, this study emphasizes the importance of implementing a vitamin and mineral program in cow-calf herds, particularly in western Canada. Due to the differences that exist in feeding and management of dairy and beef animals, it is difficult to make general conclusions for all calf rearing operations: best management practices should prioritize herd health and optimal nutritional status to help mitigate the growing concern for antimicrobial resistance and to support calf health and welfare, and producer profitability. In theory, oral supplementation, compared to injections, is justified as the superior approach to ensure minimal pain and stress to calves, avoid lesions or injection-site damage, and facilitate the process for unskilled users. However, the effectiveness of oral products, such as *VitaFerst-Care,* needs to be improved significantly beyond the results observed in this study before widespread producer adoption can be recommended.

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