Strategies to monitor and optimize the transfer of passive immunity in newborn dairy calves

by

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ABSTRACT

STRATEGIES TO MONITOR AND OPTIMIZE THE TRANSFER OF PASSIVE IMMUNITY IN NEWBORN DAIRY CALVES

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The assessment and adequate management of transfer of passive immunity in newborn calves is dependent on various factors, with the majority of research focusing primarily on immunoglobulin G. Even though there are clear recommendations for calf rearing protocols, colostrum feeding practices can still be improved. Therefore, the objectives of this thesis were to 1) evaluate the accuracy of STP measurements to estimate FTPI in calves fed CR in comparison to calves fed MC, 2) Determine if different CR feeding frequencies affect serum IgG levels and AEA, 3) Evaluate if reducing the total solids, and osmolality of CR by increasing dilution amount has an effect on colostral IgG absorption or abomasal emptying rates in newborn calves, and 4) Investigate if low and high-quality MC can be enriched with bovine dried CR to achieve adequate serum IgG levels at 24 h. Results from Chapter 2 demonstrated that current threshold points used for STP inflate the proportion of calves estimated to have FTPI when they are fed CR, but correctly classifies FTPI for calves fed MC. As a result, the efficacy of STP to estimate serum IgG in CR-fed calves needs to be elucidated to correctly assess calf health on-farm. Data from Chapter 3 shows that feeding three CR meals within the first 12 h of life did not result in added benefits to serum IgG or AEA levels as compared to calves fed the same volume (12 %

birth BW) in two meals. In addition, results indicate that an upper limit of IgG absorption does not occur when feeding less than 300 g of IgG at birth. Chapter 4 concluded that that feeding one CR meal high in TS and osmolality at birth might not influence the incidence of abnormal fecal scores, decreasing TS increased abomasal emptying rate, and decreasing TS tended to increase IgG absorption. Lastly, data from Chapter 5 indicates that low-quality colostrum can be enriched with CR and achieve acceptable serum IgG levels at 24h in newborn calves without affecting AEA. Overall this thesis provides insight on colostrum feeding strategies that can enhance IgG absorption in newborn dairy calves.

DEDICATION

I dedicate this thesis to my mom, dad, and brother: Melba Cabus, Jose Lopez, and Daniel Lopez.

And to all my family members that have passed away since I left my house in 2014.

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LIST OF SYMBOLS, ABBREVIATIONS OR NOMENCLATURE

°C – Degrees Celsius
ADG – Average Daily Gain
ADG_{48h} – Average Daily Gain at 48 h relative to colostrum feeding
ADG_{7d} – Average Daily Gain at day 7
AEA – Apparent Efficiency of Absorption
AUC – Area Under the Curve
BRD – Bovine Respiratory Disease
BS – Bovine Serum
BW – Body Weight
Cmax – Maximum Concentration
CR – Colostrum Replacer
Cr-EDTA – Chromium EDTA
CV – Coefficient of Variation
FA – Fatty Acids
FcRn – Neonatal Fc Receptor
FL – Final Volume
FTPI – Failed Transfer of Passive Immunity
HTST – High Temperature Short Time
IgA – Immunoglobulin A
IgE – Immunoglobulin E
IgG – Immunoglobulin G
IgG_{0h} – Serum IgG at 0 h
IgG_{24h} – Serum IgG at 24 h relative to colostrum feeding
IgG_{7d} – Serum IgG at day 7 of life
IgM – Immunoglobulin M
kAB – Abomasal Emptying Rate
kABh – Abomasal Emptying Rate per Hour
kELh – Acetaminophen Elimination from Blood
LBC – Lyophilized Bovine Colostrum
LTLT – Low Temperature Long Time
MC – Maternal Colostrum
MR – Milk Replacer
OS – Oligosaccharides
Osm – Osmolality
RID – Radial Immunodiffusion
ROC – Receiver Operator Characteristic
SCCL – Saskatoon Colostrum Company Ltd
SD – Standard Deviation
SEM – Standard Error of the Mean
STP – Serum Total Protein
STP_{0h} – Serum Total Protein at 0 h
STP_{24h} – Serum Total Protein at 24 h relative to colostrum feeding

STP_{d7} – Serum Total Protein at day 7 of life
STPI – Successful Transfer of Passive Immunity
TM – Transition Milk
Tmax – Time to Reach Maximum Concentration
TRT – Treatment
TS – Total Solids

1 Literature Review

1.1 Introduction

Calf management practices involve various factors, with colostrum feeding playing a major role (Godden et al., 2019). Newborn dairy calves need to ingest colostrum to absorb IgG plus other immune factors (Lombard et al., 2019) that will benefit their performance and health (Godden et al., 2019). This literature review will focus on the overall management of newborn dairy calves, which is imperative to a successful dairy herd (Hammon et al., 2020). In addition, it will discuss specific topics, such as the importance of colostrum ingestion for the acquisition of transfer of passive immunity (Lombard et al., 2020) and its consequences (Raboisson et al., 2016; Shivley et al., 2018).

The transfer of passive immunity is mainly derived from the ingestion and absorption of IgG; however, this review will also describe additional classes of immunoglobulins present in colostrum (Butler, 1969), as well as other components, such as micronutrients and bioactive factors (Blum and Hammon, 2000). This review will further discuss the storage of surplus colostrum and feeding alternatives, such as colostrum replacers, when high-quality colostrum is not available. Furthermore, techniques to assess and measure transfer or passive immunity on-farm will be described. Lastly, different techniques that can increase total IgG delivered to a newborn calf, such as increasing feeding frequency, enriching low-quality colostrum, or increase IgG absorption by reducing total solids in colostrum replacer will be discussed.

1.2 Management of Dairy Calves

Rearing healthy and productive dairy calves is essential for dairy herds (Hammon et al., 2020). Therefore, appropriate calf nutrition and management is crucial (Urie et al., 2018a), especially in regard to colostrum feeding (Davis and Drackley, 1998). Throughout the years, research has provided new strategies that include improved colostrum management, colostrum heat-treatment, whole milk or milk replacer feeding regimens, increased rates of milk feeding, energy effects on growth, and optimal grain feeding (Kertz et al., 2017; Heinrichs et al., 2020). Moreover, the use of precision dairy technologies for the assessment of behavioral (i.e., lying time, steps) and physiological (rumination, temperature monitoring) traits continue to be researched as an adequate tool for calf management (Costa et al., 2021).

Calves are born with a naïve immune system and are required to consume colostrum to acquire transfer of passive immunity (Bush and Staley, 1980). Calves that do not acquire passive transfer are more prone to diseases during the preweaning period, which increases their odds of mortality by 4.7 times (Urie et al., 2018b). Therefore, this transfer should be monitored as it affects the morbidity and mortality rates of the newborn (Lombard et al., 2020). Even though the prevalence of improper transfer of passive immunity in Canada has improved, there is still opportunity to improve current practices (Renaud et al., 2020a). At present, dairy calf morbidity and mortality poses a large threat to the future of the global dairy industry, with morbidity rates of up to 38% reported throughout pre-weaning period in Canadian farms (Urie et al., 2018a). In terms of mortality, studies have reported mortality rates of 6.4 % during the preweaning period, and 2.4% from weaning and first calving in U.S farms (Winder et al., 2018). As a result, appropriate management during the first weeks of life is crucial, as up to 66% of deaths occur

during the first 3 wk of age (Winder et al., 2018). Of all the management practices, colostrum feeding is one of the most important (McGuirk and Collins, 2004). Thereafter, milk and grain feeding during the pre-weaning has impacts in later life, where milk provides the majority of nutrients required until significant dry feed intake is achieved (Kertz et al., 2017).

Milk feeding programs vary across farms, but restricted milk feeding has been traditionally used, with calves being fed twice daily at 10% BW/d (Vasseur et al., 2010). Even though 33% of farmers still feed less than 6 L/d of milk at peak milk intake, average milk intake in Canada is ~8.2 L/d (Winder et al., 2018). Providing sufficient milk is crucial as it has been reported that 19.6 % of the first-lactation yield variation is related to the amount of milk fed during the preweaning period (Gelsinger et al., 2016). To date, there have been various reports on the efficacy of increased milk feeding regimens on milk production (Gelsinger et al., 2016). A report by Soberon and Van Amburgh (2013) found that milk production in the first lactation is influenced by nutrient intake from milk or MR. This study led other groups to do further investigations on the appropriate pre-weaning milk feeding regimen (Heinrichs et al., 2020). It is believed that calves are underfed milk as it is evident that they can consume large volumes during their first weeks of life (Dairy Cattle Code of Practice Scientist's Committee, 2009). Usually, milk feeding protocols state to feed 4-6 L per day, but recent recommendations state that calves should be fed at least 20% of BW milk intake/day (~8L for a 40 kg calf; Khan et al 2011). This practice of feeding greater volumes of milk is defined as intensified milk feeding (Khan et al., 2011), and has been implemented widely in the dairy industry to ensure appropriate growth and organ development during the pre-weaning period (Hammon et al., 2020). Limited milk feeding protocols do not completely nourish an animal, which leads to animal welfare

concerns as it does not follow the codes established by the World Organization for Animal Health (OIE, 2017). An appropriate colostrum feeding program should be followed by an intensive milk feeding program (Hammon et al., 2020), plus, it has been reported that it does not affect post weaning feed consumption (Parsons et al., 2021). Also, it permits calves to reach their growth potential (Drackley, 2005). In addition, having ad-libitum feeding programs can also have long-term performance benefits and prevent hunger, with calves fed ad-libitum milk having less unrewarded visits to feeders (De Paula Vieira et al., 2008; Hammon et al., 2020). However, specific protocols should be adapted to each individual farm's capacity and needs. Overall, close attention needs to be provided at every stage from birth until 6 mo of age, as nutrient requirements change as calves grow (Mitchell et al., 2020).

Feeding whole milk from the tank is considered the highest quality liquid feed for raising dairy calves (Moore et al., 2009). Surveys from US have reported that 38.5 % of dairy operations use MR (Urie et al., 2018b), however, large variations exist in the quality and digestibility of different MRs (Medina et al., 1983). As a result, some producers decide to feed non-saleable milk (Godden et al., 2005), commonly known as "waste milk", as an economical alternative to MR or whole milk, despite potentially critical quality problems (Moore et al., 2009). Nevertheless, some research has suggested that the use of pasteurized non-saleable milk is an alternative to conventional milk replacer and results in calves having a greater weaning weight and lower morbidity rates (Godden et al., 2005). The differences in weight could be attributed to the higher energy and protein consumed by calves fed non-saleable whole milk, which also contained transition milk. For example, Godden et al. (2005) used a MR with 20% of protein and fat, while the non-saleable milk had approximate values of 25.6 and 29.6%, respectively (Davis

and Drackley, 1998). In addition, whenever the percentage of the total solids is low in the whole milk given to calves, producers have started to add MR or milk balance powder to whole milk to increase total solids (Burgstaller et al., 2017). This has been shown to increase growth rates before weaning (Glosson et al., 2015), which could be accredited to the higher crude protein, fat, and energy consumed with the enriched milk meal.

The pre-weaning period is one of the most challenging stages (van Niekerk et al., 2021). During this stage, calves are exposed to disease-causing organisms and are most susceptible to rotavirus, *Cryptosporidium* spp., resulting in diarrhea, especially when successful transfer of passive immunity is not achieved (Lora et al., 2018). The most common pathogens responsible for diarrhea during their first month of life are caused by *E. coli*, rotavirus, coronavirus, *C. parvum*, and *S. enterica subsp. enterica* serovars (Maunsell and Donovan, 2008; McGuirk, 2008, Constable, 2009). The relationship between TPI and occurrence of enteric diseases and mortality is well established and understood (Lora et al., 2018). Due to mortality risk by diseases (McGuirk, 2008), colostrum feeding at birth is one of the most critical practices that affect calf health (Godden, 2008; Lora et al., 2018). Most calves suffer from digestive disorders, which is associated with the majority of deaths (Urie et al., 2018a) and high antimicrobial use (Constable, 2009) during the pre-weaning period. The use of antimicrobials, more commonly used to treat diarrhea, remains controversial due to its over-use; but other alternatives, such as proper colostrum feeding, and use of oral rehydration solutions could help with diarrhea events (Constable, 2004) while reducing societal and animal welfare concerns. Recent research has demonstrated the utility of supplementation of CR to MR feedings in resolving diarrhea in young calves (Carter et al., 2022), demonstrating the wide array of colostrum bioactive compounds and

nutrients that assist in maintaining and promoting a healthy gastrointestinal tract. Unfortunately, this practice is not common on-farm at present, and thus the use of antimicrobials during the pre-weaning period remains high.

It has been accepted that providing oral electrolyte solutions or intravenous fluids help treat diarrhea, however, the use of antimicrobials remains controversial (Constable, 2004). The issue with overuse of antimicrobials lies in concern surrounding the potential for development of antimicrobial resistance and its repercussions on animal health (Tang et al., 2017). Strategic use of antimicrobials should be implemented given that a recent cross-sectional study reported that 74% and 96% of Canadian producers use antimicrobials to treat diarrhea and respiratory diseases, respectively (Uyama et al., 2022). In addition, less than 40% of producers have established written protocols to treat diseases, leaving the decision-making process unclear and somewhat arbitrary (Uyama et al., 2022). Nevertheless, specific antimicrobials can effectively treat diarrhea when used with established protocols and when the antimicrobial used targets specific causing agents (Constable, 2004). In severe cases, calves with diarrhea can develop a compromised immune system due to bacteremia and in these cases, the controlled used of antimicrobials is recommended (Constable, 2004).

After one month of age, respiratory disease is one of the most common challenges calves suffer from (USDA, 2002). The impact of BRD, also known as enzootic calf pneumonia (ECP), affecting the respiratory tract of dairy calves (Cantor and Costa, 2022) could be reduced by ensuring an adequate immunity in calves by the feeding of high-quality colostrum (Gordan and Plummer, 2010). Furthermore, the consequences of respiratory diseases include costs due to prevention, treatment, and productivity lost (Gorden and Plummer, 2010). The presence of BRD

during the preweaning period has been shown to reduce milk and starter intake (Cantor and Costa, 2022), and reduced milk production during the first lactation (Dunn et al., 2018, Buczinski et al., 2021). In addition, heifer calves identified with BRD have 2.85 higher odds of dying before first lactation and an ADG reduced by 0.067 kg/day (Buczinski et al., 2021). Overall, mortality and reduced ADG are related to BRD and proper management during the preweaning period is needed (Buczinski et al, 2021).

1.3 Importance of Colostrum Ingestion

Bovine maternal colostrum is the first secretion a cow produces after involution and is accumulated during pregnancy (Baumrucker and Bruckmaier, 2014). Colostrum is composed of a mixture of lacteal secretions that are accumulated in the mammary gland during the dry period, before parturition (Foley and Otterby, 1978). Newborn calves are born without circulating IgG, defined as "agammaglobulinemic" (Chase et al., 2008), due to the structure of the bovine placenta which does not allow for the transfer of antibodies (Davis and Drackley, 1998). This placenta is syndesmochorial, with three maternal (endometrium) and three fetal layers (chorioallantois, amnion), creating an impenetrable barrier for immunoglobulins (Hoffmann et al., 1976; Blum and Baumrucker, 2008; Peter, 2013). Therefore, calves are dependent on colostrum feeding to acquire transfer of passive immunity at birth (Bush and Staley, 1980). In addition, colostrum has to be fed immediately after birth, as the ability of the intestine to absorb immunoglobulins decreases with time and is thought to cease at approximately 24 h (Stott et al., 1979a). More recently, it was also shown that IgG absorption decreases when colostrum feeding is delayed from 45 mins to 6 and 12 h after birth (Fischer et al., 2018a).

Immunoglobulin G is transported to the mammary secretory lumen by a receptor complex known as FcRn during colostrogenesis (Rojas and Apodaca, 2002). Mammary cells internalize and transcytose the IgG1 molecule for transport to luminal secretions (Barrington et al., 2001). Transcytosis activity, as well as recycling, is performed by FcRn and is responsible for IgG1 appearance during colostrum formation (Ollivier-Bousquet, 1998). In brief, IgG1 binds FcRN on the basolateral side of the epithelial cells of the alveoli, which is then released to mammary gland secretions (Hammer et al., 1969; Kemler et al., 1975). Even though the transport of IgG during colostrogenesis has been researched, the transport mechanisms of IgG in the intestine of a newborn calf have not been characterized. It is thought that FcRN is present in the calf's intestine, similar to rodents (Rodewald, 1976), where enterocytes bind IgG1 and enclose the complex to form an endosome, which is either transcytosed or recycled to the apical or basal side of the cell (Tzaban et al., 2009). Even though the existence of FcRN mRNA in the calf's intestine has been reported (Rodewald, 1976), it might not be the primary mechanism involved in IgG1 absorption (Baumrucker and Bruckmaier, 2014).

1.4 Transfer of Passive Immunity

Successful transfer of passive immunity (STPI) occurs when a calf consumes enough IgG and attains serum IgG concentrations > 10 g/L at 24 h. Conversely, failed transfer of passive immunity (FTPI) occurs when a calf fails to attain the cutoff of < 10 g/L (Weaver et al., 2000; Shivley et al., 2018). New recommendations classify STPI according to four serum IgG concentration categories: poor (<10 g/L), fair (10.0–17.9 g/L), good (18.0–24.9 g/L), and excellent (≥25.0 g/L; Lombard et al., 2020). It is crucial to avoid FTPI because calves with this condition are more prone to develop respiratory diseases, diarrhea, and have increased mortality

rates (Weaver et al., 2000; Raboisson et al., 2016). It is important to improve current colostrum management practices, as FTPI prevalence in the US and Canada (Ontario) are approximately 13.0 and 23.6 %, respectively (Urie et al., 2018a; Renaud et al., 2020a). Previous colostrum management recommendations have stated that calves should be fed 3-4 L of high-quality maternal colostrum (> 50 g/L IgG; McGuirk and Collins, 2004). Nevertheless, updated recommendations focus on total IgG mass and aim to feed above 150-200 g at birth (Godden et al., 2019). The purpose of increasing IgG mass fed to calves comes from data suggesting that calves with higher circulating serum IgG concentrations at 24 h have reduced risks of morbidity and mortality (Lombard et al., 2020).

1.5 Classes of Immunoglobulins in Colostrum

Calves need to ingest IgG to ensure passive immunity until their active immune system develops (Davis and Drackley, 1998) and starts producing endogenous IgG at approximately 3 wk of age (Devery et al., 1979; Kertz et al., 2017). However, this endogenous production can vary as calves that are colostrum deprived or have low serum IgG concentrations after colostrum feeding start their endogenous production earlier (Logan et al., 1974a; Pauletti et al., 2003). The IgG class is subdivided into IgG1 and IgG2, with IgG1 playing a major role in FTPI (Butler, 1969). The IgG1 and IgG2 subclasses result from antigenic and physiological differences in the heavy polypeptide chains (Butler, 1969). The subclass IgG1 appears in higher quantities in comparison to IgG2, with a ratio of approximately 7:1 in bovine colostrum (Butler et al., 1974). In addition to IgG, bovine maternal colostrum (MC) is also a source of immunoglobulin M (IgM) and A (IgA; Butler et al., 1974). The proportion of IgG, IgM, and IgA in colostrum is 85-95%, 7%, and 5%, respectively (Butler, 1969; Sasaki et al., 1976). All these classes of

immunoglobulins belong to the same family of proteins with high molecular weight and are polymers composed of a four-polypeptide chain molecule with two light and two heavy chains (Butler, 1969). Specifically, the role of IgG, IgM, and IgA is to prevent enteric infections, protect against septicemia and overgrowth of bacteria in the intestinal lumen, respectively (Bywater and Logan, 1974; Logan et al., 1974a, b, c; Thatcher and Gershwin, 1989). In addition to these major types of immunoglobulins, other studies have reported the presence of immunoglobulin E (IgE) in colostrum, which might play a role in fighting intestinal parasites (Thatcher and Gershwin, 1989). The role of all these immunoglobulins is to identify and fight pathogens and help develop the calf's immature immune system (Davis and Drackley, 1998). However, even calves with STPI are still susceptible to diseases or death if farm management activities, hygiene controls, and feeding regimens are not adequate (Godden, 2008; Murray et al., 2016; Barry et al., 2019), as well as if calves are exposed to stress, inadequate housing, or have inadequate nutrition (Lombard et al., 2020).

1.5.1 Other Components in Colostrum

The quality of (MC) plays an important role in a newborn's development. Before the components of colostrum are discussed, it has to be noted that MC is a secretion with valuable microflora (i.e., *Lactobacillus casei* and *Bifidobacterium pseudolongum*; probiotic strains; Lindner et al., 2011). The core microbiome of bovine MC are the microbes present regardless of the dam's genetics and diet (Ley et al., 2006; Turnbaugh and Gordon, 2009). However, a consensus has not yet been established about the existence of a core commensal microbiota in bovine milk (Ruegg, 2022). The importance of the colostrum microbiome relies on its possible use as a taxonomic marker of mammary gland health (Lima et al., 2017).

Usually, colostrum quality is based on IgG concentration (Garry et al., 1996); however, other factors can also affect colostrum quality. For example, its bacterial population can be detrimental to calf health and IgG absorption; as it has been suggested that IgG and microbes could compete for a common receptor in the intestine (Staley and Bush, 1985). However, research has shown that up to 90% of total bacterial and coliform counts can be reduced by heating colostrum 30 to 60 minutes at 60 °C (Elizondo-Salazar et al., 2010). Beyond important immunoglobulins, colostrum is rich in various components that are beneficial to the calf (Hurley and Theil, 2013; Mehra et al., 2021). Colostrum is a source of sugars (i.e. lactose, glucose, fructose; Gopal and Gill, 2000), lipids, energy (~ 130 kcal/100 mL; Godhia and Patel, 2013), carbohydrates, vitamins, minerals, hormones, growth factors, enzymes, cytokines (McGrath et al., 2016), leukocytes, and antimicrobial factors (Foley and Otterby, 1978; Concha et al., 1980). These components are present at a higher DM percentage in comparison to mature milk, except for lactose, which increases as colostrum transitions into mature milk (Foley and Otterby, 1978; McGrath et al., 2016). Beyond the roles of protein, lipids, and energy for muscle development and helping thermoregulation (Quigley and Drewry, 1998; Morrill et al., 2012), growth factors and hormones in colostrum may aid in the maturation of the gastrointestinal tract (Hammon et al., 2013). The composition of colostrum varies due to distinct factors (Foley and Otterby, 1978) such as parity, dry-period nutrition, and past disease exposure (Parrish et al., 1948; Mann et al., 2016). More specifically, the starch source offered in the peripartum diet could affect IgG concentrations in colostrum (Fatahnia et al., 2012). However, the general composition of first-milking bovine colostrum is approximately 6.7% fat, 14.92% protein, 2.49% lactose, 27.64% total solids, 0.05% ash, 34.96% IgG1, 6.00% IgG2, and 0.82 g/L lactoferrin (Kehoe et al., 2007).

Other components in colostrum are of importance; including micronutrients and bioactive factors as they promote physiological maturation and calf growth (Blum and Hammon, 2000; Fischer-Tlustos et al., 2021). From these macronutrients, fat plays a big role as it is the largest source of energy for a newborn (Fischer-Tlustos et al., 2021), its FA oxidation is involved in maintaining glucose homeostasis (Hammon et al., 2012), and its phospholipids provide protection against gastrointestinal diseases (Sprong et al., 2002). In addition, colostral fat is elevated in certain fatty acids (FA; Contarini et al. 2014; O'Callaghan et al. 2020), which help in oxidation status and immune response (Opgenorth et al. 2020). It is important to feed a newborn first-milking colostrum as its composition changes with short-chain saturated FA increasing and long chain FA decreasing from parturition up to day 5 (Contarini et al., 2014). In addition, cholesterol and phospholipids content are higher at d 1 post-calving (Contarini et al., 2014). Besides fat, oligosaccharides (OS) are another component of interest in colostrum. The OS content of colostrum is 15-72 times greater in comparison to mature milk (Fischer-Tlustos et al., 2020). In contrast to most components that are higher in colostrum than mature milk, lactose content is 1.8x lower in colostrum (Fisher-Tlustos et al., 2020) However, the OS present in colostrum are mainly composed of a lactose core (Engfer et al., 2000), and it has been suggested that this may be the reason as to why free lactose is low, but lactose-containing OS are high in bovine colostrum (Fischer-Tlustos et al., 2021). The OS from colostrum may be involved in promoting a healthy gut microbiome by serving as prebiotics for microbiota in the newborn calf's intestine (Yu et al., 2013; Fischer et al., 2018b) and also protect the intestinal mucosa by adhering to bacteria (Martín et al., 2002). In general, OS seem to play an important role in calf development and future research will provide more insight on its mechanisms.

Colostrum also has a high content of hormones, including insulin (Blum and Hammon, 2000) and insulin growth-like factor (IGF-1; Bühler et al., 1998), which assist in gastrointestinal cells proliferation (Fisher-Tlustos et al., 2021). Besides, its protein content is also high differing from mature milk, as it drastically decreases from 15% to approximately 3% (Playford and Weiser, 2021). The concentration of protein in colostrum is constituted of whey proteins (soluble fraction) and caseins (insoluble fraction), where casein constitutes 75% of total protein content (Ginger and Grigor, 1999). This high proportion of casein is important as a source of energy and for its immune-regulatory properties, such as helping in the absorption of other biologically active peptides (Playford et al., 1993). Peptides in colostrum are critical as they perform antimicrobial activities (Playford and Weiser, 2021). In the whey protein faction, α-Lactalbumin represents 40% of the total content and is a source of essential amino acids (Kuken and Pearson, 1949).

1.6 Storage of Maternal Colostrum

The storage of colostrum represents an opportunity for dairy farms to use their surplus colostrum, which in past years would be considered as unmarketable (Foley and Otterby, 1978). One method that is commonly used is to freeze or refrigerate surplus colostrum (Foley and Otterby, 1978; Arthington et al., 2000a). This method preserves colostrum in the short-term and helps create an on-farm "colostrum bank" (Abdelsattar et al., 2022). However, refrigerated colostrum should not be kept for more than 48 h, as refrigeration does not stop bacteria proliferation (Stewart et al., 2005), and frozen colostrum should not be kept more than one year (Godden et al., 2019). Overall, the best way to preserve colostrum is via freezing, spray-drying, or freeze-drying (Abdelsattar et al., 2022). Stored frozen colostrum is commonly thawed using

water baths, and the water bath should not exceed 60°C as it can result in a 26% loss of IgG1 (Balthazar et al., 2015). As a result, it has been reported that thawing at 40-60°C could be a more conservative approach, with only 8% loss of IgG (Balthazar et al., 2015). Besides the use of water baths, colostrum is also often thawed in microwaves. It has been reported that colostrum IgG1 is better preserved by microwaving it for 30 min at 200 W in comparison to 30 min at 350 W, where IgG reductions were approximately 20 and 31%, respectively (Balthazar et al., 2015). However, the use of a microwave should be avoided as it could create "hot spots" in colostrum above 60°C that can denature IgG molecules (Denholm, 2022). Early research has evaluated the effects of multiple freeze-thawing cycles on colostrum, highlighting that up to 8 freeze-thawing cycles at 37°C did not decrease IgG, IgM, or IgA concentrations (Haines et al., 1992). However, a more recent report discussed that two and three freezing-thawing cycles can decrease IgG concentration by 7.8 and 7.7%, respectively (Morrill et al., 2015).

In addition to the possible denaturation of IgG by multiple freeze-thawing cycles, other immunological and bioactive components could be affected, as reviewed by Robbers et al. (2021). Maternal leukocytes present in colostrum remain viable at approximately 37°C, but temperatures above 42°C causes denaturation of the cells (Robbers et al., 2021). Also, the freezing process creates intracellular crystals that can damage viable cells (Robbers et al., 2021), as a result, cellular components are not maintained during freezing (McGuirk and Collins, 2004). It has been reported that no viable cells were found in colostrum that was previously exposed to a freeze-thawing cycle, demonstrating that leukocytes and viable cells are damaged during this process (Donovan et al., 2007; Novo et al., 2017a,b). Overall, freezing-thawing cycles should be minimized and standardized protocols should be established for thawing colostrum.

1.7 Colostrum Replacer Products

Colostrum replacers (CR) are products that aim to replace whole bovine colostrum, supplying enough IgG and nutrients to a newborn calf. In brief, bovine colostrum is altered from a liquid to a powdered form (Gomes et al., 2021), which could affect the different components of colostrum when processed. Research has shown that exposing colostrum to high-temperature and short-time pasteurization (HTST, 72 °C, 15 s) denatures 48% of IgG (Chatterton et al., 2020). However, exposing bovine colostrum to low-temperature and long-time pasteurization (LTLT, 63 °C, 30 min) followed by spray drying is an adequate technique to preserve bioactive proteins and anti-inflammatory proteins, such as serpin anti-proteinases (Chatterton et al., 2020). Other reports have also shown that exposing colostrum to a heat treatment of 60°C for 30 or 60 min does not affect IgG concentration and reduces bacterial count (Johnson et al., 2007; Elizondo-Salazar et al., 2010; Gelsinger et al. 2015b). Even though LTLT is the most appropriate procedure, it can reduce lactoperoxidase and lactoferrin content by 56% and 20%, respectively (Chatterton et al., 2020).

These products are mainly classified as CR or colostrum supplements (CS). The main difference is that CR's are meant to replace a whole colostrum meal and give the newborn calf all the nutrients and IgG it needs (Jones and Heinrichs, 2006). In contrast, CS are products that need to be given in conjunction with MC and are not intended to completely replace a high-quality MC meal (Jones and Heinrichs, 2006). Current standards state that for a product to be considered a colostrum "replacer", it has to provide or contain at least 100 g of IgG in one single dose (McGuirk and Collins, 2004; Foster et al., 2006). Nevertheless, colostrum feeding protocols should always aim to feed at least 150-200 g IgG mass at birth (Godden et al., 2019).

Historically, CRs were created for situations when dairy farms do not have a sufficient volume of high-quality or low-pathogen maternal colostrum and want to achieve STPI in their calves (Foster et al., 2006; Lopez et al., 2020). Feeding fresh maternal colostrum could expose a calf to *E. coli*, *Salmonella spp*., *Mycoplasma* spp., and *Mycobacterium avium* ssp. *paratuberculosis* (MAP), which causes Johne's disease (Swan et al., 2007). These pathogenic microbes come from either infected cow mammary glands, feces in colostrum, or improper colostrum collection, handling, or storage (Stewart et al., 2005). As such, CR offers a consistent and clean alternative to MC. However, its use is less common than MC, as it was reported that only 6 dairy operations out of 104 surveyed in US routinely use CR (Urie et al., 2018a.) Colostrum replacer products available on the market have changed throughout the years. Previously, CRs had lower IgG concentrations and differing composition compared to MC. For example, Mee et al. (1996) used a whey protein concentrate as a colostrum substitute, derived from freeze-dried cheese, with an IgG concentration of 3.4%. Now, it is more common for CR products to contain higher levels of IgG, with some products with IgG levels as high as 40.64% (Lopez et al., 2020). Often, these high IgG concentrations are obtained through removal of fat and casein (Lopez et al., 2020).

It has been stated that heat-treatment followed by a drying method, either freeze or spray-drying, reduces microbial presence in colostrum with a low impact on its bioactive components (IgG or lactoferrin) or antioxidant properties (Salar et al. 2021). Overall, there are various CR products and manufacturing procedures used among distinct companies. The various CR available can be categorized as derived from lacteal, blood or serum, egg, or whole bovine colostrum sources (Quigley, 2004; Swan et al., 2007). The effectiveness of spray-drying of bovine MC was first examined by Chelack et al. (1993), who reported that this procedure and

final product can be used as an effective CR and achieve acceptable serum IgG levels in newborn calves. Besides the use of CRs to completely replace a meal of high-quality colostrum, it has also been used as a therapy for diarrhea (Carter et al., 2022) or to mimic transition milk (TM; Van Soest et al., 2020). Currently, there is interest in bovine colostrum byproducts and how they can be used to develop food or pharmaceutical products to aid in gastrointestinal and respiratory diseases in other species, including people (Mehra et al., 2021). The various components of colostrum have distinct roles that aid in health: its enzymes facilitate digestion of food (Fuquay et al., 2011), proteinases hydrolyze peptide bonds (Larsen et al., 2006), antioxidant that are involved in the production of antimicrobial compounds (Farkye, 2002), lipases that could be used to treat obesity (Mead et el., 2002), and trypsin which prevents the decay of growth and immune factors (McGrath et al., 2016).

1.8 Methods to Assess FTPI

There are various ways to measure FTPI and STPI, including direct or indirect methods that estimate serum IgG concentrations. Usually, these estimates are recommended for use at a herd level (Buczinski et al., 2018). The most common laboratory-based methods (Sutter et al., 2020) are radial immunodiffusion (RID; direct method) and ELISA (indirect method; Coons et al., 2012; Sutter et al., 2020). These laboratory assays are complex and require trained personnel (Davis and Giguère, 2005; Hogan et al., 2015). However, the gold-standard reference method most commonly used in the past few years is RID (Ameri et al., 2008; Sutter et al., 2020), as it accurately estimates serum IgG levels in calves (Beam et al., 2009). RID can be expensive and time-consuming, and as such, ELISA has been used as an economical alternative (Gelsinger et al., 2015a). However, Gelsinger et al. (2015a) determined that IgG concentrations in bovine

plasma were considerably lower when measured with ELISA rather than RID. Also, they reported that a direct comparison should not be made between the two methods due to the low correlation of r = 0.59. Besides ELISA and RID, literature has published numerous assays aiming to assess FTPI at the farm level. The majority try to estimate serum total protein (STP) through the use of refractometers (Tyler et al., 1996), as well as measurements of zinc sulfate (Hudgens et al., 1996), sodium sulfate turbidimetry (Tyler et al., 1996), and some immunoassays (Quick Test Calf IgG Kit; Dawes et al., 2002). Other assays have been used in other species, including glutaraldehyde coagulation, semiquantitative immunoassays, and a quantitative immunoassay, but still conclude that these are not as precise as the reference RID assay (Davis and Giguère, 2005).

The use of RID as the reference method to estimate FTPI and serum IgG concentrations is expensive and not practical on farm (Dawes et al., 2002; Deleen et al., 2014). Dairy farmers or veterinarians need immediate results to make clinical decisions on-farm (Tyler et al., 1996). As a result, other alternatives, such as the use of digital and optical refractometers, have been evaluated (McBeath et al., 1971; Deleen et al., 2014; Elsohaby et al., 2015). The use of a refractometer to estimate serum IgG was first evaluated by McBeath et al. (1971), who reported an existent positive correlation between STP and IgG, measured with radial immunodiffusion; however, the relationship was not high (r = 0.72) as refractometers measure the total protein present in the blood rather than directly measuring IgG. However, immunoglobulins, including IgG, represent a large proportion of total proteins in a calf's bloodstream (Calloway et al., 2002). After early research by McBeath et al. (1971) on the use of refractometry as a method to assess FTPI, more studies have evaluated its efficiency and defined accurate cutoff values. Tyler et al.

(1996) investigated the most suitable STP cutoff point to accurately assess STPI, as defined by a serum IgG concentration of 10 g/L at 24 h. The authors reported that the proportion of calves correctly classified with FTPI using the cutoff values of 5.0 and 5.5 g/dL were 83 and 82%, respectively. Thereafter, they performed a regression equation and showed that a STP threshold of 5.2 g/dL was equivalent to a serum IgG concentration of 10 g/L. The cutoff value of 5.2 g/dL (Tyler et al., 1996) is still widely used by many farms to assess FTPI, and is also used as a reference threshold in various research manuscripts.

Literature has also evaluated the efficacy of distinct refractometers to assess FTPI. Calloway et al. (2002) reported that the use of temperature-compensating or non-temperature compensating refractometer performed similarly in the accuracy of estimating FTPI. The limitation of using a non-temperature compensating refractometer is that they need to be used at room temperature (~ 22 °C) and there is a delay time between sample placement and result to allow for temperature equilibration (Calloway et al., 2002). This time frame will vary depending on the manufacturer's instructions or the device used. In addition, Calloway et al. (2002) showed that when using a STP cutoff point of 5.2 g/dL, the temperature and non-temperature compensating refractometers had sensitivities of 93% and 89%, respectively, to predict FTPI. In addition, the specificities to predict FTPI were 80% and 84%, respectively. Overall, Calloway et al. (2002) concluded that a test cutoff point of 5.2 g/dL was adequate to assess FTPI with either a temperature or non-temperature compensating refractometer. It is important to note that the proportion of calves adequately classified as having FTPI or STPI will depend on the prevalence of FTPI in the population samples (Calloway et al., 2002). Lastly, when we decide to use a STP cutoff point to assess for passive immunity, factors such as sensitivity and specificity should also be considered.

Similar to Calloway et al. (2002), Dawes et al. (2002) reported the efficacy of refractometry as a predictor of a serum IgG value of 10 g/L. Dawes et al. (2002) utilized a temperature-compensating refractometer that had a sensitivity and specificity of 71% and 83%, respectively, which is lower than the data reported by Calloway et al. (2002). However, even though sensitivities and specificities will vary depending on FTPI prevalence, herd assessment, and sampling protocol, refractometry use has been widely accepted due to its direct relationship with IgG and ease of measurement. Nevertheless, a systematic review by Buczinski et al. (2016) aimed to establish consensus on a suitable threshold to assess FTPI for Brix and STP, recommending an STP cut-off value of 5.5 g/dL to minimize the prevalence of false-negatives and suggesting that using 5.2 g/dL would be better to rule out FTPI in herds.

Brix refractometers have also been used to estimate IgG with Brix % readings. Brix refractometers are used in the food industry to measure sucrose concentrations, but when used in non-sucrose solutions, they give an estimate of total solids percentage (Quigley et al., 2013; Deleen et al., 2014). Research has compared Brix measurements directly to serum IgG analyzed with RID. Morrill et al. (2013) evaluated Brix to estimate FTPI and concluded that it can predict serum IgG concentrations (10 g/L) and assess FTP using a cut-off point of < 7.8%, but they did not directly compare if Brix had any relationship with STP measurements. Deleen et al. (2014) evaluated the efficacy of Brix compared with STP readings to estimate STPI and reported that Brix % was highly correlated with STP (r = 1.00) and that a cutoff value of 8.4% was the most suitable with a sensitivity and specificity of 88.9% and 88.9%, respectively. In addition, Deleen et al. (2014) reported that Brix is also highly correlated with serum IgG (r = 0.93), which could serve as an on-farm tool to monitor colostrum management.

Beyond the use of refractometry to assess FTPI, Quigley et al. (2013) stated that Brix refractometry is an easy and inexpensive tool to estimate colostral IgG concentrations on-farm. Brix does not directly estimate IgG but it could estimate TS, which have a strong relationship with IgG (Quigley et al., 1994). Quigley et al. (2013) reported that a threshold value of 21% is equivalent to MC with > 50 g/L of IgG concentration. Before the use of Brix refractometers to measure colostrum quality and IgG concentration, the use of colostrometers was common. Fleenor and Stott (1980) developed the colostrometer which is a hydrometer that measures specific gravity and gives an estimate of colostrum quality. However, its correlation with IgG is low as some studies report R^2 values of 0.69 and 0.47 (Fleenor and Stott, 1980; Pritchett et al., 1994). More recent reports have stated that colostrometer data is highly correlated with IgG measured with RID ($r = 0.77$), however, it was suggested that a Brix refractometer is a more reliable tool to assess colostrum IgG concentration (Bartier et al., 2015). Some limitations of a colostrometer include that it is a fragile tool made of glass, colostrum needs to be at 20 - 22 °C to obtain an accurate measurement (Mechor et al., 1992; Bartier et al., 2015), and that colostrometer measurements vary ~0.8 g/L for every Celsius degree change (Mechor et al., 1992). In addition, colostrometers measure specific gravity rather than IgG, so the proportion of non-IgG protein can affect results (Bartier et al., 2015). Overall, in comparison to the colostrometer, a refractometer is a more suitable on-farm tool to measure IgG in colostrum, while additionally having a dual-purpose to asses FTPI in calf serum (Deleen et al., 2014; Bartier et al., 2015). Recent research has concluded that Brix values do not correlate with colostral IgG ($R^2 = 0.127$) and proposed a new method to quantify colostrum IgG has which consists of an optimized Western blot assay that uses quantitative detection of IgG heavy chain (IgG-H;

Schalich et al., 2021). This new proposed method challenges currently used techniques, RID and ELISA, and the validity of Brix refractometer to estimate colostral IgG concentration. It has to be noted however that this report challenging current Brix standards only used a sample size of 27 and only had samples with high IgG concentration, in comparison to reports by Bielman et al. (2010) and Bartier et al. (2015), which evaluated 288 and 569 samples, respectively. In addition, their samples included an IgG range of 22.4 to 196.9 g/L and 8.3 to 128.6 g/L IgG, respectively. Even though this report debates the accuracy of Brix refractometer, it is a validated on-farm tool that can be used on farm to estimate colostral IgG concentration.

1.9 Factors Contributing to IgG Absorption

Colostral IgG absorption in the gut epithelium is reduced as time from birth increases, and is thought that IgG absorption ceases at approximately 24 – 36 h after birth (Brambell, 1958; Deutsch and Smith, 1957; Stott et al., 1979a). However, some reports state that IgG could be absorbed up to 27 h (Penhale et al., 1970). In addition, Smith and Erwin found that y-globulins were absorbed when colostrum was dosed at 6 h and 18h but not at 48 h or 60 h after birth. The exact time at which IgG stops being absorbed is unknown. It has also been reported that AEA is reduced from 51.8 to 35.6% when delaying colostrum feeding from birth until 6 h, respectively (Fischer et al., 2018a). As a result, serum IgG concentrations were also affected and reduced from 22.3 to 15.2 g/L (Fischer et al., 2018a). Nevertheless, it must be considered that calves are still able to absorb IgG when fed at 6 or 12 h after birth (Fischer et al., 2018a). It is clear that there is a gradual decrease in the ability of the gut to absorb different classes of immunoglobulins as time after birth increases, but the exact time at which this occurs is currently unknown (Penhale et al., 1970).

Besides time, IgG absorption is affected by bacterial contamination, where it has been thought that bacteria compete with IgG for binding receptors in the intestine, affecting its absorption (Staley and Bush, 1985; Gelsinger et al., 2014). However, the exact mechanism remains unknow (Shivley et al 2018) Some bacteria can be reduced by heat-treating colostrum (60°C for 30-60 min), which also benefits colostral IgG absorption (Gelsinger et al., 2015b; Saldana et al., 2019). In addition, one study showed that heat-treating colostrum does not affect neonatal immune response in terms of absorbing cytokines such as IFNγ (Gelsinger and Heinrichs, 2017). Even if heat treatment of colostrum benefits IgG, Mann et al. (2020) reported that 38 proteins were lower in the serum of calves fed heat-treated colostrum (60°C for 60 min) compared with calves fed raw colostrum. In addition, Mann et al. (2020) noted that calves fed heat-treated colostrum had lower abundances of enzymes involved in glycolysis or glycogenolysis. However, a recent nationwide wide report from US farms by Shivley et al. (2018) reported that calves fed heat-treated colostrum, temperature and time not specified, had higher serum IgG concentrations than calves fed untreated colostrum, which benefits their immune system and highlights the importance of colostrum pasteurization to reduce bacteria content.

1.10 Feeding Frequency of Colostrum

Traditionally, colostrum feeding recommendations state to feed 4 L of high-quality MC (> 50 g/L) or at least 150-200 g of IgG within 2 h after birth to a newborn calf (Weaver et al., 2000; Godden et al., 2019). However, recent guidelines promote the achievement of serum IgG concentrations higher than 10 g/L to ensure an adequate STPI level in order to significantly reduce mortality and morbidity rates (Lombard et al., 2020). This could be achieved by

increasing total IgG mass fed either with the use high-quality colostrum or feeding extra colostrum meals in the first 24 h. Morin et al. (1997) evaluated the effects of feeding low or high-quality colostrum at 0 and 12 h and concluded that calves fed high-quality colostrum had higher serum IgG concentrations, but this was expected as they consumed more IgG mass. They also reported feeding a group of calves three times – at birth, 6 h, and 12 h of life – in comparison to calves fed only at birth and 12 h. They fed the same total IgG mass to both treatment groups (143.7 g) and found no differences in serum IgG concentrations at 24 h for calves fed either two or three times (10.0 g/L and 11.2 g/L, respectively). This suggests that calves fed three times and consequently less IgG mass at birth (first meal), when IgG absorption is stated to be highest, were able to continue absorbing IgG up to 12 h and achieve similar IgG levels as calves fed a higher mass at birth.

Recent work by Abuelo et al. (2021) concluded that feeding a second meal of colostrum at 6 h (2 L), in addition to an initial meal of 3 L, in comparison to calves only fed once at birth, are less likely to have FTPI. Also, Abuelo et al. (2021) reported on an retrospective study that calves fed a second colostrum meal had greater pre-weaning ADG and less probability to be treated for respiratory disease or diarrhea. Other studies have also researched different colostrum feeding frequencies. Jaster (2005) compared the effects of feeding fist-milking high-quality colostrum (84 g/L IgG) or second- and third-milking low-quality colostrum (31.2 g/L IgG): 4 L at birth or 2 L at birth followed by 2 L at 12 h. They reported that feeding two feedings of high-quality colostrum (at birth and 12 h) resulted in higher serum IgG concentrations at 24 h (45.8 g/L) compared to calves fed once at birth (39.6 g/L). However, they reported opposing results when calves were fed low-quality colostrum (31.2 g/L), were Jersey calves resulted in lower serum

IgG concentrations at 24 h (10.9 g/L) when fed twice (0 and 12 h) compared to calves fed low-quality colostrum once (0 h; 15.8 g/L). They discussed that whenever low-quality colostrum is divided into two meals, IgG protection is not adequate. It has to be considered that these experimental meals were offered to Jersey calves and results could vary with the use of Holstein calves.

1.11 Osmolality and Total Solids in Colostrum and Milk Replacer

Osmolality is a measurement that refers to the number of osmoles per kg of water and is expressed as mOsm/kg (McGuirk, 2003). Osmolarity refers to the number of osmoles dissolved in 1 litre (mOsm/L; Debnam, 2005). Osmolarity is used more often than osmolality because the volume is influenced by temperature (Debnam, 2005). One osmole is equivalent to one gram of molecular weight/mass (1 mol) of any non-dissociating substances (i.e., glucose, lactose, urea; McGuirk, 2003, Debnam, 2005). In addition, osmolality also depends on the content of osmotically active particles in solutions, such as electrolytes, oligo- and monosaccharides, AA, and fatty acids (Pearson et al., 2013). As such, the number of osmoles in a particular solution will reflect the lactose or protein content (McGuirk, 2003). This parameter can be measured with a machine called an osmometer, which gives mOsm/kg values using a freezing-point depression method and measuring solutes in relation to their concentration (McGuirk, 2003). Before considering the importance of osmolality and its effects on colostrum absorption, the osmolality of intra- and extra-cellular fluids needs to be considered. The osmotic pressures of intra- and extra-cellular fluids are similar (285-295 mOsm/kg) but have different ionic compositions, however, they are critical for fluid movement across cell walls (Debnam, 2005).

Different fluids or liquids have distinct osmolarities that classify them as hypertonic or isotonic. Hypertonic meals are normally described as having an osmolarity of approximately 600 mOsm/L, while isotonic solutions have around 300 mOsm/L (Sen et al., 2006). The tonicity of a solution, and concentration of non-penetrating solutes (Debnam, 2005), are detrimental to gastric emptying rates (Sen et al., 2006). This is directly related to how fast a colostrum meal will get to the absorption site - the intestine – and will ultimately affect how colostral IgG is absorbed (Cabral et al., 2014; Fischer et al., 2018a). Osmolality has been extensively researched in whole milk, milk replacers (MR), or oral rehydration programs, but not in colostrum feeding protocols. The osmolality of MR tends to vary depending on the manufacturer and reports have shown ranges from 278 to 1,210 mOsm/L, with mean and median values of 500 and 525 mOsm/L, respectively (McGuirk, 2003). For oral rehydration solutions, osmolarity can range from 300 to 720 mOsm/L (Sen et al., 2006). These studies have focused on how osmolality affects abomasal emptying rate, which is the time the chymus stays in the abomasum until it reaches the intestine (Burgstaller et al., 2017). In addition, this process is important to calf health, as milk feeding protocols that prolong abomasal emptying rate could lead to gastrointestinal diseases (Glenn Songer and Miskimins, 2005). The abomasal emptying rate is affected by increased osmolality (Constable et al., 2009; Cabral et al., 2014). The rate at which a colostrum meal leaves the abomasum or reaches the small intestine could explain 22% of the AEA variation (Mokhber-Dezfooli et al., 2012). Sen et al. (2006) concluded that hypertonic meals are emptied slower than isotonic meals. Overall, it seems that osmolality plays a major role in abomasal emptying, therefore, it also affects the time at which IgG is absorbed in the small intestine. It is clear that osmolality plays a major role in gastric emptying, however, it is affected by other factors in the

solution or meal, such as meal volume, energy density, content, and source of protein (Burgstaller et al., 2017).

Overall, high total solids and osmolality levels can lead to problems in dairy calves. It is recommended to check these levels; specifically, total solids can be estimated using a refractometer (Chigerwe and Hagey, 2014). Even though threshold values for total solids to prevent gastrointestinal diseases, such as abomasal bloat, do not exist, it is recommended to maintain total solids below 15% or equivalent to approximately 650 mOsm/L (Burgstaller et al., 2017). However, this value would be considered highly hypertonic (Wilms et al., 2019) and could affect abomasal emptying rate (Seigel et al., 1982; Sen et al., 2006). More research is needed to identify which specific TS and osmolality values in colostrum could have effects on IgG absorption, as well as possibly on the incidence of scours or other health events.

1.12 Enrichment of Bovine Maternal Colostrum

The current colostrum feeding management recommendations state that calves should ingest more than 150-200 g of IgG at birth (Godden et al., 2019; Lombard et al., 2020). Nevertheless, this is not possible when a farm has cows producing colostrum with low IgG concentrations or not enough volume to be fed to a newborn – situations that compromise a calf's ability to achieve STPI (Shivley et al., 2018). It has been demonstrated that dairy farmers have the option to enrich or enhance the IgG concentration and overall quality of MC by simply adding CR (Lopez et al., 2020). Even though research on colostrum enrichment is not extensive, there have been some projects evaluating its applicability. Early research added CR products to MC, but it was not defined as "colostrum enrichment". Zaremba et al. (1993) compared feeding calves either 3 kg of pooled MC (95 g IgG/kg) or pooled MC plus 85 g of a dried colostrum

powder (113 mg/g IgG). They reported that calves fed the enriched MC did not have higher serum IgG concentrations than calves fed just the pooled MC. However, it is important to note that the addition of 85 g of the dried colostrum powder only added 9.6 g of extra IgG. As a result, we would expect that circulating IgG would not show any significant increase.

Arthington et al. (2000b) enriched MC of different qualities and evaluated feeding 2 L of pooled high-quality colostrum (95.8 g IgG; 0% from bovine serum (**BS**)), 2 L of pooled medium-quality colostrum enriched with spray-dried bovine serum (BS-28.8% IgG; 95.2 g IgG; 47% from BS), or 2 L of pooled low-quality colostrum enriched with BS (98.8 g IgG; 70% from BS). They found that feeding either medium or low-quality MC with BS resulted in higher serum IgG concentrations at 24 h (9.6 and 9.6 g/L; respectively) compared to feeding high-quality MC (6.2 g/L). In addition, they reported that the apparent efficiency of absorption (AEA) was greater for calves fed the enriched medium- and low-quality MC (37 and 38%, respectively) than calves fed the high-quality, but not enriched MC (25%).

Another study conducted by Kindlein et al. (2017) examined the effects of a second meal of MC (12 h; 120 g/L IgG) enriched with lyophilized bovine colostrum (LBC; 506 mg/g IgG) that was defatted. Before the second colostrum meal, newborn calves were fed at 5% of birth body weight (BW) of either a low (30 g/L) or high (100 g/L) IgG concentrated colostrum and reported that calves fed the enriched meal at their second feeding had reduced villus height and decreased mucous layer thickness in the proximal and distal jejunum. In addition, they noted that the second colostrum meal had an effect on the villus height to crypt depth ratio of the medium and distal jejunum and ileum. Data from Kindlein et al. (2017) suggests that feeding enriched MC could affect absorptive area of the intestine. Furthermore, it has also been reported that high

total solids could affect villus height (Moretti et al., 2010), which is something that happens when a colostrum meal is enriched. However, Kindlein et al. (2008) reported that feeding MC enriched with lyophilized colostrum or MC with high IgG concentration (> 100 g/L IgG) benefitted the maturation of intestinal mucosa compared to calves fed colostrum with an IgG concentration (< 30 g/L IgG). It has also been investigated how feeding colostrum from birth until d 3 of life increases the villus area of 8 d old calves and that lack of colostrum affects epithelial growth (Blätter et al., 2001).

The benefits and methods to perform colostrum enrichment have not been clearly defined in previous research. In addition, the effects of adding CR powder to MC for a newborn calf must be elucidated. For example, the addition of CR could affect the TS of the meal, resulting in gastrointestinal implications (Moretti et al., 2010). The associated decrease in the abomasal emptying rate that could occur may also be linked to the fact that colostrum will take more time to reach the absorption site: the intestine (Cabral et al., 2014). Delayed abomasal emptying rate affects IgG uptake due to decreased absorption and intestinal permeability that exist as time after birth increases, and it ceases completely at 24 h (McCoy et al., 1970; Stott et al., 1979a). Further experiments should clarify the effects of increasing TS by enriching colostrum. Lastly, it should be clarified which colostrum IgG concentrations can be enriched and up to what final IgG concentration without affecting IgG absorption.

1.13 Conclusion and Thesis Objectives

Feeding colostrum is considered the most crucial management factor for a newborn calf. A newborn calf will absorb immunoglobulins that will allow them to fight diseases until their naïve immune system develops and starts to produce endogenous immunoglobulins. In addition,

colostrum provides numerous nutrients, bioactives, growth factors, and energy that a calf requires to survive during the first hours and days of life. In this literature review, various factors that influence IgG absorption and methods to assess adequate colostrum feeding management were discussed. There are tools, such as STP refractometry, to assess good or poor colostrum feeding protocols that will allow dairy farmers to improve management and perform preventive or proactive practices. In addition, there are strategies, including CR feeding, additional MC meals within 12 h after birth, or enrichment of colostrum, that can help attain acceptable STPI rates whenever a farm does not have enough colostrum or has colostrum with low IgG concentrations.

Thus, the overall objective of this thesis is to improve current colostrum feeding practices by providing new strategies that correctly identify failure of transfer of passive immunity and enhance IgG mass fed and its absorption. Chapter 2 presented in this thesis evaluates the accuracy of refractometry to assess FTPI when calves are fed CR. Chapter 3, 4, and 5 evaluated distinct colostrum feeding strategies to improve IgG ingestion and absorption in newborn Holstein calves.

As a result, the specific objectives of the chapters presented in this thesis are as follows:

The objective of Chapter 2 was to determine the accuracy of STP measurements by refractometry to estimate FTPI in newborn calves fed CR in comparison to animals fed MC. Given the differences in protein concentrations between CR and MC, and that current FTPI cutoff points were developed with data from calves fed MC, we hypothesized that a low

correlation would exist between STP readings with refractometry and serum IgG analyzed with RID for calves fed with CR instead of MC.

The objective of Chapter 3 was to determine if distinct CR feeding frequencies and the number of meals during the first 12 h of life of a newborn calf, affect serum IgG concentrations at 24 and AEA. It has been stated that an upper limit of IgG absorption might exist within a certain time period. Usually, this happens when high total IgG masses are fed in one single colostrum meal. Thus, we hypothesized that reducing total IgG mass fed in one single meal at birth by increasing the number or frequency of colostrum meals would increase AEA.

The objective of Chapter 4 was to evaluate if reducing the TS of CR by dilution affects IgG absorption and abomasal emptying rates in newborn calves. Literature has reported that milk or colostrum meals with high TS or osmolality reduce abomasal emptying, thus, increasing the time at which IgG reaches the intestine. As a result, we hypothesized that diluting the CR meal with additional water for reconstitution would decrease TS, osmolality and increase IgG absorption.

The objective of Chapter 5 was to investigate if low- and moderate-quality colostrum can be enriched with CR to achieve adequate serum IgG concentrations without compromising AEA or abomasal emptying rates. The addition of CR to low-quality colostrum is a method that dairy farmers are currently using to increase the total IgG mass fed to calves. We hypothesized that low- and moderate-quality colostrum has the potential to be enriched with CR to achieve acceptable serum IgG levels at 24 or similar to those from calves fed a high-quality MC.

2 Hot topic: Accuracy of refractometry as an indirect method to measure failed transfer of passive immunity in dairy calves fed colostrum replacer and maternal colostrum »

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2.1 Abstract

Serum total protein (STP) refractometry is a widely used indicator of failed transfer of passive immunity (FTPI), defined as serum IgG concentrations of <10 g/L or STP levels <5.2 g/dL measured at 24 h of life. However, recent reports have demonstrated that refractometry could be inaccurate at estimating serum IgG concentrations and FTPI when calves are fed colostrum replacer (CR). The objective of this study was to evaluate the accuracy of STP measurements to estimate FTPI in calves fed CR compared with calves fed maternal colostrum. Blood was collected from dairy calves fed maternal colostrum (n = 927) or colostrum- derived CR (n = 1,258) and analyzed for STP and serum IgG. Serum total protein was measured with a digital refractometer, whereas radial immunodiffusion was used to determine IgG concentrations. Calves fed maternal colostrum had a mean STP of 5.80 ± 0.72 (standard deviation) g/dL and a mean IgG concentration of 22.81 ± 10.14 g/L, respectively, whereas calves fed CR had a mean STP and IgG concentration of 5.14 ± 0.50 g/dL and 12.78 ± 4.60 g/L, respectively. Rates of FTPI for calves fed maternal colostrum or CR were 4.2% and 27.26%, respectively. Calves were considered to have FTPI if their IgG post-colostrum feeding was <10 g/L. Logistic and linear regression analyses were performed to determine cutoff points and existent relationships between STP and IgG. Serum total protein and IgG for calves fed maternal colostrum were highly correlated. In contrast, STP and IgG for calves fed CR were lowly correlated. A receiver operator characteristic curve analysis demonstrated that an STP cutoff point that could predict FTPI when calves are fed CR would be 4.9 g/dL (sensitivity = 0.68; specificity = 0.75). This study suggests that current cutoff points used for STP inflates the number of calves estimated to have FTPI when they are fed CR. »

2.2 Introduction

Calves are born with a naïve immune system and must acquire specific immune defense mechanism through colostrum feeding (Bush and Staley, 1980). Transfer of passive immunity is the process by which calves absorb immunoglobulins via colostrum feeding (Davis and Drackley, 1998). Calves are categorized as having a failed transfer of passive immunity (FTPI) when their serum IgG concentration is <10 g/L or their serum total protein (STP) is <5.2 g/dL at 24 h of life (Calloway et al., 2002; Quigley, 2004; Godden, 2008). This categorization is based on trials that demonstrated that heifer calves with serum IgG concentrations <10 mg/ mL had higher risks of mortality rates (Besser et al., 1991; Wells et al., 1996; Furman-Fratczak et al., 2011), and has not changed in several years (Gay, 1983). In addition, recent studies conducted by Gelsinger et al. (2015b), Cummins et al. (2017), Lago et al. (2018), and Saldana et al. (2019) have used this threshold to classify FTPI in newborn calves.

A FTPI is not a disease, but a condition that makes calves susceptible to diseases during the first weeks of life (Stott et al., 1979c; Weaver et al., 2000). A recent meta-analysis highlighted this increased susceptibility, identifying that calves with FTPI had an increased risk of mortality, bovine respiratory disease, and diarrhea as well as a decreased ADG (Raboisson et al., 2016). Moreover, Raboisson et al. (2016) identified increased on-farm costs associated with calves with FTPI; especially the increased use of antimicrobials to control the health disorders, namely diarrhea and bovine respiratory disease.

Failed transfer of passive immunity can be determined by measuring IgG concentrations either by direct or in direct methods. The most commonly used method that directly measures IgG is radial immunodiffusion assay (RID); however, ELISA can also directly measure IgG

(Gelsinger et al., 2015a). The RID is considered to be the reference standard for serum IgG measurement (Tyler et al., 1996; Wilm et al., 2018). When using RID, FTPI is considered as serum IgG concentrations <10 g/L at 24 h after birth (Shivley et al., 2018); it has been previously defined by Besser et al. (1991) and Furman-Fratczak et al. (2011). Despite been the reference standard test, this assay is time consuming, expensive, not applicable on-farm, requires laboratory procedures and gives results in approximately 24 h (Deelen et al., 2014; Renaud et al., 2018a). Consequently, practical and simple indirect measures are often used to estimate levels of serum IgG or FTPI (Hernandez et al., 2016). The most common indirect method is STP using refractometry (Deelen et al., 2014). Refractometers use the degree of refraction to estimate total proteins in solutions (Chavatte et al., 1998), which is valuable because immunoglobulins constitute a major proportion of protein in newborn serum (Calloway et al., 2002). In addition, as the amount of non-immunoglobulin proteins in serum is considered to be constant (Calloway et al., 2002), and thus the result of refractometry should directly relate to the levels of immunoglobulins transferred into circulation.

It has been reported that STP is highly correlated with serum IgG and, as such, is commonly used to classify FTPI (McBeath et al., 1971; Deelen et al., 2014; Renaud et al., 2018a). A wide range of STP thresholds (4.0–8.0 g/dL; Buczinski et al., 2018) have been used to classify FTPI. Despite this variation, Buczinski et al. (2018) concluded that an STP threshold of <5.2 g/dL (sensitivity = 76%; specificity = 89%) was the most accurate for classifying FTPI in calves fed maternal colostrum. However, the authors did not include studies within the meta-analysis where CR had been fed (Buczinski et al., 2018), and consequently their STP threshold of <5.2 g/dL may not accurately represent FTPI in calves fed CR. Similarly, an earlier study by

Quigley et al. (2002) suggested that current STP thresholds were developed from calves fed maternal colostrum; therefore, the cutoff point to classify FTPI in calves fed CR is unclear.

Colostrum replacers (CR) have been developed as an alternative for farmers who lack high quality (IgG content), pathogen-free maternal colostrum or an adequate volume of maternal colostrum (Lopez et al., 2020). More producers are starting to use CR, with 19.1% of all operations in the United States using CR (NAHMS, 2014); however, low STP readings in newborn calves are reportedly becoming more frequent even when calves have serum IgG concentrations ≥10 g/L. In addition, Quigley et al. (2002), Lago et al. (2018) and Lopez et al. (2020) highlighted that when calves are fed CR, the STP threshold of ≤ 5.2 g/dL is inaccurate to predict FTPI. This threshold facilitates a misconception in which calves are falsely assumed to have FTPI (Donovan et al., 1998; Calloway et al., 2002; Priestley et al., 2013). This inaccuracy in STP readings could be due to different non-immunoglobulin proteins in CR compared with maternal colostrum. Specifically, Priestley et al. (2013) and Leeflang (2014) found that CR have protein profiles that differ from maternal colostrum and would affect serum refractometry readings of CR-fed calves.

Thus, the objective of this cross-sectional diagnostic accuracy study was to evaluate the ability of STP refractometry measures to estimate serum IgG and FTPI in calves fed maternal colostrum and CR. We hypothesized that a low correlation would exist between STP and serum IgG for calves fed CR in contrast to a high correlation for calves fed maternal colostrum. »

2.3 Materials and Methods

This study used pre- and postcolostrum STP and serum IgG data (Saskatoon Colostrum Company Ltd.; SCCL; Saskatoon, SK, Canada) from calves fed maternal colostrum (n = 927) or SCCL colostrum-derived CR (n = 1,258). Data included samples from male and female newborn Holstein calves; there was no selection based on sex. The sample size of this study was based on the number of samples available in an existing data set provided by SCCL

2.3.1 Animals and Experimental Design

Data were collected from 85 dairy farms located in the United States (WI, MN, and CA) and Canada (BC, AB, SK, MB, ON, and QC) from 2009 to 2017. The SCCL colostrum-derived CR fed to calves were as follows: Calf's Choice Total Gold (IgG: ≥26%; CP: 58%, crude fat: 18%; DM: 94%), Calf's Choice Total (IgG: ≥21%; CP: 55%, crude fat: 20%; DM: 94%), and Calf's Choice Total HiCal (IgG: ≥14, CP: 48%; crude fat: 23%; DM: 94%). Colostrum feeding protocols likely differed in the commercial farms, but STP and serum IgG samples were retrospectively collected for the purpose of validating FTPI thresholds in commercial dairy farms. The inclusion criteria did not change over the course of the trial, as all calves that were sampled at 0 h after birth and 24 to 48 h after colostrum feeding were included in the analyses.

Precolostrum blood samples were collected immediately before colostrum feeding, and postcolostrum blood samples were collected between 24 and 48 h after birth. Blood samples were taken via jugular venipuncture using serum vacutainer tubes without anticoagulant, left at room temperature for 3 h to coagulate, then centrifuged at 3,000 × g for 15 min at room temperature (~20°C) to separate serum. Thereafter, serum was transferred to 2-mL microtubes and frozen at −20°C. Serum samples were then sent to SCCL for further laboratory analysis for

STP and serum IgG. Serum total protein was measured using a digital refractometer (PA2020X Misco Palm Abbe Dual Scale, Solon, OH), and serum IgG was analyzed by an RID performed by SCCL, as described by Chelack et al. (1993), and more recently by Shivley et al. (2018). In brief, RID is an assay that quantifies IgG by the reaction between antibodies in a plate and antigens present in serum samples. After 24 h, a precipitin ring is formed in a well at room temperature. The ring's diameter is then measured using a scale loupe, and the measurement is used in a logarithmic formula to determine the IgG concentration of the sample.

2.3.2 Statistical Analyses

Statistical analysis was conducted using SAS (University Edition; SAS Institute Inc., Cary, NC). Data were imported from Microsoft Excel (version 16.16.21; Microsoft Corporation., Redmond, WA) into SAS. Calves with pre-colostrum serum IgG ≥2 g/L were presumed to have nursed before colostrum feeding and were removed from the analysis, according to SCCL guidelines. Moreover, other researchers have also removed calves with serum IgG above 0 g/L before colostrum feeding, with the assumption that they might have suckled colostrum from their dam, which could affect serum IgG analyses at 24 h (Gelsinger et al., 2015b; Saldana et al., 2019). This exclusion criterion has been used because serum IgG values at birth are almost nonexistent (0.1–0.3 g/L; Gelsinger et al., 2015b). The complete data set provided by SCCL had a total of 950 and 1,339 blood samples from calves fed maternal colostrum and CR, respectively. However, 23 (2.42%) calves fed maternal colostrum and 81 (6.04%) calves fed CR were removed based on serum IgG ≥2.0 g/L before colostrum feeding. As a result, only 927 and 1,258 blood samples from calves fed maternal colostrum and CR, respectively, were used in the final analysis. No additional calves were removed from the study, as calves with severe dehydration or

sickness at the postcolostrum feeding sampling time point did not have samples taken. Calves were diagnosed for dehydration whenever they had severe diarrhea (i.e., eye recession, prolonged skin tent, difficult in rising) and were considered sick if they presented diarrhea or septicemia in the first hours of life.

Data were analyzed for normality and descriptive statistics using the UNIVARIATE procedure of SAS. Normality was declared using Shapiro-Wilk as criterion. The final model only included blood samples collected after maternal colostrum or CR feeding. The STP and serum IgG values postcolostrum feeding were analyzed using PROC MEANS. Significance was declared at $P < 0.05$. Additionally, STP and serum IgG postfeeding data were analyzed using the PROC CORR. The regression procedure from SAS was used to determine whether a linear relationship existed between serum IgG concentration and STP concentration, with STP as the predictor variable and serum IgG as the outcome variable. Serum total protein and serum IgG were also analyzed using receiver operator characteristic (ROC) curves to determine the STP cutoff point necessary to predict FTPI, defined as a serum IgG <10 g/L. Youden's index was used to determine the cutoff point that maximized both sensitivity and specificity (Youden, 1950). A subset of data including calves with a serum IgG concentration ≥10 g/L was created. This subset was analyzed using the frequency procedure to visualize the distribution of STP values when calves achieved STPI, defined as a serum IgG concentration ≥10 g/L. All values are reported as mean ± standard deviation. Additionally, an interval likelihood ratio analysis was done using the PROC GLIMMIX and PROC FREQ in SAS.

2.4 Results

A total of 39 (4.20%) calves fed maternal colostrum had FTPI after colostrum feeding, defined as serum IgG <10 g/L via RID. Serum total protein for calves fed maternal colostrum was 5.80 ± 0.72 g/dL, whereas their mean serum IgG was 22.81 ± 10.14 mg/ mL. Postcolostrum STP and serum IgG showed a high, positive correlation, with STP explaining 81% of the variation in the serum IgG concentrations ($R^2 = 0.81$; $P < 0.0001$) (Figure 2.1a). These results demonstrated that STP explained a substantial portion of the variation of IgG, and that it was an adequate predictor of serum IgG in calves fed maternal colostrum. When evaluating calves fed maternal colostrum, a ROC analysis reported that these data had an area under the curve of 0.96 (95% confidence interval: 0.94–0.98). According to the ROC analysis and using the Youden Index, the STP cutoff point for FTPI was determined to be 5.0 g/dL, with a sensitivity of 0.92 (95% confidence interval: 0.74–0.95) and specificity of 0.90 (95% confidence interval: 0.35–0.61), for calves fed maternal colostrum. These results demonstrated that a high correlation exists between STP and serum IgG when evaluating calves fed maternal colostrum. This high correlation was corroborated by the meta-analysis performed by Buczinski et al. (2018), where the STP cutoff points of 5.2 g/dL and 5.5 g/dL were defined as the most accurate to predict FTPI, with sensitivities and specificities of 76.1 and 86.3% for 5.2 g/dL, and 88.2 and 77.9% for 5.5 g/dL.

A total of 343 (27.26%) calves fed CR had FTPI after colostrum feeding, defined as serum IgG <10 mg/ mL via RID. Serum total protein for calves fed CR was 5.14 ± 0.50 g/dL, whereas their mean serum IgG was 12.78 g/L ± 4.60 g/L. A low correlation was found between STP and serum IgG, with STP only explaining 40% of the variation in the level of serum IgG (R^2

= 0.40; $P < 0.0001$; Figure 2.1b). The coefficient of determination between STP and serum IgG found in this study was lower than previously reported ($R^2 = 0.59–0.62$; Mowrey, 2001; Quigley et al., 2002) for calves fed CR. However, these results collectively demonstrate that STP does not explain the majority of the variation in serum IgG, and consequently does not accurately predict serum IgG concentrations in calves fed CR. The ROC analysis in this study reported that these data had an area under the curve of 0.78 (95% confidence interval: 0.75–0.80), which is lower than the area under the curve reported for calves fed maternal colostrum. According to the ROC analysis and using the Youden Index, the STP threshold was determined to be 4.9 g/ dL, with a sensitivity of 0.68 (95% confidence interval: 0.54–0.81) and specificity of 0.75 (95% confidence interval: 0.32 to 0.61). These results suggested that a lower STP cutoff point should be used to predict FTPI in calves fed SCCL colostrum-derived CR compared with calves fed maternal colostrum. However, even with a lower threshold, the low sensitivity and specificity of this threshold further compromises the use of STP use as an indicator of FTPI for CR-fed calves. The low sensitivity will result in a greater proportion of false negatives, with those classified as FTPI actually having STPI. The cutoff point of 4.9 g/dL found in this study will not greatly improve the correct identification of true positives and true negatives, but does underline that an alternate STP cutoff point should be developed to ensure a high sensitivity and specificity when classifying FTPI in calves fed CR. Due to the inadequate sensitivities and specificities from the ROC analysis performed in this study, an additional analysis of interval likelihood ratios was performed (Table 2.1). Interval likelihood ratio is defined as the probability of the test result when the condition is present divided by the probability of the test result when the condition is absent. The interval likelihood ratio calculation for 1 specific range (i.e., 4.0–4.5) was done by

dividing the percentage (%) of calves with FTPI by the percentage of calves with STPI that fell in that same range. From the interval likelihood ratio calculation in Table 2.1, we concluded that the likelihood that STP will identify FTPI in calves fed maternal colostrum is high in the ranges from 4.0 to 5.0. In contrast, the likelihood ratios from calved fed CR are considerably low from the ranges of 4.0 to 6.5. This led to the assumption that the probability of identifying FTPI condition in calves fed CR is low; which is in concordance with the correlation between STP and IgG. Overall results suggested that STP refractometry is not suitable for correcting identifying FTPI in calves fed CR. As a result, refractometry should only be used to identify trends and should not be the only assessment tool in a calf-rearing program that feeds a significant amount of CR products.

2.5 Discussion

Although the majority of CR-fed calves (72.74%) had serum IgG values ≥10 g/L, some had an STP below the FTPI threshold of 5.2 g/dL (Calloway et al., 2002). The low STP concentrations could lead to FTPI misinterpretation if producers rely solely on the use of refractometry to determine FTPI. The results indicated that CR-fed calves can achieve successful immunity and serum IgG values above 10 g/L, despite having low STP concentrations. The frequency distributions of STP values for calves with serum IgG concentrations ≥10 g/L, either fed maternal colostrum (n = 888) or CR (n = 915), are presented in Figure 2.2a and b, respectively. Only 14.9% of calves fed maternal colostrum with successful transfer of passive immunity had STP values below 5.2 g/dL (Figure 2.2a), supporting that STP is a good predictor of successful immunity when calves are fed maternal colostrum. In contrast, 41.2% of CR-fed calves had STP protein values <5.2 g/dL (Figure 2.2b), even with successful transfer of passive

immunity, further highlighting that currently established thresholds are inaccurate to predict FTPI in calves that have consumed CR.

The incongruency of STP as an adequate predictor of serum IgG concentration and FTPI has also been discussed by Quigley et al. (2002), Lago et al. (2018), and Lopez et al. (2020). Similar to our study, others have reported that although CR-fed calves had serum IgG concentrations ≥10 g/L, STP was less than 5.0 or 5.2 g/dL (Quigley et al., 2002; Lago et al., 2018; Lopez et al., 2020). Quigley et al. (2002) reported that calves fed CR had a mean STP of 5.0 g/L and serum IgG of 13.6 g/L. Lago et al. (2018) reported that calves fed CR had a mean STP of 5.2 g/dL, despite serum IgG being 19.6 g/L. Similarly, Lopez et al. (2020) reported an STP of 4.5 g/dL, lower than the mean reported in the current study, and serum IgG was 16.9 g/L. The low STP values found in CR-fed calves led Quigley et al. (2002), Lago et al. (2018), and Lopez et al. (2020) to conclude that the STP cutoff point of 5.2 g/dL was inaccurate at predicting FTPI. As a result, Quigley et al. (2002) and Lopez et al. (2020) suggested new STP cutoff points. In concordance with our study, these authors also mentioned that lower cutoff points should be used when calves are fed CR. Quigley et al. (2002) suggested that a STP <4.9 g/dL would be a better predictor of serum IgG concentrations <10 mg/ mL, which is similar to the 4.9 g/dL STP cutoff value determined in our study. Using a ROC analysis between STP and serum IgG, Lopez et al. (2020) found that a STP threshold of 4.2 g/dL (sensitivity = 1.0; specificity = 0.58) at 24 h of life would be a better predictor of FTPI when calves are fed CR products. However, it is important to note that STP cutoff points developed using a specific CR or performance of one CR should not be extrapolated to other CR products (Godden et al., 2009), due to their different manufacturing techniques and nutritional compositions.

The inconsistency of STP by refractometry as a method to adequately identify FTPI in newborn calves is complex (Buczinski et al., 2018) and not completely understood. Research has shown that different CR products have different protein profiles (Priestley et al., 2013; Leeflang, 2014) and fat contents (Lopez et al., 2020) compared with maternal colostrum, which may contribute to the observed inconsistencies. For example, Lago et al. (2018) fed calves whey-derived CR and stated that the low STP values found in blood of CR-fed calves could be attributed to the lesser quantities of protein present in the CR. As a result, they hypothesized that STPI could be achieved at lower refractometry readings when calves were fed CR (Lago et al., 2018).

Colostrum replacers differ widely in composition. They can be derived from blood or serum, egg, and lacteal sources (colostrum, whey, or milk; Quigley, 2004) and their manufacturing varies depending on the fractioning techniques implemented, ratio and type of non-IgG proteins, and amount of lactose (Mee et al., 1996; Arthington et al., 2000a; Quigley et al., 2001). For example, Quigley et al. (2002) used a plasma-derived CR with a composition of IgG concentrate (21.2%), lactose, high fructose corn syrup, and dry fat blend (7% CP, 60% fat), whereas Lopez et al. (2020) used a whey-based CR with a higher IgG concentration of 40.64% that was devoid of fat and casein content. However, for the present study, the CR were all derived from natural bovine colostrum with IgG concentrations of 26, 21, and 14% with protein (48–58%) and fat contents (18, 20, and 23%) not having major variation. These differing compositions could provide some rationale for the low performance of STP as a measure of serum IgG and FTPI when CR is fed.

In addition to CR composition and manufacturing processes, refractometry readings can be affected by additional factors, such as dehydration (Buczinski et al., 2018). In dehydrated calves, blood components and protein contents become more concentrated and, as a result, their total STP is increased and might appear satisfactory (Buczinski et al., 2018). As a consequence, calves could be mistakenly classified as having a successful transfer of passive immunity based on their elevated STP due to dehydration. Also, sick calves (i.e., septicemia or diarrhea) experience systemic inflammatory processes that could elevate their IgG levels, causing elevated serum IgG concentrations (Fecteau et al., 2013). Sick calves could also be wrongly classified as having a successful transfer of passive immunity because the serum refractance will be increased due to the higher presence of inflammatory markers (Fecteau et al., 2013).

Assays to determine IgG that require laboratory work, such as the zinc sulfite turbidity test, γ-glutamyl transferase, whole-blood glutaralehyde coagulation test, sodium sulfate turbidity test, and ELISA, have been widely used to predict serum IgG concentrations (Tyler et al., 1996; Parish et al., 1997; Weaver et al., 2000). However, these tests are not available to most producers that need rapid results (Tyler et al., 1996; Parish et al., 1997; Weaver et al., 2000). Currently, simple on-farm IgG tests that accurately predict FTPI are scarce, inaccurate, or have not been tested thoroughly. Some of the on-farm IgG tests available include semiquantitative antibody test (ZAPvet Bovine IgG test, NOW Diagnostics Toronto, Ontario, Canada), quick IgG test (Plasma Calf IgG Midland Quick Test Kit, Midland Bioproducts Corporation, Boone, IA), and IgG Check Calf IgG Test Kit (PortaCheck, Moorestown, NJ). Elsohaby and Keefe (2015) evaluated the effectiveness of semiquantitative antibody test to detect FTPI, where 202 blood samples were analyzed and compared the FTPI rates obtained with the semiquantitative antibody test versus

RID assay. They reported that it has the potential to be a rapid on-farm test to detect FTPI (sensitivity = 0.82 and specificity = 0.65). However, Renaud et al. (2018a) evaluated the same test in calves arriving at a milk-fed veal facility and stated that this test poorly detected FTPI (sensitivity = 0.77; specificity = 0.44). Another on-farm test that has been evaluated is a commercial quick IgG test. Stilwell and Carvalho (2011) found the Quick Test Kit blood IgG immunoassay accurately detects serum IgG concentrations ≥10 g/L (sensitivity = 0.93; specificity = 0.88); however, to the author's knowledge, this test has not been widely evaluated. Moreover, another alternative that could be used to estimate serum IgG is Brix refractometry (Deelen et al., 2014), as Brix values are highly correlated with IgG (r = 0.93; Deelen et al., 2014). Future studies should evaluate whether a similar low correlation is found between Brix values and IgG when a CR is fed. As producers require simple, quick, and affordable on-farm tools to assess FTPI in calves, it is necessary to develop or validate an accurate on-farm test that directly measures serum IgG, especially when calves are fed CR, as STP is an inadequate predictor of FTPI.

2.6 Conclusions

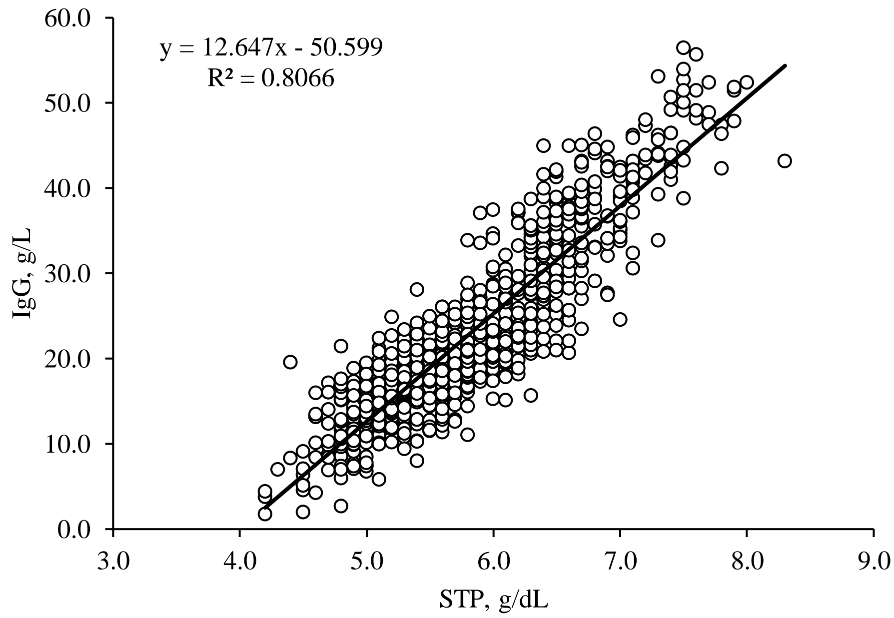
In conclusion, the results of this study demonstrated that even low STP concentrations in CR-fed calves do not correlate well with FTPI, as defined by serum IgG ≥ 10 g/L. Further investigation is needed to clearly develop an alternate STP threshold to define FTPI for CR-fed calves. The discrepancy between STP and serum IgG for FTPI thresholds in CR-fed calves needs to be clarified to correctly assess calf health on-farm and effectively manage calf rearing programs.

Table 2.1 Serum total protein (STP) threshold ranges in newborn calves fed maternal colostrum (n=927) or colostrum replacer (n=1,258): likelihood ratios.

Source	STP	C[1]	FTPI[2]		Success[3]		Likelihood ratio	95% CI	
			#	%	#	%		Lower	Upper
Maternal colostrum	<4.0	0	-	-	-	-	-	-	-
	4.0 – <4.6	6	5	12.82	1	0.11	113.85	0.04	0.08
	4.5 – <5.0	78	26	66.67	52	5.86	11.38	0.30	0.36
	5.0 – <5.6	250	8	20.51	242	27.25	0.75	0.59	0.66
	5.5 – <6.0	259	0	0.00	259	29.17	0.00	0.77	0.83
	6.0 – <6.6	158	0	0.00	158	17.79	0.00	0.89	0.93
	6.5 – <7.0	100	0	0.00	100	11.26	0.00	-	-
	7.0 – 8.0	76	0	0.00	76	8.56	0.00	-	-
Colostrum replacer	<4.0	7	5	0.55	2	0.58	0.94	0.00	0.00
	4.0 – <4.6	82	60	6.56	22	6.41	1.02	0.02	0.04
	4.5 – <5.0	375	168	18.36	207	60.35	0.30	0.22	0.28
	5.0 – <5.6	483	87	9.51	396	115.45	0.08	0.65	0.71
	5.5 – <6.0	237	20	2.19	217	63.27	0.03	0.91	0.94
	6.0 – <6.6	66	3	0.33	63	18.37	0.02	0.98	1.00
	6.5 – <7.0	7	0	0.00	7	2.04	-	-	-
	7.0 – 8.0	1	0	0.00	1	0.29	-	-	-

[1]C=counts
[2]FTPI= defined as a serum IgG concentration < 10 g/L
[3]Success= defined as a serum IgG concentration >10 g/L

a.



b.

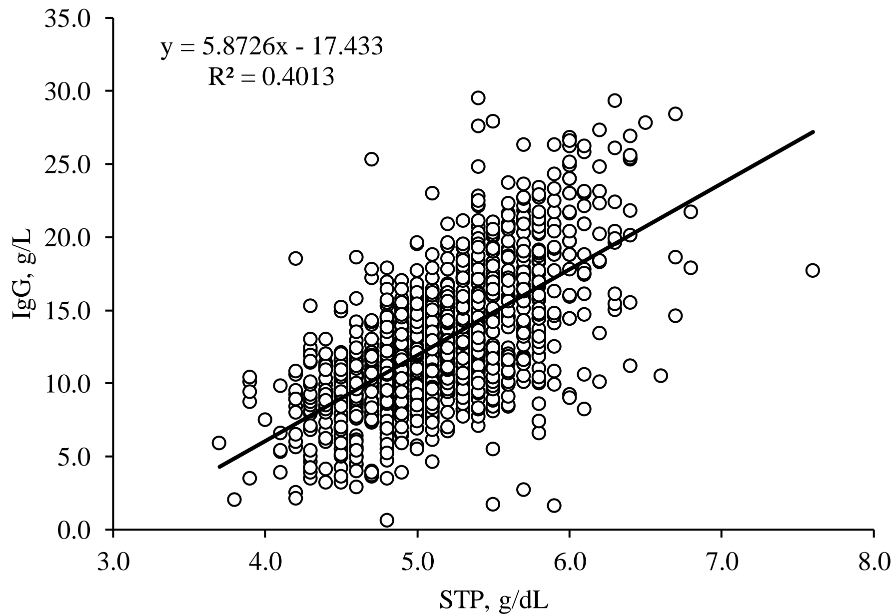
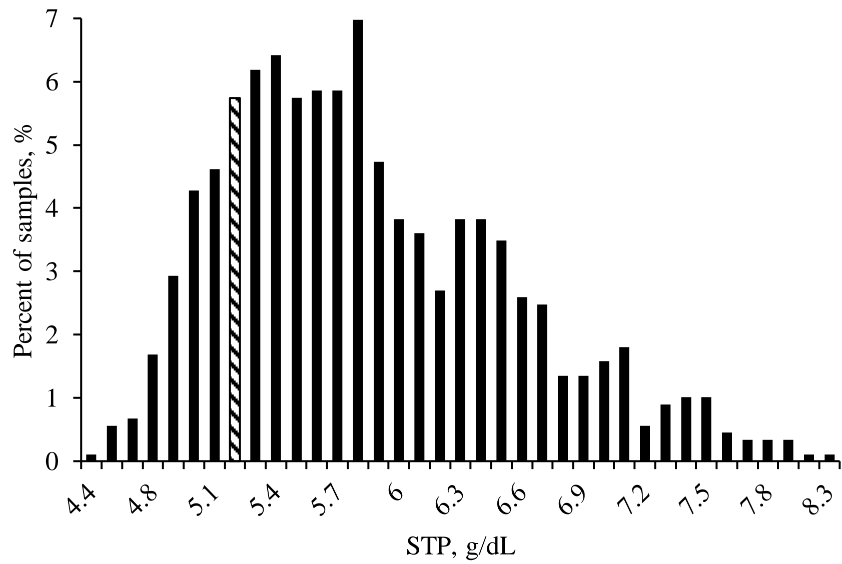


Figure 2.1 Linear regression relationship between serum total protein (STP) and serum immunoglobulin G (IgG) for 927 calves fed maternal colostrum (panel a) or 1,258 calves fed colostrum replacer (panel b). Slope for calves fed CR was 5.87, which is less than the slope for calves fed maternal colostrum (12.65).

a.



b.

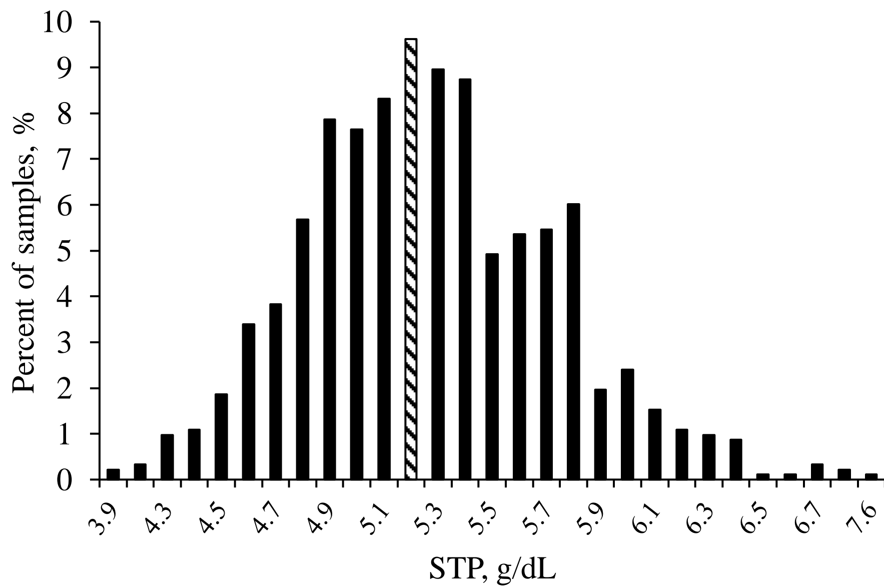


Figure 2.2 Frequency distributions of serum total protein (STP) for maternal colostrum-fed calves (n=888, panel a) or colostrum replacer-fed calves (n=915, panel b) with serum immunoglobulin G (IgG) concentrations >10 g/L (STPI). The dashed column represents a STP value of 5.2 g/dL.

3 Effects of a low- or high-frequency colostrum feeding protocol on immunoglobulin G absorption in newborn calves »

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3.1 Abstract

Calves might experience an upper limit of IgG absorption from colostrum ingestion at birth, but it is not clear whether the total IgG mass fed in the first meal or feeding frequencies can saturate the IgG transport mechanism and therefore limit IgG absorption. The objective of this study was to determine whether different colostrum replacer (CR) feeding frequencies affect serum IgG levels or apparent efficiency of absorption (AEA) in neonatal calves. Male Holstein calves (n = 40) were separated from their dams immediately after parturition and randomly assigned to receive CR [12% of birth body weight (BW)], following either (1) a low- frequency (LF; n = 20) or (2) a high-frequency (HF; n = 20) feeding protocol. Low-frequency calves received 2 CR meals (8% and 4% birth BW within 1 h after birth and 12 h after first CR feeding, respectively), whereas HF calves received 3 CR meals (4% of BW for each meal; within 1 h after birth, 6, and 12 h after first CR feeding). The CR powder fed had a dry matter IgG concentration of 30% and an IgG concentration of 70.5 g/L when reconstituted. All CR was fed via esophageal tube within 1 h after birth. Calves were bottle-fed pasteurized milk (5% birth BW) at 24, 36, and 48 h after the first CR feeding. Blood was collected before first CR feeding and at the following intervals post-CR feeding: every 2 h until 18 h; every 3 h from 18 to 30 h; and every 6 h from 30 to 48 h after the first CR feeding. Serum IgG values at 24 h did not differ between LF and HF (25.79 ± 0.93 and 25.66 ± 0.88 g/L, respectively). In the first meal, calves fed LF ingested a higher total IgG mass than HF (257.98 ± 4.16 g and 126.72 ± 4.05 g, respectively); however, AEA at 24 h did not differ for calves fed HF or LF (27.68 ± 1.16% and 27.63 ± 1.26%, respectively). The IgG area under the curve (AUC) at 24 h was greater for calves fed LF than HF (443.13 ± 15.17 and 379.59 ± 13.99 g of IgG/L × h, respectively).

Additionally, AUC at 6 h, 12 h, and 48 h were greater for calves fed LF than HF. These results indicate that, although LF calves had a greater AUC, HF calves were still able to absorb IgG in the second and third meal, allowing HF calves to achieve serum IgG levels similar to those of LF calves at 24 h. In addition, the provision of 3 meals at 70.5 g/L of IgG within the first 12 h of life did not result in added benefits to serum IgG or AEA levels.

3.2 Introduction

Newborn calves depend on colostrum ingestion to acquire circulating IgG during their first days of life (Lombard et al., 2020). Successful transfer of passive immunity is achieved when calves reach serum IgG concentrations above 10 g/L; in contrast, concentrations <10 g/L are considered as failed transfer of passive immunity (Quigley, 2004; Shivley et al., 2018). To achieve this threshold, it is recommended that calves consume 10% of their birth BW of colostrum at birth (3 to 4 L; Besser et al., 1991), although new reports recommend calves' ingest 150 - 300 g of IgG (Quigley et al., 2002; Godden et al., 2019) within 2 h after birth and achieve transfer of passive immunity. This mass of Ig should be fed within 2 h after birth, as delayed feeding negatively affects IgG absorption (Fischer et al., 2018), but guidelines for the number of colostrum meals and volume of colostrum fed per meal have not been clearly established. Although transfer of passive immunity is achieved at a serum IgG concentration of 10g/L, recent recommendations state that calves should achieve a much higher serum IgG concentration (Lombard et al., 2020). As a result, higher colostral IgG ingestion than current recommendations would need to be delivered in the first colostrum meal.

Although it is known that delaying colostrum feeding by 6 and 12 h reduced IgG absorption by 26% compared with calves fed immediately after birth (Fischer et al., 2018), it is not known how the different number of meals affects transfer of passive immunity or IgG absorption. Notwithstanding, it has been reported that in the United States, small farms (30 to 99 cows) feed ~3.79 L in the first 24 h, and large farms (≥500 cows) feed ~5.67 L or more (NAHMS, 2014). However, this data does not specify the number of meals fed. Jaster (2005) evaluated the effects of feeding one versus two colostrum meals within 12 h to Jerseys calves. The author reported that feeding 2 L of high-quality colostrum (84 g IgG/L) in 2 meals (0 h and 12 h) instead of 1 meal (4 L at 0 h) resulted in higher apparent efficiency of absorption (AEA) and serum IgG levels at 24 h. In contrast, other reports state that Holstein calves fed 1 meal of 4 L (60.1 g IgG/L), followed by a 2-L meal at 12 h, resulted in higher serum IgG levels than two meals of 2 L (Morin et al., 1997). In addition, Abuelo et al. (2021) compared the effects of one versus two meals of colostrum, and, although possible saturation level was not the focus of the study, it was reported that calves fed a second colostrum meal at 5 to 6 h had lower odds of failed transfer of passive immunity than calves fed just 1 meal at birth. Given these findings, multiple colostrum meals may aid in improving successful transfer of passive immunity; however, the effects of multiple meals on absorption of IgG are not well understood.

Total IgG mass is directly related to IgG concentration and increases as greater volumes of colostrum are fed, but AEA simultaneously decreases and is negatively correlated with the amount of IgG fed (Besser et al., 1985; Saldana et al., 2019). This decrease in AEA, when high total masses of IgG are fed at birth, is thought to occur due to the physiological limitation of the intestine to absorb IgG within a determined time (Besser et al., 1985). This phenomenon has also

been defined as “gut saturation” or achieving an upper limit of IgG absorption (Saldana et al., 2019). Besser et al. (1985) proposed 2 possible hypotheses for this upper limit of absorption when high amounts of IgG are fed. First, that shared macromolecular transport mechanisms of IgG are saturated, or, second, that the calf regulates its serum IgG concentrations when a threshold level is met (Besser et al., 1985). In support of the competitive binding hypothesis, Davenport et al. (2000) reported that adding casein to colostrum supplements or maternal colostrum reduced IgG absorption, and Lopez et al. (2020) found that the removal of casein in colostrum replacer (CR) increased AEA. Davenport et al. (2000) discussed that intake of further proteins or casein above a limited macromolecular transport would not be absorbed, and, as a result, circulating IgG in the blood could decrease. In consequence, AEA can be compromised.

Thus, we hypothesized that reducing total IgG mass fed at birth by increasing the number of colostrum meals would increase AEA. Thus, the objective of this study was to evaluate the effects of two different CR feeding frequencies, low and high, by delivering a determined volume according to birth BW in two or three meals. Also, we aimed to evaluate whether an upper limit of IgG absorption exists or whether AEA is decreased when a larger meal is fed at birth.

3.3 Materials and Methods

The animal use protocol was approved by the Animal Care Committee of the University of Guelph (Guelph, ON, Canada; AUP no. 4488). This study was conducted at a commercial dairy farm with a total herd of ~3,500 animals and ~1,600 milking cows in Aylmer, Ontario, Canada. This farm was chosen due to their willingness to participate in the research project and the

availability of a high frequency of calvings over a short period, which would reduce any calf-to-calf variation that may have occurred in different seasons.

3.3.1 Calving and Neonatal Calf Management

Primiparous and multiparous Holstein cows were separated from the close-up pen and moved to a maternity pen approximately one to two days before parturition. Maternity pens were bedded daily with shavings and sanitized between calvings. Cows were monitored during the calving process, and assistance was provided when needed. Male Holstein calves born in the maternity pen were immediately separated from their dams to prevent colostrum suckling. Afterward, calves were thoroughly dried with 3 clean towels, the navel was dipped with 7 % iodine tincture (Iodine Tincture Stronger, Dominion Veterinary Laboratories Ltd.; Winnipeg, Manitoba, Canada), and a navel clamp was placed on the distal aspect of the navel. Calves were weighed with a digital scale (Brecknell Scales, PS1000, Avery Weigh-Tronix, LLC) and moved to individual calf hutches bedded with shavings (Starter Pen, AGRI-6000B, Agri-Plastics). In between calves, pens were cleaned and disinfected with alcohol (70%) and a chlorhexidine solution (Germi-Stat 4%, Germiphene Corporation). Sanitized pens were allowed to dry for 1 d before a new calf was assigned.

3.3.2 Animal Experiment, Colostrum Replacer, and Milk Feeding

Male Holstein calves (n = 40; 20 per treatment) born from November 2020 to December 2020 were enrolled in this experiment. All newborn calves were randomly assigned to treatment to receive CR following either (two meals) a low-frequency (**LF**) or (three meals) a high-frequency (**HF**) feeding protocol. Calves in the LF treatment were offered CR within 1 h after birth (8% of birth BW) and 12 h (4% of birth BW) after the first CR feeding. Meanwhile, the HF

calves received CR within 1 h after birth, and 6 and 12 h after the first CR feeding (4% of birth BW per meal). Regardless of feeding frequency treatment, all calves were fed CR only at a total of 12% of birth BW. The randomization sequence was generated in Microsoft Excel (version 16.16.21, Microsoft Corporation) using the RAND function per block. The CR powder used was derived from bovine dried colostrum, contained IgG at 30% DM, and was reconstituted with water to 70.5 g of IgG/L when fed (Saskatoon Colostrum Company Ltd.). The composition analysis of the CR is presented in Table 3.1. In addition, the Quality Assurance Laboratory from Saskatoon Colostrum Company Ltd. (Saskatoon, SK, Canada) reported that coliforms, Salmonella spp., and Escherichia coli were absent. Reconstitution of CR was performed as follows: (1) weighing CR powder, (2) weighing warm (40°C) water, (3) reconstituting the solution by adding CR powder to a bowl and slowly adding water while mixing with a whisk for approximately 2 min, and (4) weighing the reconstituted solution to verify it matched with the final mass to be fed. After verification, reconstituted CR was transferred to a 4-L esophageal tubing bottle. All CR meals were offered via an esophageal tube, and calves were not allowed to voluntarily consume colostrum. The first CR feeding was fed to all calves 1 h after birth. After the corresponding CR meals, all calves were fed (5% of BW) pasteurized waste milk at 24, 36, and 48 h after the first CR feeding via nipple bottle. Milk was heated to ~39°C in a warm water bath before feeding. Refused milk was weighed and recorded for every feeding. Calves were weighed at 48 h immediately after the last milk feeding when their experimental enrollment concluded.

3.3.3 Blood Sampling

At approximately 10 to 20 min after birth, a jugular catheter was placed, as described by Fischer et al. (2018), to enable frequent blood sampling for 48 h. Before catheter placement, one side of the calf's neck was shaved with clippers (Wahl Basic Pet Clipper Kit, model no. 58108), scrubbed with a chlorhexidine solution (Germi-Stat 4%, Germiphene Corporation), and sprayed with isopropanol (70%). Thereafter, a 2-inch, 16-gauge catheter (Thermo Fisher Scientific) was placed in the jugular vein. As soon as the catheter was placed, a baseline blood sample was taken (0 h). Blood samples (7 mL per collection) were then collected at the following intervals: every 2 h until 18 h; every 3 h from 18 to 30 h; and every 6 h from 30 to 48 h after the first CR feeding. All blood samples that coincided with a CR or milk meal were taken before feeding. Blood samples (7 mL) were transferred to a serum blood collection tube (Vacutainer; Becton, Dickinson and Co.). Immediately after every collection, 7 mL of saline and 1 mL of heparinized saline were flushed back into the catheter. Samples in serum collection tubes were allowed to clot at room temperature for approximately 1 to 2 h before centrifugation. Samples were centrifuged at 3,000 × g at 4°C for 20 min. Serum collected after centrifugation was transferred into three 1.5-mL microcentrifuge tubes in equal aliquots and frozen at −20°C until further analysis.

3.3.4 IgG and Serum Total Protein Analyses

Serum was thawed and re-centrifuged at 3,000 × g for 20 min at 4°C to separate the supernatant, which was then transferred to a new 1.5-mL microcentrifuge tube. Samples were then refrozen at −20°C and shipped overnight on ice to the Saskatoon Colostrum Company Ltd. Quality Assurance Laboratory (Saskatoon, SK, Canada) for IgG and serum total protein

analyses. Serum IgG was determined by radial immunodiffusion, as described by Chelack et al. (1993), with modifications according to Shivley et al. (2018). Serum IgG values were determined for all time points and used to represent IgG dynamics during the first 48 h of life. The AEA formula used was adapted from Quigley and Drewry (1998), Quigley et al. (2002), and Saldana et al. (2019), where:

$$\boldsymbol{AEA} = \frac{\textbf{birth body weight (kg)} \times \mathbf{0.09} \times \textbf{serum IgG} \left(\frac{\mathbf{g}}{\mathbf{L}} \mathbf{24}\boldsymbol{h}\right)}{\textbf{total IgG fed (g)}} \times \mathbf{100}$$

Additional parameters calculated with serum IgG data included the following: time to reach maximum concentration, and maximum concentration reached. Also, the positive incremental area under the curve (AUC) was calculated using the trapezoidal rule (Cardoso et al., 2011) at 6 h (AUC_6), 12 h (AUC_{12}), 24 h (AUC_{24}), and 48 h (AUC_{48}) after the first CR feeding.

3.3.5 Sample Size

An anticipated mean serum IgG concentration was selected from Fischer et al. (2018) due to the similarity of the colostrum feeding methodology. Fischer et al. (2018) reported an average serum IgG value of 22.30 g/L with a standard error of the mean of 1.40 g/L. The standard deviation (SD) was determined to be 4.20 g/L, which was used to calculate a coefficient of variation of 18.83%. Using this value, we calculated the total replicates needed per treatment group using an 80% power and α of 0.05, as outlined by Berndtson (1991). To detect a difference of 20% in serum IgG values compared with the control, 19 animals were needed per group, for a total sample size of 38 calves. To account for 5% mortality, it was then determined that 20 animals per treatment were needed, for a total of 40 newborn calves.

3.3.6 Statistical Analyses

Data were analyzed using SAS (University Edition, SAS Institute Inc.). A total of 40 male Holstein calves were included in the analysis (n = 20 per treatment), but one calf was removed due to an error when reconstituting CR powder and consequently total IgG consumed. The final data set therefore included 19 calves fed LF and 20 calves fed HF. Residual distribution for each variable was assessed for normality (using Shapiro-Wilk as a criterion) and homoscedasticity using the UNIVARIATE and PLOT procedures. Statistical significance was declared at $P \leq 0.05$. All values are reported as mean ± SD. Effects of colostrum treatment on serum IgG values collected over time were analyzed as a repeated measures design using the GLIMMIX procedure. The model contained the fixed effects of treatment, time, and their interaction, and the random effect of calf. Time (hour) was specified as the repeated measure. Non-repeated measurements were analyzed using the MIXED procedure. The model included the fixed effect of treatment, time, and an interaction of treatment and time, and the random effect of calf. Least squares means are presented with the respective standard errors. Differences between least squares means were compared using the SLICE option, and contrast statements were performed for birth BW, BW at 48 h, serum total protein concentrations at 24 h after colostrum feeding, serum IgG concentrations at 24 h after colostrum feeding, AEA, and baseline IgG.

3.4 Results

3.4.1 Body Weight, Colostrum, and Milk Feeding

Birth BW for LF and HF calves did not differ ($P = 0.50$; Table 3.2). Additionally, BW was not different ($P = 0.53$) between treatments at 48 h after colostrum and milk feedings.

Calves fed LF were fed a total IgG mass of 257.9 and 128.9 g in the first (0 h) and second (12 h) CR meals, respectively, whereas HF calves were fed 126.7, 126.7, and 126.7 g at the first (0 h), second (6 h), and third (12 h) CR meals, respectively. Overall, LF and HF were fed a total IgG mass of 386.9 and 380.1 ± 8.1 g ($P = 0.53$), respectively. A milk consumption effect was found for all calves ($P < 0.001$), where calves drank more milk in the second (36 h) and third (48 h) milk feedings than in the first milk feeding (24 h). Although LF calves were fed a higher amount of colostrum at birth (8% of BW) than HF calves (4% of BW), milk consumption in the first milk feeding did not differ ($P = 0.96$).

3.4.2 Effect of Two Feeding Frequencies on IgG Absorption

Baseline serum IgG concentrations at birth were negligible and not different ($P = 0.78$; Table 3.2) between LF and HF calves. Feeding colostrum twice versus 3 times did not affect serum IgG values at 24 h ($P = 0.92$; Figure 3.1; Table 3.2). According to serum IgG categories specified by Lombard et al. (2020), 55.56 and 44.44% of LF calves were in the good (18.00 to 24.90 g/L) and excellent (≥ 25.00 g/L) categories, respectively, whereas 38.10% and 61.90% of HF calves were in the good and excellent categories, respectively. The percentages of calves in either excellent or good category were not different between LF or HF calves ($P > 0.05$). None of the calves fed either LF or HF were classified as having poor (<10.00 g/L) or fair (10.00 to 17.90 g/L) transfer of passive immunity. Additionally, serum total protein values at 24 h were not affected ($P = 0.92$; Table 3.2) by feeding LF or HF colostrum treatments (Table 3.2). We found a treatment by time effect ($P = 0.003$) on serum IgG concentrations throughout the experimental period (Figure 3.1). However, simple sliced effects demonstrated mean differences only in serum IgG between treatments at 8 h ($P < 0.01$; Figure 3.1).

Apparent efficiency of absorption at 24 h did not differ ($P = 0.97$) between LF and HF calves (Table 3.2). In addition, calves fed either LF or HF did not have a different maximum concentration ($P = 0.11$; Table 3.2). Similarly, time to reach maximum concentration for LF and HF did not differ ($P = 0.44$; Table 3.2). Calves fed LF had a higher ($P < 0.01$) AUC_6 than HF calves (Table 3.2). Similarly, LF calves had a higher ($P < 0.01$) AUC_{12} than HF calves (Table 2). The same scenario was observed for AUC_{24}, where LF calves had a higher value ($P < 0.01$) than HF calves (Table 3.2). In addition, LF calves had a higher AUC_{48} ($P < 0.01$) than HF calves (Table 3.2). Overall, it seems that HF calves were able to absorb enough IgG from meals offered at 6 and 12 h and equalize serum IgG concentrations at 24 h compared with calves fed LF, even though it is known that IgG absorption decreases as time after birth increases (Stott et al., 1979a).

3.5 Discussion

We hypothesized that increasing CR feeding frequency from two to three meals would increase serum IgG concentrations at 24 h and AEA. This possible improvement in IgG absorption could be related to a more efficient IgG absorption when feeding lower masses of IgG compared with higher IgG masses in a certain period of time that could surpass an upper limit of absorption (Besser et al., 1985; Saldana et al., 2019). Nevertheless, results from this study show that serum IgG and AEA levels were not affected by feeding newborn calves either two or three CR meals postnatally. This might indicate that two or three colostrum meals already reduce the upper limit of IgG absorption a calf might experience compared with when they are fed one large meal at birth.

Apparent efficiency of absorption of colostral IgG seems to decrease when large colostrum meals or high masses of IgG are fed at birth (Saldana et al., 2019), but this was not seen in the current study. It has to be considered that we did not have a treatment where calves were fed only one meal at birth, which limits some comparisons with other studies. Saldana et al. (2019) showed that calves fed a single meal at birth using a lower total IgG mass [IgG concentration of 52.3 g/L (198.74 g of IgG fed)] from maternal colostrum had higher AEA (37.3%) than calves fed colostrum with an IgG concentration of 98.1 g/L (372.78 g of IgG fed; AEA: 20.8%). Even though all calves in the present study were fed colostrum to a total of 12% of their BW in their first 12 h of life, as recommended by Godden et al. (2019), our results indicate that calves fed a decreased total IgG mass in the first meal (4% of BW instead of 8% of BW) did not have an increased AEA. These results disagree with findings from Saldana et al. (2019) and Lopez et al. (2020), who reported decreased AEA when higher doses of IgG were fed. However, it has to be considered that total IgG masses fed by Saldana et al. (2019) in a single meal at 0 h (351.5 g of IgG) were higher than meals in our study. Furthermore, Lopez et al. (2020) also found that calves fed a single meal, with a lower IgG mass of 154 g, within 2 h after birth had an increased AEA (54.4%) compared with calves fed a higher IgG dose of 401 g, which had a lower AEA of 24.4%. In both Saldana et al. (2019) and Lopez et al. (2020), it is important to note that the reported reduction in AEA occurred when calves were fed a higher total IgG mass at birth, delivering a high amount of total IgG (> 300g). This might indicate that a higher IgG mass needs to be fed in a single meal to induce a possible upper limit of IgG absorption.

In addition to the studies performed by Saldana et al. (2019) and Lopez et al. (2020), which reported IgG absorption when calves were fed only a single colostrum meal after birth, other

studies have also researched colostrum feeding frequencies involving more than one meal (Morin et al., 1997; Jaster, 2005; Cabral et al., 2012, 2014). Morin et al. (1997) evaluated feeding one or two colostrum meals. Morin et al. (1997) studied the effects of feeding 4 L at birth or 4 L at birth followed by a 2-L meal of maternal colostrum with an IgG concentration of 60.1 g/L at 12 h. The authors reported that feeding two meals (4 L at birth plus 2 L at 12 h) resulted in higher serum IgG concentrations. The current study, however, did not compared feeding two or three meals against one single meal, where possible reductions of IgG absorption have been observed (Morin et al., 1997; Saldana et al., 2019). Similar to the two feeding frequencies studied by Morin et al. (1997), Cabral et al. (2012) compared the effects of feeding CR at 0 h (191.4 g of IgG fed) or at 0 and 6 h (127.6 and 63.8 g of IgG fed, respectively) to newborn Holstein calves. Cabral et al. (2012) reported that serum IgG was not different for calves fed once or twice (15.8 and 16.8 g/L, respectively). In addition, they did not find an increase in AEA when calves were fed twice (32.9%) compared with calves fed once (32.1%). Their results contrast the findings of Morin et al. (1997) but are in agreement with our results, where decreasing total IgG fed at birth by increasing number of meals did not affect IgG absorption. Cabral et al. (2012) discussed that the 6-h meal did not significantly contribute to additional IgG absorption due to decreased or depleted pinocytotic activity, which is responsible for macromolecular transport of IgG after the lumen has initial contact with colostrum at 0 h (Stott et al. 1979b). However, our AUC values indicate that calves fed a lower IgG mass at birth, followed by two more colostrum meals (6 and 12 h) were able to achieve the same IgG levels as calves fed higher IgG doses at birth. These findings suggest that meals offered at 6 and 12 h for HF calves contributed to additional IgG absorption. In a follow-up study, Cabral et al. (2014) reevaluated the effects of feeding colostrum

once versus twice in the first 6 h of life. They compared feeding CR at 0 h (184.5 g of IgG fed) or at 0 and 6 h (123.0 and 61.5 g of IgG fed, respectively). Again, they did not find differences in serum IgG concentrations, AEA, nor AUC at 24 h for calves fed once or twice. In contrast to Cabral et al. (2012) and Cabral et al. (2014), we found that calves fed a higher mass of IgG at birth (LF) had a higher AUC throughout the whole sampling period (6, 12, 24, and 48 h). Nevertheless, even though calves fed LF (colostrum fed at 0 and 12 h) had a consistently greater AUC than HF calves (colostrum fed at 0, 6, and 12 h) up to 24 h, serum IgG values at 24 h were not different. It was not directly measured in this study, but the higher AUC for LF fed calves could imply that the rate of absorption was higher during the first hours of life due to the greater total mass fed compared with HF calves: 257.9 and 128.9 g, respectively. Although AUC was higher for LF calves, serum IgG concentrations at 24 h did not differ. This suggests that HF calves were still able to effectively absorb the IgG fed at 6 and 12 h and still attain the serum IgG concentrations of LF calves, which were fed a higher total IgG mass at birth. From these observations, it could be suggested that calves still absorb IgG efficiently at 6 and 12 h, as they reach sufficient levels of IgG to achieve successful transfer of passive immunity, even when high-quality colostrum feeding is delayed to 6 or 12 h after birth (Fischer et al., 2018). Hare et al. (2020) also found that calves fed prolonged colostrum meals after a first meal at birth (every 12 h from 12 until 72 h) had increased serum IgG concentrations and demonstrated an apparent IgG persistency of 88.2%. From these experiments studying feeding regimens, only Jaster (2005) fed a high dose of IgG at birth (>300 g) and consequently found a response in IgG uptake and AEA. Jaster (2005) evaluated feeding 4 L of high-quality maternal colostrum (84 g/L of IgG) once (0 h; 336 g of IgG fed) or feeding 2 L at 0 h (168 g of IgG fed) and 2 L at 12 h (168 g of IgG fed) to

newborn Jersey calves. They reported that serum IgG concentrations at 24 h were higher for calves fed 2 L twice (45.8 g/L) rather than feeding one meal of 4 L (39.7 g/L). In addition, AEA at 48 h was increased when calves were fed two separate meals of 2-L meals (31.2%) instead of a single 4-L meal of high-quality colostrum (24.6%). Overall, providing a second colostrum meal to newborn calves seems to contribute to circulating serum IgG concentrations at 24 h.

Jaster (2005) fed a considerably higher total IgG mass (336 g) to calves in a single meal compared with the doses fed by Cabral et al. (2012; 191.4 g), Cabral et al. (2014; 184.5 g), and our study (128.9 g). This leads us to speculate that high doses of IgG are needed to decrease AEA and IgG absorption. Saldana et al. (2019) and Lopez et al. (2020) reported decreased AEA when high total IgG masses, above 300 g, were fed in a single meal after birth. In contrast, the present study only fed calves either 258 or 126.7 g of IgG at birth. Besser et al. (1985) noted that newborns have a physiological limit to the amount of Ig they can absorb from a given volume of colostrum. Moreover, Saldana et al. (2019) suggested that a decrease in AEA is due to an upper limit of absorption in a given period. Although total IgG mass fed is a limiting factor, total volume fed is also an important consideration. Stott and Fellah (1983) reported that calves absorb IgG more efficiently when a determined total IgG mass is fed in 1 L rather than 2 L of colostrum. Specifically, Stott and Fellah (1983) stated that 1 L of colostrum with an IgG concentration of 100 g/L is better absorbed than 2 L of colostrum with an IgG concentration of 50 g/L. Moreover, Jaster (2005) also discussed how large volumes of colostrum are absorbed less efficiently.

Colostrum replacer AEA varies between different products due to different manufacturing techniques (Godden et al., 2009; Lopez et al., 2020). The AEA from maternal colostrum can

range from 20 to 35% (Jones and Heinrichs, 2006); however, these ranges vary, with Halleran et al. (2017) reporting a range from 7.7 to 59.9% in dairy heifers fed a standardized meal, whereas Quigley et al. (2019) and Lopez et al. (2020) reported AEA ranges from 15.0 up to 40.1% for calves fed CR products. The reasons why AEA varies between calves remain unclear, but colostrum composition can be a factor. For example, the removal of casein and fat in CR resulted in AEA values of 40 and 41% (Lopez et al., 2020), whereas high doses (> 500 g) of casein added to colostrum affected colostral Ig absorption (Davenport et al., 2000). Finally, other factors, such as maternal colostrum pasteurization and the presence of bioactive hormones (e.g., IGF-1), have been shown to positively and negatively affect AEA (Gelsinger et al., 2014, 2015b; Hammon et al., 2020; Pyo et al., 2020). Although CR composition is variable, in this study we used dried bovine colostrum derived from whole colostrum; thus, we anticipate our results would be similar to calves fed maternal colostrum.

Although we did not find any benefit to feeding 3 meals of colostrum, none of the calves in either of the two feeding regimens in this study had failed transfer of passive immunity or were categorized as having poor (<10 g/L) or fair (10.0 to 17.9 g/L) IgG levels, as proposed by Lombard et al. (2020). This demonstrates that neither of these feeding frequencies negatively affected serum IgG levels or compromised transfer of passive immunity. Therefore, increasing colostrum feeding frequencies in the first 12 h of life when feeding volumes according to industry standards, > 10% of birth BW, with colostrum containing an IgG concentration of 70.5 g/L, does not result in added benefits, such as increased serum IgG concentrations at 24 h. This underlines that colostrum management does not require dairy farmers to offer 3 colostrum meals in the first 12 h of life.

3.6 Conclusions

These results indicate that additional meals of CR with a 70.5 g/L IgG concentration do not result in added benefits to serum IgG concentrations or AEA levels. Feeding newborn calves a total IgG mass of 258 g in the single first feeding does not appear to influence a possible upper limit of IgG absorption or decrease AEA; thus, a higher IgG mass fed at birth is needed to negatively affect AEA or IgG absorption. These results indicate that feeding additional meals of high-quality colostrum do not result in added benefits to serum IgG or AEA levels.

Table 3.1 Composition analysis of colostrum replacer (CR) used to feed calves assigned to either LF or HF feeding frequency treatments.

Test Description	Specification	Result[1]
IgG, % (Min)	30	30
Crude Protein, % (Min)	50	60.7
Crude fat, % (Min)	14	14.3
Lactose, %	N/A	8.0
Moisture, % (Max)	7.0	6.4

[1]Composition analyses were performed by the Quality Assurance Laboratory from the Saskatoon Colostrum Company Ltd. (SCCL; Saskatoon, SK, Canada).

Table 3.2 Parameters (mean ± SEM) in newborn calves fed low frequency (LF; n = 19) or high frequency (HF; n = 20) colostrum replacer within the first 12h post-first CR feeding.

Item	Treatment[1]		SEM	Contrast
	LF	HF		*P*-value
BW at birth, kg	46.5	45.6	0.96	0.50
BW at 48 h, kg	48.1	47.3	0.95	0.53
Baseline serum IgG, g/L	0.8	0.5	1.27	0.78
IgG_{24},[2] g/L	25.8	25.7	1.28	0.92
STP_{24},[2] g/dL	6.3	6.3	0.26	0.92
AEA, %	27.6	27.7	1.26	0.97
FPTI[3], %	0	0	-	-
IgG C_{max}, g/L	29.8	27.7	0.91	0.11
IgG T_{max}, h	21.0	22.0	0.94	0.43
AUC_6	36.5	25.1	2.60	0.003
AUC_{12}	145.2	105.9	5.91	<0.01
AUC_{24}	443.1	379.6	15.17	0.004
AUC_{48}	997.8	866.8	33.5	0.008

[1]LF = colostrum replacer fed (total of 12% birth BW) in 2 meals (8 and 4% birth BW; within 1h after birth and 12 h post-first CR feeding, respectively) and HF = colostrum replacer fed (total of 12% BW) in 3 meals (4% BW; within 1 h after birth, and 6, and 12 h post-first CR feeding).

[2] IgG_{24} = serum IgG concentrations at 24 h after colostrum feeding, STP_{24} = serum total protein concentrations at 24 h after colostrum feeding, AEA = apparent efficiency of absorption calculated as= [(birth weight (kg) × 0.09 × serum IgG (g/L 24 h))/total IgG fed (g)] × 100, IgG C_{max}= maximum concentration, IgG T_{max}= time to reach maximum concentration, and AUC_6, AUC_{12}, AUC_{24}, AUC_{48} = incremental area under the curve during the first 6, 12, 24, and 48 h after colostrum feeding, respectively.

[3]FPTI= failure of passive immunity (serum IgG_{24} < 10 g/L)

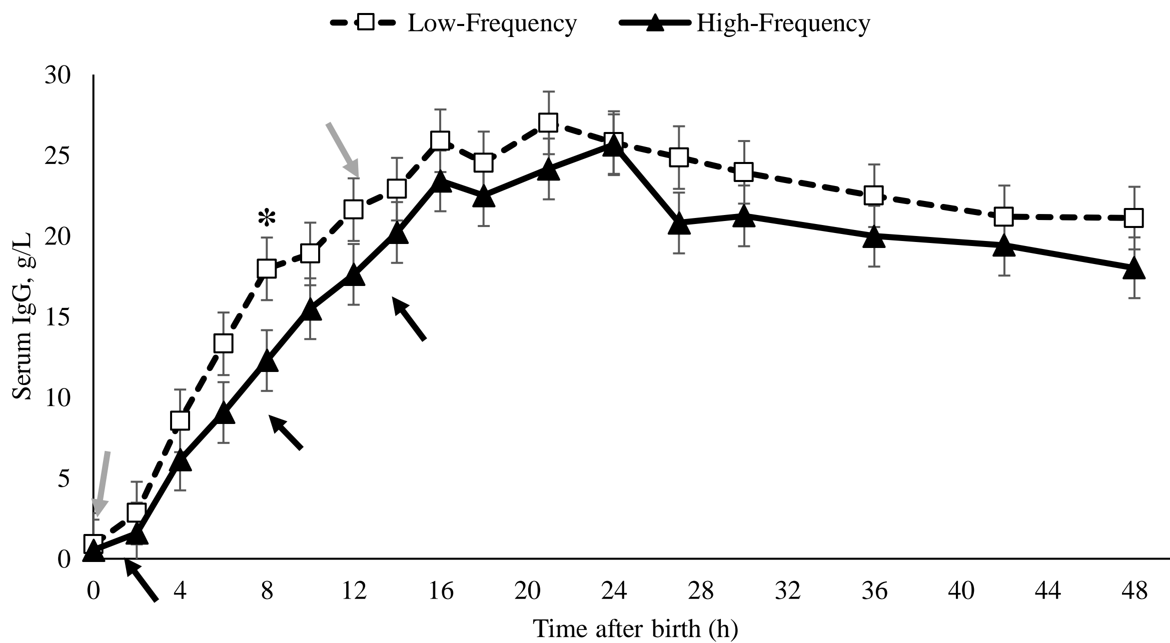

Figure 3.1 Serum IgG concentrations (mean ± SEM) for calves fed low frequency (LF; within 1 h after birth and 12 h post-first CR feeding) or high frequency (HF; within 1 h after birth, 6, and 12 h post-first CR feeding). Grey arrows represent colostrum meals fed within 1h after birth (8% BW) and 12 h post-first CR feeding (4% BW) for calves assigned to LF. Black arrows represent colostrum meals fed within 1 h after birth (4% BW), 6 (4% BW), and 12 h post-first CR feeding (4% BW) for calves assigned to HF. Asterisks indicate differences between treatments at a given time ($P < 0.01$)

4 Effects of reducing total solids in colostrum replacer with different dilutions on IgG absorption in newborn Holstein calves

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4.1 Abstract

Newborn calves are required to consume immunoglobulin G (**IgG**) at birth, but its absorption depends on various factors, such as abomasal emptying rate and total solids (**TS**) of the meal. Thus, the objective of this study was to determine if reducing TS and osmolality of colostrum replacer (**CR**) by increased dilution has any effect on colostral IgG absorption, abomasal emptying rates or gut permeability in calves. Newborn male Holstein calves (n = 48; 12/treatment) with birth body weights (**BW**) of 40-52 kg were randomly assigned to be fed 949 g (150 g IgG) of CR with different final volumes (**FL**) within 1 h after birth. The TS (g/L) were equally spaced and treatments were defined as a dose-response with 4 levels: **Control** = 1.8 kg water, FL: 2.6 L, 360 g/L CR; Dilution 1 (**D-1**) = 2.1 kg water, FL: 3.0 L, 320 g/L CR; Dilution 2 (**D-2**) = 2.6 kg water, FL: 3.4 L, 280 g/L CR; or Dilution 3 (**D-3**) = 3.1 kg water, FL: 4.0 L, 240 g/L CR. Half of the calves enrolled (n = 24; 6/treatment) were randomly assigned to have a catheter placed at birth to allow frequent blood sampling at 1, 2, 3, 4, 5, 6, 8, 10, 12, 24, 36, and 48 h relative to initial colostrum feeding to estimate abomasal emptying rates using acetaminophen. In addition, this subset of calves was administered a mixture of lactulose, D-mannitol, and Cr-EDTA at 24 h via esophageal feeder immediately after the 24 h milk replacer feeding; and blood sampled at 26, 28, 30, and 32 h relative to CR feeding to measure gut permeability. Unless otherwise stated, the results for all measurements are presented in the following order: Control, D-1, D-2, and D-3. The osmolality for the treatment meals was significantly reduced by adding more water: 835.1, 714.8, 514.0, and 410.3 ± 23.32 mOsm/kg. Serum IgG concentrations at 24 h tended to linearly increase with reduced TS levels: 15.5, 12.2, 17.3, and 18.4 ± 1.75 g/L. In addition, the apparent efficiency of absorption (AEA) tended to

increase with decreasing TS: 43.9, 35.1, 49.5, and 51.2 ± 4.77 %. Also, the abomasal emptying rates linearly increased with reduced TS: 0.09, 0.11, 0.13, and 0.13 ± 0.017. No differences between treatment groups were observed in lactulose, D-mannitol, and Cr-EDTA concentrations during the gut permeability sampling period. Lastly, no differences were observed in abnormal fecal scores or respiratory scores until d 7 of life for calves fed the different dilutions. These results suggest that the decrease of TS may benefit IgG absorption.

4.2 Introduction

Newborn calves need to absorb immunoglobulins, specifically immunoglobulin G (IgG), to achieve successful transfer of passive immunity (STPI; Shivley et al., 2018) allowing them to better combat disease (Godden et al., 2019). In general, STPI is defined when calves have a serum IgG concentration equal or more than10 g/L at 24 h after birth (Shivley et a., 2018). Ruminant neonates absorb IgG via colostrum at birth (Davis and Drackley, 1998); however, its absorption efficiency depends on multiple factors, including colostrum quality (IgG concentration; Weaver et al., 2000), volume fed (Besser et al., 1985; Saldana et al., 2019), feeding quickness (Fischer et al., 2018), and bacterial contamination (Johnson et al., 2007; Gelsinger et al., 2015b; Malik et al., 2022). In addition, other factors, such as abomasal emptying rate (kAB) and colostrum osmolality, have been shown to have an effect on colostral IgG absorption. The mechanisms by which high osmolality (> 300 mOsm/kg; Lopez et al., 2023) in colostrum meals affects IgG absorption are unknown. It has been reported that an increased osmolality can slow down kAB (Constable et al., 2009); therefore, IgG absorption may be affected due to the delay at which IgG appears in the small intestine (Cabral et al., 2014).

Osmolality has been shown to varying tremendously depending on what is being fed to calves. Specifically, values of 300, 330, 325 to 410, and 278 mOsm/kg have been reported for CR (Cabral et al., 2014), bovine maternal colostrum (Quigley et al., 2019; Lopez et al., 2023), milk replacer (MR; Constable et al., 2005), and mature bovine milk (Constable et al., 2005), respectively. However, these values will vary as final osmolality from CR or MR will depend on the dilution used. Data from oral rehydration solutions in monogastric animals report that hypertonic solutions (> 300 mOsm/L) decrease kAB in comparison to isotonic solutions and that profound inhibition of kAB occurs when osmolality is greater than 600 mOsm/kg (Seigel et al., 1982; Sen et al., 2006). However, the osmolality level in which colostral IgG absorption starts to be compromised is unknown. More recently, Cabral et al. (2014) reported that colostral IgG absorption is decreased when osmolality of CR is increased from 300 to 516 mOsm/kg. These results suggest that hypertonic solutions affect how IgG is absorbed. Thus, we hypothesized that diluting CR with additional water would decrease total solid percentage and osmolality, thereby, increasing IgG absorption and gastric abomasal emptying. The primary objective of this study was to determine if the reduced TS by increased dilutions had any effect on colostral IgG absorption and abomasal emptying rates in newborn calves. Secondary objective was to evaluate the effects of high TS and osmolality on gut permeability at 24 h.

4.3 Materials and Methods

All animal experimental procedures and protocols were executed under the Canadian Council of Animal Care (CCAC; Olfert et al., 1993), and were approved by the Animal Care Committee of the University of Guelph (AUP #4497). The current project was performed at a commercial dairy farm located in Aylmer, Ontario, Canada. At the time of study execution, the

farm had approximately 1,600 milking cows and a total herd of ~3,000 animals. This project was conducted at this farm due to an existing relationship between the University of Guelph, our research group, and the farm. In addition, this commercial farm has proper animal facilities to conduct research and a high number of calvings, which allowed us to enroll multiple calves in a short period of time (July to September 2022), reducing calf-to-calf variation throughout different seasons.

4.3.1 Parturition and Calving Procedures

Newborn calves enrolled in this experiment were born from primi- (n= 4) and multiparous (n = 44) Holstein cows. The pregnant cows on this farm were housed in close-up pens and were transferred to maternity pens when calving signs were obvious or the calving process had started. Cows were constantly monitored during the calving process and research staff was noticed immediately after a calf was born. All twins and heifer calves were excluded from the experiment.

4.3.2 Animal Enrollment

Holstein male calves born from July to September 2022 were immediately separated from their dam to prevent any MC consumption. After birth, newborns were weighed using a digital scale (Global Industrial® Digital Floor Scale, Global Equipment Company Inc.; Richmond Hill, Ontario, Canada). Only animals with a birth body weight (BW_{0h}) of 40 to 52 kg were enrolled (n = 48). This BW range was calculated using previous BW data from animals born on the same farm (Lopez et al., 2023). After birth BW recording, a vigor score based on Villettaz Robichaud et al. (2017) was performed on all calves. Then, the calves were thoroughly dried and their navels were dipped in 7 % iodine tincture (Iodine Tincture Stronger, Dominion Veterinary

Laboratories Ltd.; Winnipeg, Manitoba, Canada) to prevent further contamination. Thereafter, the subset of calves (n = 24) had a catheter placed, and then all calves (n = 48) were fed their respective CR treatment, depending on their random allocation. Then, calves were moved to individual hutches (L: 100cm W: 64cm H: 105.5cm; Starter Pen: AGRI-6000B, Agri-Plastics, Young Street, Grassie, ON, Canada) deeply bedded with sawdust. Throughout this period, wet bedding and feces were removed and new sawdust was added as needed. Calves remained in these hutches for 48 h and then were moved to a calf-rearing facility (pen dimensions; L: 215cm W: 86cm H: 97cm). After a calf was moved at 48h, hutches were washed and disinfected with isopropyl alcohol (70%) and chlorhexidine (Germi-Stat 4%, Germiphene Corporation, Brantford, ON, Canada), and dried with clean towels before a new calf was placed in the hutch. The sanitized hutches were allowed to dry for 1 d before a new calf was allocated in.

4.3.3 Experiment Design, Colostrum Replacer Meals, and Milk Replacer Feeding

The whole bovine dried CR (15.8% IgG; Table 4.1) used was donated by the Saskatoon Colostrum Company Ltd. (Saskatoon, SK, Canada; SCCL) and all CR used came from the same batch. The composition and description of the experimental CR meals fed to all calves is presented in Table 4.2. Colostrum replacer samples were sent to Lactanet Canada (Guelph, Ontario, Canada) for analysis by near-infrared refractance (NIR) spectroscopy (Gagnon et al., 2020). Brix was measured with a digital refractometer (PAL-1 Refractometer, Atago) and osmolality was determined using an osmometer (The Advanced TM Micro Osmometer Model 3300, Advanced Instruments Inc. Norwood, Massachusetts, USA). Calves were randomly assigned to be fed one of four distinct CR replacer meals. The amount of CR powder fed to all calves was 949 g (150 g IgG) but the volume of water used to reconstitute the CR differed.

However, the control treatment was based on the mixing instructions of the manufacturer in terms of grams of powder mixed per litre of water. The TS were the baseline parameters for all treatments fed to calves and were equally spaced. As result, treatments were defined as a dose-response with 4 levels of TS: **Control** = 1.791 kg water, final volume: 2.64 L, TS: 360 g/L CR; Dilution 1 (**D-1**) = 2.083 kg water, final volume: 2.97 L, TS: 320 g/L CR, Dilution 2 (**D-2**) = 2.574 kg water, final volume: 3.39 L, TS: 280 g/L CR, or Dilution 3 (**D-3**) = 3.110 kg water, final volume: 3.96 L, TS: 240 g/L CR within 1 h after birth. The detailed composition of all treatments fed is presented in Table 4.2. The randomized allocation for treatment feeding was generated using Microsoft Excel (version 16.16.21; Microsoft Corporation, Redmond, WA) using the RAND function.

All CR meals were fed using a clean esophageal feeder (Handi Grip™ 4-Qt. Feeder and Storage Bottle with Storage Cap & Plastic Esophageal Probe Assembly, Spectrum Educational Supplies Limited; Newmarket, ON, Canada). Besides CR feeding, calves were offered 3.0 L (150 g/L) of MR (26 % protein, 18% fat, and 41% lactose; Grober Nutrition, Cambridge, ON, Canada). Milk replacer meals were offered via nipple bottle at 12, 24, 36, and 48 h relative to CR feeding. In addition, from 3 to 7 d, 3.0 L of MR were offered twice daily at 0700h and 1600h. At each feeding, MR refusals were recorded to calculate milk intake. Lastly, all calves were weighed after the 48 h CR meal and on day 7 after the 0700h meal.

4.3.4 Acetaminophen Feeding, Abomasal Emptying, and Gut Permeability Test

A subset of calves (n = 24; 6 per treatment) was randomly assigned to be fed acetaminophen (A5000, Sigma-Aldrich; MilliporeSigma Canada Ltd; Oakville, ON, Canada) using the RAND and INDEX functions from Excel. This marker was mixed with the CR meal

and fed at a dose of 150 mg/kg $BW^{0.75}$ to estimate abomasal emptying rates per hour (kABh) based on the kinetics of its appearance in blood after absorption in the small intestine (Schaer et al., 2005). The acetaminophen marker was selected, as its absorption is directly related to abomasal emptying (Burgstaller et al., 2017) and it has been used to estimate abomasal emptying rates in calves by MacPherson et al. (2016). The subset of calves fed acetaminophen were also catheterized at birth to enable frequent blood sampling for abomasal emptying calculations. For these calculations, plasma samples collected at different time points were analyzed for acetaminophen concentrations using a Paracetamol Assay Kit-K8002 (Cambridge Life Sciences Ltd., Cambridgeshire, UK), as per Welboren et al. (2021).

At 24 h post-colostrum feeding, and immediately after MR feeding, the same subset of calves assigned to be fed acetaminophen were also assigned to be dosed with a mixture of lactulose, D-mannitol, and Chromium EDTA (Cr-EDTA) to estimate gut permeability. Lactulose was dosed at a rate of 0.2 g/kg birth BW, D-mannitol at 0.12 g/kg birth BW, and Cr-EDTA at birth 0.1 g/kg birth BW (Mellors et al., 2023). All samples were analyzed by a Thermo Vanquish™ Duo, tandem UHPLC system coupled to TSQ Altis™, triple quadrupole mass spectrometer (Thermo Fisher Scientific). Samples were stored in an autosampler at 10 °C and 1.5 µL was injected onto one of two Poroshell 120 HILIC-Z (PEEK lined 100 × 2.1mm; 2.7µm) maintained at 45°C with a flow rate of 450 µL min-1. The overall method was 5.1 min, including equilibration time, which takes place on one column, while analytes are being resolved on the second column. Mobile phase A (10 mM ammonium formate, 90% acetonitrile; Optima LC-MS Grade, Sigma-Aldrich, Oakville, Ontario, Canada) was held at 100% for 0.5 min. Mobile phase B (10 mM H2O; Optima LC-MS Grade, Sigma-Aldrich, Oakville, Ontario, Canada) was then

increased to 50% over 2.5 min. The flow rate and mobile phase B composition were immediately altered to 300 μL min^{-1} and 70% and held for 1.75 min before B was returned to 0% over 0.25 min. While analytes were being resolved on one column, the second, identical column was re-equilibrated at 300 μL min^{-1} mobile phase A in preparation for the subsequent injection. The OptaMax NG H-ESI source was operated in a negative ionization mode source with a capillary voltage of 3.8 kV, ion transfer tube temperature of 315 °C, and vaporizer temperature of 400 °C. The sheath, auxiliary, and sweep gases were set to 47, 15, and 1 arbitrary unit respectively. D-mannitol (Sigma-Aldrich, Oakville, Ontario, Canada), lactulose (Sigma-Aldrich, Oakville, Ontario, Canada), and their corresponding internal standards (13C6 mannitol, Cambridge Isotope Laboratories, Inc. and 13C12 lactulose, Omicron Biochemicals Inc., Tewksbury, MA, US) were monitored. Quantification was performed in Thermo TraceFinder 5.0. The extraction recoveries (Re%) were determined by spiking an equal amount of internal standard (0.3 µg) before and after the protein precipitation and extraction step. The Re% in plasma was 103% and 104% for lactulose and D-mannitol, respectively. The method detection limit for both analytes in serum was 30 ng mL-^{1}.

4.3.5 Blood Sampling

The amount and timing of blood samples depended on whether animals were catheterized or not. Catheterized calves were assigned to be dosed with acetaminophen to estimate abomasal emptying and administered lactulose/D-mannitol/Cr-EDTA markers to measure gut permeability, as described by Mellors et al. (2023). Calves without a catheter were sampled via jugular venipuncture (7 mL for plasma and 7 mL for serum) with a 20-gauge × 2.5 cm needle (Greiner Bio-One International GmbH; Kremsmünster, Austria) at: birth (0h, baseline), 12, 24,

36, and 48 h relative to colostrum feeding, and daily from 3 d to 7 d after MR feeding in the morning. Calves with a jugular catheter were sampled at: birth (0 h; baseline), 1, 2, 3, 4, 5, 6, 8, 10, 12, 24, 36, and 48 h relative to colostrum feeding. At 48 h the catheter was removed and blood samples were collected daily from 3 d to 7 d via jugular venipuncture after MR feeding in the morning. The lactulose/D-mannitol/Cr-EDTA mix was fed using an esophageal feeder immediately after the 24 h MR feeding; thereafter, samples to measure gut permeability were taken at: 26, 28, 30, and 32 h. All blood samples were collected with two syringes (7mL of blood each) through an extension set attached to the catheter and then transferred to serum (Vacutainer; Becton, Dickinson, and Co., Franklin Lakes, NJ) and plasma/sodium heparin collection tubes (Vacutainer; Becton, Dickinson and Co., Franklin Lakes, NJ). Immediately after every 14 mL blood collection from calves, 14 mL of saline was flushed back via the extension set. In addition, 1 mL of heparinized saline was flushed back to prevent blood coagulation. After collection, samples in serum collection tubes were allowed to clot for approximately 20 min at room temperature. For plasma collection tubes, 3.5 µl of aprotinin was added and then stored at 4°C until centrifugation. Collection tubes were centrifuged at 3,000 x *g* at 4°C for 20 min. Finally, serum and plasma collected after separation were transferred into 1.5 mL microcentrifuge tubes and stored at −20°C until further analysis.

4.3.6 IgG and Serum Total Protein Analyses

Frozen serum samples were packaged with ice packs and shipped overnight to SCCL Quality Assurance Laboratory (Saskatoon, SK, Canada) for IgG and serum total protein (STP) analyses. Serum IgG was determined by a radial immunodiffusion (RID) assay, which was previously described by Chelack et al. (1993) and Shivley et al. (2018). Serum IgG

concentrations at 24 h post-colostrum feeding (IgG_{24h}) were used to determine apparent efficiency of absorption (AEA) using the following formula adapted from Quigley and Drewry (1998), Quigley et al. (2002), and Saldana et al. (2019):

$$AEA = \frac{\textbf{birth body weight (kg)} \times \mathbf{0.09} \times \textbf{serum IgG} \left(\frac{\mathbf{g}}{\mathbf{L}} \mathbf{24}\boldsymbol{h}\right)}{\textbf{total IgG fed (g)}} \times \mathbf{100}$$

In addition, the positive incremental area under the curve (AUC) was calculated using the trapezoidal rule (Cardoso et al., 2011) at 24 h ($IgGAUC_{24}$) and 48 h ($IgGAUC_{48}$) post-colostrum feeding and at day 7 of life ($IgGAUC_{d7}$).

4.3.7 Health Scoring

All calves had a health score assessment once daily from birth until day 7 of life. Health score data included overall respiratory and fecal scores. The scoring included fecal consistency to determine if osmolality had any effect on scours/diarrhea, and respiratory scoring to detect signs of respiratory disease. The fecal scoring system used was: 0 = normal, firm not hard; 1 = soft, piles and spreads slightly, does not hold form; 2 = runny, spreads easily; and 3 = watery, devoid of any solid material, splatters (Renaud et al. 2020b). An abnormal fecal score was classified as a calf that had a fecal score equal or more than 2. The respiratory score assessment was done following the University of California-Davis scoring system: eye discharge (2 points), abnormal nasal discharge (4 points), spontaneous or induced cough (2 points), rapid or laboured respiration rate (2 points), elevated rectal temperature (≥ 39.5°C; 2 points), and the presence of

droopy ears or head tilt (5 points). All these scores were added to get a total respiratory score, where a score of ≥ 5 indicated respiratory disease (Love et al., 2014).

4.3.8 Sample Size

The sample size was determined following Berndtson et al. (1991) guidelines. Our main variable of interest was serum IgG, using a selected serum IgG value of reference from Fischer et al. (2018a) due to the similarity of the colostrum feeding methodology. The selected serum IgG value was 22.3 g/L with an SEM of 1.40 g/L. The standard deviation was calculated using the SEM and resulting in a standard deviation of 4.87 and coefficient of variation (CV) of 18.01%. Using those values, Table 2 from Berndtson et al. (1991) was used to calculate the total replicates needed per treatment group for experiments of 80% power with an alpha of 0.05. The number of replicates needed to detect a difference of 25% in serum IgG values compared to the control was 12 animals per group. As a result, we utilized 12 animals per treatment group in order to have a total sample size of 48.

4.3.9 Statistical Analyses

All data were analyzed using SAS (University Edition; SAS Institute Inc., Cary, NC). The final data included blood parameters, body weights, respiratory and fecal scores, colostrum, and milk intake from a total of 48 calves (n = 12 / treatment). Residual distribution for each variable was assessed for normality using the Shapiro-Wilk test as a criterion and homoscedasticity using the UNIVARIATE and PLOT procedures. Linear and quadratic regressions were performed to analyze the relationship between the four CR treatment groups and the studied variables using the PROC MIXED. All values are reported as least square means using the GLIMMIX procedure. Tukey's adjustment means were used for variables that were

significant for the dose-response analysis, either linear or quadratic. Serum IgG, acetaminophen, lactulose, D-mannitol, and Cr-EDTA concentrations were analyzed as repeated measures using the GLIMMIX procedure. This model included the fixed effects of treatment, the interaction of treatment and time, and the random effect of calf. In addition, covariance structures were used to account for uneven or even sampling collection timepoints. The best fit model was determined by selecting the model with smaller AICC. For the abomasal emptying analysis, the NLMIXED procedure was used for each individual calf to estimate their kABh according to given constants and their acetaminophen concentrations during the 48 h sampling period. Thereafter, the GLIMMIX procedure was used to calculate the kABh for each treatment. All calculations for kABh were based of Schaer et al. (2005) procedures. Respiratory and fecal score data were analyzed as repeated measures using the GENMOD and GLIMMIX procedures. Statistical significance was declared at $P \leq 0.05$, and tendencies toward significance were declared when $0.05 < P \leq 0.10$.

4.4 Results

4.4.1 Colostrum Replacer and Experimental Meals Composition

The certificate of analysis of the bovine-dried CR used in this study is presented in Table 4.1. The main component of interest was IgG concentration, in which a total of 949 g of CR powder was included in all meals to ensure all calves were fed a total IgG mass of 150 g. The final composition of all CR dilution treatments, including fat, protein, and lactose, is presented in Table 4.2. The results from Table 4.2 demonstrate that osmolality was reduced for D-1, D-2, and D-3, where it was greatest for Control and lowest for D-3 ($P < 0.01$). Also, the addition of water

significantly decreased the Brix content of all diluted meals, which was greatest for Control and lowest for D-3 ($P < 0.01$).

4.4.2 Body Weight, Vigor Score, Milk Replacer Feeding, and Water Intake

Birth BW (BW_{0h}, Table 4.3) and lactation from the dam at calving (Table 4.3) did not differ among different treatment groups ($P > 0.10$). Similarly, the vigor score did not differ ($P = 0.57$) for calves enrolled in Control, D-1, D-2, and D-3: 4.7, 3.6, 4.8, and 4.3 ± 0.67; respectively. Body weight at 48 h relative to colostrum feeding (BW_{48h}) and BW at d 7 of life (BW_{7d}) did not differ between treatments (Table 4.3; $P > 0.10$). Similarly, the average daily gain (ADG) at 48 h relative to colostrum feeding (ADG_{48h}) and at d 7 (ADG_{7d}) did not differ in relation to TS levels (Table 4.3; $P > 0.10$). For MR intake, there was no interaction between treatment and feeding timepoint effect ($P = 0.98$). Average consumption for 12, 24, 36, and 48 h MR meals for all calves were 2.2, 2.3, 2.1, and 2.5 ± 0.12 L, respectively ($P = 0.12$). After 48 h relative to colostrum feeding, or from d 3 to d 7, calves were transported to another facility but remained under an experimental methodology that included blood sampling, milk feeding, and *ad libitum* water access. From d 3 to d 7, calves were offered 3.0 L of MR at 0700 and 1600 h and daily consumption was not different ($P = 0.68$) for the treatment and day interaction. Overall, total daily MR consumption for d 3, 4, 5, 6, and 7 was 5.5, 5.9, 5.9, 5.7, and 5.7 ± 0.15 L ($P < 0.05$). In addition, water consumption was not different for the interaction of treatment and feeding time at 24, 36, and 48 h relative to CR feeding ($P = 0.54$). However, overall water consumption for all calves was higher at 48 h compared to 36 h (0.4 and 0.2 L, respectively; $P = 0.02$).

4.4.3 Serum Total Protein, Serum IgG, Apparent Efficiency of Absorption, and Area Under the Curve

Serum total protein (STP) concentrations at birth (STP_{0h}) and at 24 h relative to colostrum feeding (STP_{24h}) did not differ depending on treatment group (Table 4.3; $P > 0.05$). Baseline serum IgG concentrations collected at birth (IgG_{0h}) did not differ between treatments (Table 4.3; $P > 0.05$). Serum IgG levels at 24 h relative to colostrum feeding (IgG_{24h}) tended to have a linear increase with D1, D2 and D3 relative to the C group (Table 4.3; $P = 0.10$). In addition, serum IgG concentration at d 7 (IgG_{7d}) linearly increased with D1, D2 and D3 ($P = 0.01$). Overall, we did not observe an interaction of treatment and time between serum IgG concentrations from birth until 48 h post-colostrum feeding ($P = 0.24$; Figure 4.1a). Similarly, the interaction for treatment and time for serum IgG concentrations from birth until day 7 of life was not significant ($P = 0.22$; Figure 4.1b). Serum IgG maximum concentrations (IgG C_{max}) tended to linearly increase with D1, D2 and D3 ($P = 0.07$; Table 4.3). In contrast, the time to reach serum IgG concentrations did not differ ($P = 0.11$; Table 4.3). It was found that AEA tended to linearly increase with D1, D2 and D3 ($P = 0.09$; Figure 4.1c). In addition to serum IgG and AEA, we also evaluated the IgG area under the curve (AUC) at 24 h ($IgGAUC_{24}$) and 48 h ($IgGAUC_{48}$) post-colostrum feeding and at day 7 ($IgGAUC_{d7}$). We found that $IgGAUC_{24}$ tended to linearly increase with D1, D2 and D3 ($P = 0.08$; Table 4.3) but $IgGAUC_{48}$, and $IgGAUC_{d7}$ linearly increased when calves were fed a CR meal with D1, D2 and D3 ($P = 0.02$, $P = 0.02$; respectively).

4.4.4 Abomasal Emptying and Gut Permeability Test

Besides analyzing plasma acetaminophen concentrations to estimate abomasal emptying rate, plasma samples were used to create acetaminophen concentration dynamics from birth (0 h)

until 48 h relative to colostrum feeding (Figure 4.2). In general, we did not find a treatment and time effect (*P* = 0.63) on acetaminophen concentrations throughout the 48 h blood sampling period (Figure 4.2a). However, we found a linear increase of kABh for calves fed the CR meals D1, D2 and D3 (*P* = 0.03; Figure 4.2b). Lactulose, D-mannitol, and Cr-EDTA concentrations after performing the gut permeability test at 24 h relative to colostrum feeding and right after MR feeding were used to elaborate concentration dynamics in Figure 4.3a, b, and c. We did not find an interaction for treatment and time for lactulose (*P* = 0.51; Figure 4.3a), D-mannitol (*P* = 0.66; Figure 4.3b), and Cr-EDTA concentrations. (Figure 4.3c; *P* = 0.54). These markers were also analyzed for Tmax and Cmax; and area under the curve (**AUC**) at 26, 28, 30, and 32 h post-colostrum feeding (2, 4, 6, and 8 h post-dosage; Table 4.4). The Cr-EDTA Tmax (**CrTmax**) and D-mannitol Tmax (**MTmax**) showed a linear decrease (*P* = 0.01 and *P* = 0.03, respectively; Table 4.4). There were no linear nor quadratic effects for AUC at 26, 28, 30, and 32 h post-colostrum feeding for Cr-EDTA, lactulose, and D-mannitol concentrations (*P* > 0.05; Table 4.4).

4.4.5 Health Score

We did not find any differences among treatments in the presence of an abnormal fecal score (2 or 3; *P* = 0.28), the first day to have an abnormal fecal score (*P* = 0.20), and days to max abnormal fecal score (*P* = 0.21). In addition, the total days with an abnormal fecal score (*P* = 0.69) for calves fed Control, D-1, D-2, and D-3 were not different: 1.3, 1.0, 0.8, 1.1 ± 0.31, respectively. Similarly, we did not find differences in respiratory score parameters. The day of life when the highest respiratory score was reached did not differ (*P* = 0.37) between treatment groups: Control, D-1, D-2, and D-3: 2.6, 2.7, 3.8, and 3.3 ± 0.54, respectively. Also, the total amount of days with a respiratory score ≥ 5 was not significant for calves fed Control, D-1, D-2,

and D-3: 0.5, 0.6, 0.7, and 0.4 ± 0.21, respectively. In general, we did not find any differences in respiratory and fecal scores for the first 7 days of life for calves fed the different CR dilution treatments.

4.5 Discussion

Previous research has investigated different levels of TS and osmolality in MR or oral rehydration solutions and its effects on the calf's gut health (Azevedo et al., 2016; Amado et al., 2019; Wilms et al., 2019). In regard to maternal colostrum or CR, there is limited research investigating meals with high or low TS or osmolality and its effect on IgG absorption, gut health, water intake, and fecal scoring (Cabral et al., 2014) in newborn dairy calves. To the author's knowledge, no previous studies have investigated the effects of decreasing the percentage of TS and osmolality by diluting CR meals with additional water. As a result, we hypothesized that diluting CR meals with additional water would decrease the percentage of total solids and osmolality, thereby, increasing IgG absorption and leading to a faster gastric abomasal emptying. The findings of this study suggest that decreasing the percentage of TS or osmolality in CR may benefit IgG absorption, however, volume differences could be a confounding factor in the study design. In addition, one hypertonic meal does not seem to impact the incidence of abnormal fecal scores up to 7 d of life.

The growth parameters were not different between CR meals. These measurements were not expected to be affected directly by experimental treatments unless a hypertonic CR meal induced diarrhea event over several consecutive days, as a high osmolality can lead to diarrhea in calves (Muller et al., 1974). Fecal scores were not different across treatments. It has been

reported that calves exposed to hypertonic milk meals could experience nutritional diarrhea (Hof, 1980). This nutritional diarrhea could be linked to the intestine's limited capacity to absorb water when a meal is highly hypertonic (Glosson et al., 2015). It has to be considered that in our study newborn calves were only exposed to one single hypertonic meal at birth. Some concern arises when calves are continuously exposed to feeding regimens such as MR feeding twice a day.

Early research by Muller et al. (1974) reported that calves fed 3.6 kg of colostrum once daily until weaning at 4 wk of age tended to have a greater incidence of days with scours (2) in comparison to calves fed whole milk (TS: 12.5%). Colostrum fed by Muller et al. (1974) had a TS of 13.7%, which is even lower than TS for calves fed D-3 in this study. Azevedo et al. (2016) found that increasing TS up to 20.4 % (533 mOsm/L; osmolarity) of whole milk by adding MR increased body frame measurements pre- and post-weaning without compromising solid feed intake. Overall, results in this suggest that one hypertonic CR fed at birth does not affect the calf's fecal score, respiratory score, water intake, or MR intake during the first 7 d of life.

Similar to growth parameters, milk replacer and water intake were not affected by treatment meals. In general, we would expect calves getting a dense or high TS/osmolality colostrum meal would consume more water, which could be the result from dehydration as a consequence of an increased fecal score which is an indicator of diarrhea (Renaud et al., 2020b). However, no differences in water intake were found. All CR treatment meals in this experiment had an osmolality $\geq$ 300 mOsm/kg and would be classified as slightly hypertonic (> 300 mOsm/kg; Amado et al., 2019) and others as highly hypertonic ($\geq$ 450 mOsm/kg; Wilms et al. 2019). As a result, for this Control, D-1, and D-2 would be classified as highly hypertonic and D-3 as slightly hypertonic.

Data showing the effects of TS and osmolality on IgG absorption is limited. Lopez et al. (2023) showed that colostral IgG absorption was compromised when osmolality increased from 331 to 612.5 mOsm/kg. As a result, we expected to see an increase in serum IgG_{24h} concentrations and AEA with a reduction of osmolality, which was decreased with reduced TS levels. We did not find any treatment by time differences in serum IgG concentrations from birth until d 7 of life. However, we found that serum IgG_{24h} and serum IgG_{7d} tended to increase when TS decreased. Nevertheless, it remains unclear why D-1 had a decreased IgG absorption than Control even though its osmolality was lower. It is possible that having a treatment with lower TS or osmolality around 300 mOsm/kg could have improved the interpretation of our results. It has to be considered that Lopez et al. (2023) increased TS percentage from 22.7% (227 g/L) to 29.4 % (294 g/L) and saw a decrease in AEA and in serum IgG_{24h} concentrations. The TS percentages increments by Lopez et al. (2023) also coincided with an osmolality increase from 331 to 612.5 mOsm/kg by adding bovine-dried CR (620 g) to maternal colostrum. In that experiment, there were various components contributing to the increase in total solids percentage as a solute (CR powder) was being added to maternal colostrum. In contrast, the TS changes in this study occurred by the addition of water. Similar to Lopez et al. (2023), Cabral et al. (2014) found that IgG absorption was reduced by increasing osmolality from 301 to 516, however, their experiment design aimed to evaluate pH changes by adding $NaHCO_3$. More research is needed to elucidate the effects of osmolality on IgG absorption.

Besides the possible effects of osmolality on IgG absorption, we did not find relevant effects on gut permeability markers. It was only found that CrTmax and MTmax linearly decreased with reduced TS. However, we did not find an effect on LTmax or any other

parameters for lactulose dosage. This delay in Tmax for both markers could be related to the decreased kABh found in the treatments with higher TS and osmolality. An increased absorption of large molecules, lactulose or Cr-EDTA, suggest a diminished intestinal barrier function, which results from increased paracellular permeability in the gut (Wilms et al., 2019; Welboren et al., 2021). Usually, this could happen when the intestine is exposed to hypertonic meals that disrupt intercellular tight junctions (Ussing, 1966, 1969). However, this effect of permeability was not found in this study. Even though we saw effects on CrTmax, we can observe in Figure 3a, b, and c that lactulose, D-mannitol, and Cr-EDTA postprandial concentrations did not differ between CR meals offered. Differences in length of exposure to hypertonic meals could also explain these differences, as Wilms et al. (2019) offered a hypertonic meal of CR for consecutive days, whereas, in this study calves were only exposed to one hypertonic meal of CR.

It is known that hypertonic milk replacer meals can decrease abomasal emptying rate (Sen et al., 2006). Data shows a linear increase of kABh as TS decreased for calves fed Control, D-1, D-2, or D-3. Nevertheless, these results could also be interpreted as increased kABh with higher TS and osmolality levels. These results align with Lopez et al. (2023), Constable et al. (2009), and Sen et al. (2006), where authors found that kABh decreased with increasing TS or osmolality in various solutions offered to calves at different ages. In addition, Mokhber-Dezfooli et al. (2012) reported that abomasal emptying rate can explain 22% of the variation in AEA. Overall, these results suggest that one hypertonic meal offered at birth might not impact gut permeability in the first 24 h after birth. Also, reducing TS and osmolality could benefit colostral IgG absorption, although further research is needed to clarify its mechanisms and address the confounding factor of volume, as calves enrolled in this experiment were fed different volumes.

4.6 Conclusions

Data from this experiment indicate that reducing osmolality of CR could improve IgG absorption. In addition, these results suggest that offering one hypertonic colostrum meal to newborn calves at birth might not interfere with gut permeability or contribute to abnormal fecal score incidences. However, more research is needed to clarify which specific factors or components affect IgG absorption when TS and osmolality are reduced in colostrum meals. In addition, further experiments including lower or higher TS levels are needed.

Table 4.1 Certificate of analysis of colostrum replacer used for experimental meals fed to newborn calves (n = 48).

Item	Specification	Result[1]
Immunoglobulin G (IgG), %	≥ 14	15.8
Crude Protein (min), %	40	47.1
Crude Fat (min), %	10	16.4
Moisture (max), %	7.0	6.2
Total viable aerobic count, CFU/g	$\leq 5 \times 10^5$	9.5×10^4
Pathogens coliforms	Absent	Absent
Salmonella sp.	Absent	Absent

[1]Results and certificate of analysis from the bovine dried colostrum replacer used for all experimental meals were provided by the Saskatoon Colostrum Company Ltd. (SCCL) Quality Assurance Laboratory (Saskatoon, SK, Canada)

Table 4.2 Composition analysis and reconstitution description of colostrum replacer treatments fed to calves.

Item	Treatment[1]				SEM	*P*-value
	C	D-1	D-2	D-3		
Fat, %	5.2[a]	4.7[ab]	4.0[bc]	3.6[c]	0.16	< 0.01
Protein, %	14.9[a]	13.7[ab]	13.0[ab]	11.6[b]	0.48	0.03
Lactose, %	8.4[a]	8.4[a]	6.0[b]	5.2[b]	0.28	< 0.01
Total Solids, %	31.4[a]	29.9[ab]	26.0[bc]	23.3[c]	0.92	0.01
Osm[2], mOsm/kg	835.1[a]	714.8[b]	514.0[c]	410.3[d]	23.32	< 0.01
Brix, %	33.7[a]	30.2[b]	26.2[c]	22.9[d]	0.21	< 0.01
IgG, g/L	56.88	50.56	44.24	37.92	-	-

[1]C1 = control (949 g colostrum replacer / 150 g IgG); D-1: dilution 1 (949 g colostrum replacer / 150 g IgG); D-2: dilution 2 (949 g colostrum replacer / 150 g IgG); D-3: dilution 3 (949 g colostrum replacer / 150 g IgG). All colostrum replacer used was from the Saskatoon Colostrum Company Ltd (SCCL) and had an IgG concentration of 15.8 % IgG on a DM basis. The reconstitution and mixing for all treatments were performed with the use of warm water at 40 °C.
All composition analyses, except for osmolality and Brix, were performed by Lactanet (Guelph, ON, Canada).
[2]Osmolality (Osm): measured with the use of an osmometer (The AdvancedTM Micro Osmometer Model 3300, Advanced Instruments Inc. Norwood, Massachusetts, USA).
[a-d] Values within colostrum replacer treatments with different superscripts are different ($P < 0.05$) after accounting for multiple comparisons by Tukey's adjustment.

Table 4.3 Body weight and blood parameters of newborn calves fed colostrum treatments (n = 80; 16/ treatment).

Item	Treatment[1]				SEM	*P*-value	
	C	D-1	D-2	D-3		Linear	Quadratic
BW_{0h},[2] kg	46.6	47.9	47.1	46.0	1.09	0.57	0.25
BW_{48h},[2] kg	49.3	49.5	49.1	48.5	1.08	0.57	0.73
BW_{7d},[2] kg	52.0	52.5	52.3	51.2	1.08	0.59	0.44
Lactation,[2] #	1.1	1.7	1.1	1.4	0.23	0.73	0.62
ADG_{48h},[2] g	1,341	775	970	1,291	201.15	0.96	0.03
ADG_{7d},[2] g	772.6	655.9	747.6	752.4	70.40	0.92	0.39
IgG_{0h}, [3] g/L	0.4	0.6	0.7	0.5	0.16	0.60	0.12
IgG_{24h}, [3] g/L	15.5	12.2	17.3	18.4	1.75	0.10	0.21
IgG_{7d}, [3] g/L	8.7	8.1	10.5	11.9	1.10	0.01	0.33
IgG C_{max},[3] g/L	16.7	13.9	19.2	19.3	1.54	0.07	0.37
IgG T_{max},[3] h	13.8	14.0	15.8	17.8	1.91	0.11	0.63
$IgGAUC_{24}$	191.6	156.7	225.4	221.2	19.3	0.08	0.39
$IgGAUC_{48}$	139.4	130.1	184.1	180.4	18.5	0.02	0.87
$IgGAUC_{d7}$	9.3[ab]	8.2[b]	10.8[ab]	12.6[a]	1.17	0.02	0.19
STP_{0h},[3] g/dL	4.4	4.4	4.4	4.3	0.09	0.20	0.64
STP_{24h},[3] g/dL	5.0	4.8	5.3	5.0	0.15	0.54	0.87

[1] **Control** = 1.791 kg water, final volume: 2.64 L, 360 g/L CR; Dilution 1 (**D-1**) = 2.083 kg water, final volume: 2.97 L, 320 g/L CR, Dilution 2 (**D-2**) = 2.574 kg water, final volume: 3.39 L, 280 g/L CR, or Dilution 3 (**D-3**) = 3.110 kg water, final volume: 3.96 L, 240 g/L CR within 1 h after birth. All TRT meals were reconstituted using 949 g of CR (150 g IgG). All colostrum replacer used was from the Saskatoon Colostrum Company Ltd (SCCL) and had an IgG concentration of 15.8 % IgG on DM basis.

[2] BW_{0h}= body weight at birth (0 h); BW_{48h}= body weight at 48 h after colostrum feeding; BW_{7d}= body weight at 7 d, Lactation= number of dam lactation at calving; ADG_{48h}= average daily gain at 48 h post-colostrum feeding; ADG_{7d}= average daily fain at day 7 of life.

[3] IgG_{0h}= baseline serum IgG concentrations at birth (0h); IgG_{24h}= serum IgG concentrations at 24 h post-colostrum feeding; IgG_{7d}= serum IgG concentrations at 7 d; STP_{0h}= serum total protein at birth (0h); STP_{24h} = serum total protein concentrations at 24 h after colostrum feeding; IgG C_{max}= serum IgG maximum concentration, IgG T_{max}= time to reach maximum IgG concentration.

Table 4.4 Lactulose, D-mannitol, and Cr-EDTA parameters evaluated during the gut permeability test (n = 80; 16/ treatment).

Item	Treatment[1]				SEM	*P*-value	
	C	D-1	D-2	D-3		Linear	Quadratic
CrTmax,[2] h	7.6	7.3	7.3	5.7	0.47	0.01	0.15
CrCmax,[2] ug/mL	1.3	1.3	1.5	1.4	0.22	0.67	0.81
$CrAUC_{26}$[2]	0.4	0.5	0.6	0.6	0.11	0.20	0.51
$CrAUC_{28}$[2]	1.8	1.6	2.1	2.3	0.31	0.15	0.50
$CrAUC_{30}$[2]	3.6	3.4	4.3	3.9	0.74	0.61	0.85
$CrAUC_{32}$[2]	5.3	4.9	6.0	4.6	1.00	0.79	0.54
LTmax,[3] h	7.5	6.4	7.2	6.3	0.77	0.41	0.91
LCmax,[3] ug/mL	33.4	31.7	26.7	35.5	3.2	0.75	0.09
$LAUC_{26}$[3]	13.5	14.9	15.0	17.0	2.56	0.31	0.90
$LAUC_{28}$[3]	23.3	24.2	24.2	31.1	4.09	0.21	0.45
$LAUC_{30}$[3]	29.8	29.3	24.3	34.1	3.09	0.54	0.10
$LAUC_{32}$[3]	33.3	29.3	25.9	30.4	2.91	0.44	0.11
MTmax,[4] h	8.0	8.0	7.6	7.0	0.37	0.03	<0.01
MCmax,[4] ug/mL	44.4	58.4	50.6	50.0	8.3	0.90	<0.01
$MAUC_{26}$[4]	8.2	10.1	11.7	10.1	1.62	0.25	0.22
$MAUC_{28}$[4]	22.0	19.4	29.6	24.2	5.21	0.37	0.55
$MAUC_{30}$[4]	35.1	47.0	45.1	41.1	7.86	0.65	0.30
$MAUC_{32}$[4]	44.4	52.6	50.2	30.9	8.28	0.24	0.08

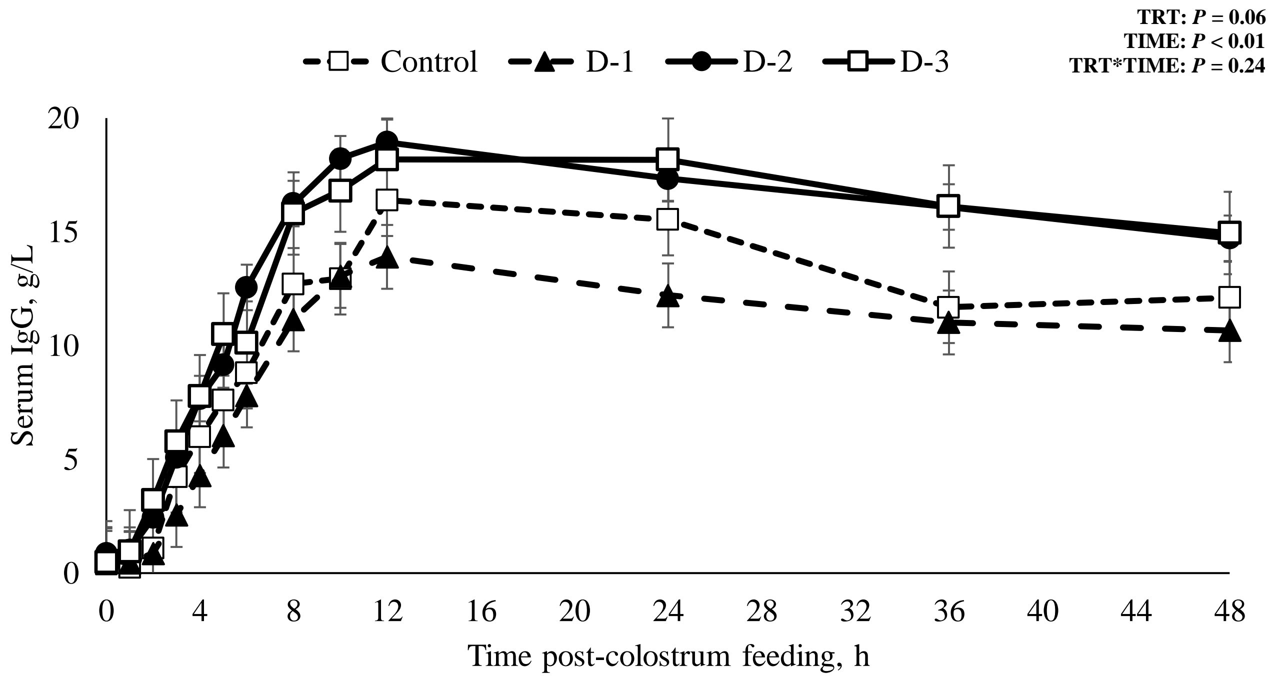
[1] **Control** = 1.791 kg water, final volume: 2.64 L, 360 g/L CR; Dilution 1 (**D-1**) = 2.083 kg water, final volume: 2.97 L, 320 g/L CR, Dilution 2 (**D-2**) = 2.574 kg water, final volume: 3.39 L, 280 g/L CR, or Dilution 3 (**D-3**) = 3.110 kg water, final volume: 3.96 L, 240 g/L CR within 1 h after birth. All TRT meals were reconstituted using 949 g of CR (150 g IgG). All colostrum replacer used was from the Saskatoon Colostrum Company Ltd (SCCL) and had an IgG concentration of 15.8 % IgG on a DM basis.

[2]CrTmax= time to reach maximum plasma Cr-EDTA concentration; CrCmax= plasma Cr-EDTA maximum concentration; $CrAUC_{26}$, $CrAUC_{28}$, $CrAUC_{30}$, and $CrAUC_{32}$ = Cr-EDTA area under the curve at 26, 28, 30, and 32 h post-colostrum feeding. Cr-EDTA was dosed at 24 h post-colostrum feeding after MR feeding with an esophageal feeder.

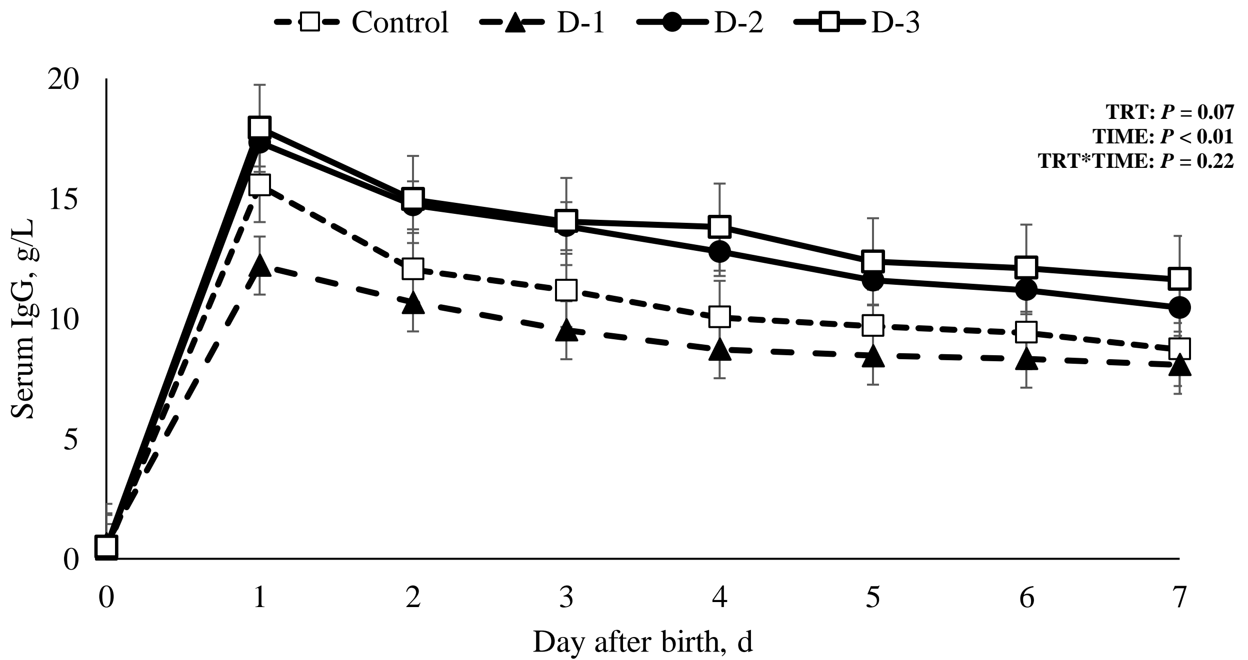
[3]LTmax= time to reach maximum plasma Lactulose concentration; LCmax= plasma Lactulose maximum concentration; $LAUC_{26}$, $LAUC_{28}$, $LAUC_{30}$, and $LAUC_{32}$ = Lactulose area under the curve at 26, 28, 30, and 32 h post-colostrum feeding. Lactulose was dosed at 24 h post-colostrum feeding after MR feeding with an esophageal feeder.

[4]MTmax= time to reach maximum plasma D-mannitol concentration; MCmax= plasma D-mannitol maximum concentration; $MAUC_{26}$, $MAUC_{28}$, $MAUC_{30}$, and $MAUC_{32}$ = D-mannitol area under the curve at 26, 28, 30, and 32 h post-colostrum feeding. D-mannitol was dosed at 24 h post-colostrum feeding after MR feeding with an esophageal feeder.

a.



b.



c.

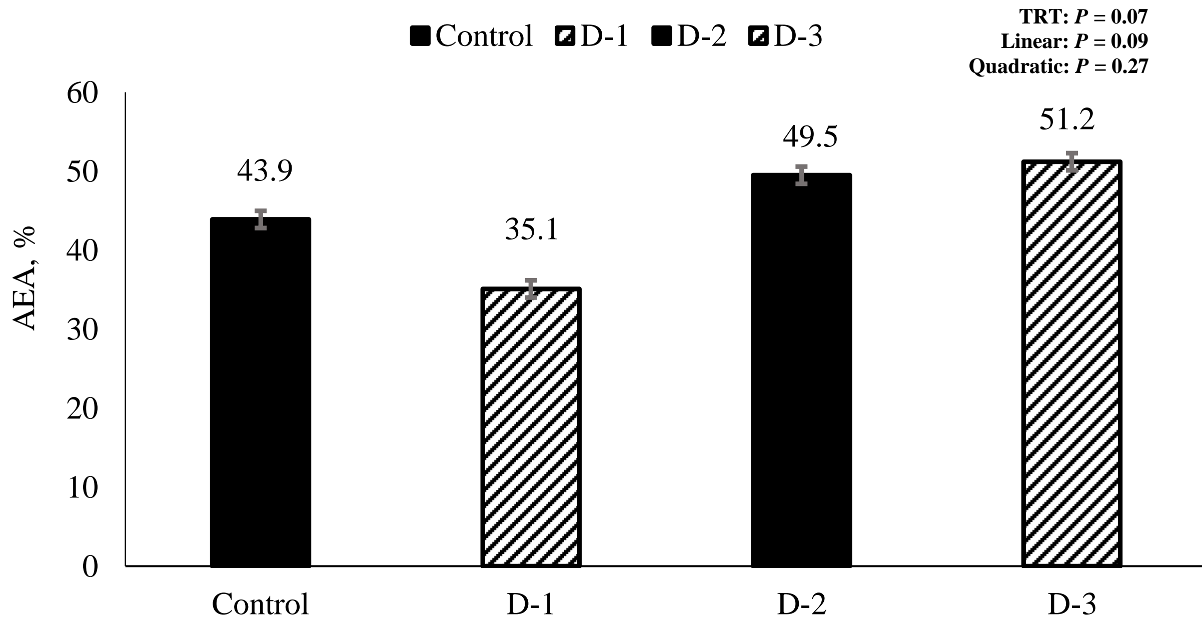
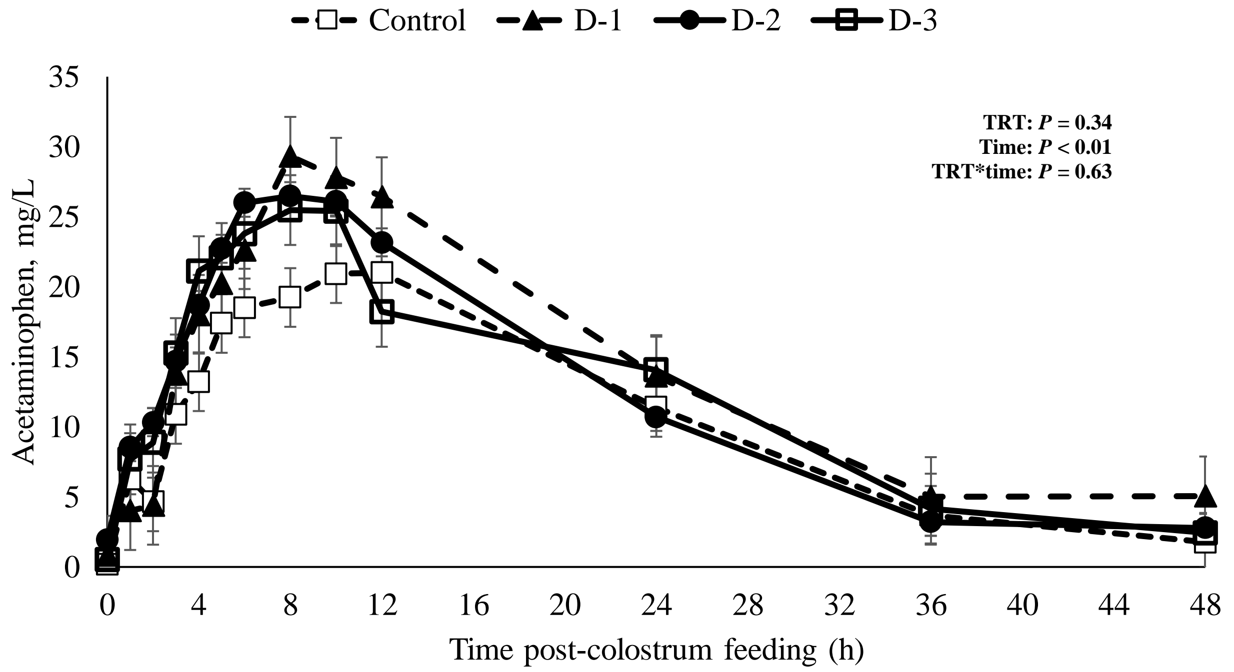


Figure 4.1 Serum IgG concentration dynamics during the first 48 h blood sampling period (mean + SE; **a**), serum IgG concentrations from birth until day 7 of life (mean + SE; **b**), and apparent efficiency of absorption at 24 h post-colostrum feeding (AEA; **c**) values for calves fed: Control = 1.791 kg water, final volume: 2.64 L, 360 g/L CR; Dilution 1 (D-1) = 2.083 kg water, final volume: 2.97 L, 320 g/L CR, Dilution 2 (D-2) = 2.574 kg water, final volume: 3.39 L, 280 g/L CR, or Dilution 3 (D-3) = 3.110 kg water, final volume: 3.96 L, 240 g/L CR within 1 h after birth. All TRT meals were reconstituted using 949 g of CR (150 g IgG). All colostrum replacer used was from the Saskatoon Colostrum Company Ltd (SCCL) and had an IgG concentration of 15.8 % IgG on a DM basis.

a.



b.

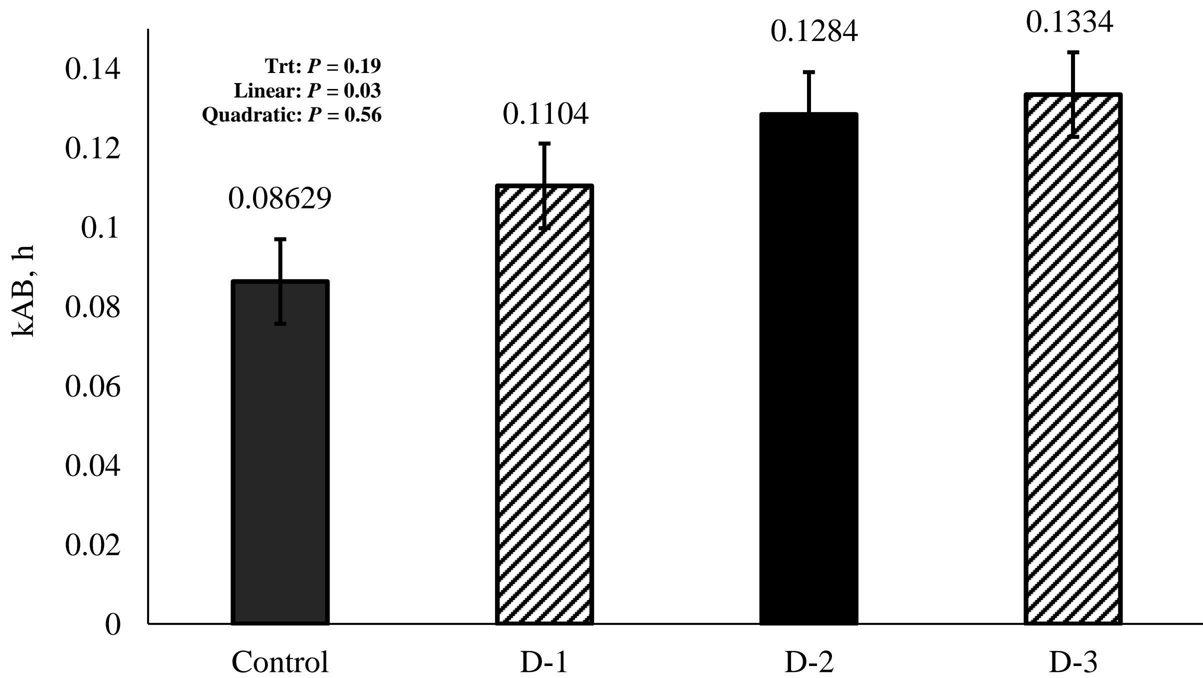
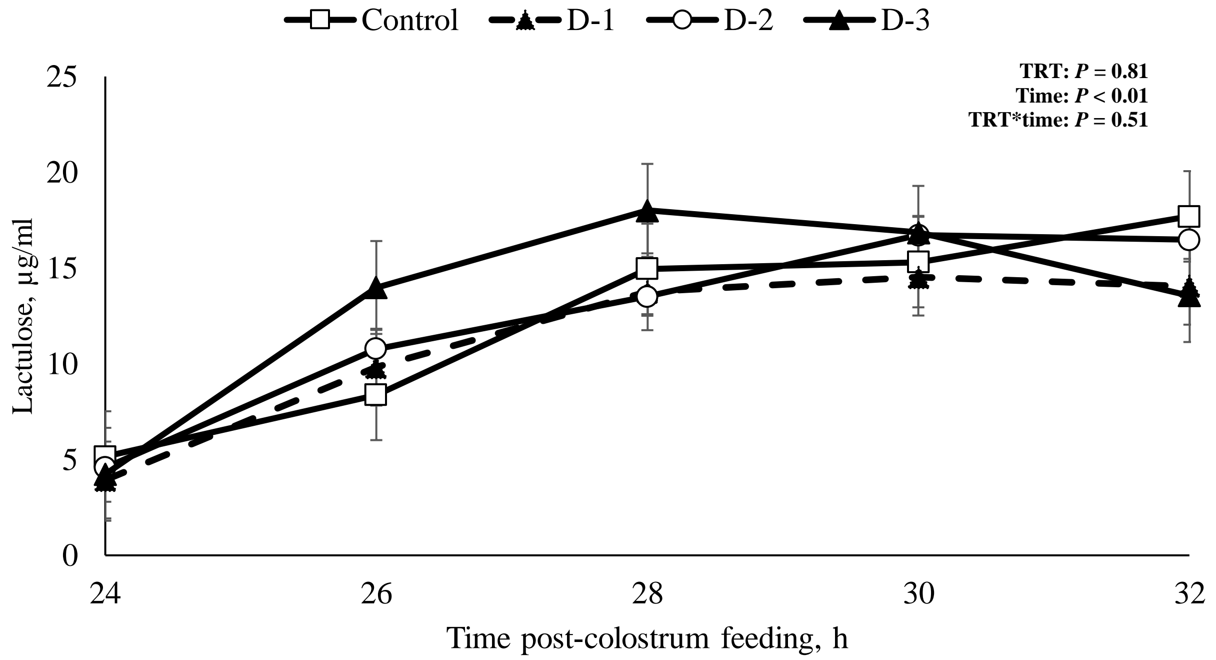
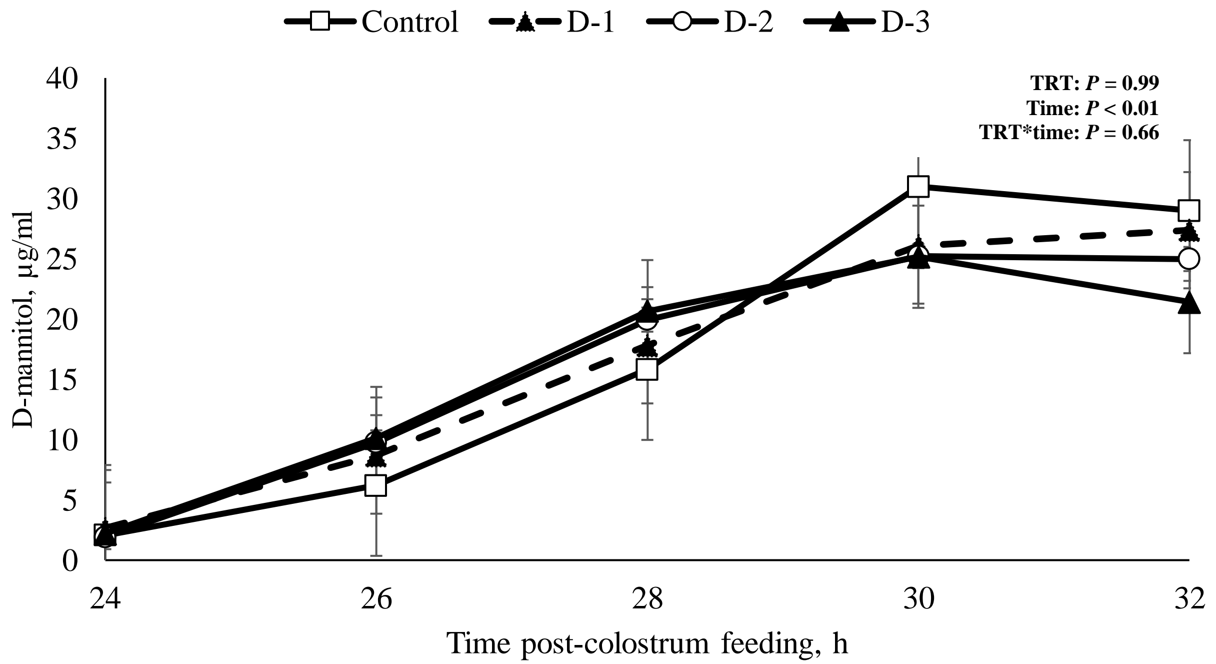


Figure 4.2 Plasma acetaminophen dynamics throughout the 48 h blood sampling period (mean + SE; **a**), and abomasal emptying rates per hour (kABh; mean + SE; **b**) for calves fed: Control = 1.791 kg water, final volume: 2.64 L, 360 g/L CR; Dilution 1 (D-1) = 2.083 kg water, final volume: 2.97 L, 320 g/L CR, Dilution 2 (D-2) = 2.574 kg water, final volume: 3.39 L, 280 g/L CR, or Dilution 3 (D-3) = 3.110 kg water, final volume: 3.96 L, 240 g/L CR within 1 h after birth. All TRT meals were reconstituted using 949 g of CR (150 g IgG). All colostrum replacer used was from the Saskatoon Colostrum Company Ltd (SCCL) and had an IgG concentration of 15.8 % IgG on a DM basis.

a.



b.



c.

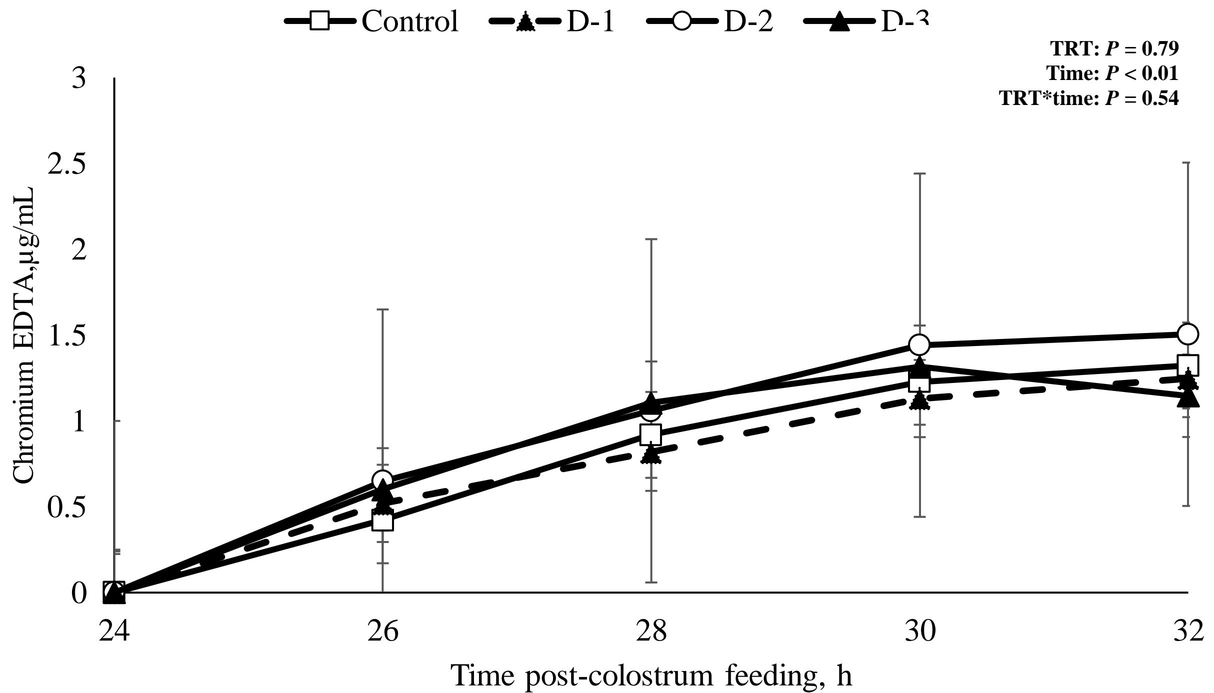


Figure 4.3 Lactulose concentration dynamics after dosage at 24 h post-colostrum feeding after MR feeding (mean + SE; **a**), D-mannitol concentration dynamics after dosage at 24 h post-colostrum feeding after MR feeding (mean + SE; **b**), chromium EDTA (Cr-EDTA) concentration dynamics after dosage at 24 h post-colostrum feeding after MR feeding (mean + SE; **c**) for calves fed: Control = 1.791 kg water, final volume: 2.64 L, 360 g/L CR; Dilution 1 (D-1) = 2.083 kg water, final volume: 2.97 L, 320 g/L CR, Dilution 2 (D-2) = 2.574 kg water, final volume: 3.39 L, 280 g/L CR, or Dilution 3 (D-3) = 3.110 kg water, final volume: 3.96 L, 240 g/L CR within 1 h after birth. All TRT meals were reconstituted using 949 g of CR (150 g IgG). All colostrum replacer used was from the Saskatoon Colostrum Company Ltd (SCCL) and had an IgG concentration of 15.8 % IgG on a DM basis.

5 Effects of enriching IgG concentration in low- and medium-quality colostrum with colostrum replacer on IgG absorption in newborn Holstein calves

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5.1 Abstract

Ingestion and absorption of greater quantities of IgG are required to increase serum IgG levels in newborn calves. This could be achieved by adding colostrum replacer (CR) to maternal colostrum (MC). The objective of this study was to investigate whether low and high-quality MC can be enriched with bovine dried CR to achieve adequate serum IgG levels. Male Holstein calves (n = 80; 16/treatment) with birth body weights (BW) of 40 to 52 kg were randomly enrolled to be fed 3.8 L of the following combinations: 30 g/L IgG MC (**C1**), 60 g/L IgG MC (**C2**), 90 g/L IgG MC (**C3**), C1 enriched with 551 g of CR (60 g/L; **30-60CR**), or C2 enriched with 620 g of CR (90 g/L: **60-90CR**). A subset of 40 calves (8/treatment) had a jugular catheter placed and were fed colostrum containing acetaminophen at a dose of 150 mg/kg of metabolic body weight, to estimate abomasal emptying rate per hour (kABh). Baseline blood samples were taken (0 h), followed by sequential samples at 1, 2, 3, 4, 5, 6, 8, 10, 12, 24, 36, and 48 h relative to initial colostrum feeding. Results for all measurements are presented in the following order, unless otherwise stated: C1, C2, C3, 30-60CR, and 60-90CR. Serum IgG levels at 24 h were different among calves fed C1, C2, C3, 30-60CR, and 60-90CR: 11.8, 24.3, 35.7, 19.9, and 26.9 g/L ± 1.02 (mean ± SEM), respectively. Serum IgG at 24 h increased when enriching C1 to 30-60CR, but not from C2 to 60-90CR. Similarly, apparent efficiency of absorption (AEA) values for calves fed C1, C2, C3, 30-60CR, and 60-90CR were different: 42.4, 45.1, 43.2, 36.3, and 33.4% ± 1.93, respectively. Enriching C2 to 60-90CR reduced AEA, and enriching C1 to 30-60CR tended to decrease AEA. The kABh values for C1, C2, C3, 30-60CR, and 60-90CR were also different: 0.16, 0.13, 0.11, 0.09, and 0.09 ± 0.005, respectively. Enriching C1 to 30-60CR or C2 to 60-90CR reduced kABh. However, 30-60CR and 60-90CR have similar kABh compared

with a reference colostrum meal (90 g/L IgG, C3). Even though kABh was reduced for 30-60CR, results indicate that C1 has the potential to be enriched and achieve acceptable serum IgG levels at 24 h without affecting AEA.

5.2 Introduction

The absorption of immunoglobulins from colostrum provides protection against common diseases until the calf's immune system is fully developed (Godden et al., 2019). Therefore, newborn calves need to ingest high-quality colostrum and absorb IgG to achieve successful transfer of passive immunity at 24 h (**STPI**: serum IgG >10 g/L; Shivley et al., 2018). In contrast, failure to provide appropriate colostrum feeding has been linked to higher risk of mortality in newborn calves (Renaud et al., 2018b). The serum IgG cutoff threshold of 10 g/L has been widely accepted and used; however, recent reports state that this cutoff point should be reconsidered to ensure better protection against disease and reduce mortality (Urie et al., 2018a; Lombard et al., 2020). It has recently been demonstrated that calves with serum IgG concentrations >15 g/L compared with 10 g/L at 24 h of life had reduced mortality and morbidity rates (Urie et al., 2018a). Moreover, a recent consensus has declared 4 different categories to classify STPI or failed transfer of passive immunity (**FTPI**) in newborn calves: excellent (≥25.0), good (18.0–24.9), fair (10.0–17.9), and poor (<10.0 g/L; Lombard et al., 2020). Therefore, it is critical to ensure that calves achieve STPI and, if possible, reach the "excellent" STPI category.

To increase serum IgG concentrations, newborn calves need to consume greater total quantities of IgG at birth. One strategy to provide calves with more IgG is by feeding colostrum meals with relatively high concentrations of IgG. However, a large variation of ranges exists due

to low and high IgG1 concentrations among individual cows (Baumrucker et al., 2010). This variation in colostral IgG concentrations limits producers' ability to consistently feed greater total quantities of IgG in the first meal. An alternative and promising solution could be to enrich low-quality colostrum harvested on farm with a determined amount of colostrum replacer (**CR**) powder. Lopez et al. (2020) reported that enriching low-quality colostrum with an IgG concentration of 30 g/L to 41 g/L resulted in no cases of FTPI, and calves had a mean serum IgG concentration of 22.33 g/L at 24 h of life. Lopez et al. (2020) increased the IgG concentration of maternal colostrum (**MC**) from 30 to 41 g/L by adding 104.3 g of whey-derived CR powder (40 g of IgG, or 40.64% IgG). Their results indicated that serum IgG concentration at 24 h benefit from enriching MC with CR. However, it was not clear how adding CR powder to MC could affect abomasal emptying or whether it is possible to enrich MC with higher IgG concentrations than 30 g/L.

Thus, we hypothesized that supplementing low- and medium-quality colostrum with bovine dried CR would result in serum IgG levels similar to these of calves fed medium- and high-quality MC, respectively. The objective of the present study was to investigate whether low- (30 g/L IgG) and moderate-quality colostrum (60 g/L IgG) can be enriched to achieve serum IgG concentrations comparable to calves fed high-quality MC (90 g/L IgG), without having any negative effects on abomasal emptying or apparent efficiency of absorption (AEA).

5.3 Materials and Methods

The animal experiment procedures and protocols were conducted in accordance with the Canadian Council of Animal Care (CCAC; Olfert et al., 1993) were approved by the Animal Care Committee of the University of Guelph (AUP no. 4488). The present study was executed at

a commercial dairy farm located in Aylmer, Ontario, Canada. The farm had a total herd size of ~3,500 animals and ~1,600 milking cows at the time of the experiment. The project was conducted at this farm due to previously established relationships and the proper animal facilities to execute the experiment. In addition, the high number of pregnant cows allowed us to enroll multiple calves in a short period (June to August 2021), reducing calf-to-calf variation throughout different seasons.

5.3.1 Parturition and Calving Procedures

Newborn calves used for this experiment were from primiparous and multiparous Holstein cows, and treatments were balanced for parity. The pregnant cows were housed in a close-up pen but were moved to a maternity pen at approximately 12 to 24 h before parturition, when calving signs were evident or the calving process was initiated. Cows were constantly monitored during the calving process, and assistance was provided when needed.

5.3.2 Animal Enrollment

Male Holstein calves born from primiparous and multiparous cows in the maternity pen were immediately separated from their dams, to prevent MC ingestion. Thereafter, calves were translocated to a digital weighing scale (Global Industrial Digital Floor Scale, Global Equipment Company Inc.) for birth BW recording. Only animals with a birth BW range of 40 to 52 kg were enrolled in the study, because a fixed meal size was fed. The BW range was calculated using previous BW data from animals born on the same farm. Previous BW data were analyzed using the MIXED and UNIVARIATE procedures of SAS (University Edition; SAS Institute Inc.), whereby it was determined that the mean BW for calves born on this farm was 46.0 ± 4.15 kg (mean ± SD). Then, we decided to use the 90% and 10% quantiles to determine the BW range of

40 to 52 kg. After weighing a calf, a vigor score based on Villettaz Robichaud et al. (2017) was assessed before any other procedures. Calves were thoroughly dried with clean towels, navels were dipped with 7 % iodine tincture (Iodine Tincture Stronger, Dominion Veterinary Laboratories Ltd.; Winnipeg, Manitoba, Canada), and a navel clamp was placed to prevent any further contamination. Then, calves were fed their respective colostrum treatments and moved to individual hutches (100 cm long, 64 cm wide, and 105.5 cm high; Starter Pen AG- RI-6000B, Agri-Plastics) deeply bedded with sawdust. Between calves, hutches were cleaned and disinfected with isopropyl alcohol (70%) and a chlorhexidine solution (Germi-Stat 4%, Germiphene Corp.). Then, hutch walls were dried with clean towels. Sanitized hutches were allowed to dry for 1 d before a new calf was allocated in. From d 3 to d 7 of life, calves were moved to another calf-rearing facility within the same dairy farm, where pens had the following dimensions: 215 cm long, 86 cm wide, and 97 cm high.

5.3.3 Experimental Design, Maternal Colostrum, Colostrum Replacer, and Milk Feeding

Male Holstein calves (n = 80; 16/treatment) born from June 2021 to August 2021 were enrolled in the experiment. All MC and CR powders used in this experiment were provided by the Saskatoon Colostrum Company Ltd. (Saskatoon, SK, Canada; SCCL). The composition analyses of the colostrum treatments and CR can be found in Table 5.1. All analyses, except for osmolality and brix, were performed by Lactanet Canada (Guelph, ON, Canada) by NIR spectroscopy (Gagnon et al., 2020). Osmolality was measured using an osmometer (Advanced Micro Osmometer Model 3300, Advanced Instruments Inc.), and brix was determined using a refractometer (PAL-1 Refractometer, Atago). For MC collection, SCCL pasteurized pooled MC at their facilities and then created 3 batches of 30-, 60-, and 90-g/L MC. After that, 3.8 L of MC

was stored in Perfect Udder Colostrum Management Bags (Dairy Tech Inc.). Finally, colostrum was frozen and shipped to the commercial farm where the experiment was executed. A bovine dried CR powder (SCCL) with IgG concentration of 27% (DM basis) was used to increase IgG concentration of enrichment meals. Calves were randomly enrolled to be fed 3.8 L of the following combinations: 30 g/L IgG MC (C1), 60 g/L IgG MC (C2), 90 g/L IgG MC (C3), C1 enriched with 551 g of CR (60 g/L; 30-60CR), or C2 enriched with 620 g of CR (90 g/L: 60-90CR). Overall, calves assigned to C1, C2, C3, 30-60CR, and 60-90CR were fed total IgG masses of 114, 228, 342, 228, and 342 g, respectively. The randomized allocation was generated using Microsoft Excel (version 16.16.21; Microsoft Corp.) using the RAND function in blocks of 16.

Colostrum feeding was performed by first thawing the frozen colostrum in a warm water bath (~50°C). When the colostrum was completely thawed and had reached a temperature of 40°C, it was remeasured to verify a constant volume of 3.8 L and then transferred to a clean esophageal feeder (Handi Grip 4-Qt. Feeder and Storage Bottle with Storage Cap and Plastic Esophageal Probe Assembly, Spectrum Educational Supplies Ltd.). For calves fed 30-60CR or 60-90CR, 551 or 620 g of CR powder, respectively, was added once MC was completely thawed, and thoroughly mixed with a whisk. Due to the addition of CR powder, a volume increase occurred. However, after the mixture was completed, 3.8 L of the total mixture (~ 40°C) was remeasured and transferred to an esophageal feeder for further feeding. All colostrum treatments were fed within 2 h after birth. After colostrum feeding, 3 L (150 g/L) of milk replacer (MR; 26% protein, 18% fat, and 41% lactose; Grober Nutrition) was offered per feeding to all calves at 12, 24, 36, and 48 h relative to colostrum feeding via nipple-bottle. Refusals were

measured, to allow milk consumption calculations. Calves were weighed after their last milk feeding at 48 h relative to colostrum feeding and at 7 d. In addition, all calves were offered 4 L of water starting at 12 h after colostrum feeding. At 24 h, refusals were measured, and 4 L of fresh water was offered. Finally, water refusals were measured again at 48 h relative to colostrum feeding. Solid feed was not provided during the duration of the study.

5.3.4 Acetaminophen Feeding and Abomasal Emptying

Using the RAND and INDEX functions, a subset of calves (n = 40; 8/treatment) were randomly assigned to be fed acetaminophen (A5000, Sigma-Aldrich, MilliporeSigma Canada Ltd.) in their colostrum meal and catheterized. Acetaminophen absorption by the small intestine was used as a marker to measure abomasal emptying rate (**kABh**) from the kinetics of its concentration appearance in blood (Schaer et al., 2005); as its time to maximal absorption concentration is directly related to abomasal emptying (Burgstaller et al., 2017). Based on MacPherson et al. (2016), acetaminophen was added to 3.8 L of MC (C1, C2, or C3) or enriched MC (30-60CR or 60-90CR) at a dose of 150 mg/kg of metabolic body weight. Thereafter, the colostrum meal was thoroughly mixed with a whisk for approximately 1 min and then transferred to an esophageal feeder for feeding. Calves fed acetaminophen were catheterized at birth, and blood was sequentially sampled for 12 h after colostrum feeding to measure abomasal emptying, as will be described. Plasma samples taken at distinct time points were analyzed for acetaminophen concentrations with a Paracetamol (acetaminophen) Assay Kit-K8002 (Cambridge Life Sciences Ltd.), which has been previously used for these measurements (Welboren et al., 2021).

5.3.5 Blood Sampling

The blood sampling protocol varied depending on whether calves were fed acetaminophen or not. For calves not fed acetaminophen, blood samples (7 mL for plasma and 7 mL for serum) were collected via jugular venipuncture with 20-gauge × 2.5-cm needles (Greiner Bio-One International GmbH) at 0 (baseline), 12, 24, 36, and 48 h relative to colostrum feeding. In addition, a blood sample was collected at 7 d after the morning feeding from all calves enrolled in this study. The acetaminophen-fed calves were catheterized at ~30 min after birth, as described by Fischer et al. (2018a) and Lopez et al. (2023) to enable frequent blood sampling. Once the catheter was placed, one blood sample was collected as the baseline (0 h), and subsequently samples were collected at 1, 2, 3, 4, 5, 6, 8, 10, 12, 24, 36, and 48 h relative to colostrum feeding. All blood samples that coincided with a colostrum or MR meal were taken before feeding. All blood samples were collected with 2 syringes (7 mL of blood each) through the jugular catheter and were transferred to serum collection tubes (Vacutainer, Becton, Dickinson and Co.) and sodium heparin collection tubes (Vacutainer, Becton, Dickinson and Co.). After blood collection, 14 mL of saline and 1 mL of heparinized saline were flushed back into the catheter. Samples in serum collection tubes were allowed to clot at room temperature for approximately 1 h before centrifugation. Thereafter, tubes were centrifuged at 3,000 × g at 4°C for 20 min. Then, serum and plasma collected after centrifugation were transferred into 1.5-mL microcentrifuge tubes and frozen at −20°C until further analysis.

5.3.6 IgG and Serum Total Protein Analyses

Serum frozen samples were transferred from −20°C to a −4°C freezer. Thereafter, samples were left overnight in the −4°C freezer and then moved to a refrigerator at 4°C. Samples

were left in the refrigerator for approximately 12 h. Then, thawed samples were re-centrifuged at 3,000 × g for 10 min at 4°C to separate the supernatant, which was then transferred to a new 1.5-mL microcentrifuge tube. Samples were refrozen at −20°C and immediately shipped overnight with ice packs to SCCL Quality Assurance Laboratory (Saskatoon, SK, Canada) for IgG and serum total protein (STP) analyses. Serum IgG measurements were determined by radial immunodiffusion, as described by Chelack et al. (1993), with modifications according to Shivley et al. (2018). Serum IgG values were determined for all time points and used to represent IgG concentration dynamics during the first 48 h of life. In addition, serum IgG values at 24 h after colostrum feeding (**IgG_{24h}**) were used to calculate AEA at 24 h. The AEA formula used was adapted from Quigley and Drewry (1998), Quigley et al. (2002), and Saldana et al. (2019), where

$$\boldsymbol{AEA} = \frac{\textbf{birth body weight (kg)} \times \mathbf{0.09} \times \textbf{serum IgG } (\frac{\mathbf{g}}{\mathbf{L}}\mathbf{24}\boldsymbol{h})}{\textbf{total IgG fed (g)}} \times \mathbf{100}$$

Additional parameters calculated with serum IgG data included the time to reach maximum concentration (**IgGTmax**) and the maximum concentration reached (**IgGCmax**).

5.3.7 Sample Size

The required number of replication (animals) was determined following the recommendations of Berndtson (1991). A serum IgG value of reference was selected from Fischer et al. (2018a) due to the similarity in colostrum feeding methodology. Fischer et al. (2018a) reported an average serum IgG value of 22.3 g/L with standard error of the mean (SEM) of 1.40. The SEM was converted to standard deviation, which was 4.87 g/L, and thereafter, the

coefficient of variation (CV) was calculated. The resulting CV was 18.01%. Using those values, we calculated the total replicates needed per treatment group for experiments of 80% power with an α of 0.05. As a result, using a reference CV value of 18% from Table 1, the number of replicates needed to detect a difference of 20% in serum IgG values compared with the control was 14 animals per group. Finally, we decided to use 16 animals per treatment group, to have a total sample size of 80 and allow 2 additional animals for possible experimental errors or animal loss. The 16 animals per treatment group provided us 80% power with an α of 0.05 to detect differences of 20% compared with the control (Berndtson, 1991).

5.3.8 Statistical Analyses

Data were analyzed using SAS (University Edition). The final data set included blood and BW parameters from a total of 80 calves (16/treatment). Residual distribution for each variable was assessed for normality using the Shapiro-Wilk test as a criterion and for homoscedasticity using the UNIVARIATE and PLOT procedures. All values reported are least squares means with the respective standard errors. All means reported were separated by Tukey's adjustment. The effect of colostrum treatment on serum IgG concentration over time was analyzed as a repeated measures model using the GLIMMIX procedure. This model included the fixed effects of treatment, time, the interaction of treatment and time, and the random effect of calf. In addition, a covariance structure was used to account for uneven sampling collection time points. The GLIMMIX procedure was used to analyze AEA, STP values at birth (**STP_{0h}**), STP concentration at 24 h after colostrum feeding (**STP_{24h}**), IgGTmax, IgGCmax, (determined by using all serum IgG samples collected from calves from 0 to 7 d) and BW at 0 and 48 h, and 7 d. Overall, models included the fixed effects of treatment, time, the interaction of treatment and

time, and the random effect of calf. The NLMIXED procedure was used for each individual calf to estimate their kABh and acetaminophen elimination from blood per hour (**kELh**) according to given constants and their acetaminophen concentrations during the 48-h sampling period. After that, kABh and kELh mean differences were analyzed using the GLIMMIX procedure. In addition, STP_{24h} and serum IgG_{24h} data were analyzed using the CORR and REG procedures in SAS. The regression procedure from SAS was used to determine whether a linear relationship existed between serum IgG_{24h} and STP_{24h} concentrations. The STP_{24h} values were used as the predictor variable and serum IgG_{24h} as the outcome variable. Statistical significance was declared at $P \leq 0.05$, and tendencies toward significance were declared when $0.05 < P \leq 0.10$.

5.4 Results

5.4.1 Colostrum and Colostrum Replacer Composition

The analyzed composition of colostrum treatments and CR is presented in Table 5.1. In general, C1 had the lowest fat, protein, and TS ($P < 0.01$), whereas C3 had the lowest lactose content ($P < 0.01$). The 60-90CR enrichment meal was the highest in protein and TS ($P < 0.01$), and the C3 treatment was the highest in fat ($P < 0.01$). Lactose was highest for 30-60CR and lowest for C3 ($P < 0.01$). The osmolality was highest for 60-90CR and lowest for C1 ($P < 0.01$).

5.4.2 Body Weight, Vigor Score, Colostrum Feeding, Milk Feeding, and Water Intake

Birth BW (**BW_{0h}**, $P = 0.88$; Table 5.2) and dam lactation ($P = 0.11$, Table 5.2) did not differ among treatments ($P = 0.88$; Table 5.2). The BW at 48 h relative to first colostrum feeding (BW_{48h}; $P = 0.91$) and at 7 d of age (BW_{7d}; $P = 0.85$) were not different (Table 5.2). Similarly, average daily gain at 48 h relative to first colostrum feeding (**ADG_{48h}**; $P = 0.66$) and ADG at 7 d of age (**ADG_{7d}**; $P = 0.35$) did not differ among colostrum treatments (Table 5.2). Additionally,

we did not observe any difference in mean vigor score between treatments before enrollment in C1, C2, C3, 30-60CR, or 60-90CR: 5.3, 5.0, 5.4, 5.4, and 5.2, respectively ($P = 0.98$). Overall, we did not observe a treatment ($P = 0.85$) or treatment × feeding ($P = 0.35$) effect on MR consumption (Table 5.3), but there was a time effect ($P < 0.01$). Overall, milk consumption for feedings at 12, 24, 36, and 48 h relative to first colostrum feeding were 2.13, 1.66, 2.10, and 2.61 L, respectively ($P < 0.01$). Specifically, milk consumption was higher at 12 h compared with 24 h (P < 0.05) but similar when compared with 36 h ($P = 0.99$). In addition, milk consumption was the highest at 48 h compared with all other milk feedings ($P < 0.01$). Finally, water consumption was low and did not differ between treatments ($P = 0.93$). On average, calves assigned to C1, C2, C3, 30-60CR, and 60-90CR consumed 0.4, 0.4, 0.3, 0.2, and 0.3 ± 0.18 L of water in the first 48 h of life relative to first colostrum feeding.

5.4.3 Serum Total Protein, Serum IgG, and Apparent Efficiency of Absorption

Serum total protein values at birth (**STP_{0h}**) were not different among enrolled animals at birth ($P = 0.59$; Table 5.2). In contrast, STP_{24h} differed significantly ($P < 0.01$) between treatments (Table 5.2). Calves fed C1 had the lowest STP_{24h}, whereas calves fed C3 had the highest STP_{24h} concentrations. All blood samples taken immediately after birth were used as baseline concentrations (0 h). Serum IgG concentrations at birth (**IgG_{0h}**) were almost negligible and did not differ ($P = 0.67$) between treatments (Table 5.2). Overall, we observed an interaction of treatment and time ($P < 0.01$) for serum IgG concentrations from birth until 48 h (Figure 5.1a). To further understand and visualize the benefit of increasing IgG mass ingestion or enriching colostrum, serum IgG_{24h} concentrations are presented in Figure 5.1b. Notably, serum IgG_{24h} concentrations increased as total IgG mass ingestion increased. The enrichment of C1 to

30-60CR increased serum IgG_{24h} concentrations ($P < 0.01$; Figure 5.1b), but the enrichment of C2 to 60-90CR did not significantly increase levels ($P = 0.39$; Figure 5.1b). In addition, serum IgG_{24h} was highest for calves fed C3 and lowest for C1 ($P < 0.01$). Moreover, it is important to highlight that all calves fed C3 were classified in the "excellent" serum IgG category (≥ 25.0 g/L IgG; Table 5.4) from Lombard et al. (2020), which would be a reference meal in a colostrum feeding program. Even though the 60-90CR enrichment did not significantly increased serum IgG_{24h} concentrations, the FTPI categories (Lombard et al., 2020) were improved in comparison to calves fed C2. Specifically, the percentage of calves classified in the "excellent" category increased, and none were classified in the poor or fair categories (Table 5.4; $P < 0.01$). Furthermore, the correlation values between STP_{24h} and serum IgG_{24h} for calves fed C1, C2, C3, 30-60CR, and 60-90CR were 0.55, 0.82, 0.50, 0.64, and 0.41. In addition, the regression analyses showed that the adjusted R^2 for calves fed C1, C2, C3, 30-60CR, and 60-90CR were respectively 0.26, 0.66, 0.20, 0.36, and 0.11.

Furthermore, AEA tended to decrease when enriching C1 to 30-60CR ($P = 0.06$; Figure 5.1c), but it was reduced ($P < 0.01$) when C2 was enriched to 60-90CR (Figure 5.1c). However, AEA was not different between calves fed either C1, C2, or C3 ($P > 0.05$; Figure 5.1c). Finally, IgG_{Tmax} did not differ ($P = 0.12$) between colostrum treatments (Table 5.2), but IgG_{Cmax} was different ($P < 0.01$; Table 5.2). Specifically, calves fed C3 had the highest IgG_{Cmax}, and those fed C1 had the lowest IgG_{Cmax} ($P < 0.01$; Table 5.2).

5.4.4 Abomasal Emptying

In general, we observed a treatment effect ($P < 0.01$; Figure 5.2a) on acetaminophen concentrations during the 48-h sampling period. The kABh was reduced ($P < 0.01$; Figure 5.2b)

when calves were fed the enrichment meals of 30-60CR and 60-90CR compared with C1 and C2 ($P < 0.01$; Figure 5.2b), respectively. However, the kABh from the 30-60CR ($P = 0.39$; Figure 5.2b) and 60-90CR enrichments ($P = 0.65$; Figure 5.2b) were not different from C3, which would be considered a very high-quality MC meal (> 50 g/L IgG) in a colostrum feeding protocol. Finally, kELh did not differ between colostrum treatments ($P = 0.16$).

5.5 Discussion

A limited number of studies have investigated the enrichment of colostrum, specifically IgG, with CR. We hypothesized that low- and medium-quality MC could be enriched with bovine dried CR to achieve similar serum IgG_{24h} to that of calves fed high-quality colostrum. The findings from this study show that low-quality colostrum with an IgG concentration of 30 g/L (C1) can be enriched to 60 g/L IgG (30-60CR) and increase serum IgG_{24h}. However, we also found that enriching a 60 g/L IgG colostrum (C2) to 90 g/L IgG (60-90CR) did not significantly increase serum IgG_{24h} and decreased AEA.

Milk feeding was not different between treatments; however, we found that calves consumed less milk at 24 h in comparison to 12 h. The combined colostrum meal fed within 2 h (3.8 L) after birth plus the MR feeding at 12 h (3 L) could have impacted the 24-h MR meal. In regards to colostrum feeding effect, our results show the increment of serum IgG concentrations at 24 h without an effect on AEA as total IgG mass fed increased with C1 (114 g), C2 (228 g), and C3 (342 g), and persisted until 48 h. Therefore, increasing the total IgG mass fed to newborns calves is recommended. Previous research recommended feeding 100 g of IgG at birth, but new research suggests feeding more than 150 to 200 g of IgG (Chigerwe et al., 2008; Godden et al., 2009; Lago et al., 2018). Moreover, new colostrum feeding recommendations by Lombard

et al. (2020) highlight the importance of maximizing IgG consumption and concentrations in the bloodstream. Therefore, the enrichment of MC with CR may be an important colostrum feeding strategy to achieve this objective. This was previously demonstrated by Lopez et al. (2020), who enriched MC but used a whey-based CR instead of a bovine dried CR, as in our study. Lopez et al. (2020) enriched a 30 g/L IgG MC to 41 g/L, resulting in high serum IgG levels and preventing FTPI. However, it is important to emphasize that the current study did not use a whey-derived CR like Lopez et al. (2020). Also, Lopez et al. (2020) did not report abomasal emptying data. As a result, more research is needed to understand the effects of enriching MC with CR developed with different manufacturing techniques.

In the present study, none of the calves fed the enrichment meal of 30-60CR had FTPI, whereas 3 calves (18.75%) fed C1 had FTPI (Table 5.4). Furthermore, the enrichment of C1 to 30-60CR increased the IgGCmax of calves, which aligns with the goal of producers to increase IgG concentrations in newborn calves. In contrast to our results, others have reported no added benefit to enriching MC. Specifically, Abel Francisco and Quigley (1993) found that enriching 4 L of MC (59.2 g/L IgG) with 125 g of CR (11.4% IgG) from a blend of lyophilized and dried bovine colostrum did not increase serum IgG concentrations in calves at 24 h. In fact, Abel Francisco and Quigley (1993) reported that calves fed enriched MC (13.0 g/L) had lower serum IgG_1 concentrations at 24 h than calves fed only MC (18.7 g/L). Therefore, it is important to analyze different factors that can influence the effectiveness of enrichments. For example, our enrichments were made with 27% IgG concentrated CR and aimed to provide an extra 114 g of IgG, whereas the enrichments of Abel Francisco and Quigley (1993) and Morin et al. (1997) used CR with lower IgG concentrations of 11.4% (125 g of CR powder) and 14.34% (136 or 272

g of CR powder), respectively. Another factor to consider is that our enrichment consisted of one meal of 3.8 L at birth, whereas Abel Francisco and Quigley (1993) and Morin et al. (1997) fed 2 meals of 2 L each (0 and 12 h after birth). As a result, calves in our study ingested a greater total quantity of IgG earlier, which could have affected colostral IgG absorption (Fischer et al., 2018a). In addition, our study supplemented a total of 114 g to either 30-60CR or 60-90CR, which is higher in comparison to 14.25 and 39.6 or 82.4 g of IgG that Abel Francisco and Quigley (1993) and Morin et al. (1997) added to their enrichments, respectively. Morin et al. (1997) used MC with an IgG concentration of 25.7 g/L IgG, which is close to our C1 meal and 30-60CR enrichment, but did not find a benefit. Various factors can affect how colostral IgG is absorbed, but we suggest that our 30-60CR enrichment significantly increased serum IgG_{24h} due to the high amount of IgG mass supplemented with the CR Moreover, the addition of CR powder to C1 (30-60CR) or C2 (60-90CR) reduced the correlation between STP_{24h} and serum IgG_{24h}. Also, the regression model showed that the ability of STP_{24h} to predict serum IgG_{24h} was reduced when CR powder was added to C1 or C2. The adjusted r-square was reduced from C1 to 30-60CR and from C2 to 60-90CR. These results are in agreement with the findings of Lopez et al. (2021), who noted that STP_{24h} inaccurately predicts FTPI, as defined by serum IgG $\geq$ 10 g/L, when calves are fed CR. Inasmuch, the addition of bovine dried CR to both enrichment meals, 30-60CR or 60-90CR, could affect FTPI predictions if we rely solely on STP_{24h} values.

Apparent efficiency of absorption tended to decrease when enriching C1 to 30-60CR, and the enrichment of C2 to 60-90CR significantly decreased AEA. Similarly, Morin et al. (1997) reported a decrease in IgG_1 absorption from 32.76 to 18.13% when colostrum was enriched with 272 g (compared with 136 g) of bovine dried CR. Similar to the C1 meal, Morin et al. (1997)

used colostrum with an IgG1 concentration of 25.7 g/L and found that AEA was decreased in their highest enrichment (272 g of CR). This result also occurred in our study, although the highest enrichment used a different base colostrum (60 g/L IgG) and a higher inclusion of CR powder (620 g). These results suggest that for each specific colostrum, there is a limit on how much CR powder can be added before it starts to negatively affect the efficacy of IgG absorption.

The colostrum batches used in the current study were standardized by IgG concentration; however, this resulted in variations in the composition of colostrum, which could influence colostral IgG absorption or abomasal emptying rate (Hunt and Stubbs, 1975; Davenport et al., 2000; Burgstaller et al., 2017). High protein contents, specifically casein, can affect colostral IgG absorption (Davenport et al., 2000), where excessive protein intake results in macromolecular transport saturation, which could compromise IgG absorption. However, this was not measured in the present study, so no conclusion can be made in regards to casein concentration.

A delay in abomasal emptying could slow the rate at which colostrum appears at its absorption site, the small intestine, affecting nutrient and IgG uptake (Cabral et al., 2014). Furthermore, delaying the appearance of colostrum could decrease IgG absorption, as the intestine loses the ability to absorb Ig as time after birth increases (Stott et al., 1979a; Fischer et al., 2018a). Overall, it is important to understand that a balance between a delay or increase in abomasal emptying could be beneficial to IgG absorption. Our results indicate that kABh was reduced when enriching C1 to 30-60CR and C2 to 60-90CR. However, the kABh for 30-60CR and 60-90CR did not differ from C3. As a result, even if kABh is reduced by enriching MC, it is still similar to the kABh of a reference MC meal of 90 g/L IgG. The lower kABh for the

enrichment meals, 30-60CR and 60-90CR, could be linked to their higher caloric densities and osmolality (Hunt and Stubbs, 1975; Burgstaller et al., 2017), resulting from adding bovine dried CR powder. Data from CR feeding regimens reported that an osmolality increment of CR from 301 to 516 mmol/ kg, by the addition of NaHCO3, decreased serum IgG_{24h} concentrations (16.08 to 12.61 g/L) and reduced AEA (31.1 to 24.8%; Cabral et al., 2014). Even though high osmolality likely reduced the abomasal emptying rate (Constable et al., 2009), to the authors' knowledge, the colostral osmolality value at which IgG absorption or abomasal emptying rate are compromised remains unclear. For the current study, the colostrum meal (C1) with fastest kABh ($P < 0.05$; Figure 5.2b) also had the lowest osmolality (302.83 mOsm/L), which agrees with the idea that abomasal emptying is affected by meal osmolality (Constable et al., 2009). In addition, the osmolality of C1, the colostrum treatment with the lowest IgG concentration (30 g/L), is similar to the osmolality values reported for mature bovine milk (292 ± 2 mmol/kg; Constable et al., 2009). Even though kABh was affected by enriching MC, the final emptying rate did not differ from a 90 g/L IgG MC meal. This suggests that the decrease in IgG uptake by calves fed 60-90CR might be attributable to other factors, such as meal composition, rather than abomasal emptying rate. However, Mokhber-Dezfooli et al. (2012) reported that calves fed erythromycin, a drug used to increase abomasal emptying rate, experienced increased AEA and plasma IgG from 9 h up to 7 d of life. Even though it was clearly demonstrated that 30 g/L IgG can be effectively enriched with bovine dried CR, more research is needed to understand how the addition of CR powder affects IgG uptake.

5.6 Conclusions

Results from this study indicate that low-quality colostrum, C1, has the potential to be enriched with bovine dried CR to achieve acceptable serum IgG levels at 24 h in newborn calves. In addition, we found that enriching 60 g/L MC to 90 g/L did not significantly increase serum IgG concentrations at 24 h and reduced AEA. In addition, the high osmolality of this meal was associated with a decrease in the abomasal emptying rate. Further research is needed to evaluate how the composition of enriched colostrum affects IgG absorption, but these primary results suggest that the enrichment of maternal colostrum presents an opportunity for farms that produce low-quality colostrum.

Table 5.1 Composition analysis of colostrum replacer and colostrum treatments fed to calves (n = 12 samples).

Item	Treatment[1]					CR	SEM	*P*-value
	C1	C2	C3	30-60CR	60-90CR			
Fat, %	2.6[e]	3.5[d]	5.3[a]	4.0[c]	4.4[b]	3.7[d]	0.04	<0.01
Protein, %	8.9[e]	12.9[d]	14.4[b]	14.6[b]	16.6[a]	13.6[c]	0.11	<0.01
Lactose, %	3.0[c]	2.4[d]	2.0[e]	5.4[a]	4.4[b]	4.3[b]	0.03	<0.01
Total Solids, %	18.4[e]	22.7[d]	25.6[c]	27.9[b]	29.4[a]	25.5[c]	0.17	<0.01
Osmolality, mOsm/kg	302.8[d]	331.0[c]	339.0[c]	518.4[b]	612.5[a]	-	7.63	<0.01
Brix, %	15.8[f]	20.3[e]	22.6[d]	26.4[b]	31.3[a]	24.6[c]	0.13	<0.01

[1]C1 = maternal colostrum (30 g/L IgG); C2: maternal colostrum (60 g/L IgG); C3: maternal colostrum (90 g/L IgG); 30-60CR: maternal colostrum (30 g/L IgG) enriched with 551 g of CR to elevate the IgG concentration to 60 g/L; 60-90CR: maternal colostrum (60 g/L IgG) enriched with 620 g of CR to elevate the IgG concentration to 90 g/L; CR: colostrum replacer powder from SCCL with an IgG concentration of 27% on DM basis (values reported are from its reconstitution just for composition analyses purposes:750 mL of water and 225 g of powder).
[a-d] Values within colostrum treatments with different superscripts are different ($P < 0.05$) after accounting for multiple comparison by Tukey's adjustment.
All composition analyses, except for osmolality and Brix, were performed by Lactanet (Guelph, ON, Canada).

Table 5.2 Body weight and blood parameters of newborn calves fed colostrum treatments (n = 80; 16/ treatment).

Item	Treatment[1]					SEM	*P*-value
	C1	C2	C3	30-60CR	60-90CR		
BW_{0h},[2] kg	45.4	46.4	45.5	45.7	46.4	0.92	0.88
BW_{48h},[2] kg	47.2	48.2	48.0	48.0	48.6	1.00	0.91
BW_{7d},[2] kg	46.7	47.6	47.6	48.1	47.9	0.93	0.85
Lactation,[2] #	1.8	1.4	1.2	1.9	1.1	0.25	0.11
IgG_{0h},[3] g/L	0.5	0.5	0.5	0.5	0.6	0.06	0.67
IgG_{7d},[3] g/L	9.5[d]	20.3[b]	27.7[a]	15.5[c]	23.1[b]	0.95	<0.01
ADG_{48h}, g	931.3	893.8	1246.9	1181.3	1100.0	199.03	0.66
ADG_{7d}, g	189.3	167.0	297.3	344.6	218.8	70.77	0.35
STP_{0h},[3] g/dL	4.5	4.7	4.5	4.8	4.7	0.13	0.59
STP_{24h},[3] g/dL	4.84[c]	5.79[ab]	6.22[a]	5.32[bc]	5.73[ab]	0.17	<0.01
FTPI,[3] %	18.8	0.0	0.0	0.0	0.0	-	-
IgG C_{max},[3] g/L	14.2[d]	26.0[bc]	38.2[a]	21.9[c]	28.9[b]	1.04	<0.01
IgG T_{max},[3] h	14.6	18.0	15.5	19.5	23.3	2.51	0.12
kEL,[3] h	0.11	0.09	0.10	0.09	0.08	0.005	0.16

[1]C1 = maternal colostrum (30 g/L IgG); C2: maternal colostrum (60 g/L IgG); C3: maternal colostrum (90 g/L IgG); 30-60CR: maternal colostrum (30 g/L IgG) enriched with colostrum replacer for a final concentration of 60 g/L IgG; 60-90CR: maternal colostrum (60 g/L IgG) enriched with colostrum replacer for a final concentration of 90 g/L IgG.
[2]BW_{0h}= body weight at birth (0 h); BW_{48h}= body weight at 48 h after colostrum feeding; BW_{7d}= body weight at 7 d, Lactation= number of dam lactation at calving
[3] IgG_{0h}= baseline serum IgG concentrations at birth (0h); IgG_{7d}= serum IgG concentrations at 7 d; STP_{0h}= serum total protein at birth (0h); STP_{24h} = serum total protein concentrations at 24 h after colostrum feeding; FTPI: failure transfer of passive immunity (serum IgG concentrations at 24 < 10 g/L); IgG C_{max}= maximum concentration, IgG T_{max}= time to reach maximum concentration; kEL= acetaminophen elimination from blood per h following first-order kinetics.
[a-d] Values within colostrum treatments with different superscripts are different ($P < 0.05$) after accounting for multiple comparison by Tukey's adjustment.

Table 5.3 Milk consumption for calves fed milk replacer at 12, 24, 36, and 48 h post-colostrum feeding (n = 80; 16/ treatment).

Item	Treatment[1]					SEM	*P*-value
	C1	C2	C3	30-60CR	60-90CR		
Meal #1,[2] L	2.5	2.2	2.4	1.7	1.8	0.22	0.06
Meal #2,[2] L	1.4	1.6	1.8	1.7	1.9	0.28	0.75
Meal #3,[2] L	2.3	2.1	2.0	2.3	1.9	0.25	0.70
Meal #4,[2] L	2.6	2.4	2.7	2.7	2.7	0.18	0.69

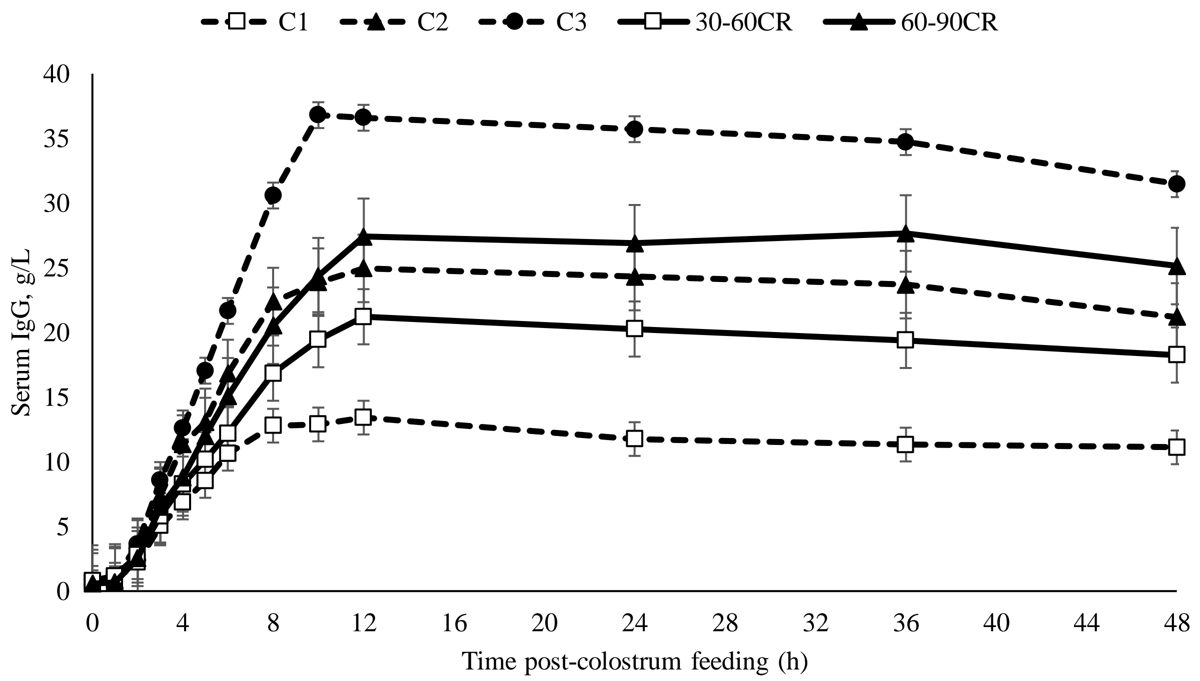
[1]C1 = maternal colostrum (30 g/L IgG); C2: maternal colostrum (60 g/L IgG); C3: maternal colostrum (90 g/L IgG); 30-60CR: maternal colostrum (30 g/L IgG) enriched with colostrum replacer for a final concentration of 60 g/L IgG; 60-90CR: maternal colostrum (60 g/L IgG) enriched with colostrum replacer for a final concentration of 90 g/L IgG.
[2]All calves were offered 3 L (150 g/L) of milk replacer (26 % protein, 18% fat, and 41% lactose) at 12 (#1), 24 (#2), 36 (#3) and 48 (#4) h post-colostrum feeding

Table 5.4 Transfer of passive immunity categories at 24 h for newborn calves (n = 80; 16/ treatment).

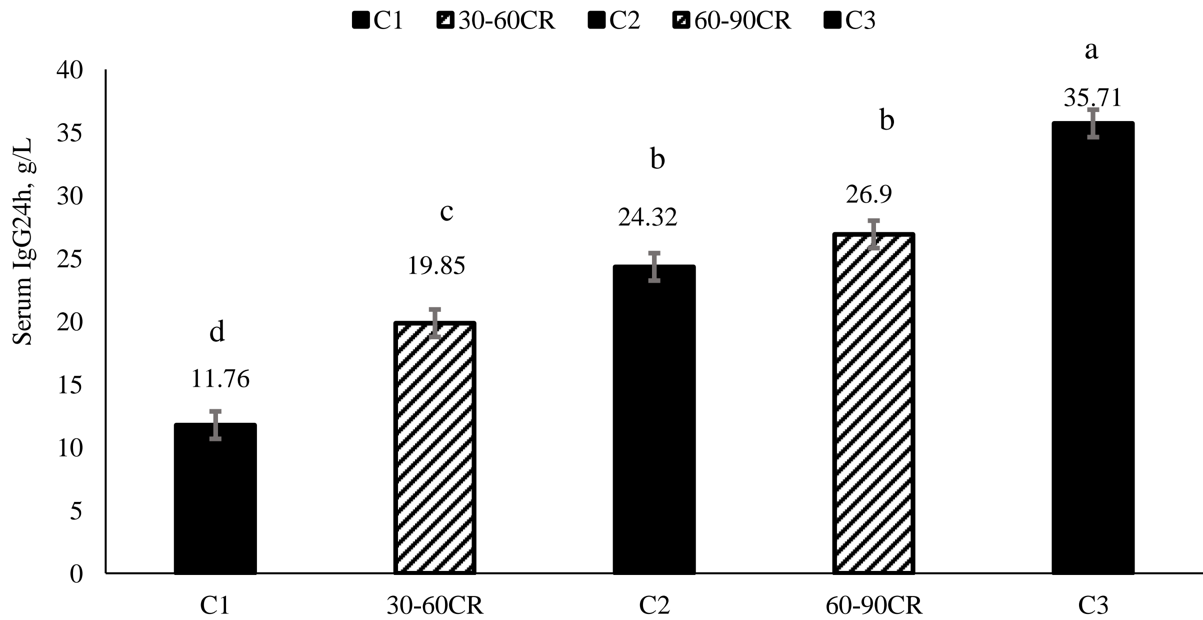
Category	Treatment[1]					SEM	*P*-value
	C1	C2	C3	30-60CR	60-90CR		
Excellent[2], %	0	50	100	6.25	62.50	0.04	<0.01
Good[2], %	0	43.75	0	62.50	37.50	0.11	<0.01
Fair[2], %	81.25	6.25	0	31.25	0	0.03	<0.01
Poor[2], %	18.75	0	0	0	0	0.17	<0.01

[1]C1 = maternal colostrum (30 g/L IgG); C2: maternal colostrum (60 g/L IgG); C3: maternal colostrum (90 g/L IgG); 30-60CR: maternal colostrum (30 g/L IgG) enriched with colostrum replacer for a final concentration of 60 g/L IgG; 60-90CR: maternal colostrum (60 g/L IgG) enriched with colostrum replacer for a final concentration of 90 g/L IgG
[2]Failed transfer of passive immunity (FTPI) or successful transfer of passive immunity (STPI) categories classified by serum IgG values at 24 h after birth by Lombard et a. (2020). Excellent = ≥ 25.0, good =18.0 to 24.9, fair = 10.0 to 17.9, and poor = < 10.0 g/L IgG.

a.



b.



c.

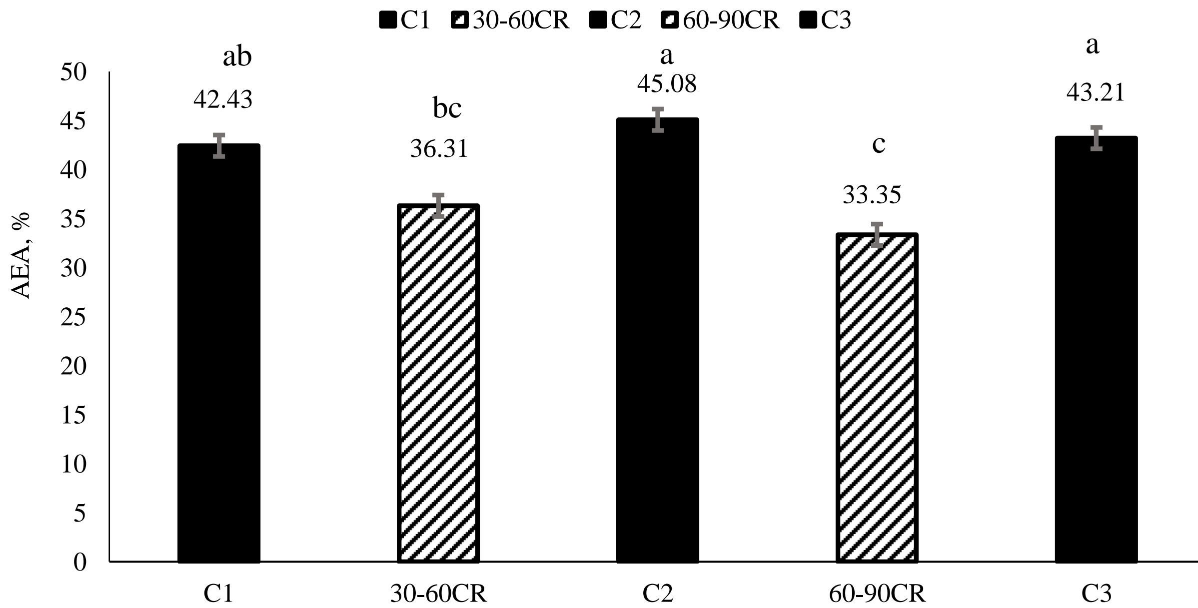
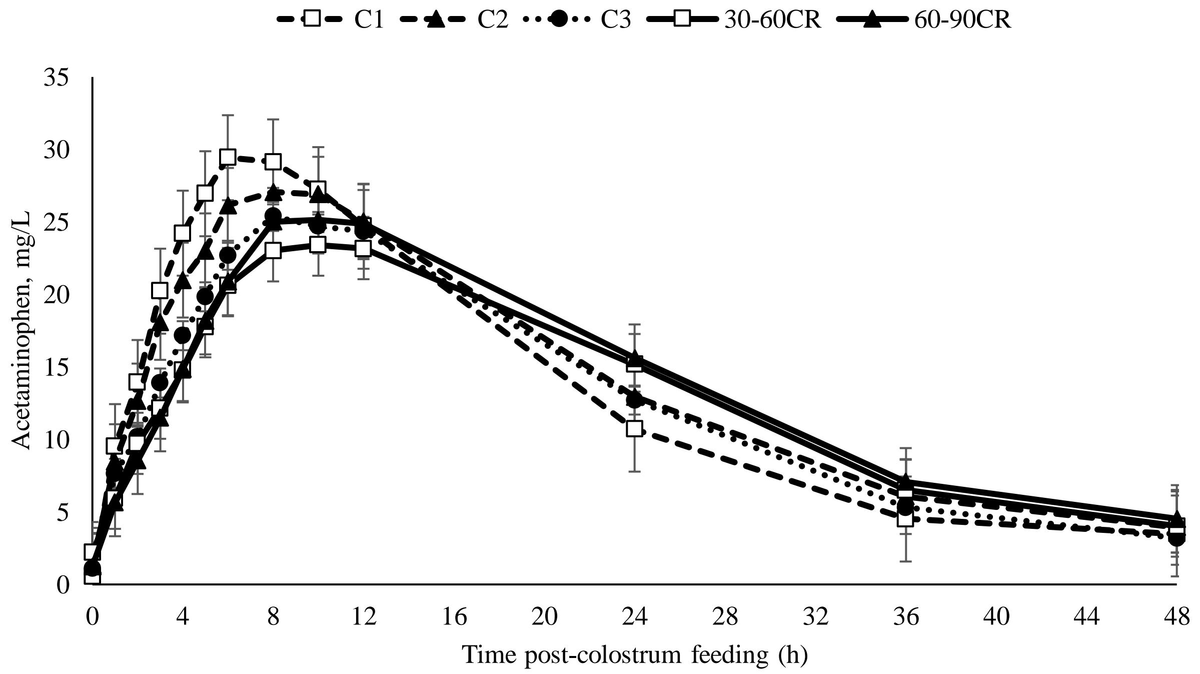


Figure 5.1 Serum IgG concentration dynamics throughout the 48 h blood sampling period (mean + SE; **a**), serum IgG concentrations at 24 h relative to colostrum feeding (mean + SE; **b**), and apparent efficiency of absorption (AEA; C) values for calves fed either C1: maternal colostrum (30 g/L IgG), C2: maternal colostrum (60 g/L IgG), C3: maternal colostrum (90 g/L IgG), 30-60CR: maternal colostrum (30 g/L IgG) enriched with colostrum replacer for a final concentration of 60 g/L IgG, or 60-90CR: maternal colostrum (60 g/L IgG) enriched with colostrum replacer for a final concentration of 90 g/L IgG. Differences were detected for colostrum treatment, time, and their interaction ($P < 0.01$). [a-d] Values within colostrum treatments with different superscripts are different ($P < 0.05$) after accounting for multiple comparisons by Tukey's adjustment.

a.



b.

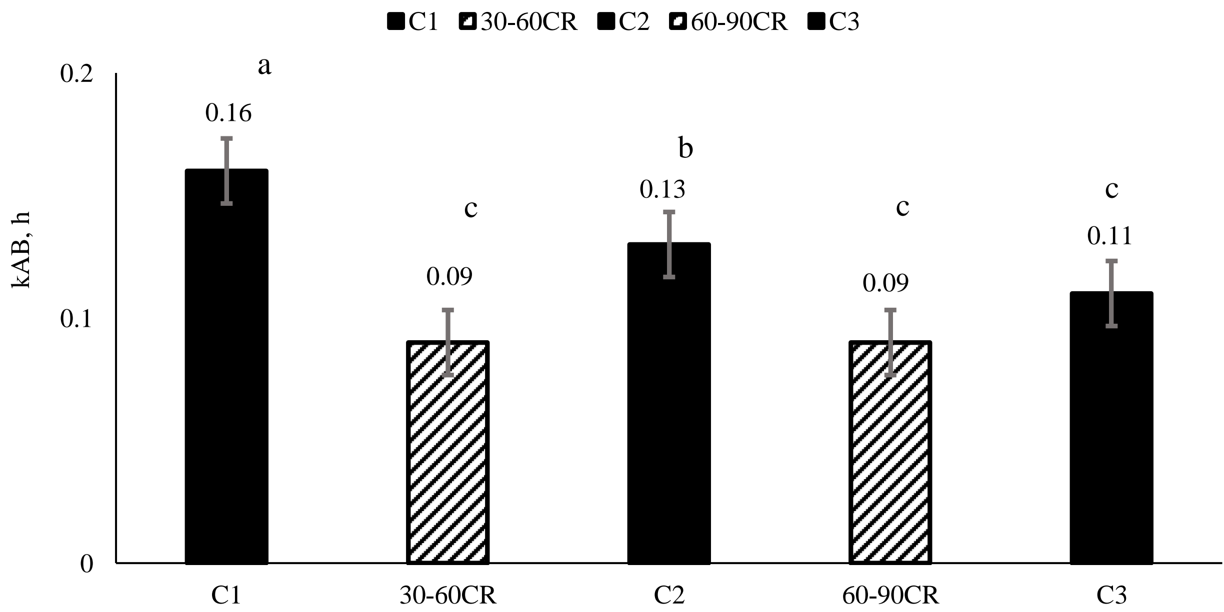


Figure 5.2 Plasma acetaminophen dynamics throughout the 48 h blood sampling period (mean + SE; **a**), and abomasal emptying rates per hour (kABh; mean + SE; **b**) for calves fed either C1: maternal colostrum (30 g/L IgG), C2: maternal colostrum (60 g/L IgG), C3: maternal colostrum (90 g/L IgG), 30-60CR: maternal colostrum (30 g/L IgG) enriched with colostrum replacer for a final concentration of 60 g/L IgG, or 60-90CR: maternal colostrum (60 g/L IgG) enriched with colostrum replacer for a final concentration of 90 g/L IgG. Differences were detected for colostrum treatment, time, and their interaction ($P < 0.01$). [a-d] Values within colostrum treatments with different superscripts are different ($P < 0.05$) after accounting for multiple comparisons by Tukey's adjustment.

6 General Discussion

6.1 Main Findings

The investigations and results from this thesis provide insight into different strategies and management techniques that can be implemented on-farm to enhance colostral IgG absorption, total IgG mass fed, and adequately assess the transfer of passive immunity in newborn calves. This thesis explains how STP can be used with accuracy in calves fed MC in comparison to calves fed CR. In addition, this thesis also evaluated new methods to improve current colostrum feeding practices. These methods include techniques that increase the total IgG mass fed to newborn calves by increasing the number of colostrum meals or by taking advantage of low-quality colostrum and enriching it with CR. Lastly, this thesis aimed to explain how factors such as TS, osmolality, and abomasal emptying affect IgG absorption and AEA.

It is well understood that newborn calves need to ingest colostrum to acquire passive immunity. This condition can be detected by taking a blood sample at 24 h after birth and analyzing it for serum IgG concentrations. Nevertheless, this technique requires trained laboratory technicians and is expensive. As a result, dairy farmers have used refractometry, an indirect method that estimates IgG by measuring STP or Brix. This method has been validated by research experiments (Deelen et al., 2014; Chigerwe et al., 2014; Buczinski et al., 2018) but most data used to develop these cutoff points were from calves fed MC. The protein profile of calves fed distinct CRs available in the market will be different (Quigley et al., 2002). As a result, new cutoff points need to be developed to assess FTPI in calves fed CR. This also creates an opportunity for CR manufacturing companies to develop unique thresholds for their specific products. Chapter 2 of this thesis evaluated a data set from calves fed CR or MC in U.S. and

Canada, including their serum IgG and STP values between 24 and 48 h after birth. A regression analysis determined that a low correlation exists between STP and serum IgG whenever calves are fed CR, but not for calves fed MC. A receiver operator characteristic curve analysis was performed to determine a suitable STP cutoff point to predict a serum IgG value of 10 g/L to assess FTPI. For that specific data set, a STP value of < 4.9 g/dL was reported to indicate FTPI, which is lower than most thresholds used to define FTPI (5.2 or 5.5 g/L; Buczinski et al., 2018). Even though a new cutoff was suggested in Chapter 2, its sensitivity and specificity were not high and could affect the detection of true positives and true negatives. Data from this chapter proves that current cutoff points to assess FTPI are not accurate when calves are fed CR instead of MC. Refractometry can still be used on-farm when calves are fed MC as the correlation is still high, but new cutoff point should be investigated.

Recent colostrum feeding recommendations state the importance of feeding more IgG to newborn calves and aim to increase serum IgG concentrations at 24 h (Godden et al., 2019; Lombard et al., 2020). It is clear that feeding more total IgG mass is beneficial to a calf, but research by Besser et al. (1985) and Saldana et al. (2019) reported that there might be a limited amount of IgG that a calf can absorb in one single colostrum meal. Usually, AEA starts to decrease when calves are fed more than 300 g of IgG (Saldana et al. 2019). Thus, Chapter 3 explains an experiment that compared feeding the same total mass of IgG, but divided into different frequencies. It was found that decreasing the amount of total IgG mass fed at birth compared to a high dose did not increase AEA or improve serum IgG concentration at birth. Those results indicate that if an upper limit of absorption exists, it would be found when > 300 g of IgG are fed in one single meal. However, it was found that calves are still able to absorb

immunoglobulins at 6 and 12 h after birth. Calves fed less IgG at birth, but the same total mass as the other treatment meal, were able to achieve the same level as calves fed a higher dose at birth. These results suggest that even if a high-quality colostrum meal is not offered at birth, there is still a possibility that dairy producers can achieve acceptable calf serum IgG levels if they feed another colostrum meal at 6 or 12 h after birth or at both time points. That said, it is always recommended to feed high-quality colostrum and sufficient IgG mass to a calf within 2 h after birth.

In Chapter 4, as expected, it was found that adding additional water to reconstituted CR decreased TS and osmolality. The reduction of TS tended to linearly increase serum IgG concentrations at 24 h and AEA; and significantly increased abomasal emptying rate. It was hypothesized that diluting CR meals with additional water would decrease TS and osmolality, thereby, increasing IgG absorption and gastric abomasal emptying. In addition, it was expected that reduced fat, protein, and TS would affect this passage, as these components inhibit gastric emptying, with the degree of inhibition proportional to their concentration (Coke, 1975). Our data shows that IgG tended to linearly increase with reduced TS/osmolality, but more research is needed with additional dilutions or testing different colostrum enrichments and colostrum qualities; which differ in composition such as TS and osmolality. Furthermore, calves assigned to different treatments were fed different final volumes, which could have affected our results and their interpretation. This will clearly elucidate the possible benefits of reduced TS/osmolality on IgG absorption.

The feeding of low-quality colostrum, which is usually defined as having a low IgG concentration, is an issue on a dairy farm. Alternatively, some farms have decided to supplement

their existing MC with the addition of CR powder, known as "colostrum enrichment". As a result, in Chapter 5 investigated if supplementation of maternal colostrum with CR enrichment had significant scientific importance. It was found that low-quality colostrum (30 g/L Ig) can be successfully enriched with CR powder. Calves fed the enriched colostrum compared to calves fed the same colostrum without enrichment had their serum IgG concentrations significantly increased. It was further found that enriching moderate-quality colostrum (60 g/L IgG) did not significantly increase serum IgG concentrations and compromised IgG absorption. Various factors could have affected this behavior, mostly the decrease in the abomasal emptying rate. The results from Chapter 5 show that the increased osmolality by adding a solute (CR powder) to MC affected IgG absorption and abomasal emptying rate, but results from Chapter 4 show that IgG absorption only tended to be affected by reduced TS and osmolality. However, both Chapter 4 and 5 demonstrated that osmolality affects abomasal emptying rate, thereby, affecting IgG absorption.

6.2 Relevance of Research

Even though the cutoff points to assess the transfer of passive immunity with STP and Brix has been well-established for calves fed maternal colostrum (Buczinski et al., 2018), they are not suitable for calves that are fed different CRs available on the market. In addition, these cutoff points have not been investigated whenever calves are fed enriched colostrum. These thresholds may also not be suitable for this scenario. A low correlation between STP and serum IgG was reported when calves are fed CR. This statement is necessary to make dairy farmers aware of how they could be erroneously classifying calves with FTPI even though they attained serum IgG concentrations > 10 g/L at 24 h. Also, CR manufacturers would be aware that using their

products can lead to low STP concentrations, even if their product achieves acceptable serum IgG levels or STPI rates. In response, colostrum replacer manufacturers could develop alternative, more accurate STP cutoff points for their product, although these should not be extrapolated to other CRs.

The results from Chapter 3 helped to emphasize the importance of feeding additional meals of colostrum to increase the total IgG mass consumed by a newborn calf. Immunoglobulin G absorption decreases when colostrum feeding is delayed (Fischer et al., 2018a). However, this chapter demonstrated that calves were still absorbing IgGs at 6 and 12 h of life and helped them achieve similar serum IgG concentrations at 24 h to calves fed a higher total mass at birth. It is recommended to always feed a high dose of IgG at birth, but if colostrum is not available or an issue arises, there is still an opportunity to feed at 6 and 12 h. Chapter 4 aimed to prove that TS could affect IgG absorption and also impact abnormal fecal scores or gut permeability in newborn dairy calves. The results of this chapter found that serum IgG concentrations tend to linearly increase with reduced TS but more research is needed to clarify the role of TS on IgG absorption. Perhaps, more dose-response studies with a wide range of TS levels could further clarify current results. Besides, results from Chapter 4 suggest that feeding only one meal with high TS, or osmolality, does not affect abnormal fecal scores up to 7 d of life or gut permeability at 24 h.

Chapter 5 helped to clarify that low-quality maternal colostrum can be enriched with CR and that this enrichment helped calves achieve acceptable serum IgG concentrations at 24 h. This data provides dairy farmers with another option to improve calf serum IgG when their herd is producing colostrum with low IgG concentrations. In addition, it also represents an opportunity

for companies that sell CRs, where they can recommend dairy farmers to take advantage of their low-quality colostrum and enrich it by adding CR, instead of doing a complete replacement. Overall, all these studies provide insight into strategies to improve current colostrum feeding practices in newborn dairy calves.

6.3 Study Limitations

Although results presented in this thesis provide useful colostrum feeding strategies and recommendations to assess FTPI when calves are fed CR, experiments mainly focused on IgG and did not evaluate other components or bioactives in colostrum. These other components such as fat, insulin growth factor-1 (IGF-1), insulin and nucleotides aid on thermoregulation, intestinal development, post-natal growth and gut development, and immune function (Fischer Tlustos et al., 2021). Another limitation is that calves were not evaluated for growth parameters or health until weaning which could have explained the short-term effects of the distinct colostrum feeding strategies proposed. Chapter 5 could have contributed to clarify other benefits of enriching colostrum. In addition, it would have been of interest to investigate if calves receiving additional fat with the enrichment had improved thermoregulation.

While this thesis provides many relevant and important results, there are factors that limited some of the data analysis, interpretation, and conclusions. As such, further research on colostrum feeding and management practices is needed to wholly benefit newborn calves. Even though it was demonstrated that using STP to estimate serum IgG concentrations is inaccurate when calves are fed CR, a new suggested cut-off point was provided from one specific data set. This cutoff should not be extrapolated for calves fed CRs from other manufacturers and more data is needed from different CRs to develop a suitable cutoff point. In addition, the cutoff

presented did not have a high sensitivity or specificity. As such, FTPI prevalence should be assessed at a herd level instead of emphasizing on individual calves. One specific threshold value could be developed for each CR or manufacturer until a consensus is established. In the second experiment presented in this thesis, one of the limitations was to not include an experimental treatment where calves were fed only one meal at birth, with this meal having a higher IgG mass dose. Previous studies have shown that AEA drops when calves are fed > 300 g IgG in one single meal after birth (Saldana et al., 2019). This study failed to provide a significant load of IgG mass (> 300 g) in one single meal, which could have led to an upper limit of absorption.

In Chapter 4, serum IgG concentrations at 24 h and AEA tended to increase with decreasing TS/osmolality. In addition, from those results it could be suggested that one meal high in TS/osmolality does not affect abnormal fecal score appearance in newborn calves. Our results suggest that TS and osmolality plays a role in how IgG is absorbed, but more research is needed to find significant results and the mechanism behind it. A limitation of this chapter is that health scoring was only performed until 7 d of life. Therefore, a study evaluating the effects of one hypertonic meal up to weaning is needed. Lastly, an experiment feeding the same volume to all calves, but with distinct TS and osmolality, would further clarify our current results.

Chapter 5 of this thesis was able to demonstrate the possibility of enriching the IgG concentration of low-quality MC. However, enriching MC with an IgG concentration of 60 g/L to 90 g/L IgG was not successful and compromised colostral IgG absorption. Possibly, enriching to a final IgG concentration of 90 g/L was too high and enriching to lower concentrations, 70 or 80 g/L, could have significantly increased serum IgG concentrations at 24 h. Also, the specific characteristics of the MC used in that experiment could have affected the results. The Brix

concentrations were lower than expected. For example, an expected IgG concentration of 50 g/L is expected from a 22 % Brix but, instead, the one MC treatment resulted with 90 g/L IgG while having 22.6% Brix. Thus, more enrichments with different colostrum sources at different IgG concentrations or Brix should be tested.

6.4 Future Research

The results from this thesis clarified that using refractometry to estimate STP and FTPI in calves fed CR is inaccurate, but more research and analysis of distinct data sets from calves fed different CRs available in the market is needed. Perhaps different cutoff points could be developed for specific CR products. The threshold provided in our study had a sensitivity and specificity below 80%, which could be improved with larger data sets and analyses. New cutoff points are needed for distinct CR available on the market or a consensus need to be defined for whenever calves are fed CR rather than MC. This is needed, as dairy farmers use STP refractometry, a widely used on-farm tool to evaluate their overall colostrum management practices. Besides, future search could evaluate or develop IgG test that can be used on-farm without the necessity of expensive equipment or trained laboratory technicians. The FTPI could be monitored by evaluating the mean STP values calves achieve when using one specific CR product. In addition, it has not been evaluated the accuracy of STP when calves are fed colostrum that has been enriched with CR. Therefore, a regression analysis between serum IgG and STP should clarify how protein levels behave when calves are fed enriched colostrum. Lastly, it also has to be evaluated if diluting CR meals, as in Chapter 4, has any effects on STP readings as a method to asses FTPI in newborn calves.

Results from Chapter 3 suggest that feeding a third meal of colostrum in comparison to feeding two meals, does not result in added benefits to IgG absorption. Future studies need to evaluate different IgG masses fed either in one, two or three colostrum meals. In addition, future research needs to evaluate high doses of IgG fed at birth, to determine at what level IgG absorption starts to decrease. Evaluating dose-effect experiment that includes feeding 200, 300, 400, 500, and 600 g IgG in one single meal would provide more details about how AEA behaves with high loads of IgG mass fed at birth. Clarifying this concept will lead to more efficient colostrum feeding practices. The current colostrum feeding recommendations state calves should be fed a minimum of 150-200 g IgG (Godden et al., 2019). Chapter 3 showed that feeding 257 g did not compromise IgG uptake, which leaves opportunity to increase current colostrum recommendations without affecting AEA. In addition, cows can produce 5-10 L of colostrum (Moore et al., 2009), which is more than what is currently being feed to newborns.

The results from Chapter 4 support the hypothesis that reduced TS or osmolality increases abomasal emptying rate. Even though IgG uptake tended to decrease with reduced TS; more research evaluating different TS and osmolality levels is needed to better understand how it affects IgG absorption. Nevertheless, data from Chapter 5 revealed that colostrum's increase in TS and osmolality due to the addition of CR to enrich the IgG concentration significantly decreased AEA. Results from Chapter 5 provide insight that high TS and osmolality levels could play a major role in how IgG is absorbed. The specific level at which TS and osmolality in the first colostrum meal impact IgG absorption, abomasal emptying rates, AEA, or abnormal fecal score onset remains unclear. More research focusing on these areas would help clarify effects and the mechanisms behind them. The industry needs to know which colostrum IgG

concentration besides 30 g/L can be enriched and significantly increase serum IgG concentrations at 24 h. As a result, new recommendations on colostrum enrichment and utilization of low-quality could be established by clarifying to dairy producers which colostrum qualities, in terms of IgG concentration, can be enriched. In addition, more strategies to utilize colostrum with low-IgG concentration need to be defined. For example, data from Chapter 3 demonstrates that calves are able to absorb colostrum if fed at 6 and 12 h and still achieve similar serum IgG concentrations as calves fed higher IgG doses at birth. This could lead to further research evaluating the enrichment of colostrum meals offered at 6 or 12 h after birth. This could also demonstrate the capacity of enriching transition milk which is sometimes fed as a second or third colostrum feeding. In addition, transition milk is not commonly fed while it still represents a significant amount of total colostrum IgG yield (Schalich et al., 2021).

In summary, this thesis provided insight into new colostrum feeding strategies that: increase total IgG mass fed, improve serum IgG concentrations at 24 h, strategically use on-farm low-quality colostrum, and provides guidance on how to accurately assess passive immunity in calves that are fed CR. In addition, recommendations that were proposed can be easily adapted on-farm. Overall, it is concluded that there are various ways to improve colostrum feeding practices that can increase the acquired immunity of a newborn calf. Data from all this research emphasizes the need to evaluate new methods and strategies for maximizing total IgG mass fed and absorption in newborn calves.

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