

The Effect of Different Management Factors on the Health and Performance of Pre-Weaned
and Post-Weaned Dairy Calves

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AUTHORIZATION TO SUBMIT THESIS

This thesis of Bekir Ozer, submitted for the degree of Master of Science with a major in Animal and Veterinary Science and titled “The Effect of Different Management Factors on the Health and Performance of Pre-Weaned and Post-Weaned Dairy Calves,” has been reviewed in final form. Permission, as indicated by the signatures and dates below, is now granted to submit final copies to the College of Graduate Studies for approval.

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ABSTRACT

The main purpose of the work described in this thesis is to identify management strategies for maximizing IgG levels in calves on modern dairies. It is well established that 25 to 35 % of dairy cows on US dairies must be replaced annually to maintain herd size and improve genetics (Harris and Shearer, 2005). The cost of raising dairy heifers increases if inadequate management results in a higher than normal morbidity and/or mortality. Colostrum management has a very large impact on dairy calf health because calves are born without significant number of immunoglobulins (antibodies). The objectives of these studies were to 1) determine the effect of adding a serum derived colostrum supplement to maternal colostrum and its effects on serum immunoglobulin concentration in calves, health and performance of pre-weaned and post-weaned dairy calves, 2) determine the effect of quantity and frequency of colostrum feeding on serum immunoglobulin concentration, health parameters, and growth in Holstein calves, 3) determine the correlation between total serum protein in calves and first lactation milk performance.

Adding a supplement to maternal colostrum did not achieve any positive effect on performance and health parameters of dairy calves. Feeding Holstein calves 2 separate feedings of maternal colostrum will improve passive transfer (PT) and might lead to some health benefits. The effect of colostrum feeding quantity and frequency on respiratory scores (RS) needs to be investigated further. In the third study, calves that had total serum protein (TSP) below 5.4 mg/d produced $10,551 \pm 230$ kg, those with TSP between 5.4 and 6.4 mg/dL produced $10,499 \pm 229$ kg, and those with TSP above 6.4 mg/dL produced $10,445 \pm 230$ kg in the first lactation. There was no relationship ($P=0.13$) between TSP and future milk production.

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DEDICATION

To my major professor, family and to my friends, for their never-ending support and for encouraging me to never give up.

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CHAPTER 1: LITERATURE REVIEW

COLOSTROGENESIS IN DAIRY COWS

Colostrum is defined as the first lacteal secretion of the mammary gland prior to parturition and up to 3 days afterward (McGrath et al., 2016). The production of colostrum is termed colostrogenesis and comprises the prepartum transfer of immunoglobulins and other serum proteins from maternal serum to mammary gland secretions. Immunoglobulins in mammary secretions are humoral, which arise from the blood stream, and local which arise from production by plasmacytes in the mammary gland (Larson et al., 1980). Immunoglobulins are transferred in utero or via the colostrum and they primarily belong to the immunoglobulin G (IgG) class. Immunoglobulin A (IgA) and immunoglobulin M (IgM) classes are locally produced by plasmacytes located adjacent to the secretory epithelium. The bovine colostrum contains small amounts of IgA and IgM. The transport of IgG into colostrum begins 5 weeks before parturition with the highest concentration occurring 1-3 days before parturition in the cow (Brandon et al., 1971; Sasaki et al., 1976; Barrington et al., 2001; Baumrucker et al., 2014). The large amounts of IgG are transported by a process of transcytosis across the mammary epithelial cells during colostrogenesis. These lacteal secretions are facilitated by mammary gland secretory epithelial receptors (Larson et al., 1980; McGuirk and Collins, 2004; Baumrucker et al., 2010). Immunoglobulin G exists in the forms IgG1 and IgG2, the mammary transport of IgG into colostrum is very specific and transfer accumulates only IgG1 in high concentrations in colostrum but not IgG2 (Larson et al., 1980). Immunoglobulin G1 is transported across mammary epithelial cells by a receptor specific for IgG1 (Barrington et al., 1997) which causes a 10-fold increase in its concentration in the colostrum compared to its concentration in the bovine maternal serum (Besser and Gay, 1994). Throughout

colostrogenesis, up to 500 g/week of IgG1 are transferred into mammary secretions (Brandon et al., 1971) and IgG1 constitutes approximately 85% of the total immunoglobulins in colostrum (Larson et al., 1980). In addition, colostrum includes maternal leukocytes, growth factors, hormones, cytokines nonspecific antimicrobial factors, and nutrients (Roy, 1970; Roy, 1990; Godden, 2008). The immune components of colostrum in dairy cattle are immunoglobulins and immunologically active maternal leukocytes which average 1×10^6 cells/mL. These leukocytes include macrophages, T and B lymphocytes, and neutrophils (Larson, 1980). Eventhough the leukocytes' functional role is unknown, it has been suggested that their role is to enhance lymphocyte response to nonspecific mitogens, increase phagocytosis and bacteria killing ability, and stimulate humoral immune response (Donovan et al., 2007). Colostrum nutrient content is also very important for the new born calf. The total solid content (%) in colostrum is increased incomparison to normal milk (Table 1). Most of this increase in colostrum solid content is due to protein and casein content. Protein content is increased due to the simultaneous accumulation of IgG. The crude fat content in colostrum is also significantly higher than normal milk. (Table 1). The increase of fat content contributes additional energy that is critical for thermogenesis and body temperature regulation of the calf (Godden, 2008).

As seen in Table 1, many constituents present in whole milk are changed in colostrum to cover the needs of the newborn calf. Colostrum quality is determined by IgG level because the absorption of adequate amounts of colostral immunoglobulin by calves during the first 24 h of life is critical for the calf's ensuing resistance to infectious disease (Besser and Gay, 1994). Variation in IgG level between cows and cow breeds have been identified (Gulliksen et al., 2008, Conneely et al., 2013). A minimum level of 50g of IgG/L colostrum has been suggested to be the threshold value for high quality colostrum. This recommendation is based on studies

Table 1. Compositional analysis of colostrum of samples collected from cows in Pennsylvania (Kehoe et al., 2007), compared with Foley and Otterby (1978) compositional analysis of colostrum and milk.

Compositional analysis of colostrum from samples collected from cows in Pennsylvania (Kehoe et al., 2007)						Foley and Otterby, 1978	
Item	n	Mean	SE	Minimum	Maximum	Mean Colostrum	Milk
Fat, %	54	6.7	4.16	2	26.5	6.7	4
Protein, %	55	14.92	3.32	7.1	22.6	14	3.1
Lactose, %	55	2.49	0.65	1.2	5.2	2.7	5
Total solids, %	55	27.64	5.84	18.3	43.3	23.9	12.9
Ash, %	55	0.05	0.01	0.02	0.07	1.11	0.74
IgG, mg/mL	—	—	—	—	—	32	0.6
IgG1, mg/mL	55	34.96	12.23	11.8	74.2	—	—
IgG2, mg/mL	55	6	2.82	2.7	20.6	—	—
IgA, mg/mL	55	1.66	0.99	0.5	4.4	—	—
IgM, mg/mL	55	4.32	2.84	1.1	21	—	—
Lactoferrin, mg/mL	55	0.82	0.54	0.1	2.2	—	—
Retinol, µg/g	55	4.9	1.82	1.4	19.3	2.8	—
Tocopherol, µg/g	55	2.92	3.65	0.6	10.4	—	—
β-Carotene, µg/g	55	0.68	0.63	0.1	3.4	—	—
Vitamin E, µg/g of fat	55	77.17	33.51	24.2	177.9	84	15
Thiamin, µg/mL	54	0.9	0.28	0.3	2.1	0.58	0.38
Riboflavin, µg/mL	54	4.55	0.31	2.4	9.2	4.83	1.47
Niacin, µg/mL	54	0.34	1.57	0	1.6	0.96	
Vitamin B12, µg/mL	5	0.6	0.35	0.2	1.1	0.049	0.006
Folic acid, µg/mL	—	—	—	—	—	0.008	0.002
Pyridoxal, µg/mL	54	0.15	0.07	0.1	0.3	—	—
Pyridoxamine, µg/mL	54	0.21	0.07	0.1	0.5	—	—
Pyridoxine, µg/mL	5	0.04	0.07	0	0.2	—	—
Pantothenic acid, µg/mL	—	—	—	—	—	1.73	3.82
Ca, mg/kg	55	4,716.10	1,898.00	1,775.10	8,593.50	2,600	1,300
P, mg/kg	55	4,452.10	1,706.29	1,792.40	8,593.50	—	—
Mg, mg/kg	55	733.24	286.07	230.3	1,399.60	400	100
Na, mg/kg	55	1,058.93	526.02	329.7	2,967.80	700	400
K, mg/kg	55	2,845.89	1,159.89	983.2	5,511.40	1,400	1,500
Zn, mg/kg	55	38.1	15.9	11.2	83.6	12.2	3
Fe, mg/kg	55	5.33	3.09	1.7	17.5	2	0.5
Cu, mg/kg	55	0.34	0.14	0.13	0.64	0.6	0.1
S, mg/kg	55	2,595.67	904.97	889.4	4,143.70	—	—
Mn, ¹ mg/kg	23	0.1	0.11	0	0.36	0.2	0.04

¹Part of the samples were quantified as <0.05 and therefore not included in averages.

showing that colostrum containing IgG below 50g of IgG/L had significant rates of inadequate serum IgG concentrations in calves (Besser et al., 1991, Hopkins and Quigley, 1997; Korhonen et al., 2000; Biemann et al., 2010).

Five IgG classes in bovine colostrum were described by Butler (1969), IgG1, IgG2, IgM, IgA and IgE. The colostrum distribution approximates 85 to 90% IgG, 7% IgM, and about 5% IgA while the IgG1 accounts for about 80 to 90% of the total IgG. Immunoglobulin E is also present in colostrum but in very small amounts (McGuirk and Collins, 2004). The immunological function of the IgGs is comprehensive and depends on the Ig class (Kohornen et al., 2000). Immunoglobulin G and IgM have an array of functions among which activation of bacteriolytic reactions are most important. They also enhance the recognition and phagocytosis of bacteria by leucocytes. Immunoglobulin M is considered to be more effective than IgG in the mentioned functions but is present in smaller amounts. Although present in even smaller amounts, IgA exerts an important local protection against intestinal disorders by preventing adhesion of enteropathogenic bacteria to mucosal epithelial cells. Also, it neutralizes viruses and bacterial toxins and agglutinates antigens (Kohornen et al., 2000).

PLACENTAL STRUCTURE

Placental structures vary between animal species. The primary function of the placenta is to act as an interface between the dam and fetus. It performs many important functions during gestation. These functions include attaching the developing fetus to the uterine wall, mediating maternal immune tolerance, O₂ / CO₂ exchange, providing nutrients for the fetus and removing waste products during embryonic development.

Placenta types are classified according to the histologic relationship established between the chorion and uterine wall. The ruminant placenta is an epitheliochorial type which is very superficial and lacks significant invasion of the uterine lining (Weaver et al., 2000; Frukawa et al., 2014). This type of placenta is non-invasive due to 8 membranes separating the fetal and maternal blood circulations (Fowden, 2006). A ruminant's placenta is also considered to be cotyledonary where the chorionic villi are concentrated in cotyledons which originate from the chorion and attach to the caruncle of the uterus. The transport of nutrients with large molecular weights depends on the uterine lining and how many membranes separate the fetal and maternal blood supplies (Senger, 1999). This separation between the maternal and fetal blood supplies in cattle prevents the transmission of immunoglobulins in utero, therefore calves are born agammaglobulinemic, rendering the ingestion and absorption of adequate amounts of colostral immunoglobulins essential for establishing passive immunity (Weaver et al., 2000).

COLOSTRUM NUTRIENT COMPOSITION

Neonatal calves have a relatively mature gastrointestinal tract, but it still requires morphological and functional changes. The intake of colostrum with its nutrient and non-nutrient components aids in gastrointestinal development and function. The colostrum provides immunoprotection and nutrients which are essential for the survival of calves during the neonatal period (Blum, 2006). The physical properties and the composition of colostrum are highly variable due to many factors, such as; breed, parity, pre-partum nutrition, length of the dry period of cows and time post-partum (Parrish et al., 1950; McGrath et al., 2016). Colostrum contains less lactose and more fat, protein, peptides, non-protein nitrogen, ash, vitamins and minerals, hormones, growth factors, cytokines and nucleotides than mature milk; except in the case of lactose, the levels of these compounds decrease rapidly during the first 3 days of lactation (Blum, 2006;

McGrath et al., 2016). It is essential that the newborn calf receives an adequate supply of colostrum as both the concentration of immunoglobulins and the permeability of the gut decrease rapidly over the first 24h following parturition (Weaver et al., 2000). Close to 60% of maternal colostrum produced on US dairy farms does not meet the minimum requirements for passive transfer and bacteriological standards. Up to 30% of maternal colostrum contained <50 mg of IgG/mL and 60.6 % had total plate count of >100,000 cfu/mL, respectively (Morrill et al., 2012a).

Adequate colostrum management and feeding is important to reduce neonatal mortality, strengthen immunity and increase animal life span (Besser et al., 1988; Besser and Gay, 1994; Quigley et al., 1998). Delaying colostrum feeding decreases immunoglobulin absorption and fat-soluble vitamins' absorption (Zanker, 2000). Delaying first colostrum feeding by more than 12–13 h after birth impaires the plasma beta-carotene, retinol and alpha-tocopherol status during the 1st month of life compared to calves that receive colostrum within 7 h (Zanker, 2000). The composition of colostrum is important in satisfying the nutritional requirements of neonatal dairy calves, principally for nutrients which only minimally cross the placenta, such as fat-soluble vitamins (Spielman et al., 1946).

DEVELOPMENT OF IMMUNITY IN THE CALF

The placenta of the cow does not allow the transfer of maternal immunoglobulins to the fetus in utero (Weaver et al., 2000). Consequently, the bovine neonate's serum is agammaglobulinemic at birth (Weaver et al., 2000). Immunoglobulin G is indispensable due to its protective role against infectious agents, thus the neonate relies on the passive immunity derived from colostrum ingestion (Kruse, 1983; Weaver et al., 2000). Passive acquisition of

maternal immunoglobulins is facilitated by the ability of the neonatal enterocyte to non-selectively absorb immunoglobulins and other macromolecules during the first 24 to 36 h after birth, by pinocytosis (Kruse, 1983; Weaver et al., 2000). Subsequently, the transepithelial absorption of macromolecules is low (Kruse, 1983). In addition, during this period the proteolytic activity within the digestive tract is low and trypsin inhibitors present in colostrum limit the destruction of immunoglobulins and other biologically active macromolecules (Kruse, 1983; Godlewski et al., 2005).

Once the neonatal enterocyte absorbs immunoglobulins, then immunoglobulins are transported across the cell wall into the lymphatic system and from there they enter the bloodstream via the thoracic duct (Kruse, 1983; Weaver et al., 2000). In nature, the calf normally stands within 30 minutes of birth and consumes its first colostrum meal shortly afterward. In dairy based colostrum management programs calves are immediately removed from their dams, and the recommended optimal time for colostrum ingestion is 8 to 12 h after birth (Weaver et al., 2000). Feeding colostrum via an esophageal feeder is recommended to ensure optimal colostrum ingestion (Molla, 1978; Weaver et al., 2000). While the exact mechanism of intestinal closure remains unknown, it appears to be initiated by colostrum ingestion and is believed to be under some endocrine influence coordinated by digestive enzyme development (Stott et al., 1979; Kruse, 1983). The highly vacuolated immature enterocyte population undergoes marked proliferation and is replaced by a mature microvillus surface devoid of pinocytotic capability (Kruse, 1983; Kaup et al., 1996; Weaver et al., 2000; Godlewski et al., 2005). Time of ingestion, quality and quantity of colostrum ingested, method of colostrum administration, fetal stress and respiratoric acidosis status of the calf can influence the passive transfer of colostrum immunoglobulins (Molla, 1978; Stott et al., 1979; Kruse, 1983; and Weaver et al., 2000).

Adequate transfer of colostral immunoglobulin in the calf is represented by a serum IgG concentration of 1,000 mg/dL or greater. This compares with a serum total protein concentration greater than or equal to 5.2 g/dL (Tyler et al., 1998). Pathogen elimination during the pre-colostral post-natal period is dependent upon innate immune mechanisms, primarily phagocytic leukocytes and complement proteins (Osburn, 1981).

In comparison with adults, neutrophils and key components of the complement system function are much less efficient in neonatal calves (Mueller et al., 1983). Not only are circulating levels of the C3 complement protein low but neutrophil Fc-receptor expression and bactericidal activity are less efficient, resulting in inherent susceptibility to bacterial infections (Osburn, 1981; Mueller et al., 1983). Due to the newborn calf's limited antigenic recognition, the immune system of the bovine neonate is characterized by having a limited ability to recognize and target lipopolysaccharide (LPS) prior to 4 weeks of age (Osburn, 1981; Mueller et al., 1983). The bovine neonatal immune system antibody production starts 3 weeks after birth with endogenous IgG1 production (Devery et al., 1979). However, the rate of production was minimal (1 g/day) and is invariably dependent on antigenic exposure (Devery et al., 1979). The bovine neonate also has a high proportion of antigen-presenting cells with defective co-stimulatory function, and depressed T helper cell 1(Th1) cytokine production (Nonnecke et al., 2005).

The bovine colostrum contains nutrients such as vitamins and essential fatty acids, as well as non-nutrient components including immunoglobulins, leukocytes, lysozyme, tri-peptide growth factors and anti-oxidants which have demonstrated roles in innate immunity (Migliore Samour et al., 1992; Weaver et al.; 2000; Blum, 2006); peptides such as transforming growth factor-beta (TGF- β), insulin-like growth factor-1 (IGF-1) and cytokines. While colostrum-replete

calves are less likely to develop neonatal respiratory and enteric disease, failure of passive transfer does not guarantee mortality in affected calves (Tyler et al., 1998; Weaver et al., 2000). However, some studies have demonstrated that the disadvantages associated with failure of passive transfer often persist later in life (Robison et al., 1988; DeNise et al., 1989; Tyler et al., 1998). For example, calves with failure of passive immunity, or those which have failed to ingest an adequate volume of colostrum within the appropriate time-frame (24h serum IgG concentration < 5.2 g/dL), have significantly increased relative risk of mortality. A risk that persists at least until 10 weeks of age (Tyler et al., 1998). Additionally, decreased productivity and longevity has been observed throughout the lifetime of affected animals within the herd (Robison et al., 1988; DeNise et al., 1989). In the 2001 National Animal Health Monitoring System (NHAMS) survey of pre-weaning mortality rates among live heifer births, 8.9 % of deaths were attributed to factors related to impaired host immunity (USDA, 2001).

COLOSTRUM QUALITY

To meet industry quality recommendations, maternal colostrum's IgG levels should be >50mg of IgG/mL, and bacterial contamination total plate count (TPC) should be <100,000 cfu/mL (McGuirk and Collins, 2004; Johnson et al., 2007; Morrill et al., 2012). Calves receiving Maternal colostrum (MC) with lower bacterial contamination had greater 24-h serum IgG concentrations compared with their counterparts (22.3 and 18.1 mg/mL, respectively). Although both groups of calves obtained adequate passive transfer, the apparent efficiency of absorption of IgG was decreased in calves fed MC with greater bacterial contamination (33 vs. 27%) (Morrill et al., 2012). Colostrum production is often lower in first lactation cattle, suggesting less mammary development and potentially reduced transport capacity for IgG into the mammary gland (Devery-Pocius and Larson, 1983).

COLOSTRUM REPLACER AND SUPPLEMENTS

Due to variation in colostrum management, non-compliance of determining colostrum quality, shortages of maternal colostrum (MC), and concerns about controlling infectious pathogens, colostrum replacers (CR) and colostrum supplements (CS) have been developed to help support calf immunity. Depending on the source of IgG (colostrum, milk, eggs, or bovine blood), the product can be very expensive, or prohibited where laws control the use of blood-derived IgG products. Colostrum replacers are designed to provide ≥ 100 g of IgG per dose, while CS are designed to provide < 100 g of IgG per dose (Quigley et al., 2002). Colostrum replacer is designed to replace a colostrum meal, whereas a supplement is designed to boost poor quality colostrum used in initial feedings. There are mixed results in terms of the efficacy of these products (Godden et al., 2009). A recent study by Poulsen et al. (2010) indicated that there was no significant difference in the risk of failure of passive transfer (FPT) when calves were fed 1 dose of replacer containing 125 g IgG within 2 h of birth followed by another dose of supplement containing 45 g IgG within 12 h of birth, compared to calves fed MC. Though the average total serum protein (TSP) and serum IgG of both groups were above the cut-off for FPT, calves fed MC had significantly higher TSP and serum IgG (5.59 g/dL and 18.68 mg/mL) compared to the other group (5.27 g/dL and 13.48 mg/mL). Godden et al. (2009b) determined the serum IgG, TSP, and risk of FPT (serum IgG < 10 mg/mL) of calves fed CR and MC. They found that 46% of calves ingesting the manufacturer recommended dosage of CR (1 dose=100 g IgG) had FPT compared to 9% of calves fed MC or 0% of calves fed two doses of CR. Calves ingesting either MC or 2 doses of CR had significantly higher TSP and serum IgG levels at 24 h compared to calves receiving 1 dose of CR. Similarly, in a separate study, calves fed MC had significantly higher serum IgG (17.6 mg/mL) and TSP (5.4 g/dL) compared to calves fed 100

g of IgG in CR (7.5 mg/mL and 4.4 g/dL) or calves fed 150 g IgG in CR (9.1 mg/mL and 4.7 g/dL). Only 5% of calves fed MC had FPT compared to 95% of calves fed 100 g IgG from CR and 76% of calves fed 150 g IgG from CR (Smith and Foster, 2007). Calves fed an additional 185 g of IgG via CS mixed into 2L of colostrum, fed at 0 and 12 h, had similar TSP levels compared to calves fed colostrum by itself (Morin et al., 1997). There are numerous other studies providing similar results, whereby calves have higher serum IgG and TSP levels when fed MC rather than CR (Swan et al., 2007; Priestley et al., 2013). This indicates that feeding MC over CR can reduce the risk of FPT and that producers should have knowledge about the proper use of replacers and supplements. There are several possible explanations for the inadequacy of replacer and supplement products. One is that these products do not contain bioactive components that are present in MC, such as colostral leukocytes, growth factors, and cytokines that may provide unknown benefit to the calf or assist in passive transfer of immunity (Godden, 2008). Other explanations are that the quality control or manufacturing protocols used to create these products are not adequate, or that the generally accepted assumption that consuming 100 g of IgG is sufficient to prevent FPT is incorrect and a higher mass of IgG needs to be consumed (Chigerwe et al., 2008a; Godden et al., 2009b). It has also been suggested that casein in colostrum supplements is the cause of reduced apparent efficiency of absorption (AEA) of IgG in calves when it is added to MC (Smith and Foster, 2007).

Recently colostrum supplements and colostrum replacers have been formulated for use in dairy calves. Effects of colostrum replacers on serum IgG concentration indicate varying results depending on the type of product used due to the differences in IgG concentrations (Quigley et al., 2001; Wereme et al., 2001; Hammer et al., 2004; Foster et al., 2006).

CHAPTER 2: ADDITION OF COLOSTRUM REPLACER TO MATERNAL COLOSTRUM AND ITS EFFECTS ON HEALTH AND PERFORMANCE OF PRE-WEANED AND POST-WEANED CALVES

ABSTRACT

The objective of this study was to determine the effect of a commercially available colostrum replacer on health parameters in Holstein dairy heifers. Fifty-seven Holstein female calves raised on a commercial facility in southern Idaho were randomly assigned to one of two treatments which consisted of maternal colostrum (MC, n=27) or maternal colostrum supplemented with bovine-serum based colostrum supplement (MCS, n=30). All colostrum treatments (3.8 L) were administered using an esophageal tube within 1 h of birth. A second feeding of colostrum (2 L) was administered 8 h following the first feeding. Blood samples were collected at 24±3 h of age and tested for total serum protein (TSP). Pre-weaned period rectal body temperature was measured every other day. Health evaluations were conducted daily by study personnel until calves were 3 months of age. Pre-weaned fecal (FC), dehydration (DH) and respiratory (RS) scores were recorded. Serum IgG and TSP concentrations were significantly greater ($p<0.002$ and $p<0.01$) in calves fed MC (IgG=23.99±0.78 g/L, TSP=6.17±0.09 g/L) compared to calves fed MCS (IgG=20.41±0.78 g/L, TSP=5.84±0.09 g/L). Rectal body temperature was recorded until calves were weaned and did not differ between MC and MCS and averaged 39.0°C±0.03. No differences were detected in pneumonia, or diarrhea incidence which averaged 77.9% and 10.5% respectively. Maternal colostrum+ supplement calves had a greater ($p<0.006$) incidence of abnormal RS score (58.5%) compared to MC (27.3%) calves. Fecal and DH scores did not differ between treatments and averaged 27.6%

and 12.7% respectively. Thus, in this study, adding a supplement to maternal colostrum did not achieve any positive effect on performance and health parameters of dairy calves.

INTRODUCTION

It is well established that 25 to 35 % of dairy cows on US dairies must be replaced annually in order to maintain herd size and improve genetics (Harris and Shearer, 2005). Therefore, quality dairy heifers must be available to replace the culled cows. The cost of raising dairy heifers increases if inadequate management results in a higher than normal morbidity and mortality. Colostrum is key in establishing immune protection because it contains immunoglobulins that increase the possibility of neonatal survival and is a very important source of essential nutrients. Failure of passive transfer (FPT) contributes to excessively high pre-weaning mortality rates and other short- and long-term losses associated with animal health and welfare. Current recommendations are feeding 3 to 4 L of high quality (>50g/L IgG and <100,000 cfu /mL of bacteria) colostrum within 6h of life (McGuirk and Collins, 2004; Godden, 2008; Chigerwe et al., 2008b). The benchmark for FPT is defined as a serum IgG concentration below 10 mg/ml (1,000 mg/dL) (Tyler et al., 1996; Faber et al., 2005; Beam et al., 2009). Tyler et al., (1996) has demonstrated that a benchmark for total serum protein (TSP) concentration of 5.2 g/dL was equivalent to 1,000 mg/dL serum IgG1, using a digital or clinical refractometer.

Studies demonstrated that calves with inadequate immunoglobulin concentrations have reduced growth rate, increased risk of disease and death, increased risk of being culled (Robison et al., 1988; Donovan et al., 1998; Furman-Fratczak et al., 2011), and decreased milk production (DeNise et al., 1989; Faber et al., 2005). Early studies conducted with calves have demonstrated that most calves deprived of colostrum develop septicemia (Smith, 1962; Gay, 1965).

Management strategies for maximizing colostrum IgG levels should be identified on modern dairies.

On dairies, limited colostrum reserves could be attributed to the variation in quality among individual cows (breed, lactation number, and colostral immunoglobulin concentration) (Swan et al., 2007; Kahoe et al., 2007.) and biosecurity programs to prevent the transmission of diseases such as bovine viral diarrhea, salmonella, *Mycobacterium avium* ssp., paratuberculosis, and bovine leukemia virus (McGuirk and Collins, 2004). Low colostrum quality, or not enough production of colostrum are the major contributing factors for failure of passive immunity. Reports point out that the prevalence of failure of passive transfer in dairy heifers has decreased from over 40% in 1991-1992 (USDA, 1993) to 19.2% in 2007 (Beam et al., 2009). Note, however, that in 2007 only healthy calves that had received colostrum were tested, while, in 1991-1992 (USDA, 1993) all calves were tested, as calf health or colostrum administration were not considered (Beam et al., 2009).

Failure of passive transfer continues to be a major factor for morbidity and mortality in dairy calves (Beam et al., 2009). The most important way to reduce calf morbidity and mortality is the early administration of adequate amounts of IgG in colostrum. The awareness of the importance of confirming successful passive transfer of immunity in neonatal calves has led dairy producers to consider colostrum supplements (CS) or colostrum replacers (CR) in addition to improving colostrum quality. These products are considered to provide supplemental IgG to the neonate during the time of macromolecular transport (Davenport et al., 2000). Generally, CS provides an additional boost of IgG (between 25 and 45 g of IgG per dose) to calves when colostrum has low Ig concentration, while CR can be used as a substitute for maternal colostrum (between 100 and 130 g of IgG per dose) (Quigley et al., 2001; Godden et al., 2009).

Commercially available colostrum supplements (CS) and colostrum replacers (CR) are derived from ultrafiltration of bovine whey, dried colostrum, or blood serum as the primary source of IgG. Studies have demonstrated that a few serum based colostrum replacers (Quigley et al., 2002; Poulsen et al., 2003; Jones et al., 2004; Pithua et al., 2010) and one colostrum-based replacer product (Foster et al., 2006) have been effective, while many other products have failed to routinely provide the necessary 1,000 mg/dL of serum IgG to dairy calves (Zaremba et al., 1993; Garry et al., 1996; Mee et al., 1996; Holloway et al., 2002; Foster et al., 2006) when fed according to label directions. In addition, most of these products do not contain bioactive components that are present in MC, such as growth factors, leukocytes and cytokines that may provide many unknown benefits to the calf or assist in passive transfer of immunity (Godden, 2008).

The objectives of this study were to determine the effect of adding a serum derived colostrum supplement to maternal colostrum on serum immunoglobulin concentration in calves as well as, calf health and performance.

MATERIALS AND METHODS

This study was conducted on a large commercial dairy in southern Idaho. The Holstein female calves (n=57) were randomly assigned to one of two treatment groups which consisted of maternal colostrum (MC, n=27) or maternal colostrum supplemented with bovine-serum based colostrum supplement (Lifeline, American protein Corp., Ames, IA) (MCS, n=30). The same batch of colostrum was used. All colostrum treatments (3.8 L) were administered using an esophageal tube within 1 h of birth. A second feeding of colostrum (2 L) was administered 8 h following the first feeding. The calves that had experienced dystocia, and twin born calves were

not included in the study. Calves were removed from their dams before colostrum ingestion. The umbilicus of each calf was treated with 7% iodine solution and the calf was weighed and the weight was recorded. The calves were placed in a straw bedded pen in the maternity barn and identified with double ear tags. After the initial two colostrum feedings, the calves were moved to individual, straw bedded wood hutches (9 x 1.8 m A = 1.62 m²) with a wood roof, a closed back, an open front, and dividers to eliminate contact between calves in the hutches. The hutches were aligned in rows from east to west with the front facing to the south. Straw bedding was re-applied to the hutches every other day, and in the 4th week the hutches were moved forward to dry ground and bedded with straw.

All calves were tissue sampled (notch from the ear) and blood sampled (jugular venipuncture) for bovine viral diarrhea (BVD). The general health of the calves was monitored daily, and all health problems and treatments were recorded.

Colostrum Preparation

All cows were milked within two hours following parturition. Samples were collected from every cow to detect mammary infections and potential pathogens (*Mycoplasma*, *Staphilococcus*, *E. coli*, etc.). The first milking colostrum from primiparous or multiparous cows was separated and poured into different cooling tanks (4°C). The colostrum was collected and picked up daily from multiple dairies in clean containers. Each container was sampled with an individual vial and the sample was sent to the laboratory. Each batch of colostrum was measured for quality using a colostrometer. The colostrum was transferred from containers to a stainless-steel tank and mixed, where the pasteurization process was performed (63°C, 45 min). After pasteurization, the colostrum was cooled and divided into two batches. The first

half batch of colostrum was bagged in single gallon plastic storage bags and no supplement was added. The colostrum supplement was added to the second half batch until the colostrometer reading was 95. The supplement was diluted in a small amount of milk and mixed well to ensure a homogenous suspension. Plastic storage bags were marked according to treatment and they were refrigerated. After processing, the colostrum was delivered back to the dairy where it was also stored in refrigerators (4°C). Colostrum was then removed from the refrigerator and warmed in water (54°C) until the colostrum temperature reached between 37.8°C and 38.9°C. The calves were fed colostrum within the 1st h of life using an esophageal feeder according to the experimental treatment group. The time of the first colostrum feeding was designated as 0h, and the second colostrum feeding were administered 8h later.

Blood Sampling and Determination of IgG Concentration of Serum and Pooled Colostrum

Blood samples from Holstein heifers were collected via jugular venipuncture into evacuated tubes 24±3 h after birth to measure TSP and serum IgG concentration. Samples were cooled to 4°C immediately following collection. Blood was allowed to clot, serum was separated by centrifugation (3000 x g for 15 minutes at 4°C) within 24 h of collection, and the serum was tested for TSP concentration. Serum was stored at -20°C for later serum IgG concentration analysis. Determination of TSP was performed using a clinical refractometer (Jorvet J-351, Jorgensen Laboratories, inc. CO). The refractometer was cleaned and calibrated with distilled water between each sample. The IgG concentration in serum and colostrum were determined by radial immunodiffusion (Prairie Diagnostic Services, Saskatoon, Saskatchewan, Canada).

Milk Preparation and Feeding

After the second colostrum feeding all calves received pasteurized hospital milk until weaning. Hospital milk was collected from dairies twice daily and stored in tanks for later pasteurization. The pasteurization process of hospital milk was performed twice a day (2:30 a.m. and 9:30 a.m.) at 60°C for 90 minutes. The pasteurized hospital milk solids were increased by adding milk replacer until solid content reached 13.5%. Solid readings were done with brix refractometer (Jorvet J0351B, Jorgensen Laboratories, Inc. CO). Milk was fed at 6:30 a.m. and 1:30 p.m. All calves were fed with bottles. Bottles were filled, and nipples were attached to bottles and chlorine was sprayed on the nipples and rinsed with water. The calves were fed 2L of milk twice a day from days 1 through 42, and 2L of milk once a day from days 43 through 50. Calves were kept in hutches for 2 additional weeks after weaning

Calva Milk Replacer (20:20) (Calva Products, LLC. Acampo, CA) was used for the milk replacer. Calves were offered starter grain and water in the hutches starting from the second day of life. Calf starter (16% protein content) and fresh clean water were offered ad libitum every day from the second day of life until calves were removed from hutches and placed in group pens. Leftovers were removed daily.

Two weeks following weaning, calves were removed from hutches, and grouped into pens of 25 where they remained for one month. During the time in the pens calves were fed 95% calf starter and 5% alfalfa hay. Calves were then moved into larger pens with a capacity of 200 calves.

Daily Observations: Health, Incidence of Abnormal Scores by Treatment (Normal or Abnormal)

The general health of the calves was monitored daily, and all health problems and treatments were recorded. The fecal, dehydration, and respiratory score tables were modified from Larson et al. (1977), Diaz et al. (2001), and Bascom et al. (2007). Fecal scores (0-3), respiratory scores (0-4), and dehydration scores (0-2) were recorded daily. The scores were recorded under the following guidelines. Fecal scores: 0 = normal; manure firm and well formed (not hard), 1 = mild diarrhea (soft, pudding-like), 2 = severe, watery diarrhea, and 3 = bloody feces; Respiratory scores: 0 = normal breathing, 1 = nasal discharge, 2 = coughing–moist, 3 = heavy thoracic breathing, 4 = abdominal breathing; Dehydration scores: 0 = eyes bright and skin flexible, 1 = mild dehydration (skin flexibility 3 to 5 second), 2 = severe dehydration (skin flexibility >5 seconds). A single observer scored all calves every day. Other health disorders were diagnosed and treated according to veterinary instructions. Intravenous fluids were administered to severely dehydrated calves. Body temperatures were recorded every other day until all calves were weaned.

Body Weight

Calves were weighed at birth, before colostrum ingestion, at day 30, at weaning on day 51, when they were moved out of hutches at day 68, and when they were moved out of group pens on day 90. All heifers were individually weighed using an electronic scale (Salter Brecknell Mod. PS 1000).

Determination of Apparent Efficiency of Absorption

Apparent efficiency of absorption (AEA) for IgG was determined as previously described (Quigley et al., 1998, 2002) using the following formula:

$$\text{AEA} = [\text{serum IgG (g/L)} \times \text{plasma volume (L)} / \text{IgG intake (g)}] \times 100.$$

The plasma volume was calculated as follows: plasma volume = $0.089 \times [\text{Body Weight at birth (BW) (kg)}]$ (Quigley et al., 1998).

STATISTICAL ANALYSES

All statistical analyses were conducted with SAS (version 9.4, SAS institute, Cary, NC). Data were analyzed using a mixed-effects model for repeated measures using Proc Mixed of SAS, except for TSP and IgG that were analyzed using the same model without the repeated measures. Proc Genmod was used to determine incidence of abnormal fecal, dehydration respiratory scores, health incidence, and multiple health incidence. Proc Corr was used to determine correlation between BW-TSP, BW-IgG.

RESULTS

IgG Concentration of Colostrum Fed to Calves

The analysis of colostrum and supplemented maternal colostrum by radial immunodiffusion assay indicated that the concentration of IgG in the colostrum before pasteurization was 53.8 g/L. Colostrum IgG concentration after pasteurization was 43.7 g/L. Colostrum IgG concentration was increased to 50.0 g/L with the addition of bovine serum derived colostrum supplement to maternal colostrum.

Serum IgG and Total Serum Protein Concentrations

Total serum protein concentrations were significantly greater ($p < 0.01$) in calves fed MC (TSP=6.17±0.09 g/dL) compared to calves fed MCS (TSP=5.84±0.09 g/dL). Serum IgG concentrations at 24 h of age were significantly higher for MC fed calves ($p < 0.01$) as compared with calves fed MCS (Table 2.3). In our study, all calves had >1,000 mg/dL of serum IgG levels and they did not experience failure of passive transfer defined as serum IgG <10 mg/mL. Total serum protein concentrations were significantly greater ($p < 0.01$) in calves fed MC (TSP=6.17±0.09 g/dL) compared to calves fed MCS (TSP=5.84±0.09 g/dL).

Body Weights and Average Daily Gains

Birth weights of calves did not differ among treatments (Table 2.3), and averaged 35.41±1.00 kg for MC, and 36.37±0.95 kg for MCS. No significant differences in growth rate were found in the calves during the 3-month study period. The average daily gain (ADG) of calves from birth to 30 days were MC=0.35±0.01 kg, and MCS=0.33±0.1 kg and averaged 0.34 kg daily gain ($p=0.37$) (Table 2.5). The average daily gain (ADG) of calves from birth to weaning (51 days) days were MC=0.46±0.01 kg, and MCS=0.45±0.1 kg and averaged 0.4 kg daily gain ($p=0.57$) (Table 2.4). The average daily gain (ADG) of calves from birth to moving out of the hutches to group pens (68 days) were MC=0.50±0.01 kg, and MCS=0.49±0.1 kg and averaged 0.50 kg daily gain ($p=0.50$) (Table 2.4). The average daily gain (ADG) of calves from birth to moving out of the first group pen (90 days) were MC=0.58±0.01 kg, and for MCS=0.57±0.1 kg and averaged 0.58 kg daily gain ($p=0.53$) (Table 2.4).

Health Scores

Rectal body temperature did not differ between MC and MCS and averaged $39.0^{\circ}\text{C}\pm 0.03$ from birth to weaning (Figure 2.1). No differences were detected in pneumonia, diarrhea incidence which averaged 77.5 %, and 10.5%, respectively (Table 2.2). Fecal scores and dehydration scores did not differ between treatments and averaged 27.9% and 12.7% respectively (Table 2.1).

Table 2.1. Effect of maternal colostrum (MC) or maternal colostrum+colostrum supplement (MCS) on health scores of Holstein female calves from birth to 3 months of age.

Health Score	MC	MCS	P
Respiratory scores ¹ (RS) %	27.3	58.5	0.0065
Fecal Scores ² (FC)%	33.3	21.9	0.27
Dehydration scores ³ (DH)%	18.1	7.3	0.15

¹Respiratory scores (abnormal) for maternal colostrum+supplement fed calves are significantly different ($p<0.05$). ²Fecal and ³Dehydration scores are not significantly different ($p>0.05$).

Table 2.2. Effect of maternal colostrum (MC) or maternal colostrum+colostrum supplement (MCS) on pneumonia and diarrhea incidence in Holstein female calves from birth to 3 months of age.

Health	MC	MCS	P
Pneumonia ¹ %	86.5	69.3	0.7
Diarrhea ² %	11.1	10.0	0.9

¹Pneumonia and ² Diarrhea incidence % from birth to 3 months of age for treatment groups are not significant ($p>0.05$)

Disease and Illness

No mortalities occurred during the study. Overall, independent of treatment, we observed a high morbidity rate of 77.9% during the experiment (77.9% of calves were treated for pneumonia) (Table 2.2). Maternal colostrum+supplement calves had a greater ($p<0.006$) incidence of abnormal RS score (58.5%) compared to MC (27.3%) (Table 2.1, Figure 2.1).

Determination of Apparent Efficiency of Absorption

Apparent efficiency of absorption (AEA) estimates efficiency of immunoglobulin absorption before cessation of intestinal absorption of immunoglobulins (Quigley et al., 1998). The mean AEA of calves fed MC (31.13%; SE=1.48%) was greater than that of calves fed MCS (23.30%; SE=0.92%) (Table 2.3).

Table 2.3. Effect of maternal colostrum (MC) or maternal colostrum+ colostrum supplement (MCS) on IgG absorption and AEA at 24 h of age

Item	MC	MCS	SE	P
N ¹	27	30		
BW ² , kg	35.4	36.3	0.98	0.4931
IgG intake, g	253	289.55	0.0	
Serum IgG ³ , g/L	23.99	20.41	0.78	0.0022
TSP ⁴ , g/L	6.17	5.84	0.09	0.0131
AEA ⁵ , %	31.13	23.30	1.2	

¹Number of calves enrolled for treatment group MC or MCS.

²Birth weights of calves averaged 35.8±0.98 kg.

³Serum IgG levels and ⁴Total serum protein levels at 24 h age significantly different (p<0.05).

⁵AEA: Apparent efficiency of IgG absorption at 24 h.

Table 2.4. Average daily gain (ADG) of maternal colostrum (MC) or maternal colostrum+ colostrum supplement (MCS) fed Holstein female calves from birth to 3 months of age

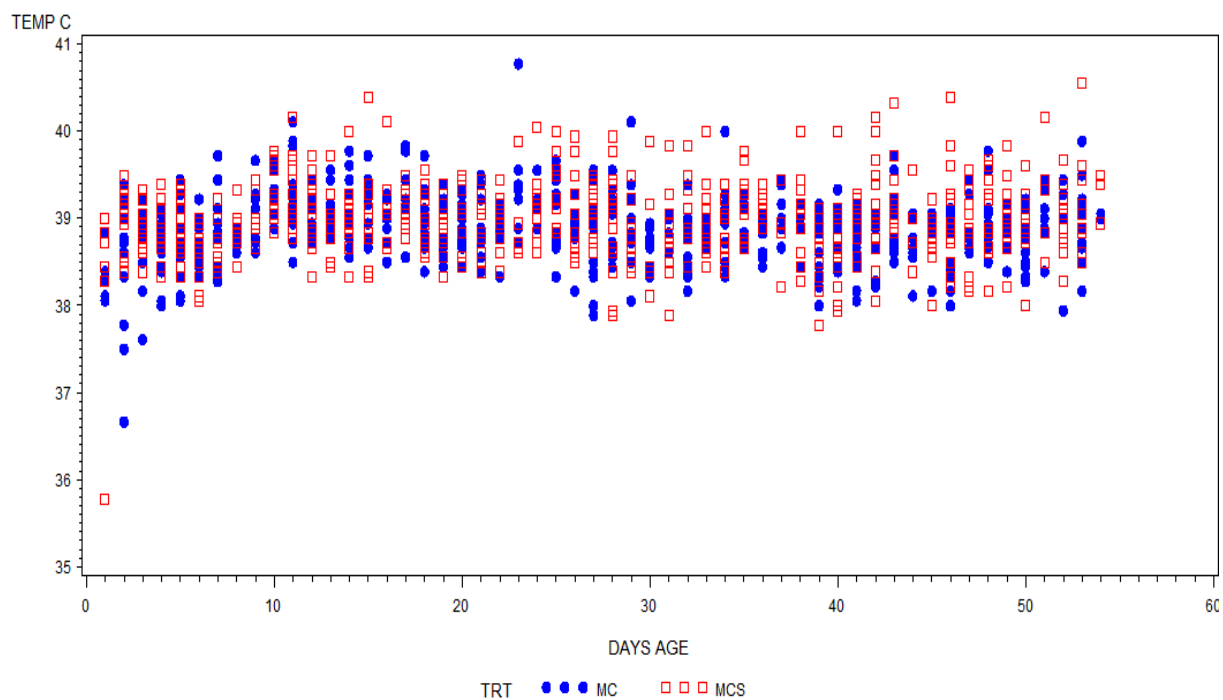
Item	MC	MCS	SE ²	P
BW ¹ , kg	35.4	36.3	0.98	0.49
ADG, kg (Birth to 30 days of age)	0.35	0.33	0.01	0.37
ADG, kg (Birth to weaning 51 days)	0.46	0.45	0.01	0.57
ADG, kg (Birth to group pen 68 days)	0.50	0.49	0.01	0.50
ADG, kg (Birth to out of group pen 90 days)	0.58	0.57	0.01	0.53

¹BW = Birth weight; ²SE = Standard error.

Table 2.5. Average daily gain (ADG) of maternal colostrum (MC) or maternal colostrum+ colostrum supplement (MCS) fed Holstein female calves from birth to 3 months of age, by period of time

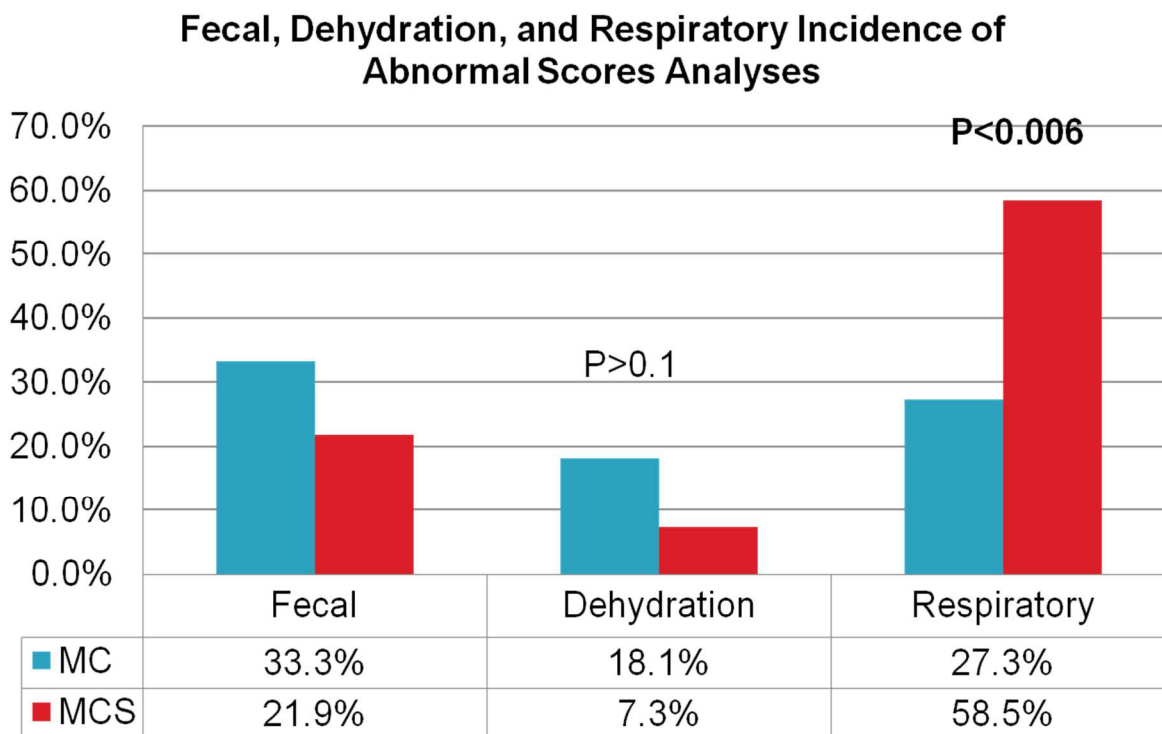
Item	MC	MCS	SE	P
Birth Weight, kg	35.4	36.3	0.98	0.49
ADG, kg (Birth to 30 days of age)	0.35	0.33	0.01	0.37
ADG, kg (30 days to weaning 51 days)	0.63	0.63	0.03	0.96
ADG, kg (51 days to group pen 68 days)	0.64	0.62	0.03	0.64
ADG, kg (68 days to out of group pen 90 days)	0.82	0.80	0.04	0.79

Figure 2.1. Body temperatures, from birth to 53 days of age, of Holstein female calves fed maternal colostrum (MC) or maternal colostrum+ colostrum supplement (MCS)



Body temperature (0 to 53 d) for MC =38.9 °C SE=0.01 MCS=39.0 °C (p>0.05).

Figure 2.2. Effect of maternal colostrum (MC) or maternal colostrum+ colostrum supplement (MCS) on health scores of Holstein female calves from birth to 90 days of age



DISCUSSION

The management of the calf is very important for future herd profitability. Herd profitability can be negatively affected by the impaired growth of the calf, transmission of diseases with the first feeding of colostrum, decreased milk production of animals that experience chronic illness during the calf period, limited opportunity for genetic selection due to high mortality of replacement animals and veterinary cost.

The IgG concentration decrease observed in our study following pasteurization coincides with the literature. Previous studies using a similar heat treatment have reported losses in colostrum IgG concentration during heat treatment (60°C for 60 min) (Johnson et al., 2007; Donahue et al., 2012). Johnson et al. (2007) reported that colostrum IgG concentration for heat-treated vs.

raw colostrum were $67.3\text{mg/mL}\pm 2.3$ vs. $72.6\text{ mg/mL}\pm 2.7$; $P= 0.45$. In this study calves fed heat treated colostrum had significantly greater serum total protein and IgG concentrations at 24h, also greater apparent efficiency of IgG absorption (TSP= 5.9 mg/dL ; IgG= 18.1 mg/mL ; apparent efficiency absorption= 35.6%) compared with calves fed raw colostrum (Total serum protein= 6.3 mg/dL ; IgG 22.3 mg/mL ; apparent efficiency absorption= 26.1%). Godden et al. (2012) demonstrated that serum IgG concentrations were significantly higher in calves fed heat treated (HT) colostrum (pasteurized at 60°C for 60 min) ($18.0\pm 1.5\text{ mg/ mL}$) compared with calves fed fresh (FR) colostrum ($15.4\pm 1.5\text{ mg/ml}$). Significant increase in risk for a treatment event (any cause) occurred in calves fed FR colostrum (36.5% , hazard ratio = 1.25) compared with calves fed HT colostrum (30.9%). In addition, they observed a significant increase in risk of treatment for scours in calves fed FR colostrum (20.7% , hazard ratio = 1.32) compared with calves fed HT colostrum (16.5%).

The National Dairy Heifer Evaluation Project, sponsored by NAHMS, reported retrospective data on 1,811 dairy farms and prospective surveillance data on 921 US dairy farms. A total of 17,011 calves were monitored (USDA, 1993). Pre-weaned calf mortality was 8.4% and 6.8% for the retrospective and prospective data, respectively. Diarrhea accounted for 52.2% of mortality, followed by respiratory problems (21.3%), trauma (2.4%), joint and navel problems (2.2%), and other (11.7%) and unknown causes (10.2%) (USDA, 1993). According to the 2007 NAHMS report, mortality for pre-weaned calves was (7.8%) and diarrhea accounted for (56.4%) of mortality, followed by respiratory problems (22.5%). In the 2011 NAHMS report, the mortality for pre-weaned calves was 4.2% , diarrhea accounted for 33.3% of mortality, and respiratory problems for 57.1% of mortality. Virtala et al., (1996) demonstrated that morbidity during the first 3 months of life was attributed to pneumonia (25%), diarrhea (29%), and

umbilical disease (14%) in data obtained from 410 dairy calves born in 1990 on 18 commercial dairy herds located in New York. The diarrhea and pneumonia reported in the previously cited study is similar to the abnormal fecal score (27.6%) reported in our study and to the abnormal respiratory score reported for the MC calves. In contrast, the MCS calves had a significantly higher incidence of abnormal respiratory score. During the 90-day observation period, only 1 calf had diarrhea in the first 14 days of life, and diarrhea incidence occurred in less than 11% of calves.

When the calf is born, it must rapidly adjust to a radical change in its environment, which requires the consumption of a high energy diet, such as that provided by colostrum. Immunoglobulins are absorbed most efficiently in the first few hours of life and absorption declines rapidly after 12 h of life (Weaver et al., 2000) and it is recommended that calves be fed colostrum before 4 h of life (Beam et al., 2009). A minimum of 150 g of IgG is required by a calf to achieve passive transfer (Chigerwe et al., 2008a), 80 to 100 g (Petrie, 1984). Thus, it has commonly been assumed that colostrum- or serum-based products containing over 100 g of IgG would be effective colostrum replacers for calves. However, simply measuring the mass of IgG provided by a replacer product is an inadequate predictor of its efficiency. The molecular mechanism in the intestine of newborn calves is capable of transferring colostral immunoglobulin and a variety of non-immunoglobulin macromolecules from the lumen to the circulatory system. If the capacity of this mechanism is limited, transfer of a large amount of non-immunoglobulin protein may interfere with transfer of immunoglobulin. For example, in a previous study, Garry et al. (1996) fed groups of calves 3 different commercially available colostrum replacer products providing an IgG mass of 107, 126, and 156 g, respectively. All three groups in this study had IgG concentrations lower than what was observed in calves fed

fresh colostrum, and the percentage of calves with failure of passive transfer was 100% in all colostrum replacer groups. In another study calves fed a product containing 100 g of IgG had a mean serum IgG concentrations of 700 mg/dL, and 90% of calves had failure of passive transfer (Foster et al., 2006).

Numerous studies have analyzed different aspects including source of IgG, method of IgG fractionation, amount and type of non-IgG protein, and presence of fat and lactose and their effects on efficiency of IgG absorption in colostrum replacers and supplements (Mee et al., 1996; Arthington et al., 2000ab; Davenport et al., 2000; Quigley et al., 2001). Likewise, research has shown that addition of some colostrum supplements reduces the absorption of IgG from natural colostrum (Stott et al., 1979; Hopkins and Quigley, 1997; Morin et al., 1997). Besser and Osborn (1993) demonstrated that efficiency of IgG1 transfer in newborn calves was reduced from 59 to 36% by the addition of bovine serum albumin (37 mg/ml) to colostrum whey, while the addition of a similar mass of amino acids in the form of acid hydrolyzed casein (37 mg/ml) did not detectably alter IgG1 transfer. It was concluded that reduced IgG1 absorption efficiency in calves fed colostrum with added bovine serum albumin is consistent with a limited capacity for the macromolecular transport mechanism in the intestine of newborn calves. In agreement, Davenport et al. (2000) showed that addition of casein to a CS product reduced the absorption of IgG by calves. Arthington et al. (2000a) used bovine serum protein (BSP), colostrum, and 2 milk-derived IgG supplements to compare AEA of IgG. At 24 h, plasma IgG levels were 12.1 g/L for colostrum, 2.2 and 3.5 g/L for the milk-derived supplements, and 6.8 g/L for BSP. Although plasma IgG levels were greatest in colostrum-fed calves, AEA was increased in calves fed the BSP. This is related to the amount of IgG initially fed to calves. Researchers hypothesized that these results indicated that in the absence of MC, BSP would be

an acceptable alternative. However, 24-h IgG levels were 6.8 g/L, which is well below the delineation of passive transfer (10 g/L of IgG at 24 h).

The ADG of calves between 0 and 30 days of age was 0.34 ± 0.01 kg, between 30 and 51 (30 days to weaning) days of age was 0.63 ± 0.03 kg, between 51 and 68 (weaning to out of hutch) days of age was 0.63 ± 0.03 kg and between 68 and 90 (group pen 20 calves) days of age 0.81 ± 0.04 kg. Average daily gain results were similar to a previous study conducted in New York where ADG during the 1st, 2nd, and 3rd months were 0.37, 0.59, and 0.71 kg, respectively. Overall, ADG for the 3-month period was 0.56 kg (Virtala et al., 1996).

Pithua et al., (2010) followed 497 calves from birth through 54 months of age and found no differences in the risk of death or culling, milk production, or reproductive performance of cows that were fed either maternal colostrum or serum-based colostrum replacer at birth. Poulsen et al. (2003) used 289 calves from 8 different dairy farms. The calves were divided into 2 groups and fed colostrum or a colostrum-replacer product containing 125 g of IgG. No differences were noted between groups in the number of calves that achieved adequate transfer of passive transfer (serum IgG concentrations $>1,000$ mg/dL) or health scores (based on fecal consistency, appetite, and attitude monitoring). Also, in other studies, there was no difference in the percentage of calves with adequate transfer of passive immunity when calves were fed fresh colostrum versus a colostrum replacer (Quigley et al., 2002; Foster et al., 2006). In another experiment where calves received the same amount of IgG from either colostrum or a serum-based colostrum replacer, no differences between the calves were observed in IgG levels, efficiency of IgG absorption, incidence of scours, or growth rate during the first month of life (Jones et al., 2004). Thus, there appears to be a number of factors that control the efficiency of IgG absorption between different colostrum-replacer products. Consequently, each colostrum

replacer and supplement product should be properly evaluated for efficacy prior to use. Examining the mass of IgG provided by the colostrum replacer is not an adequate measure of product efficiency (Smith and Foster, 2007).

In our study, the addition of bovine serum derived colostrum supplement to low IgG MC did not increase serum IgG concentration at 24 h. Calves received a total 253.00 g IgG from maternal colostrum and 289.55 g from MCS. Colostrum supplement added a total of 36.55 g of IgG per calf. In agreement with the other studies, substances in the products might have inhibited the absorption of IgG or enhanced the rate of closure, because apparent efficiency of IgG absorption at 24 h (AEA) was lower for calves that received MCS, than calves that were fed only MC. Other studies have reported similar findings for colostrum supplements containing dried colostrum or whey protein concentrate. Morin et al. (1997) demonstrated that achievement of adequate serum concentration of IgG in calves is easy if high IgG colostrum is available but when low IgG colostrum is present, increasing the volume or adding dried colostrum supplement did not achieve maximum benefits.

Respiratory Disease

A report by USDA stated 12.4% of pre-weaned calves were treated for respiratory disease and respiratory infections were responsible for 22.5% of pre-weaned dairy calf (USDA, 2007). According to the 2007 NAHMS survey, the incidence of post-weaning respiratory disease in dairy heifers was 5.9%, and it was the predominant cause of reported deaths of weaned heifers 46.5% (USDA, 2010). McGuirk (2015) reported that the incidence and prevalence of respiratory disease in calves are higher than producers report because detection is difficult. The financial toll from treatment expense, mortality, premature culling, reduced growth, fertility

and milk production in first lactation survivors of respiratory disease is reported to range from \$15 to \$36 per case. In our study, respiratory tract disease occurred in 69% of the calves and 43% of the calves had multiple incidence of respiratory tract infections. This is not consistent with the results of Brunning-Fann and Kaneene (1992), who found respiratory tract infections in 7 to 15% of calves. In addition, in our study only 2 calves had serum IgG levels less than 15 g/L, but their IgG levels were >10 g/L all other calves had IgG levels >15 g/L. Furman-Fratczk et al. (2011) showed that the morbidity and intensity of the disease course were lowest in heifer calves with serum Ig concentration exceeding 10 g/L at 30 to 60 h of life. These calves did not become ill before 14 days of age. Calves with >15 g/L gammaglobulin in serum avoided respiratory tract infections. Heifers with serum gammaglobulin levels >10 g/L at 30 to 60 h of life showed better health status. However, in our study 69% calves had respiratory tract infections, even though, IgG levels were >15g/L. The relationship between IgG levels and respiratory infections should be further investigated.

CONCLUSION

In this study, calves fed maternal colostrum achieved greater levels of total serum protein and IgG than calves fed maternal colostrum + serum derived colostrum supplement. Also, calves fed maternal colostrum experienced a lower incidence of abnormal respiratory scores than calves fed maternal colostrum + serum derived colostrum supplement. Thus, in this study, adding a supplement to maternal colostrum did not achieve any positive effect on performance and health parameters of dairy calves.

CHAPTER 3: SERUM TOTAL PROTEIN IN CALVES IS NOT CORRELATED WITH FUTURE MILK PERFORMANCE

ABSTRACT

It is well established that calves with poor passive transfer of immunoglobulins have increased risk of diarrhea, respiratory problems, and mortality. Furthermore, there are studies that have linked plasma immunoglobulin concentrations or colostrum provision early in life with improvements in future performance. The improvements have been attributed to potential lactocrine mechanisms mediated by hormones present in the colostrum. The objective of this study was to determine whether total serum protein (TSP) in calves was correlated with future milk performance in the first lactation. A total of 6,172 calves born in the same herd were fed 3 L of colostrum within 1 h of birth followed by 2 additional liters 8 h later, and blood-sampled between 24 to 48 h of life to determine TSP. Determinations of TSP were performed by an experienced veterinarian using a refractometer (Jorvet J-351, Jorgensen Laboratories, Inc. CO). Then, total milk produced by the animals in their first lactation was recorded. A categorical variable was constructed including TSP below 5.4 (n = 1,962), between 5.4 and 6.4 (n = 2,324), and above 6.4 mg/dL (n = 1,886). A mixed-effects model that accounted for the random effects of year of birth and sire (father of each heifer considered) plus the fixed effects of the 3 TSP categories and the lactation length as a covariate was run to evaluate any potential relationship between TSP and milk yield in the first lactation. In the first lactation, calves that had TSP below 5.4 mg/dL produced $10,551 \pm 230$ kg, those with TSP between 5.4 and 6.4 mg/dL produced $10,499 \pm 229$ kg, and those with TSP above 6.4 mg/dL produced $10,445 \pm 230$ kg. There was no relationship ($p=0.13$) between TSP and future milk production. These results indicate that either

TSP is not a valid proxy to assess the adequacy of colostrum feeding, or that the amount of colostrum offered during the first day of life has no impact on future animal milk production.

INTRODUCTION

It is well known that absorption of colostrum antibodies prior to cessation of macromolecular transport by the intestine is crucial for calf health and survival. Colostrum and its nutrient and non-nutrient components applies marked effects on gastrointestinal development and function, long lasting systemic effect on the nutritional status, metabolism and various endocrine systems, and on the epigenetic development of specific tissues or physiological functions (Blum, 2006; Bartol et al., 2008). The long-term effects of colostrum feeding are most likely related to important endocrine components, such as IGF-1 IGF-II, insulin, GH, epidermal growth factor, leptin, and prolactin. These hormones could impact the lactocrine mechanism to cause modifications of several hormonal accesses in the calf (Bach, 2012). In the previous study colostrum was collected 0-80 h postpartum and the content of immunoglobulins (IgG), transforming growth factor beta-2 (TGF-b2), insulin-like growth factor-1 (IGF-1) and growth hormone (GH) were analyzed. Colostrum initially contained 90mg/mL IgG1, 2.8mg/mL IgG2, 1.6mg/mL IgA, 4.5mg/mL IgM, and in later collected samples showed that these concentrations declined by 92%, 87%, 93% and 84%, respectively. Colostrum initially contained growth factors at 289–310 g/mL TGF-b2, and the concentration diminished to 66ng/mL. The content of IGF-1 and GH postpartum decreased from 870 to 150ng/mL, and from 0.17 to 0.03ng/mL, respectively. Heat treatment and freeze-drying of colostrum whey decreased the content of immunoglobulins to 75%, while the contents of IGF-1 and TGF-b2 were unaffected (Elfstranda et al., (2002). Lactocrine hypothesis describes how the effect of colostrum and milk-borne factors influence epigenetic development of specific tissues or physiological functions. (Xu,

1996; Blum et al., 2002; Bartol et al., 2008). Consequently, maternal effects on development continues after parturition. In the early neonatal period, colostrum serves as the conduit for communication of organizationally important developmental signals from the dam (Bartol et al., 2008).

Failure of passive transfer (FPT) contributes to excessively high pre-weaning mortality rates and other short- and long-term losses associated with animal health and welfare. Current recommendations are feeding 3 to 4 L of high quality (>50g/L IgG and <100,000 cfu/mL of bacteria) colostrum within 6h of life (McGuirk and Collins, 2004; Godden, 2008; Chigerwe et al., 2008b). The benchmark for failure of passive transfer is defined as a serum immunoglobulin G (IgG) concentration below 10 mg/mL (1,000 mg/dL) (Tyler et al., 1996; Faber et al., 2005; Beam et al., 2009) and the benchmark for total serum protein concentration of 5.2 g/dL was equivalent to 1,000 mg/dL serum IgG1, using digital refractometry or clinical refractometer. Therefore, failure of passive transfer was defined by values below 5.2 g/dL (Tyler et al., 1996).

Robison et al. (1988) demonstrated that dairy heifer calves with inadequate serum IgG concentrations at 24 to 48 h (<12 mg/mL) had a higher mortality rate in dairy heifer calves thorough through 6 months of age (6.78% compared with 3.33%). In addition, low IgG concentrations at birth have affected growth through 180 days of age in heifer calves. Donovan et al. (1998) confirmed that total serum protein (TSP) was a significant risk factor for mortality. The association of TSP and mortality was quadratic and showed a dramatic decrease in mortality as TSP increased from 4.0 to 5.0 g/dL, a small improvement from 5.0 to 6.0 g/dL and virtually no improvement in mortality rates as TSP increased over 6.0 g/dL. The hazard mortality ratio was constant from birth to six months, indicating that the increased risk of mortality associated with low levels of TSP was evident through six months of age.

DeNise et al. (1989) demonstrated a significant covariate between mature equivalent milk (305ME) and IgG at 24 to 48 h. Regression of ME milk on IgG concentration was 8.5 kg milk/unit IgG (mg/ml), and the regression of ME fat on IgG concentration was .28 kg fat/unit IgG (mg/ml). Faber et al. (2005) demonstrated that calves fed 4L vs. 2L of colostrum produced 550 kg more milk over the first two lactations and the economic return increased approximately \$160 per cow due to the additional milk produced.

Since immunoglobulins constitute a large proportion of the protein in colostrum and neonatal calf serum, digital brix or optical refractometer are an easy, effective and commonly used on farm tool to evaluate colostrum quality or determine serum total protein levels in calves (Calloway et al., 2002; Biemann et al., 2010; Morrill et al., 2012). These studies show a highly significant correlation between specific gravity and globulin concentration of colostrum (Fleenor and Stott, 1980; Chigerwe et al., 2008a). Therefore, in our study, a hydrometer, marketed as a colostrometer, was used to assess colostrum quality.

Since clinical refractometers can easily be used on dairy farms, producers can easily and accurately assess their management decisions. The objective of this study was to determine whether total serum protein (TSP) in calves was correlated with future milk performance in the first lactation. Analyzing the effects of TSP in these study heifer calves may be a crucial factor in determining whether a farm can enhance their replacement dairy heifer rearing program based on TSP concentration during the first 24 to 48 h of life.

Tyler et al. (1996) demonstrated that based on TSP concentration, serum IgG I concentration could be estimated by using the following regression formula: serum IgG, (mg/dL) = -3615 + [901 X total serum protein (g/dL)], $p < 0.001$ $r^2 = .76$, the 95% confidence interval of the

calculated r^2 was 0.71, 0.80. Therefore, the relationship between serum IgG concentrations and refractometer determinations of serum protein suggest that this assay is suitable for routine management assessment programs.

Measurement of TSP by refractometer as an estimate of serum immunoglobulin concentration provides practical, rapid and inexpensive test results and it is equivalent or superior to other available assay procedures, such as; sodium sulfite and zinc sulfate turbidity test relative serum immunoglobulin G1 (IgG1) concentrations determined by radial immunodiffusion. Reported r^2 values for models predicting serum immunoglobulin G (IgG) concentration range from 0.6 to 0.8 (Naylor et al., 1977; Tyler et al., 1996; Elsohaby et al., 2015). Nocek et al., (1984) showed that serum protein and IgG concentrations have a significant ($p < 0.001$) positive correlation ($r^2 = .84$) starting at 12 to 24 h after birth and maintains through day 11. However, a lower coefficient ($r^2 = .69$) at day 11 was observed. This suggests that the relationship starts to diminish with time. This is the first study to examine TSP concentration and the future milk production.

MATERIALS AND METHODS

The study was done in southern Idaho on a large commercial dairy farm. A total of 6,172 female Holstein calves were enrolled in this study. After birth, the calves were removed from their dams before they could suckle. The umbilicus of each calf was treated with 7% iodine solution, and the calves were weighed and the weights were recorded. The calves were placed in straw-bedded pens in the maternity barn and identified with double ear tags.

All cows were milked within two hours of parturition. Samples were collected from every cow to detect mammary infections and potential pathogens (*Mycoplasma*, *Staphylococcus*, *E. coli* etc.) The first milking colostrum from primiparous or multiparous cows was separated and

poured into different cooling tanks (4°C). The colostrum was collected and picked up daily from multiple dairies using clean containers each morning. Each container was sampled in an individual vial and the sample was sent to the laboratory for analysis. Each batch of colostrum was evaluated for quality using a hydrometer (colostrometer). The colostrum was transferred from containers into a stainless-steel tank, where the pasteurization process was performed (60°C, 45 min). After pasteurization, the colostrum was cooled and bagged in single gallon size freezer bags and stored in a refrigerator (4°C). After processing, the colostrum was taken back to the dairies where it was stored in refrigerators. Colostrum was then removed from the refrigerator and warmed in water (54°C) until the colostrum temperature reached a temperature between 37.8°C and 38.9°C. All calves were fed 3 L of first milking colostrum within the 1st h of life using an esophageal feeder. A second feeding of colostrum (2 L) was administered 8 h following the first feeding.

After the initial two colostrum feedings, calves were then moved to a calf raising facility and housed in individual straw-bedded hutches. Calves were fed with pasteurized hospital milk and milk replacer. The milk replacer's crude protein and crude fat contents were CP:20 and CF:20, respectively. The pasteurized hospital milk solids were increased by adding milk replacer until solid content reached 12%. Each heifer was fed twice daily (6:30 a.m. and 1:30 p.m.) with 2 L of hospital milk-milk replacer, 4L in total. Two milk-milk replacer feedings were offered from the 1st day to 46 d±3, and then was reduced to one feeding of milk-milk replacer on the 53rd day±3, and the following week milk was not offered. However, heifers were kept in the hutches during the week before they were moved to group pens (63 days±3). Calf starter pellets (16% protein content) and fresh clean water was offered ad-libitum every day from the second day of

life until they were removed from hutches and placed in group pens. Leftover pellets were removed daily.

After calves were removed from hutches, they were grouped into pens of 25 for one month. During this time, calves were fed 95% calf starter pellets and 5% alfalfa hay. Calves were kept in the weaning pens for 1 month, and then calves were moved into larger pens (200 head capacity) and fed an ad-libitum diet of alfalfa hay. Blood samples from the Holstein heifers were collected via jugular venipuncture 24 to 48 h after birth to measure TSP concentration. Samples were cooled to 4 °C immediately following collection. Serum was obtained by centrifugation from each sample within 24 h of collection and tested for TSP concentration. Determinations of TSP were performed by using a clinical refractometer (Jorvet J-351, Jorgensen Laboratories, inc., CO). The general health of the calves was monitored daily, and all health problems and treatments were recorded. Data on sire of calf and parity of dam were obtained from computerized records. The total milk produced by the animals in the first lactation was also recorded.

STATISTICAL ANALYSES

All statistical analyses were conducted with SAS (version 9.4, SAS institute, Cary, NC). Categorical variables were created and included TSP below 5.4 (n=1,962), between 5.4 and 6.4 (n=2,324), and above 6.4 mg/dL (n=1,886).

A mixed-effects model that accounted for the random effects of year of birth and sire (father of each heifer considered) plus the fixed effects of the three TSP categories and the lactation length as a covariate was run to evaluate any potential relationship between TSP and milk yield in the first lactation.

RESULTS AND DISCUSSION

The objective of the study was to assess the immunoglobulin concentrations for management practices in dairy farms. Measurement of TSP by refractometer as an estimate of serum Ig concentration provides practical, rapid and inexpensive test results and it is equivalent or superior to other available assay procedures, such as; sodium sulfite and zinc sulfate turbidity test relative serum immunoglobulin G1 (IgG1) concentrations determined by radial immunodiffusion. Reported r^2 values for models predicting serum immunoglobulin G (IgG) concentration range from 0.6 to 0.8 (Naylor and Kronfeld, 1977; Tyler et al., 1996; Elsohaby et al., 2015). Nocek et al., (1984) showed that serum protein and IgG concentrations has a significant ($p < .001$) positive correlation ($r = .84$) starting at 12 to 24 h after birth and maintains through day 11. However, a lower coefficient ($r^2 = .69$) at day 11 was observed. This suggests that the relationship between serum protein and IgG diminishes over time. Previous studies have shown that TSP concentration is highly correlated to serum IgG levels. The current study is the first to examine TSP concentration and future milk production.

Earlier studies have described a positive relationship between passive immunity of calves and future lactational performance of the cow. DeNise et al. (1989) demonstrated a significant relationship between mature equivalent milk (305ME) and IgG at 24 to 48 h. Regression of ME milk on IgG concentration was 8.5 kg milk/unit IgG (mg/ml), and the regression of ME fat on IgG concentration was .28 kg fat/unit IgG (mg/ml). However, it was noted that the association was not due to IgG directly and suggested that it was linked to other factors in colostrum that could influence subsequent production. Previous studies indicate that nutrient availability during the peripartum period does not significantly affect colostrum IgG content. Holstein cows were fed different amounts of rumen undegradable protein, which did not result in significant

differences in colostrum IgG content (Santos et al., 2001). An additional study Hough et al. (1990) demonstrated that beef cows fed nutritionally restricted diets at 57 or 100% of NRC requirements for energy and protein in their last trimester, it did not result in lower IgG concentrations in colostrum. However, calves fed colostrum from restricted cows tended to have lower serum IgG concentrations at 24 h of life.

Faber et al. (2005) demonstrated that calves fed 4L versus 2L of colostrum produced 550 kg more milk over the first two lactations, resulting in approximately \$160 per cow in additional revenue due to increased amounts of milk produced. However, in this study serum total proteins concentrations or serum IgG concentrations were not measured.

Our results did not agree with prior studies. Our study showed that in the first lactation, calves that had TSP below 5.4 mg/dL produced $10,551 \pm 230$ kg, those with TSP between 5.4 and 6.4 mg/dL produced $10,499 \pm 229$ kg, and those with TSP above 6.4 mg/dL produced $10,445 \pm 230$ kg (Figure 3.1, $p > 0.05$). There was no relationship ($p = 0.13$) between TSP and future milk production (Figure 3.2). Total serum protein (TSP) in calves was not correlated with future milk yield performance in the first lactation. Given these results, regarding future milk production based on TSP concentration in the first 24 to 48 h of calf life, we cannot affirm that TSP was a critical factor in determining whether a farm can enhance their dairy heifer replacement rearing program.

IMPLICATIONS

Total serum protein (TSP) is not a valid proxy to assess the adequacy of colostrum feeding, and the amount of colostrum offered during first day of life has no impact on future animal milk production performance.

Figure 3.1. First lactation milk yield adjusted for DIM, sire, and year of calving

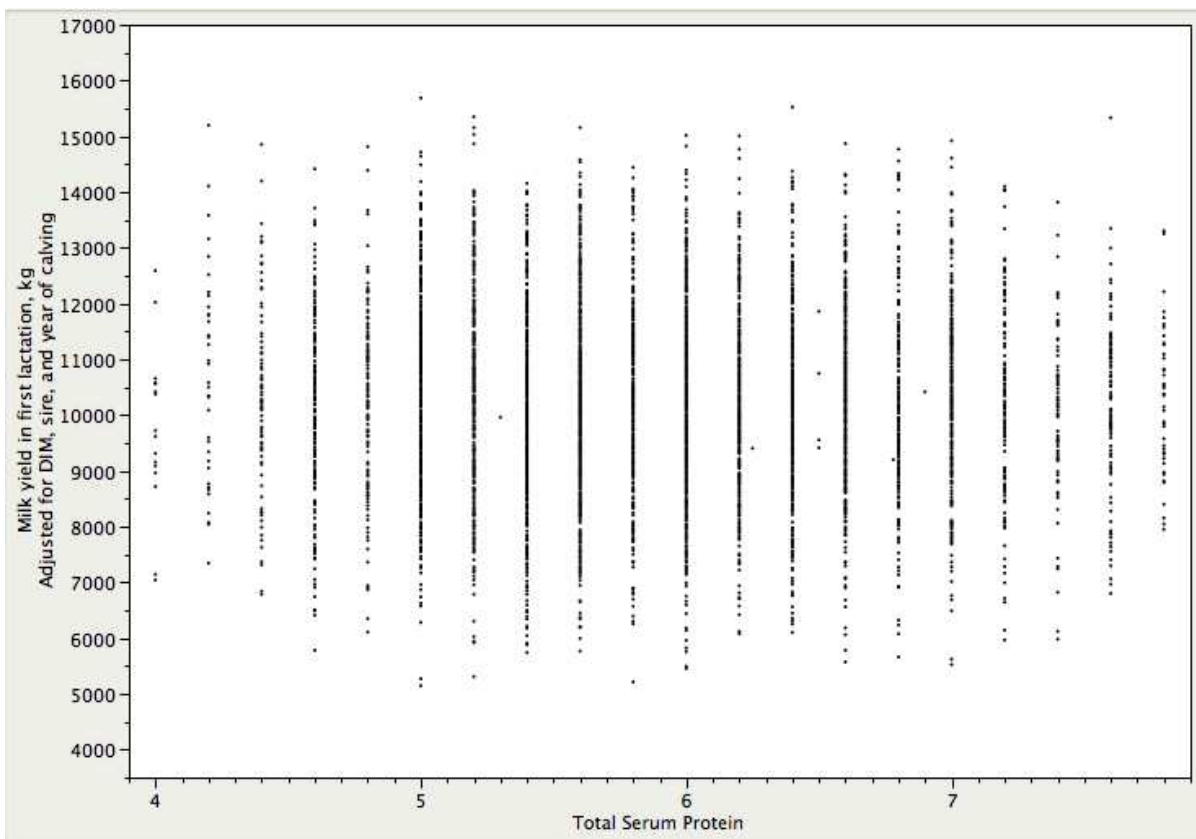
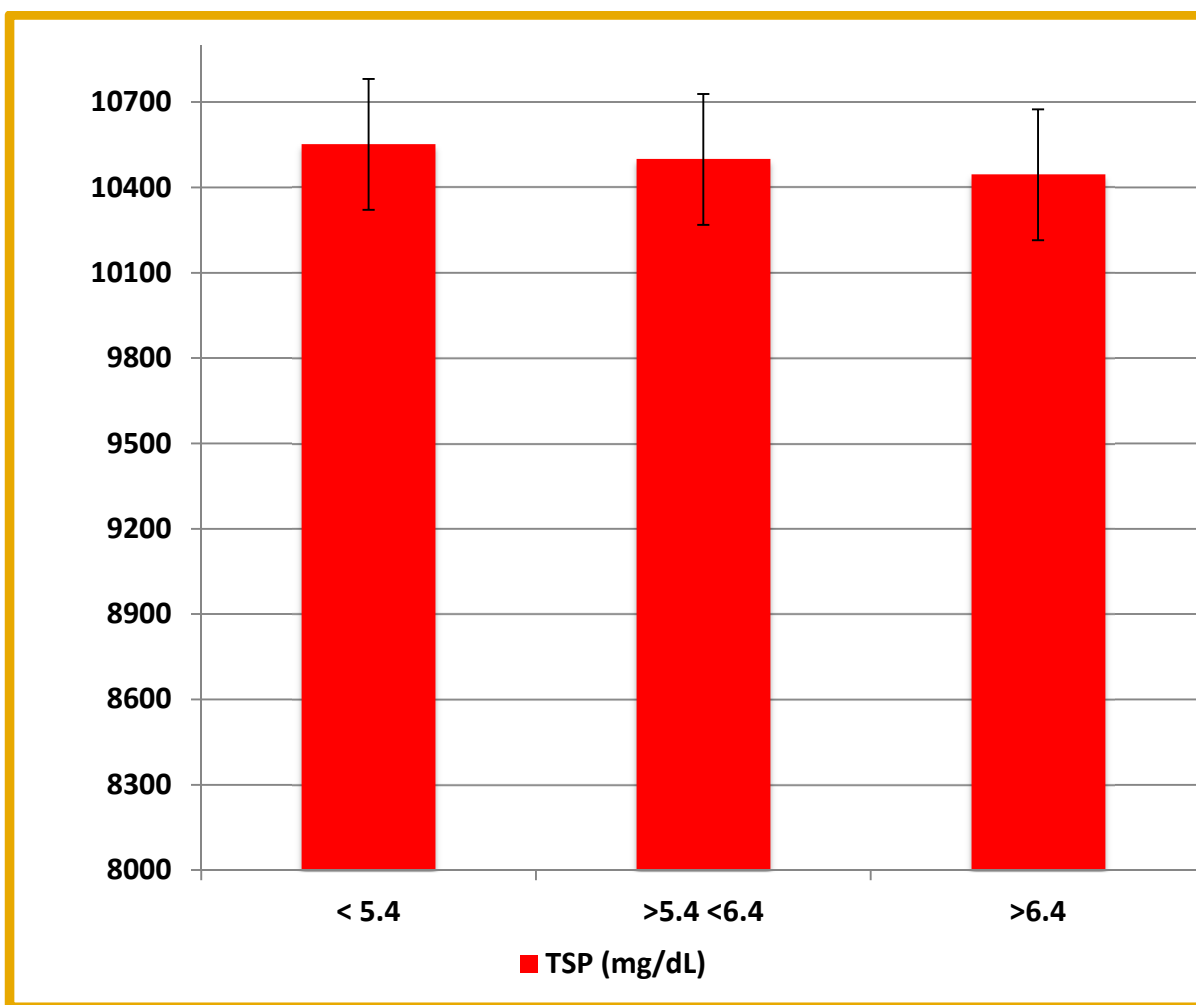


Figure 3.2. Three TSP categories and milk yield (kg) in the first lactation



CHAPTER 4: EFFECT OF QUANTITY AND FREQUENCY OF COLOSTRUM FEEDING ON HEALTH AND PERFORMANCE OF PRE-WEANED AND POST-WEANED DAIRY CALVES.

ABSTRACT

The objective of this study was to determine the effect of quantity and frequency of colostrum feeding on serum IgG concentration, health parameters, and growth in Holstein calves. Two hundred thirteen Holstein female calves raised on a commercial facility in southern Idaho were randomly assigned to one of two treatments which consisted of maternal colostrum (MC, n=107) or maternal colostrum + milk (MCM, n=106). The first feeding of colostrum (3.2 L) was administered to MC and MCM calves using an esophageal tube within 1 h of birth. A second feeding of colostrum (2 L) was administered 12 h following the first feeding to MC calves while MCM calves received 2 L of milk. Blood samples were collected at 24±3 h of age and tested for total serum protein (TSP) and serum IgG concentration. Rectal body temperature was measured twice a week during the first three weeks of age. Health evaluations were conducted daily until calves were 3 months of age. Fecal (FC), dehydration (DH) and respiratory (RS) scores were recorded during the first four weeks of age. Average daily gain was measured at 28 days of age and at weaning 51 days of age. Data were analyzed using a mixed model in SAS. Total serum protein and IgG concentrations were significantly greater ($p<0.0001$) in calves fed MC (TSP=5.74±0.05 g/dL; IgG=21.06±0.53 g/L) compared to calves fed MCM (TSP=5.17±0.05 g/dL; IgG=17.40±0.53 g/L). Rectal body temperature did not differ between MC and MCM and averaged 38.8°C±0.01. No differences were detected in pneumonia or diarrhea incidence which averaged 70.4% and 10.3% respectively. Maternal colostrum+ milk calves had a greater ($p<0.05$) incidence of abnormal FC (75.0%) and DH (30.0%) compared to

MC calves (30.3% and 12.1% respectively). Maternal colostrum calves had a greater ($p < 0.0001$) incidence of abnormal RS (69.7%) compared to MCM (20.0%). Average daily gain did not differ between MC and MCM and averaged 0.60 ± 0.01 kg/d. Results suggest that feeding Holstein calves 2 separate feedings of colostrum will improve passive transfer and might lead to some health benefits. The effect of colostrum feeding quantity and frequency on RS needs to be investigated further.

INTRODUCTION

It is well established that 25 to 35 % of dairy cows on US dairies must be replaced annually to maintain herd size and improve genetics (Harris and Shearer, 2005). Therefore, quality dairy heifers must be available to replace the culled cows. The cost of raising dairy heifers increases if inadequate management results in a higher than normal morbidity and mortality. Colostrum is key in establishing immune protection because it contains immunoglobulins that increase the possibility of the neonatal survival and is a very important source of essential nutrients. Failure of passive transfer (FPT) contributes to excessively high pre-weaning mortality rates and other short- and long-term losses associated with animal health and welfare. Studies demonstrated that calves with inadequate immunoglobulin concentrations have reduced growth rate, increased risk of disease and death, increased risk of being culled (Robison et al., 1988; Donovan et al., 1998; Furman-Fratczak et al., 2011), and decreased milk production (DeNise et al., 1989; Faber et al., 2005). Early studies conducted with calves have demonstrated that most calves deprived from colostrum develop septicemia (Smith, 1962; Gay, 1965). Current recommendations are feeding 3 to 4 L of high quality (>50 g/L IgG and $<100,000$ cfu /mL of bacteria) colostrum within 6h of life (McGuirk and Collins, 2004; Godden, 2008; Chigerwe et al., 2008b). Benchmark for failure of passive transfer (FPT) is defined as a serum

immunoglobulin G (IgG) concentration below 10 mg/ml (1,000 mg/dL) (Tyler et al., 1996; Faber et al., 2005; Beam et al., 2009) and benchmark for total serum protein concentration of 5.2 g/dL was equivalent to 1,000 mg/dL serum IgG1, using digital refractometer or clinical refractometer, therefore failure of passive transfer (FPT) was defined as values below 5.2 g/dL (Tyler et al., 1996). Reports point out that prevalence of failure of passive transfer in dairy heifers has decreased from over 40% in 1991-1992 (USDA, 1993) to 19.2% in 2007 (Beam et al., 2009).

In the dairies, limited colostrum reserves due to variation in quality among individual cows (breed, lactation number, and colostral immunoglobulin concentration) (Swan et al., 2007; Kahoe et al., 2007), biosecurity programs to prevent transmission of diseases, such as; bovine viral diarrhea, salmonella and *Mycobacterium avium* ssp., paratuberculosis, and bovine leukomia virus (McGuirk and Collins, 2004), or not enough production of colostrum are the major contributing factors for failure of passive immunity. Failure of passive transfer continues to be a major factor for morbidity and mortality in dairy calves (Beam et al., 2009). The most important way to reduce calf morbidity and mortality is the early administration of adequate amounts of IgG in colostrum. Management strategies for maximizing colostrum IgG levels should be identified on modern dairies. Feeding calves with oroesophageal tube is an easy and practical way to administer colostrum to all calves in commercial dairies. Also, it is important that all calves consume certain amount of colostrum in timely manner within a few hours after birth. The objective of this study was to determine the effect of quantity and frequency of colostrum feeding on serum IgG concentration, health parameters, and growth in Holstein calves.

MATERIALS AND METHODS

This study was conducted on a large commercial dairy in southern Idaho. The Holstein female calves (n=213) were randomly assigned to one of the treatment group which consisted of maternal colostrum (MC, n=107) or maternal colostrum+milk (MCM, n=106). The first colostrum feedings (3.2 L) were administered using an esophageal tube within 1 h of birth. A second feeding of colostrum (2 L) was administered 12 h following the first feeding to MC calves while MCM calves received 2 L of milk. An esophageal feeder was used for first and second colostrum and milk feeding. The calves that had experienced dystocia, and twin born calves were not included in the study. Calves were removed from their dams before colostrum ingestion. The umbilicus of each calf was treated with 7% iodine solution and the calf was weighed and the weight was recorded. The calves were placed in a straw-bedded pen in the maternity barn and identified with double ear tags. After the initial two feedings, the calves were moved to individual, straw-bedded wood hutches (9m x 1.8 m; A = 1.62 m²) with a wood roof, a closed back and an open front, but with dividers to eliminate contact between calves in the hutches. The hutches were aligned in rows from east to west with the front facing to south. Straw bedding was re-applied to the hutches every other day, and in the 4th week the hutches moved towards front to dry ground and bedded with straw.

All calves were sampled (notch from the ear) for BVD, and blood sampled from the jugular vein to evacuated red top tubes. The general health of the calves was monitored daily, and all health problems and treatments were recorded.

Colostrum Preparation

All cows were milked two hours after parturition. Samples were collected from every cow to detect mammary infections and potential pathogens (*Mycoplasma*, *Staphylococcus*, *E. coli* etc.). The first milking colostrum from primiparous or multiparous cows was separated and poured into different cooling tanks (4°C). The colostrum was collected and picked up daily from multiple dairies in clean containers. Each container was sampled in an individual vial and the sample was sent to the laboratory. Each batch of colostrum was measured for quality by using a colostrometer. The colostrum was transferred from containers to a stainless-steel tank and mixed, where the pasteurization process was performed (60°C, 45 min). After pasteurization, the colostrum was cooled, and colostrum was poured into 2L plastic bottles. Pooled hospital milk also was pasteurized at 60°C for 90 minutes, cooled and poured into 2L plastic bottles. Plastic bottles were marked according to treatment and they were refrigerated. After processing, the colostrum was delivered back to the dairy where it was also stored in refrigerators (4°C). Colostrum was then removed from the refrigerator and warmed in warm water (54°C) until the colostrum temperature reached between 37.8°C and 38.9°C to feed calves within 1st h of life using an esophageal feeder. For second feeding, colostrum or pasteurized hospital milk was then removed from the refrigerator and warmed in warm water (54°C) until the colostrum or milk temperature reached between 37.8°C and 38.9°C to feed calves using an esophageal feeder. The calves, on second feeding, were fed colostrum or milk according to the experimental treatment group. The time of the first colostrum feeding was designated as 0h, and the second colostrum feeding were administered 12 h later.

Blood Sampling and Determination of IgG Concentration of Serum and Pooled Colostrum

Blood samples from Holstein heifers were collected from the jugular vein into evacuated tubes 24±3 h after birth to measure total serum protein and serum IgG concentration. Samples were cooled to 4°C immediately following collection. Blood was allowed to clot, and serum was separated by centrifugation (3000 x g for 15 minutes at 4°C) within 24 h of collection and tested for TSP concentration. Serum was stored at - 20°C for later serum IgG concentration analysis. Determinations of total serum protein (TSP) were performed using a refractometer (Jorvet J-351, Jorgensen Laboratories, Inc., CO). The refractometer was cleaned and calibrated with distilled water between each sample.

The IgG concentration in serum and colostrum were determined by enzyme linked immunosorbent assay (ELISA) (Bovine IgG Elisa Quantitation Set, Cat. No. E10-118. Bethyl Laboratories, Inc., www.bethyl.com, USA).

Milk Preparation and Feeding

After the second colostrum or milk feeding all calves received pasteurized hospital milk until weaning. Hospital milk was collected from dairies twice daily and stored in tanks for later pasteurization. The pasteurization process of hospital milk was performed twice a day: at 2:30 a.m. and 9:30 a.m. Hospital milk was pasteurized at 60°C for 90 minutes. The pasteurized hospital milk solids were increased by adding milk replacer until solid content reached to 15 %. Solid readings were done with brix refractometer (Jorvet J0351B, Jorgensen Laboratories, Inc., CO). Milk was fed at 6:30 a.m. and 1:30 p.m. All calves were fed with bottles. Bottles were filled, and nipples were attached to bottles and chlorine was sprayed on the nipples and

rinsed with water. The calves were fed from day 1 to 42 days 3L twice daily, and 43- 51 days 2L once daily, after weaning calves were kept in the hutches for 2 weeks period.

Calva Milk Replacer (Crude Protein 25%, Crude Fat 20 %) (Calva Products, LLC. Acampo, CA) was used for milk replacer. Calves were offered starter grain and water in the hutches starting from the second day of life. Calf starter (16% protein content) and fresh clean water were offered ad-libitum every day from the second day of life until they were removed from hutches and placed in group pens. Leftovers were removed daily.

Two weeks following weaning, calves were removed from hutches, and then they were grouped into pens of 25, where they remained for one month. Within this time of period calves were fed 95% calf starter and 5% alfalfa hay. Calves were moved into larger pens with a capacity of 200 calves.

Daily Observations: Health, Incidence of Abnormal Scores by Treatment (Normal or Abnormal)

The general health of the calves was monitored daily, and all health problems and treatments were recorded. The fecal, dehydration, and respiratory score tables were modified from Larson et al. (1977), Diaz et al. (2001), Bascom et al. (2007). Fecal scores (0-3), respiratory scores (0-4), and dehydration scores (0-2) were recorded daily. The scores were recorded under following guidelines. Fecal scores: 0 = normal; manure firm and well formed (not hard), 1 = mild diarrhea (soft, pudding-like), 2 = severe, watery diarrhea, and 3 = bloody feces; Respiratory scores: 0 = normal breathing, 1 = nasal discharge, 2 = coughing–moist, 3 = heavy thoracic breathing, 4 = abdominal breathing; Dehydration scores: 0 = eyes bright and skin flexible, 1 = mild dehydration (skin flexibility 3 to 5 second), 2= severe dehydration (skin flexibility >5 second).

A single observer scored all calves every day until 28 days of age. Health evaluations were conducted daily by study personal until calves were 3 months of age. Health disorders were diagnosed and treated according to veterinary instructions. Intravenous fluids were administered to severely dehydrated calves. Body temperatures were recorded every other day until three weeks of age.

Body Weight

Calves were weighed at birth, before colostrum ingestion, at day 28, at weaning day 51. All heifers were individually weighed using an electronic scale (Salter Brecknell Mod. PS 1000). The electronic scale was placed in a hutch that was built out of welded mesh panels and other galvanized materials and that was equipped with rotating tires.

STATISTICAL ANALYSES

All statistical analyses were conducted with SAS (version 9.4, SAS institute, Cary, NC). Data were analyzed using a mixed-effects model for repeated measures using Proc Mixed of SAS, except for TSP and IgG that were analyzed using the same model without the repeated measures. Proc Genmod was used to determine incidence of abnormal fecal, dehydration respiratory scores, health incidence, and multiple health incidence. Proc Corr was used to determine correlation between BW-TSP.

RESULTS

Mean calf birth weight for two hundred thirteen Holstein female calves was 36.4 ± 0.5 kg (Table 4.7). Total serum protein and IgG concentrations were significantly greater ($p < 0.0001$) in calves fed MC (TSP= 5.74 ± 0.05 g/dL; IgG= 21.06 ± 0.53 g/L) compared to calves fed MCM

(TSP=5.17±0.05 g/dL; IgG=17.40±0.53 g/L) (Table 4.7, Figure 4.4). Rectal body temperature did not differ between MC and MCM and averaged 38.8°C±0.01 (Figure 4.1). No differences were detected in pneumonia or diarrhea incidence during the first 3 months of age, which averaged 70.4% and 10.3% respectively (Table 4.2), and no differences were detected in pneumonia or diarrhea incidence during the first 2 months of age, which averaged 46.0% and 10.3% respectively (Table 4.3). Also, no differences were detected in pneumonia or diarrhea multiple incidence during the first three months of age, which averaged 44.1% and 6.5% respectively (Table 4.4), and no differences were detected in pneumonia or diarrhea multiple incidence during the first 2 months of age, which averaged 23.0% and 7.0% respectively (Table 4.3).

Maternal colostrum + milk calves had a greater ($p<0.05$) incidence of abnormal fecal score (75.0%) and DH (30.0%) compared to MC calves (30.3% and 12.1% respectively). Maternal colostrum calves had a greater ($p<0.0001$) incidence of abnormal RS (69.7%) compared to MCM (20.0%) (Table 4.1, Figure 4.2). Fecal and respiratory scores frequency distribution by age value for MC and MCM was evaluated days of age between 1 to 10, 11 to 15, and 15 to 30 (Table 4.8, 4.9). Average daily gain did not differ between MC and MCM, from birth to 28 days and averaged 0.54±0.01 kg, and at weaning (Day 51) averaged 0.60±0.01 kg (Table 4.7). Birth weight and IgG levels at 24 h of age were not correlated ($p>0.05$) (Figure 4.3).

Table 4.1. Health scores of Holstein female calves, from birth to 28 days of age, receiving maternal colostrum (MC) or maternal colostrum+ milk (MCM)

Health Score	MC	MCS	P
Respiratory scores ¹ (RS) %	20.0	69.7	0.0001
Fecal Scores ² (FC)%	30.3	75.0	0.0001
Dehydration scores ³ (DH)%	12.1	30.0	0.06

¹Respiratory scores (abnormal) for maternal colostrum+milk fed calves are significantly different ($p < 0.05$).

²Fecal scores (abnormal) for maternal colostrum+milk fed calves are significantly different ($p < 0.05$).

³Dehydration scores are not significantly different ($p > 0.05$).

Table 4.2. Pneumonia and diarrhea of Holstein female calves, from birth to 3 months of age, receiving maternal colostrum (MC) or maternal colostrum+ milk (MCM)

Health	MC	MCM	P
Pneumonia ¹ %	72.9	67.9	0.4
Diarrhea ² %	8.4	12.2	0.35

¹Pneumonia and ² Diarrhea incidence % from birth to 3 months of age for treatment groups are not significant ($p > 0.05$).

Table 4.3. Pneumonia and diarrhea of Holstein female calves, from birth to 2 months of age, receiving maternal colostrum (MC) or maternal colostrum+ milk (MCM)

Health	MC	MCM	P
Pneumonia ¹ %	48.6	43.4	0.44
Diarrhea ² %	8.4	12.2	0.35

¹Pneumonia and ² Diarrhea incidence % from birth to 2 months of age for treatment groups are not significant ($p > 0.05$).

Table 4.4. Multiple pneumonia and diarrhea of Holstein female calves, from birth to 3 months of age, receiving maternal colostrum (MC) or maternal colostrum+ milk (MCM)

Health	MC	MCS	P
Pneumonia ¹ %	48.6	39.6	0.18
Diarrhea ² %	4.6	8.4	0.25

¹Pneumonia and ² Diarrhea multiple incidence % from birth to 3 months of age for treatment groups are not significant ($p > 0.05$).

Table 4.5. Multiple Pneumonia and diarrhea of Holstein female calves, from birth to 2 months of age, receiving maternal colostrum (MC) or maternal colostrum+ milk (MCM)

Health	MC	MCM	P
Pneumonia ¹ %	24.3	21.7	0.65
Diarrhea ² %	5.6	8.4	0.40

¹Pneumonia and ² Diarrhea multiple incidence % from birth to 2 months of age for treatment groups are not significant ($p>0.05$).

Table 4.6. Effect of maternal colostrum (MC) or maternal colostrum+ milk (MCM) on IgG absorption and TSP levels at 24 h of age

Item	MC	MCM	SE	P
N ¹	107	106		
Birth Weight ² , kg	36.51	36.26	0.50	0.7
Serum IgG ³ , g/L	21.06	17.40	0.53	0.0001
TSP ⁴ , g/L	5.74	5.17	0.05	0.0001

¹Number of calves enrolled for treatment group MC or MCM.

²Birth weights of calves averaged 36.4 ± 0.5 kg.

³Serum IgG levels and ⁴total serum protein levels at 24 h age significantly different ($p<0.05$).

Table 4.7. Average daily gain (ADG) for maternal colostrum (MC) or maternal colostrum + milk (MCM) fed Holstein female calves from birth to 51 days of age

Item	MC	MCM	SE	P
Birth Weight, kg	36.51	36.26	0.50	0.7
ADG ¹ , kg (Birth to 28 days of age)	0.53	0.55	0.01	0.16
ADG, kg (Birth to weaning 51 days)	0.60	0.61	0.01	0.34

¹ADG = Average daily gain

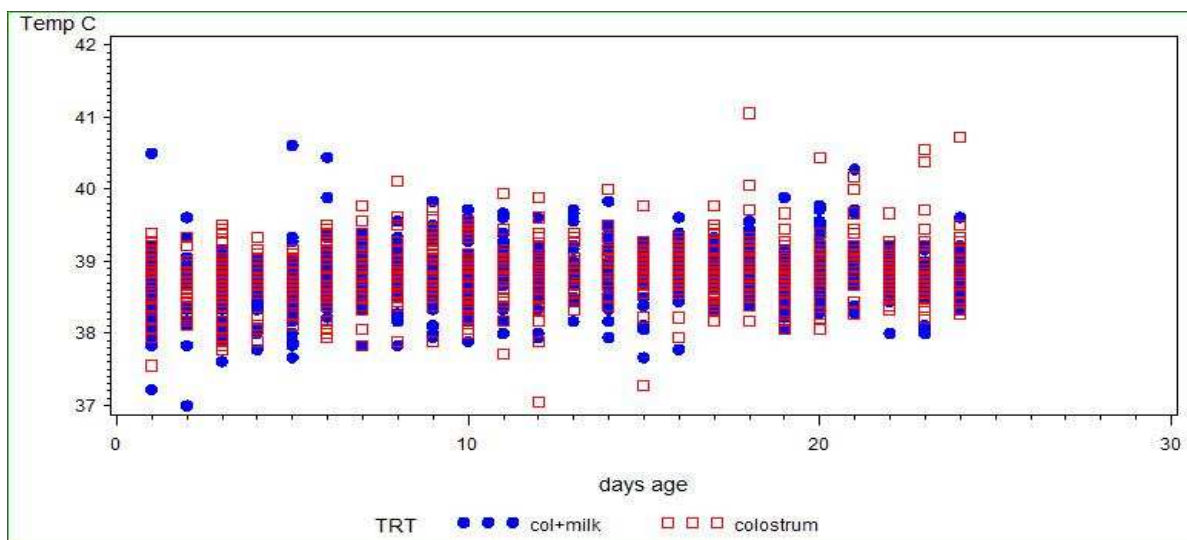
Table 4.8. Age to Fecal scores frequency distribution by age value

TRT	Age to fecal scores	Frequency (f)	Relative frequency	Percent frequency
MC	1 to 10	8	0.80	80.0
	11 to 15	1	0.10	10.0
	15 to 30	1	0.10	10.0
TOTAL		10	1	100
TRT	Age to fecal scores	Frequency (f)	Relative frequency	Percent frequency
MCM	1 to 10	24	0.80	80.0
	11 to 15	3	0.10	10.0
	15 to 30	3	0.10	10.0
TOTAL		30	1.00	100

Table 4.9. Age to Respiratory scores frequency distribution by age value

TRT	Age to respiratory scores	Frequency (f)	Relative frequency	Percent frequency
MC	1 to 10	6	0.27	27.3
	11 to 15	2	0.09	9.1
	15 to 30	14	0.64	63.6
TOTAL		22	1	100
TRT	Age to respiratory scores	Frequency (f)	Relative frequency	Percent frequency
MCM	1 to 10	2	0.29	28.6
	11 to 15	0	0.00	0.0
	15 to 30	5	0.71	71.4
TOTAL		7	1.00	100

Figure 4.1. Body temperatures, from birth to three weeks of age, of Holstein female calves fed maternal colostrum (MC) or maternal colostrum+ milk (MCM)



Body temperature for MC=38.8 °C SE=0.01 MCM=38.8 °C SE=0.01 $p>0.05$

Figure 4.2. Health scores of Holstein female calves, from birth to 28 days of age, receiving maternal colostrum (MC) or maternal colostrum+ milk (MCM)

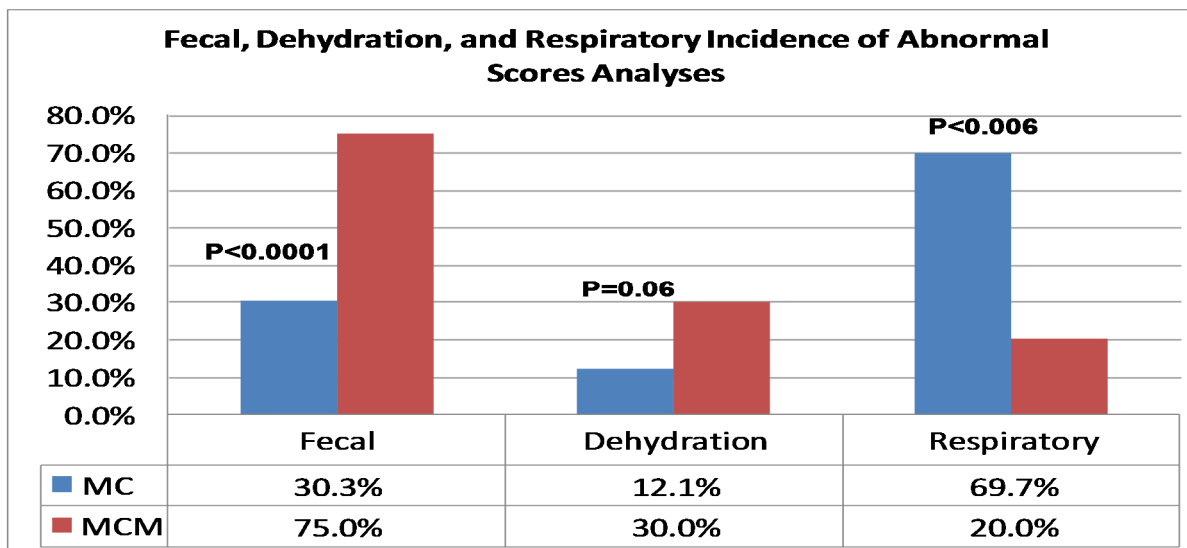
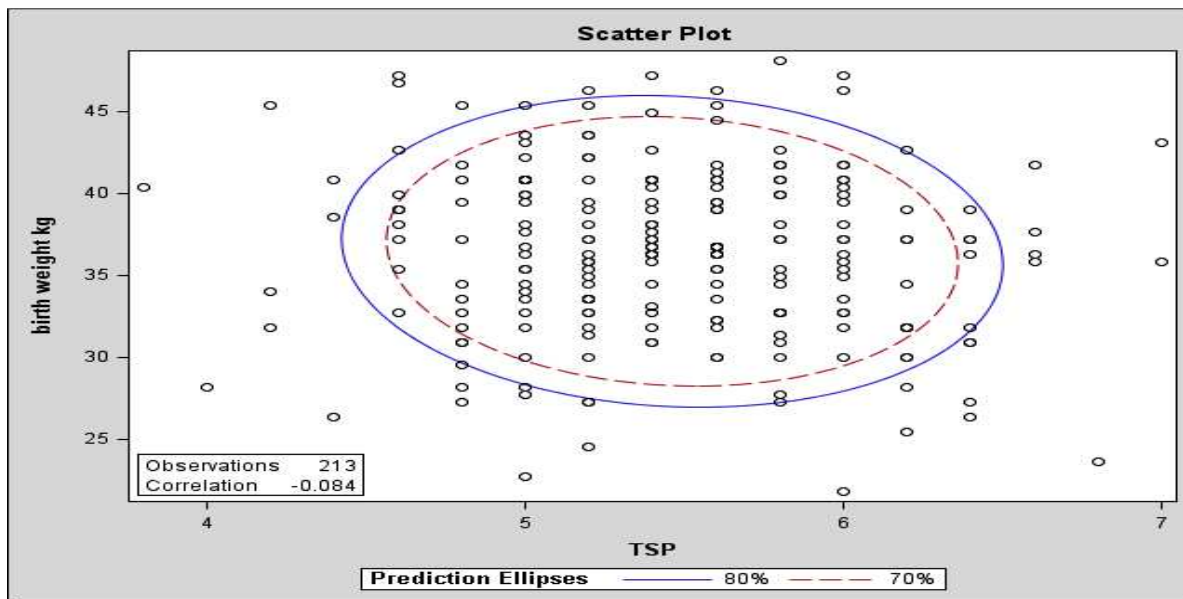
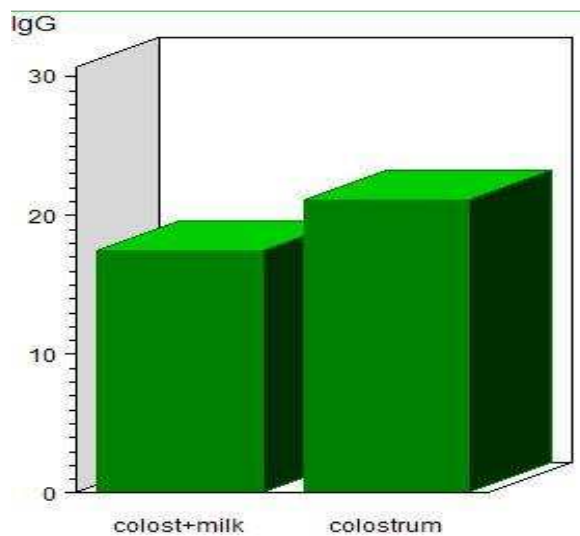


Figure 4.3. Correlation analyses between birth weight and total serum protein for maternal colostrum (MC) and maternal colostrum+ milk (MCM) fed Holstein female calves



Birth weight and TSP levels at 24 h of age were not correlated ($p>0.05$).

Figure 4.4. Serum IgG Concentration (g/L) levels at 24 h age for maternal colostrum (MC) or maternal colostrum+ milk (MCM) fed Holstein female calves



MCM (TSP=5.17±0.05 g/dL) MC (TSP=5.74±0.05 g/dL) ($P<0.05$).

DISCUSSION

Immunoglobulins are absorbed most efficiently in the first few hours of life and absorption declines rapidly after 12 h of life (Weaver et al., 2000). It is recommended that calves be fed colostrum before 4 h of life (Beam et al., 2009). A minimum of 150 g of IgG is required by a calf to achieve passive transfer (Chigerwe et al., 2008a). Petrie, (1984) recommends 80 to 100 g of IgG to achieve APT.

Pritchett et al. (1991) indicated that the average IgG concentration in colostrum of Holstein cows was 48.2 g/L, with a range of 20 to >100 g/L Quigley et al. (1994) reported that the average IgG concentration in colostrum of Jersey cows averaged 65.8 g/L of IgG, with a range of 28.4 to 114.7 g/L.

Sakai et. all. (2012) showed that apparent efficiency of absorption of IgG and serum IgG concentration at 48 h are similar in calves fed 3 or 4L of colostrum with similar colostral IgG concentrations by oroesophageal tubing. Authors suggested that dairy producers can save colostrum by feeding 3L of colostrum, as there seems to be no benefit with regards to serum IgG or AEA of IgG when calves are fed 4L of colostrum once, by oroesophageal tubing. Morin et al. (1997) confirmed that, when fixed volumes of colostrum were fed to neonatal dairy calves, higher serum IgG1 concentrations were achieved with high immunoglobulin (Ig) colostrum than with low Ig colostrum. Compared with a smaller volume (2 L), administration of a large volume (4 L) of high IgG colostrum within 3 h after birth significantly increased serum IgG1 concentration, did not reduce the efficiency of IgG1 absorption, and resulted in no apparent discomfort or disease. However, a large volume (4 L) of low Ig colostrum fed within 3 h after birth did not significantly increase serum IgG1 concentrations at 24 or 48 h over those of calves

fed a smaller volume (2 L) of colostrum. When low Ig colostrum was fed, an extra meal of colostrum (2 L at birth and 2 L at 6 h) was slightly more advantageous than a large volume (4L) of colostrum at birth. In our study, when fixed volumes of colostrum (3.2 L) were fed to the calves, additional second feeding (2 L) significantly improved serum IgG levels at 24h. However, serum IgG levels for fixed volume of 3.2 L colostrum fed calves averaged 17.4 g/L and IgG levels were well over threshold values.

A positive association between serum IgG concentration and growth performance or health status on calves has been reported in the previous studies (Nocek et al., 1984; Wells et al., 1996). In our study, no differences in body weight gain were observed among calves for any treatments at 28 or 51 d which is in agreement with Hopkins et al., (1997). Also, no differences were detected in pneumonia or diarrhea incidence at 3 months of age.

Robison et al. (1988) demonstrated that dairy heifer calves with inadequate serum IgG concentrations at 24 to 48 h (<12 mg/mL) had a higher mortality rate in dairy heifer calves thorough through 6 months of age (6.78% compared with 3.33%). In addition, low concentration IgG concentrations at birth have affected growth through 180 days of age in heifer calves. Donovan et al. (1998) confirmed that lower TSP was a significant risk factor for mortality. The association of TSP and mortality was quadratic and showed a dramatic decrease in mortality as TSP increased from 4.0 to 5.0 g/dL, a small improvement from 5.0 to 6.0 g/dL and virtually no improvement in mortality rates as TSP increased over 6.0 g/dL. The hazard mortality ratio was constant from birth to six months, indicating that the increased risk of mortality associated with low levels of TSP was evident through six months of age.

Respiratory Disease

A report by USDA stated 12.4% of pre-weaned calves were treated for respiratory disease and respiratory infections were responsible 22.5% of pre-weaned dairy calf (USDA, 2007). According to the 2007 US National Animal Health Monitoring Survey (NAHMS), the incidence of post weaning respiratory disease in dairy heifers was 5.9%, and it was the predominant cause of reported deaths of weaned heifers 46.5%. (USDA, 2010). McGuirk (2015) reported that the incidence and prevalence of respiratory disease in calves is higher than producers report because detection is difficult. The economic toll from treatment expense, mortality, premature culling, reduced growth, fertility and milk production in first lactation survivors of respiratory disease is reported to range from \$15 to \$36 per event. Furman-Fratczk et al. (2011) showed that the morbidity and intensity of a disease course were lowest in heifer calves with serum Ig concentration exceeding 10 g/L at 30 to 60 h of life; these calves did not become ill before d 14 of life. Calves with >15 g/L gammaglobulin in serum avoided respiratory tract infections. Heifers with serum gammaglobulin levels >10 g/L at 30 to 60 h of life showed better health status. However, in our study 69 % calves had respiratory tract infections, even though, IgG levels were >15 g/L which did not support previous findings. The relationship between IgG levels and respiratory infections should be further investigated.

CONCLUSION

All calves were fed 3.2 L of colostrum at birth. The calves that were fed additional 2 L of colostrum at 12 h following the first feeding had greater concentrations of IgG at 24 h than calves fed similar IgG concentrations one time at birth. Results suggest that feeding Holstein calves 2 separate feedings of fixed volumes of colostrum, within the first 14 h of life, will

improve passive transfer and might lead to some health benefits. Timely and adequate intake of colostrum will improve fecal, dehydration scores, and health of the calves. The effect of colostrum feeding quantity and frequency on RS and the relationship between IgG levels and respiratory infections need to be investigated further.

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