# The effect of tube vs. bottle feeding colostrum on IgG absorption, abomasal emptying and plasma hormone concentrations in newborn calves

by

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A thesis submitted in partial fulfillment of the requirements for the degree of

Master of Science
in
Animal Science

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**Abstract**

Colostrum management is essential for calf survival and success in the dairy herd, and current practices on farm are to feed colostrum through either an esophageal tube or a nipple bottle. Therefore, the objective of this thesis was to determine if feeding colostrum to newborn calves through an esophageal tube, compared with a nipple bottle, would delay abomasal emptying which would in turn decrease passive transfer of IgG and plasma glucose, insulin, and GLP-1 and 2 concentrations. Twenty newborn Holstein bull calves were fed 3L of colostrum replacer (200 g IgG) through either an esophageal tube or nipple bottle at 2 h after birth. The results from this thesis demonstrated that feeding method did not affect abomasal emptying, and as a result there was no treatment effect on serum IgG concentrations. Feeding with a tube resulted in higher plasma glucose and insulin area under the curve, most likely due to the decreased time to consume the colostrum meal. In addition, tube fed calves drank more milk in their first milk meal. There was no treatment effect on GLP-1 or 2, but both hormones increased after colostrum feeding. Overall findings from this thesis suggest that feeding colostrum at a volume of 3L, through either an esophageal tube or nipple bottle, accomplishes adequate passive transfer of IgG and either feeding method is viable for ensuring successful colostrum management on farm.

## Preface

This thesis is an original work by Mariah Desjardins-Morrissette. The research project in this thesis received ethics approval from the University of Alberta Research Ethics Board, project name “Bottle vs. Tube Feeding Colostrum”, AUP 00001890, July 28, 2016.

**Acknowledgements**

I would like to sincerely thank my supervisor Dr. Masahito Oba for his guidance throughout my degree and for always challenging me to think critically. I have truly grown as both a person and as a student with him as my supervisor. Words cannot describe how much I have learnt, and I will use the knowledge I have gained from my degree throughout my career.

I would also like to give a special thanks to Dr. Michael Steele for giving me the opportunity to come to the University of Alberta and introducing me both to research and the dairy industry. His passion for research inspired me to do a Masters degree.

Thank you to Dr. Lelou Guan for having your door open and joining my committee. Also, a special thanks to Dr. Vera Mazurak for being my chair and external examiner.

I would like to give a very special thanks to the de Goeij family of Breevliet Farms, without whom my study would not have been possible. Thank you Jolet Kohler, Amanda Fischer, Jennifer Haisan and Jayden MacPherson for all of your help during my study and for your friendship. From staying up all night waiting for cows to calve to sushi lunches, I would not have made it to the finish line without your support.

I would also like to extend my thanks overseas to Dr. Sugino's lab in Japan. Thank you Dr. Sugino and Dr. Oba for making my trip to Japan possible, it was the experience of a lifetime. A special thanks to Mamiko Mon and Yudai Inabu for their patience and support in the lab, I would not have been able to do my lab analysis without them.

Finally, I would like to thank Keanan Clarke for going on this crazy adventure with me and supporting me on my best and worst day, I would not be here without you. Also, I would like to thank my family who always told me the sky was the limit and encouraging me to pursue my dreams.

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**List of Abbreviations**

| | |
|---|---|
| AEA | Apparent Efficiency of Absorption |
| AUC | Area Under the Curve |
| BW | Body Weight |
| $C_{max}$ | Maximum Concentration |
| FPT | Failure of Passive Transfer |
| GIT | Gastrointestinal Tract |
| GLP | Glucagon-like Peptide |
| GLUT | Glucose Transporter |
| IGF | Insulin-like Growth Factor |
| Ig | Immunoglobulin |
| SPC | Standard Plate Count |
| $T_{max}$ | Time to Maximum Concentration |

# 1.0 Chapter 1: Literature Review

## 1.1 Introduction

Today's dairy industry continues to face the issue of high calf mortality and morbidity rates. This, combined with the reduction of antibiotic use in the industry, makes proper colostrum management even more critical. Proper colostrum management is key to the vitality of calves and life-long production but, unfortunately, many farms vary in their colostrum management and have poor transfer of immunoglobulins in their calves. Failure of passive transfer (FPT) immunity means that a calf fails to absorb sufficient amounts of immunoglobulins, which is generally accepted as <10 mg/ml in serum between 24 and 48 hours old (Beam, 2009). This is a concern for the industry as several studies show high rates of FPT on farm; a study conducted in the U.S in 2009 found 19.2% of heifer calves had FPT (Beam et al., 2009) and studies in Canada have shown FPT rates between 25-37% (Wallace et al., 2006; Trotz-Williams et al., 2008).

Current on farm practices include feeding colostrum through either an esophageal tube or nipple bottle. Previous research has shown conflicting data with regards to the impact that feeding method has on passive transfer of immunoglobulins (Kaske et al., 2005; Godden et al., 2009, Chigerwe et al., 2012). It is also unclear if feeding colostrum through an esophageal tube or nipple bottle affects abomasal emptying or release of certain hormones in response to colostrum feeding. This literature review will aim to discuss different aspects of colostrum management and how colostrum feeding affects gut development and endocrine control of metabolism in newborn calves.

## 1.2 Colostrum Management

Colostrum management is essential for raising healthy and productive calves in the dairy industry. The ingestion of colostrum is critical to build immunity in calves and must be ingested shortly after birth in order to absorb sufficient amounts of immunoglobulins (Blum and Hammon, 2000). The method used to feed colostrum to these calves could affect absorption of various nutrients in colostrum.

Current colostrum feeding strategies aim to feed at least 100 g of IgG to calves in order to achieve adequate passive transfer of immunity (Godden, 2008). In order to achieve the recommended 100 g of IgG it is currently suggested that 10-12% of body weight of colostrum be fed in the first 2 hours of life (Godden, 2008). Success of passive transfer of immunity is often assessed by calculating apparent efficiency of absorption (AEA), which is the percentage of IgG that is actually absorbed from colostrum (Godden, 2008). Several factors affect the success of this transfer including: colostrum quality, timing of colostrum feeding and volume of colostrum consumed.

### *1.2.1 Colostrum Production:*

Several weeks prior to calving initial secretion in the mammary gland is known as colostrum, which contains immunoglobulins, proteins, essential and non-essential amino acids, fatty acids, lactose, vitamins and minerals, growth factors and hormones (Blum and Hammon, 2000). Colostrogenesis is known as the transfer of immunoglobulins from maternal circulation into the mammary gland prepartum (Barrington et al., 2001). The mammary gland is actively involved in regulating concentrations of immunoglobulins in both colostrum and milk, however, the mammary epithelium does not synthesize immunoglobulins (Stelwagen et al., 2014). The

majority of immunoglobulins enter colostrum through selective receptor-mediated transport, with a receptor located in the mammary epithelial cells, called the neonatal Fc receptor (Mayer et al., 2005). This receptor plays a key role in transporting IgG into the mammary gland (Mayer et al., 2005). Although IgA and IgM are also found in colostrum they are at much lower levels and only account for 5 and 7% of total Ig in colostrum, respectively, whereas IgG accounts for 85-90% of the total Ig, thus we are more concerned with absorption of IgG from colostrum when we discuss passive transfer of immunity in calves (Larson et al., 1980).

### *1.2.2 Absorption of Immunoglobulin G*

Calves are born agammaglobulinaemic, meaning they have no immunity when born since there is no transfer of immunoglobulins in utero (Godden, 2009). Calves remain in this state due to one of the following factors: inadequate colostrum consumed, low concentrations of Ig in colostrum, delayed feeding of colostrum or early loss of absorptive capability (Stott et al., 1979a). It is critical to deliver sufficient amounts of colostrum after birth as soon as possible to ensure adequate absorption of IgG prior to gut closure which can be defined as "the cessation of absorption of macromolecules from gut to blood in neonates" (Lecce and Morgan, 1962). Many factors affect absorption of IgG, however, as mentioned previously it is primarily influenced by timing of feeding, amount fed and the quality of colostrum fed.

### *1.2.3 Timing of Colostrum Feeding*

Gut closure, defined above, is linearly influenced by timing of feeding (Stott et al., 1979a). Calves fed immediately after birth have a closure time of 21 hours of age while calves that were fed at 24 hours had a closure time of 33 hours, so although time to gut closure is

extended, overall time to absorb Ig is shortened (Stott et al., 1979a). As time to feeding was extended, the percentage of calves that failed to absorb adequate amounts of Ig increased (Stott et al., 1979a). The highest rates of absorption occur during the first 4 hours after feeding with absorption slowing after this time (Stott et al., 1979b).

### *1.2.4 Volume of Colostrum Fed*

Volume of the meal can also affect absorption of IgG in the first 24 hours after birth (Stott et al., 1979b). Calves that are fed smaller meals, 0.5 or 1 L, demonstrate an increase in absorption of IgG after a second meal within 24 hours (Stott et al., 1979b). Calves fed a larger volume of 2 L, however, do not demonstrate an increase in IgG absorption after a second meal (Stott et al., 1979b). This is similar to a result seen in piglets who when fed less than 300 ml of colostrum within 20h of birth were able to absorb macromolecules, but when fed at least 400 ml could not absorb macromolecules (Lecce, 1973). This finding parallels the results of Stott et al. (1979b). Similar results were observed by Jaster (2005), who conducted a 2x2 study where they fed 4L once versus 2L twice of either high quality colostrum (84 mg/mL) or poor quality colostrum (31.2 mg/mL). They found that when higher quality colostrum was fed in two small volumes it resulted in the highest concentration of IgG at 24 and 48 hours and also had higher AEA than the group that was also fed the higher quality colostrum but at one large volume (Jaster, 2005). This suggests that there may be a maximum of IgG that can be absorbed at one time due to a limited number of surface receptors carrying IgG from the intestinal wall to the blood stream; thus, when all of these receptors become saturated, there is no longer a means for IgG to be transported (Jaster, 2005). In addition, Conneely et al. (2004) demonstrated that feeding colostrum at 8.5% of their BW increased apparent efficiency of absorption (AEA)

compared to 10% or 7%, suggesting that there is a physiological limit for IgG absorption when feeding colostrum.

### *1.2.5 Quality of Colostrum Fed*

As discussed previously timing and quantity affect passive transfer of IgG from colostrum but another factor is quality of the colostrum which is affected by breed, parity of the dam, mastitis, dam vaccination protocol and pasteurization (Godden, 2008). The dam influences colostrum quality through breed, parity, and the vaccine protocol they underwent. The concentration of IgG can vary greatly cow to cow with a range of 9 to 186 g/L seen in individual Holstein cow's colostrum (Swan et al., 2007). This concentration can also vary between breeds with Jersey cows having the highest total Ig content (9.0%) followed by Ayrshire (8.1%), Brown Swiss (6.6%), Guernsey (6.3%) and finally Holsteins (5.6%). These variations could be due to differences in genetics or due to a dilution effect caused by more milk being produced by certain breeds, such as Holsteins (Muller and Ellinger, 1981). However, a survey conducted in the U.S, in 2012, on quality and composition of colostrum on dairy farms found no difference between colostrum from Holstein and Jersey cows, indicating that breed does not consistently effect Ig content (Morrill et al., 2012). This survey also looked at parity of the dam and found that IgG concentration increased with parity, with concentrations of 42.4, 68.6 and 95.9 mg/ml in the first, second and third plus lactations, respectively (Morrill et al., 2012). The difference between parities is due to extended exposure to pathogens as the animal ages (Godden, 2008). This is also the reason why vaccinating protocols that occur 3 to 6 weeks prior to calving can increase concentrations of protective antibodies in the colostrum (Godden, 2008).

Other factors that can influence the colostrum quality, in addition to effects contributed from the dam, include: bacterial count, pasteurization and source of colostrum. High bacterial counts is an issue on farm, as shown by a study which took samples from farms in Quebec and found 35.9% of samples were contaminated with more than 100 000 bacteria/mL (Fecteau et al., 2002). High bacteria counts in colostrum, on farms, is an issue since the bacteria in the colostrum binds to free Ig in the gut lumen or blocks uptake of Ig across the intestinal epithelial cells (Johnson et al., 2007).

Pasteurization is a method used to decrease these high bacterial counts and is usually done by heating the colostrum to 60°C for 60 minutes (Godden, 2008). Pasteurization has been shown to improve AEA in calves, in a study that compared pooled colostrum that was either pasteurized, frozen immediately (low bacterial count), or stored at 20°C for 24h and then frozen (high bacterial count) (Elizondo-Salazar and Heinrichs, 2009). The pasteurized colostrum had the lowest standard plate count (SPC) at 2.81 cfu/mL, followed by the frozen immediately group at 3.97 cfu/mL, and the frozen later group had the highest SPC at 5.61 cfu/mL (Elizondo-Salazar and Heinrichs, 2009). Treatment of the colostrum did not affect IgG concentration in the colostrum but, interestingly, they found that calves that received pasteurized colostrum had greater AEA compared to both non-pasteurized groups (Elizondo-Salazar and Heinrichs, 2009). There was no significant difference between the two non-pastuerized groups, even though bacterial count was significantly different between the two treatments (Elizondo-Salazar and Heinrichs, 2009). This indicates that pasteurized colostrum is better than non-pasteurized regardless of the difference in bacterial count between non-pasteurized treatments (Elizondo-Salazar and Heinrichs, 2009). Johnson et al. (2007) also showed that calves fed pasteurized

colostrum had higher serum protein, IgG concentrations and AEA compared with those fed raw colostrum (Johnson et al., 2007).

The type of colostrum, maternal colostrum or a colostrum replacer, could also affect absorption of IgG, . Previous research has shown that when comparing maternal colostrum with a colostrum replacer, containing an Ig concentrate derived from bovine serum, there is no difference in plasma IgG concentrations, average daily gain, health or incidence of diarrhea between groups, but calves that were fed maternal colostrum were more feed efficient in week one of life (Jones, 2004). There is conflicting data from another study showing that maternal colostrum results in significantly higher serum IgG concentration and lower failure of passive transfer rates, defined as serum IgG < 10 mg/mL (Swan et al., 2007). It is unclear if feeding colostrum replacer can result in serum IgG concentrations similar to that of calves fed maternal colostrum. Success of passive transfer of IgG can also vary greatly between products. In addition, maternal colostrum contains high levels of bioactive factors that are beneficial to the calf, in addition to IgG, therefore, when formulating colostrum replacers these bioactive factors also need to be considered.

### *1.2.6 Gastric Emptying in Calves*

It has been discussed how timing, quality and amount of colostrum fed can affect absorption of IgG. A possible mechanism that can explain differences in IgG absorption when different volumes and quality is fed is gastric emptying. Gastric emptying, also known as abomasal emptying in calves, is the rate at which contents of the abomasum enter the small intestine, where nutrient absorption occurs (Sen et al., 2006). Gastric emptying is the rate limiting step in nutrient delivery, meaning slowing or speeding up gastric emptying rate controls

the rate at which nutrients are absorbed in the small intestine (Sen et al., 2006). Large meals are emptied slower than small meals in order to spread out the nutrient load entering the small intestine (Sen et al., 2006). Calorie content also affects gastric emptying with non-caloric fluids, such as isotonic $NaHCO_3$, being emptied from the abomasum of calves in a rapid manner while caloric fluids, such as cow's milk and milk replacer, are emptied more slowly (Marshall et al., 2008). Schaer et al. (2005) also reported that feeding milk replacer resulted in a higher time to maximum concentration of acetaminophen, which indicates a slower emptying rate, compared with an electrolyte solution. The difference in empting rate is a physiologic response to ensure that nutrients are presented to the small intestine at a constant rate (Marshall et al., 2008).

*1.2.6.1 Methods for Measuring Gastric Emptying*

The two primary methods for measuring gastric emptying in the calf are ultrasonographic scanning of the abomasum and use of acetaminophen as a marker (Sen et al., 2006). Acetaminophen is widely used as an analgesic in humans and the absorption of acetaminophen has been accurately used to measure gastric emptying of liquid-phase meals in humans, horses and calves (Marshall et al., 2005). Acetaminophen is only absorbed in the small intestine, when administered orally, with its rate-limiting step being the rate at which the meal empties from the stomach into the small intestine, known as gastric emptying, thus concentrations of acetaminophen in the blood can be used to measure gastric emptying (Marshall et al., 2005).

*1.2.6.2 Meal Composition and Delivery Method*

Since gastric emptying rate is affected by amount, composition and delivery method of the meal, colostrum and the way it is fed can affect emptying rate. Colostrum is more calorically

dense than milk and has demonstrated to empty more slowly by Mokhber-Dezfooli et al. (2012), who reported that mean time to maximum acetaminophen plasma concentration ($T_{max}$) was 340 min when 3 L of colostrum was fed, while previous studies that fed 2L of cow's milk reported $T_{max}$ values of 129, 187, and 191 min (Nouri et al., 2008, Afshari et al., 2009, Constable et al., 2009) and studies that fed 2L of milk replacer reported $T_{max}$ values of 190, 201, and 206 min (Sen et al., 2006, Nouri and Constable, 2007, Marshall et al., 2008). They also added erythromycin lactobionte and ivermectin to increase the emptying rate, gentamicin to slow emptying rate, and 0.9% NaCl as a control mixed into the colostrum to determine if the different emptying rate affected IgG absorption (Mokhber-Dezfooli et al., 2012). They found AEA was higher in calves administered erythromycin while it was lower in calves administered gentamicin (Mokhber-Dezfooli et al., 2012). In addition, it appears AEA is linearly and negatively associated with emptying rate, and 22% of the variation in AEA can be explained by emptying rate (Mokhber-Dezfooli et al., 2012). These results demonstrate that emptying rate of colostrum can influence absorption of IgG in calves.

It has also been demonstrated that feeding method can affect gastric emptying rate (Schaer et al., 2005; Nouri and Constable, 2006; Sharifi et al., 2009). Since colostrum is typically fed through either a nipple bottle or esophageal tube, absorption of IgG could also be affected by gastric emptying through delivery method. Schaer et al. (2005) found that feeding an electrolyte solution with a rumen tube resulted in a higher $T_{max}$ and lower maximum concentration when compared to the nipple fed calves. The overall lower absorption of acetaminophen in tube fed calves demonstrates that there was failure of the esophageal groove and that the majority of the solution entered the rumen because acetaminophen is absorbed in the small intestine, not in the rumen (Schaer et al., 2005). Nouri and Constable (2006) also found a similar effect of tubing

versus bottle feeding oral electrolyte solutions when feeding 2L of an electrolyte solution to calves. They found calves that were tube fed had a higher time to maximum concentration than bottle fed calves (Nouri and Constable, 2006). Further evidence that feeding method can affect emptying was shown by Sharifi et al. (2009), where they used acetaminophen and radiography as diagnostic tools for evaluation of reticular groove reflex in lambs. Lambs were fed a solution containing water, acetaminophen and barium sulphate at a volume of 87-370 ml (based on BW) and they found that calves that were tube fed the solution had significantly slower emptying rates, based on acetaminophen absorption (Sharifi et al., 2009). This correlated with the failure of the reticular groove reflex that was observed with radiography in the same calves (Sharifi et al., 2009). They determined that acetaminophen concentration at time 60 min was viable for characterizing the function of the reticular groove reflex in their model (Sharfi et al., 2009). Absorption of IgG from colostrum is affected by timing of feeding, quality of colostrum and amount of colostrum fed, and could be affected by feeding method through different gastric emptying rates.

### 1.3 Gut Development in the Newborn Calf

Typically, the focus of colostrum feeding is to deliver adequate amounts of immunoglobulins, primarily IgG, to the calf to ensure successful passive transfer of immunity. However, colostrum contains many other bioactive factors that are keys for the health and development of the calf and the delivery of these factors also needs to be considered (Blum and Hammon, 2000). More specifically these bioactive factors are required for the gut development of the newborn calf. Major changes occur in the gastrointestinal tract (GIT) of neonates following birth, including growth and differentiation of the epithelial cells to acquire sufficient

digestive and absorptive ability of the GIT (Roffler et al., 2003). The presence of high concentrations of hormones, growth factors and cytokines in colostrum are shown to assist in the development of the GIT early in life (Roffler et al., 2003). Some of these factors include hormones and growth factors such as insulin and insulin-like growth factor (IGF) I and II (Blum and Hammon, 2000).

### *1.3.1 Insulin-like Growth Factors*

Insulin and insulin-like growth factors, IGF-I and II, are members of the same family of peptides known as the insulin family (Odle et al., 1996). These insulin-like growth factors have been shown to increase gut development through 2 key mechanisms: IGF-II and insulin are involved in the differentiation of intestinal epithelium while IGF-I is involved in crypt cell proliferation (Jehle et al., 1999). The somatotropic axis is responsible for regulation of these insulin-like growth factors, as well as the development of the GIT, especially in the proliferation and maturation of enterocytes (Georgiev et al., 2003). This axis is not fully developed in the neonatal calf but colostrum feeding can aid in the development of this axis due to the high levels of these growth factors in colostrum (Georgiev et al., 2003). Concentrations of insulin, IGF-I and II are significantly higher in colostrum at 65, 310 and 150 μg/L respectively, compared with whole milk which only contains 1 and <1 μg/L of insulin and IGF-I respectively, with concentrations of IGF-II not determined (Blum and Hammon, 2000). The greater concentrations of these bioactive factors in colostrum have been shown to play a key role in gut development in the neonatal calf (Blum and Hammon, 2000).

Although concentrations of these bioactive factors are high in colostrum and their effect on gut development in the calf have been shown, it is unclear if they are actually absorbed from

the colostrum by the calf. Some studies have shown no increase in insulin or decrease in glucose when insulin was administered orally prior to colostrum feeding, indicating that colostral insulin is not likely absorbed (Grutter and Blum, 1991). However, a study conducted in piglets showed evidence of absorption of insulin in the GIT (Shen and Xu, 2000). They administered 100 nmol/kg body weight of fluorescein-isothiocyanate (FITC) labeled insulin, mixed in with porcine colostrum, orally to both newborn piglets and 3-day old suckling piglets and took blood samples before and 1 and 2 h following insulin administration and then slaughtered the piglets 3 hours following administration. (Shen and Xu, 2000). They found that at 2 h after administration blood glucose had decreased by 23% and plasma insulin concentrations had increased by 64% in the newborn piglets (Shen and Xu, 2000). In addition, through microscopy, they observed uptake of the FITC-insulin throughout the small intestine, primarily in the ileum through the vesicular transport mechanism in newborn piglets – an uptake process only seen in the distal small intestine of 3-day old pigs (Shen and Xu, 2000). These results suggest that absorption of insulin can occur in neonatal pigs, but as they age and as the gut closes, the ability to absorb insulin decreases (Shen and Xu, 2000). It has not been determined if insulin is absorbed in newborn calves.

Previous studies suggest that the amount of IGF-I and II absorbed in the GIT is negligible and that the main mechanism driving their effect on gut development is through binding with their cell membrane-associated receptors in the gut (Jehle et al., 1999). There are two cell membrane-associated IGF receptors known as type I and type II receptors (Odle et al., 1996). The type I receptor has the highest affinity for IGF-I followed by IGF-II and insulin while the type II receptor has the highest affinity for IGF-II followed by IGF-I, but does not bind to insulin (Odle et al., 1996). mRNA expression of insulin, IGF-I and II receptors and IGF binding proteins

has been found in the GIT of newborn calves (Pfaffl et al., 2002), and competitive binding assays indicated that binding of IGF and insulin to their mucosal receptors can increase gastrointestinal development (Baumrucker et al., 1994). Colostrum can increase the expression of growth factor receptors, as shown by a study where they fed colostrum 6 times, colostrum only once or just milk replacer (Baumrucker et al., 1994). Calves fed colostrum 6 times had greater IGF-I receptor numbers in the ileum and total intestine than those fed colostrum once or milk replacer, and had greater IGF-II receptor numbers in the total intestine compared to the calves fed milk replacer (Hammon and Blum, 2002). They also found insulin binding was best fit by a two binding site model, and calves fed colostrum 6 times had greater insulin receptor numbers in the duodenum, ileum and total intestine when compared to calves fed just milk replacer (Hammon and Blum, 2002). Overall the binding of insulin-like growth factors to their receptors is key for gut development in newborn calves and colostrum feeding, and is increased by colostrum feeding.

The insulin-like growth factor I has another potential action of increased gut development through regulation of the hormone glucagon-like peptide-2 (GLP-2) (Dube et al., 2006). A study was conducted using knockout mice to investigate the role of IGF-I and II as mediators of GLP-2 enhanced intestinal growth (Dube et al., 2006). Their results demonstrated that there is a relationship between IGF and GLP-2, with increased expression of IGF-I mRNA when GLP-2 was administered (Dube et al., 2006). In addition, IGF-I and II positive mice responded to GLP-2 treatment with increased intestinal weight, proliferation and morphometry, whereas, IGF-I and II negative mice did not respond to GLP-2 treatment (Dube et al., 2006). It appears that certain hormones can regulate IGF expression and are involved in the development of the GIT.

*1.3.2 Glucagon-like Peptide-2*

Glucagon-like peptide-2 is a member of the glucagon-like peptide family, which has a variety of functions, including development of the gastrointestinal tract (Burrin et al., 2001). GLP-2 is a 33-amino acid peptide, which is derived from post-translational processing of the proglucagon polypeptide in enteroendocrine "L" cells (Holst, 2000). L-cells are intestinal epithelial endocrine cells that are located predominantly in the ileum and colon (Holst, 2000). They directly contact luminal nutrients through their apical surface, and neural and vascular tissue through their basolateral surface (Baggio and Drucker, 2007). Consequently, GLP-2 secretion is stimulated by a variety of nutrients, and neural and endocrine factors (Baggio and Drucker, 2007). This is confirmed in newborn piglets, where circulating GLP-2 concentrations increased approximately 4-fold within one hour after an oral feeding and was positively correlated with the level of feed intake (van Goudoever et al, 2001). The primary nutrients that stimulate GLP-2 release are carbohydrates and lipids (Xiao et al., 1999). GLP-2 has a relatively short half-life because it is rapidly inactivated by the proteolytic enzyme dipepitdyl peptidase-4 (DPP-4) (Baggio and Drucker, 2007). Dipepitdyl peptidase-4 cleaves dipeptides from the amino terminus of oligopeptides or proteins that contain an alanine or proline residue in the $2^{nd}$ position, thereby modifying or inhibiting their activity (Baggio and Drucker, 2007).

GLP-2 plays a role in stimulating gut development and is the endocrine link between nutrition and intestinal adaptation (Burrin, 2003). The GLP-2 secretory response has been shown to be functional as early as late gestation in pigs (Burrin, 2003). Both quantity and composition of feeding after birth influence parallel changes in intestinal growth and circulating GLP-2 (Burrin, 2003). A study conducted in piglets demonstrated that when piglets are fed infant formula supplemented with bovine colostrum protein concentrate, they have higher

concentrations of plasma GLP-2 when compared to piglets that received no supplement (Paris et al., 2004). Another study showed that colostrum feeding increased GLP-2 concentrations in comparison to a control diet in piglets one day after birth (Petersen et al., 2003). These studies indicate that colostrum can play an important role in circulating levels of GLP-2, which can increase gut development.

The role of GLP-2 in humans, rats and pigs has been well established but its effect in ruminants is not as clear. Recent research has shown GLP-2 receptor and DPP4 mRNA expression in the gastrointestinal tract of dairy cows, with the greatest expression being in the small intestine (Connor et al., 2010). This expression seems to be affected by developmental stage as well as location in the gut, with the lowest expression occurring in prepubertal heifers, greatest in dry cows and intermediate expression for lactating cows (Connor et al., 2010). Since GLP-2 is expressed in ruminants, several studies have examined the potential beneficial effects on gastrointestinal development. A study that administered GLP-2 subcutaneously every 12 h for 10 d found that calves administered with GLP-2 had increased superior mesenteric artery blood flow, increased small intestinal mass by 24%, by increasing epithelial mass in the jejunum and ileum, and increased villus height and crypt depths (Taylor-Edwards et al., 2011). This improved GIT development in calves administered GLP-2 could have beneficial effects with regards to reducing the negative impact of gastrointestinal diseases (Connor et al., 2013). Calves infected with Eimeria bovis and treated with GLP-2 increased large and small intestinal weights, indicating that GLP-2 treatment can help alleviate damage done to the intestinal tract during infection (Connor, 2013). Another study demonstrated increased expression of selected tight junction genes in intestinal tissues, which implies the structural integrity of the GIT is improved

when GLP-2 is administered (Walker et al., 2015). Glucagon-like peptide-2 plasma concentrations have not been measured in newborn calves after colostrum feeding.

## 1.4 Endocrine Control of Metabolism in the Newborn Calf

Thus far, it has been shown that colostrum feeding affects immunity and gut development of the calf, but it can also play an important role in metabolism of the newborn calf through influencing the release of hormones that control metabolism such as: insulin, glucagon and cortisol (Blum and Hammon, 2000).

### *1.4.1 Glucagon-like Peptide-1*

Glucagon-like peptide-1 is a 30-amino acid peptide that is also a part of the glucagon-like peptide family (Burrin et al., 2001). It is derived from the same gene, precursor peptide and cell type as GLP-2 and they are secreted in parallel (Burrin et al., 2001). Since GLP-1 and 2 are co-secreted and colostrum increases secretion of GLP-2, it is reasonable to assume that colostrum feeding could also increase GLP-1 secretion. However, no research has been done on this relationship with regards to colostrum feeding, thus far. Glucagon-like peptide-1 is primarily associated with appetite regulation through gastric emptying and insulinotropic effects (Baggio and Drucker, 2007). It is included in a class of hormones called incretins, which are secreted from the gastrointestinal tract into circulation in response to nutrient ingestion and enhance glucose-stimulated insulin secretion (Baggio and Drucker, 2007). Glucagon-like peptide-1 stimulates insulin secretion through binding to its specific receptor on pancreatic β-cells, which leads to activation of adenylate cyclase and production of cAMP (Baggio and Drucker, 2007). This subsequently depolarizes the β-cell membrane, prevents repolarization or has a direct effect

on β-cell insulin storage granule exocytosis (Baggio and Drucker, 2007). Glucagon-like peptide-1 is also shown to reduce gastric emptying while simultaneously increasing insulin secretion in humans, which indicates a relationship between gastric emptying and insulin secretion when GLP-1 is involved (Willms et al., 1996).

In calves, GLP-1 and insulin are strongly correlated both pre- and post-weaning as intravenous GLP-1 treatment increased plasma insulin concentrations and decreased plasma glucose concentrations (Fukumori et al., 2012). Another study showed elevated plasma insulin concentrations when GLP-1 and glucose were simultaneously administered to calves (Edwards et al., 1997). Glucagon-like peptide-1 appears to play an important role in glucose-stimulated insulin secretion in calves.

### *1.4.2 Insulin, Glucagon and Glucose*

Insulin is essential for regulating blood glucose concentration in the postprandial state (Khan and Pessin, 2002). Insulin regulates blood glucose concentration by stimulating glucose uptake into fat and muscle tissues to be stored as intracellular triglycerides and glycogen (Khan and Pessin, 2002). This uptake combined with insulin's action of inhibiting the release and synthesis of glucose from the liver, results in blood homeostasis of glucose (Khan and Pessin, 2002). Transport of glucose is mediated by solute carriers called the GLUT family of facilitative glucose transporters, but the GLUT-4 transporter is responsible for insulin-stimulated glucose uptake and is primarily restricted to fat and muscle tissues (Khan and Pessin, 2002). This stimulation is facilitated by the insulin receptor tyrosine kinase which promotes the movement of the GLUT-4 protein from intracellular storage to the plasma membrane, which is the rate-limiting step for insulin-regulated glucose uptake into fat and muscle tissue (Saltiel, 2001). The

action of insulin to decrease gluconeogenesis and increase storage of glucose as glycogen is also seen in preruminating calves, indicating that insulin's effects on glucose uptake are similar between calves and humans (Donkin and Armentano, 1995).

Glucagon acts in the opposite manner of insulin, with regards to glucose homeostasis, by activating glycogenolysis, the breakdown of glycogen into glucose, and gluconeogenesis, which is the production of glucose from non-carbohydrate substrates (Hammon et al., 2012). Newborn calves undergo important endocrine changes when they are born due to a complete change in energy source (Hammon et al., 2012). Prior to birth, they mainly rely on carbohydrates and amino acids through the placenta for glucose supply and then after birth their glucose supply comes from colostrum, which is high in fat and low in carbohydrates (Girard et al., 1992). This abrupt change in energy source can result in hypoglycemia since lactose content in colostrum does not meet glucose demands, thus, glycogenolysis and gluconeogenesis are critical in the newborn calf for glucose supply. Colostrum plays a critical role in the initiation of glycogenolysis and gluconeogenesis in the newborn calf (Hammon et al., 2012).

Typically, blood glucose concentrations are low following birth and remain low until 2 days of life, regardless of whether colostrum is supplied or not, indicating that age effect is critical for endogenous glucose production (Steinhoff-Wagner, 2011). This maturation of the gluconeogenic pathway is influenced by diet and ontogenic maturation (Steinhoff-Wagner, 2011). Calves that are born pre-term have lower endogenous glucose production compared with calves that are born to term, indicating development of the gluconeogenic pathway as a fetus (Steinhoff-Wagner, 2011). Diet also has an influence on development of this pathway, specifically colostrum intake plays an important role in this development, as demonstrated by many studies. A study conducted by Hammon and Blum (1998) fed colostrum 6 times, colostrum

once or just milk replacer. They found a tendency of increased glucose concentrations on day one for the milk replacer group, however, on day two both colostrum groups had significantly higher glucose concentrations. This could be due to enhanced glucose absorptive capacity of the gut when colostrum is fed (Hammon and Blum, 1998). They also found that insulin concentrations corresponded with the glucose concentrations in blood, with insulin being highest for the milk replacer treatment on day one and then for the colostrum groups on day two. Glucagon plasma concentrations were higher for both colostrum treatments on day one compared with the milk replacer group, but was higher for calves fed colostrum 6 times only on day two. Feeding colostrum compared to milk replacer increased glucose metabolism in neonatal calves.

Kuhne et al. (2000) conducted another study looking at the effects of colostrum feeding on glucose metabolism, and compared feeding of a high or low level of colostrum and a high level or low level of milk replacer. The high level groups were fed 2 meals of 1.75 L on day 1, 2 meals of 2.3 L on day 2 and 2 meals of 2.6 L on day 3 of either colostrum or milk replacer. The low level groups were fed 2 meals of 1.25 L on day 1, 2 meals of 1.7 L on day 2 and 2 meals of 1.9 L on day 3 of either colostrum or milk replacer. They found a significant increase in plasma glucose concentrations on day one in the milk replacer groups compared to the colostrum fed calves. However, postprandial glucose responses at the end of the first week were smaller in milk replacer fed calves than colostrum fed calves. This demonstrates an overall increase in glucose concentrations in colostrum fed calves compared with milk replacer fed calves. A similar effect was observed in insulin where milk replacer treatments had higher plasma insulin concentrations for the first 12 h, but on day two, insulin concentrations were higher in the colostrum groups. On day seven, calves that were fed more colostrum had increased postprandial insulin in colostrum groups but this response was not seen in the milk replacer groups, suggesting increased

pancreatic development in calves fed colostrum. Glucagon plasma concentrations correlated with glucose and insulin, with concentration being highest for the colostrum groups on day one and increasing from day two to five for the calves in the milk replacer treatment groups. Although glucose plasma concentrations were higher for the colostrum groups compared with the milk replacer groups, there was no difference between the high and low level colostrum groups. This indicates that any amount of colostrum increases glucose metabolism when compared to feeding just milk replacer.

These effects can also be observed when comparing feeding colostrum to feeding glucose monohydrate mixed in water (Hadorn et al., 1997). Higher glucose and insulin plasma concentrations were observed on day one in calves fed glucose compared to the calves fed colostrum, but on day two colostrum stimulated higher concentrations of both glucose and insulin (Hadorn et al., 1997). Further evidence of the effect colostrum has on glucose absorption is demonstrated by a xylose absorption test conducted by Hammon and Blum (1997) on day 5 of life. Calves that were fed colostrum once or 6 times, had increased xylose absorption compared to calves fed milk replacer. There was no difference between colostrum treatments, however, which could be explained by the age of the calves, since by day 5 the gut may have adapted to glucose absorption making treatment differences more difficult to detect (Hammon and Blum, 1997). These differences in xylose absorption can be explained by colostrum's ability to increase gut development which increases the absorptive capacity of the gut, thus allowing more xylose to be absorbed (Hammon and Blum, 1997). This could also explain why calves that are fed colostrum on day one have increased plasma glucose concentrations after milk feeding on day two, compared with calves that were never fed colostrum (Hadorn et al., 1997; Hammon and Blum, 1998; Kuhne et al., 2000).

### *1.4.3 Cortisol*

Cortisol is a glucocorticoid hormone that is secreted in response to stress and also plays a role in the glucoregulatory endocrine system by stimulating gluconeogenesis in the liver in a similar fashion to glucagon (Hammon and Blum, 1998). Cortisol has also been associated with colostrum feeding, with plasma cortisol concentrations decreasing after feed intake, but cortisol concentrations were higher for calves only fed milk replacer compared to calves fed colostrum (Hammon and Blum, 1998). This could be because plasma glucose concentrations were higher in calves not fed colostrum, which stimulated cortisol release to maintain glucose homeostasis, whether colostrum feeding reduces stress, however, is unclear (Hammon and Blum, 1998). There is a potential for feeding method, however, to decrease stress in calves. A study that measured cortisol response in calves following suckling or being bucket fed reported that calves who suckled had lower plasma concentrations of cortisol 30 minutes following feeding (Lupoli et al., 2001).

## 1.5 Feeding Delivery Methods

Thus far, it has been discussed how the timing, amount and quality of colostrum feeding can affect IgG absorption and how colostrum feeding can increase gut development and endocrine control of metabolism in newborn calves. However, the method by which colostrum is fed could also affect immunity, gut development and metabolism in newborn calves and should be discussed. The current industry practices are to use a nipple bottle or esophageal tube to feed colostrum to newborn calves. A survey of dairy farms in Ontario in 2008 showed that 12.5% of

dairies fed colostrum through a tube 60-100% of the time, and 69% of farms fed colostrum through a bottle 80-100% of the time (Trotz-Williams et al., 2008). A more recent survey of farms in British Columbia showed that 33% of farms fed colostrum through a tube and 66% of farms fed colostrum through a bottle (Atkinson et al., 2017). In the U.S, 25.3% of calves were still obtaining colostrum by nursing, with the remainder being hand-fed and of those hand-fed calves 82.5% were bottle fed and 13.9% were tube fed (Beam et al., 2009). Although the primary feeding method on farm is through a nipple bottle, a proportion of calves are fed with an esophageal tube.

Generally, either method is acceptable, however, many studies suggest that feeding colostrum through an esophageal feeder results in a portion of the colostrum entering the rumen which could result in a delay of delivery and overall decreased absorption of IgG and other nutrients (Kaske et al., 2005; Godden et al., 2009). This delay could be as much as 3 h according to a study conducted by Kaske et al. (2005). Another study conducted by Godden et al. (2008), fed 1.5 L or 3 L of colostrum through either nipple bottle or esophageal tube to examine the effect on passive transfer of IgG. Blood samples were collected prior to feeding colostrum and at 24 h of age (Godden et al., 2008). They observed that IgG serum concentrations were higher for calves fed 3 L regardless of feeding method (Godden et al., 2008). Calves fed 1.5 L with the nipple bottle had higher serum total protein, serum IgG, and apparent efficiency of absorption of IgG than calves fed 1.5 L with the esophageal tube feeder, but this difference was not observed when 3 L was fed (Godden et al., 2008). They suggested this was because when a small meal is fed through an esophageal tube, a large proportion of the meal flows into the rumen, while with a bigger meal only a small proportion of the meal flows into the rumen (Godden et al., 2008). This is based off the findings of Chapman et al. (1986), who found that the rumen can hold 400 mL

before the meal flows from the rumen to the abomasum. Although it is commonly accepted that the meal enters the rumen when fed with an esophageal tube, this has not been measured. Previous studies have found little to no difference in IgG AEA between the two feeding methods. however, it is unknown if this affects absorption of other nutrients from colostrum (Kaske et al., 2005; Godden et al., 2009, Chigerwe et al., 2012).

**1.6 Knowledge Gap**

Administering electrolytes to calves through an esophageal tube has been shown to cause a failure in the esophageal groove reflex and result in liquid entering the rumen (Schaer et al., 2005; Nouri and Constable, 2006; Sharifi et al., 2009). It has also been shown that electrolytes entering the rumen slowed gastric emptying, however, it is unknown if colostrum entering the rumen would slow gastric emptying rates (Schaer et al., 2005; Nouri and Constable, 2006). Previous studies suggest that colostrum entering the rumen, when fed with an esophageal tube, decreases absorption of IgG from colostrum but colostrum entering the rumen was not measured in those studies (Godden et al., 2009; Kaske et al., 2006). If colostrum enters the rumen when fed with an esophageal tube and subsequently slows gastric emptying this could decrease IgG absorption from colostrum. This could also decrease blood concentrations of glucose, insulin and GLP-1 and 2. Colostrum feeding increases glucose and insulin concentrations in neonatal calves when compared to milk feeding but how the delivery method of colostrum feeding would affect these concentrations is unclear (Hammon and Blum, 1998). Also, although colostrum has shown to increase circulating GLP-2 in piglets, it has not been determined if colostrum feeding in calves increases concentrations of GLP-1 or 2 and if the feeding method of colostrum would affect those concentrations (Paris et al., 2004; Petersen et al.,

2003). Thus the aim of this thesis is to address the knowledge gap that exists with regards to how feeding colostrum through either an esophageal tube or nipple bottle could affect not only IgG absorption but also plasma concentrations of glucose, insulin, GLP-1 and 2. Also it aims to determine if feeding colostrum through an esophageal tube would increase blood cortisol concentrations as an indicator of increased stress.

## 1.7 Literature Cited


Afshari, G., M. Nouri, E. B. Hassan, M. R. Mokhber-Dezfooli, and P.D. Constable. 2009. Effect of parenteral administration of ivermectin and erythromycin on abomasal emptying rate in suckling calves. Am. J. Vet. Res. 70:527–531.

Atkinson, D.J., von Keyserlingk, M.A.G., and D.M. Weary. 2017. Benchmarking passive transfer of immunity and growth in dairy calves. J. Dairy Sci. 100: 1-10.

Baggio, L.L., and D.J. Drucker. 2007. Biology of incretins: GLP-1 and GIP. Gastroenterology. 132: 2131- 2157.

Barrington, G.M., McFadden, T.B., Huyler, M.T., and T.E. Besser. 2001. Regulation of colostrogenesis in cattle. Livestock Production Science. 70: 95-104.

Baumrucker, C.R., Hadsell, D.L., and J.W. Blum. 1994. Effects of dietary insulin-like growth factor I on growth and insulin-like growth factor receptors in neonatal calf intestine. J. Anim. Sci. 72: 428-433.

Beam, A.L., Lombard, J.E., Kopral, C.A., Garber, L.P., Winter, A.L., Hicks, J.A., and J.L. Schlater. 2009. Prevalence of failure of passive transfer of immunity in newborn heifer calves and associated management practices on US dairy operations. J. Dairy Sci. 92: 3973-3980.

Blum, J.W., and H. Hammon. 2000. Colostrum effects on the gastrointestinal tract, and on nutritional, endocrine and metabolic parameters in neonatal calves. Livestock Production Science. 66: 151-159.

Burrin, D.G., Petersen, Y., Stoll, B., and P. Sanglld. 2001. Glucagon-like peptide 2: a nutrient-responsive gut growth factor. J. Nutr. 131: 709-712.

Burrin, D., Guan, X., Stoll, B., Petersen, Y., and P.T. Sanglld. 2003. Glucagon-like peptide 2: a key link between nutrition and intestinal adaptation in neonates. J. Nutr. 133: 3712-3716.

Chapman, H.W., Butler, D.G., and M. Newell. 1986. The route of liquids administered to calves by esophageal feeder. Can J Vet Res. 50: 84-87.

Conneely, M., Berry, D.P., Murphy, J.P., Lorenz, I., Doherty, M.L., and E. Kennedy. 2014. Effect of feeding colostrum at different volumes and subsequent number of transition milk feeds on the serum immunoglobulin G concentration and health status of dairy calves. J. Dairy Sci. 97: 6991-7000.

Chigerwe, M., Coons, DM., and J.V. Hagey. 2012. Comparison of colostrum feeding by nipple bottle versus oroesophageal tubing in Holstein dairy bull calves. J. Am. Vet. Med. Assoc. 241: 104-9.

Connor, E.E., Baldwin, R.L., Capuco, A.V., Evock-Clover, C.M., Ellis, S.E., and K.S Sciabica. 2010. Characterization of glucagon-like peptide 2 pathway member expression in bovine gastrointestinal tract. J. Dairy Sci. 93: 5167-5178.

Connor, E.E., Kahl, S., Elsasser, T.H., Baldwin, R.L., Fayer, R., Santin-Duran, M., Sample, G.L., and C.M. Evock-Clover. 2013. Glucagon-like peptide 2 therapy reduces negative effects of diarrhea on calf gut. J. Dairy Sci. 96: 1793-1802.

Constable, P. D., W. Grünberg, and L. Carstensen. 2009. Comparative effects of two oral rehydration solutions on milk clotting, abomasal luminal pH, and abomasal emptying rate in suckling calves. J. Dairy Sci. 92: 296–312.

Donkin, S.S., and L.E. Armentano. 1995. Insulin and glucagon regulation of gluconeogenesis in preruminating and ruminating bovine. J. Anim. Sci. 73: 546-551.

Dube, P., Forse, C.L., Bahrami, J., and P.L. Brubaker. 2006. The essential role of insulin-like growth factor-1 in the intestinal tropic effects of glucagon-like peptide-2 in mice. Gastroenterology. 131: 589-605.

Edwards, C.M.B., Edwards, A.V., and S.R. Bloom. 1997. Cardiovascular and pancreatic endocrine responses to glucagon-like peptide-1 (7-36) amide in the conscious calf. Experimental Physiology. 82: 709-716.

Elizondo-Salazar, J.J., and A.J. Heinrichs. 2009. Feeding heat-treated colostrum or unheated colostrum with two different bacterial concentrations to neonatal dairy calves. J. Dairy Sci. 92: 4565-4571.

Fecteau, G., Ballargeon, P., Higgins, R., Pare, J., and M. Fortin. 2002. Bacterial contamination of colostrum fed to newborn calves in Quebec dairy herds. Can. Vet. J. 43: 523-527.

Fukumori, R., Mita, T., Sugino, T., Obitsu, T., and K. Taniguchi. 2012. Plasma concentrations and effects of glucagon-like peptide-1 (7-36) amide in calves before and after weaning. Domestic Animal Endocrinology. 43: 299-306.

Georgiev, I.P., Georgieva, T.M., Pfaffl, M., and H.M. Hammon. 2003. Insulin-like growth factor and insulin receptors in intestinal mucosa of neonatal calves. Journal of Endocrinology. 176: 121-132.

Girard, J., Ferre, P., Pegorier, J.P., and P.H. Duee. 1992. Adaptations of glucose and fatty acid metabolism during perinatal period and suckling-weaning transition. Physiological Reviews. 72: 507-562.

Godden, S. 2008. Colostrum Management for Dairy Calves. Veterinary Clinic of North America: Food Animal Practice. 24: 19-39.

Godden, S.M., Haines, D.M., Konkol, K., and J. Peterson. 2009. Improving passive transfer of immunoglobulins in calves. II: Interaction between feeding method and volume of colostrum fed. J. Dairy Sci. 92: 1758-1764

Grutter, R., and J.W. Blum. 1991. Insulin and glucose in neonatal calves after peroral insulin and intravenous glucose administration. Reprod. Nutr. Dev. 31: 389-397.

Hadorn, U., Hammon, H., Bruckmaier, R.M., and J.W. Blum. 1997. Delaying colostrum intake by one day has important effects on metabolic traits and on gastrointestinal and metabolic hormones in neonatal calves. J. Nutr. 127: 2011-2023.

Hammon, H.M., and J.W. Blum. 1997. Prolonged colostrum feeding enhances xylose absorption in neonatal calves. J. Anim. Sci. 75: 2915-2919.

Hammon, H.M., and J.W. Blum. 1998. Metabolic and endocrine traits of neonatal calves are influenced by feeding colostrum for different durations or only milk replacer. J. Nutri. 128: 624-632.

Hammon, H.M., and J.W. Blum. 2002. Feeding different amounts of colostrum or only milk replacer modify receptors of intestinal insulin-like growth factors and insulin in neonatal calves. Domestic Animal Endocrinology. 22: 155-168.

Hammon, H.M., Steinhoff-Wagner, J., Schonhusen, U., Metges, C.C., and J.W. Blum. 2012. Energy metabolism in the newborn farm animal with emphasis on the calf: endocrine changes and responses to milk-born and systemic hormones. Domestic Animal Endocrinology. 43: 171-185.

Holst, J.J. 2000. Gut hormones as pharmaceuticals from enteroglucagon to GLP-1 and GLP-2. Regul. Pept. 93: 45-51.

Jaster, E.H. 2005. Evaluation of quality, quantity, and timing of colostrum feeding on immunoglobulin G absorption in Jersey calves. J. Dairy Sci. 88: 296-302.

Jehle, P.M, Fussgaenger, R.D, Blum, W.F, Angelus, N.K, Hoeflich. A.,Wolf, E., and R.J. Jungwirth. 1999. Differential autocrine regulation of intestinal epithelial cell proliferation and differentiation by insulin-like growth factor (IGF) system component. Hormone Metabolic Research. 31: 97–102.

Johnson, J.L., Godden, S.M., Molltor, T., Ames, T., and D. Hagman. 2007. Effects of feeding heat-treated colostrum on passive transfer of immune and nutritional parameters in neonatal dairy calves. J. Dairy Sci. 90: 5189-5198.

Jones, C.M., James, R.E., Quigley, J.D., and M.L. McGllllard. 2004. Influence of pooled colostrum or colostrum replacement on IgG and evaluation of animal plasma in milk replacer. J. Dairy Sci. 87: 1806-1814.

Kaske, M., Werner, A., Schberth, H.J., Rehage, J., and W. Kehler. 2005. Colostrum management in calves: effects of drenching vs. bottle feeding. Journal of Animal Physiology and Animal Nutrition. 89: 151-157.

Khan, A.H., and J.E. Pessin. 2002. Insulin regulation of glucose uptake: a complex interplay of intracellular signalling pathways. Diabetologia. 45: 1475-1483.

Kuhne, S., Hammon, H.M., Bruckmaier, R.M., Morel, C., Zbinden, Y., and J.W. Blum. 2000. Growth, performance, metabolic and endocrine traits, and absorptive capacity in neonatal calves fed either colostrum or milk replacer at two levels. J. Anim. Sci. 78: 609-620.

Larson, B.L., Heary, H.L., and J.E. Devery. 1980. Immunoglobulin production and transport by the mammary gland. J. Dairy Sci. 63: 665–71.

Lecce, J. G. 1973. Effect of dietary regimen in cessation of uptake of macromolecules by piglet

intestinal epithelium (closure) and transport into blood. J. Nutr. 103: 751.

Lecce, J. G., and D. O. Morgan. 1962. Effect of dietary regimen on cessation of intestinal absorption of large molecules (closure) in neonatal pigs and lamb. J. Nutr. 78: 265.

Lupoli, B., Johansson, B., Uvnas-Moberg, K., and K. Svennersten-Sjaunja. 2001. Effect of suckling on the release of oxytocin, prolactin, cortisol, gastrin, cholecystokinin, somatostatin and insulin in dairy cows and calves. Journal of Dairy Research. 68: 175-187.

Marshall, T.S., Constable, P.D., Crochik, S.S., and T. Wittek. 2005. Determination of abomasal emptying rate in suckling calves by use of nuclear scintigraphy and acetaminophen absorption. Am J Vet Res. 66: 364-374.

Marshall, T. S., P. D. Constable, S. S. Crochik, T. Wittek, D. E. Freeman, and D. E. Morin. 2008. Effect of suckling an isotonic solution of sodium acetate, sodium bicarbonate, or sodium chloride on abomasal emptying rate and luminal pH in calves. Am. J. Vet. Res. 69: 824–831.

Mayer, B., Doleschall, M., Bender, B., Bartyik, J., Bosze, Z., Frenyo, L., and I. Kacskovics. 2005. Expression of the neonatal Fc receptor (FcRn) in the bovine mammary gland. Journal of Dairy Research. 72: 107-112.

Mokhber-Dezfooli, M.R., Nouri, M., Rasekh, M., and P.D. Constable. 2012. Effect of abomasal emptying rate on the apparent efficiency of colostral immunoglobulin G absorption in neonatal Holstein-Friesian calves. J. Dairy Sci. 95: 6740-6749.

Morrill, K.M., Conrad, E., Lago, A., Campbell, J., Quigley, J., and H. Tyler. 2012. Nationwide evaluation of quality and composition of colostrum on dairy farms in the United States. J. Dairy Sci. 95: 3997-4005.

Muller L.D, and D.K. Ellinger. 1981. Colostral immunoglobulin concentrations among breeds of dairy cattle. J. Dairy Sci. 64: 1727–30.

Nouri, M., and P. D. Constable. 2006. Comparison of two oral electrolyte solutions and route of administration on the abomasal emptying rate of Holstein-Friesian calves. J. Vet. Intern. Med. 20: 620–626.

Nouri, M., and P.D. Constable. 2007. Effect of parenteral administration of erythromycin, tilmicosin, and tylosin on abomasal emptying rate in suckling calves. Am. J. Vet. Res. 68: 1392-1398.

Nouri, M., Hajikolace, M.R., Constable, P.D., and A. Omidi. 2008. Effect of erythromycin and gentamicin on abomasal emptying rate in suckling calves. J. Vet. Intern. Med. 22: 196-201.

Odle, J., Zijlstra, R. T., and S.M. Donovan. 1996. Intestinal effects of milkborne growth factors in neonates of agricultural importance. J. Anim. Sci. 74: 2509-2522.

Paris, M., Fuller, P.J., Carstensen, B., Nagy, E., Taylor, R.G., Sourial, M., Holst, J., Hartmann, B., and J. Binesm. 2004. Digestive Diseases and Sciences. 49: 1688-1695.

Pfaffl M.W., Georgieva T.M., Georgiev I.P., Ontsouka E., Hageleit M., and J.W. Blum. 2002. Real-time RT-PCR quantification of insulin-like growth factor (IGF)-I, IGF-I receptor, IGF-II, IGF-II receptor, insulin receptor, growth hormone receptor, IGF-binding proteins 1, 2 and 3 in the bovine species. Domestic Animal Endocrinology. 22: 91–102.

Petersen, Y.M., Hartmann, B., Holst, J., Huerou-Luron, I., Bjornvad, C.R., and P.T. Sangild. 2003. Introduction of enteral food increases plasma GLP-2 and decreases GLP-2 receptor mRNA abundance during pig development. J. Nutr. 133: 1781-1786.

Roffler, B., Fah, A., Sauter, S.N., Hammon, H.M., Gallmann, P., Brem, G., and J.W. Blum. 2003. Intestinal morphology, epithelial cell proliferation, and absorptive capacity in neonatal calves fed milk-born insulin-like growth factor-I or a colostrum extract. J. Dairy Sci. 86: 1797-1806.

Saltiel, A.R. 2001. New perspectives into the molecular pathogenesis and treatment of type 2 diabetes. Cell. 104: 517-529.

Schaer, S., Herrli-Gygi, M., Kosmeas, N., Boschung, H., and A. Steiner. 2005. Characteristics of acetaminophen absorption in healthy unweaned calves as an indirect measurement of the oroduodenal transit rate of liquid meals. J. Vet. Med. 52: 325-332.

Sharifi, K., Grunberg, W., Soroori, S., Mohri, M., and M.S. Ahrari-Khafi. 2009. Assessemtn of the acetaminophen absorption test as a diagnostic tool for the evaluation of the reticular groove reflex in lambs. Am. J. Vet. Res. 70: 820-825.

Shen, W., and R. Xu. 2000. Gastrointestinal stability and absorption of insulin in suckling pigs. Comparative Biochemistry and Physiology. 125: 389-401.

Sen, I., Constable, P.D., and T.S. Marshall. 2006. Effect of suckling isotonic or hypertonic solutions of sodium bicarbonate or glucose on abomasal emptying rate in calves. Am J Vet Res. 67: 1377-1384.

Steinhoff-Wagner, J., Gors, S., Junghans, P., Bruckmaier, R.M., Kanitz, E., and C.C. Metges. 2011. Maturation of endogenous glucose production in preterm and term calves. J. Dairy Sci. 94: 5111-5123.

Stelwagen, K., Carpenter, E., Haigh, B., Hodgkinson, A., and T.T. Wheeler. 2014. Immune components of bovine colostrum and milk. J. Anim. Sci. 87: 3-9.

Stott, G.H., Marx, D.B., Menefee, B.E., and G.T. Nightengale. 1997a. Colostral immunoglobulin transfer in calves I: period of absorption. J. Dairy Sci. 62: 1632-1638.

Stott, G.H., Marx, D.B., Menefee, B.E., and G.T. Nightengale. 1997b. Colostral immunoglobulin transfer in calves II: the rate of absorption. J. Dairy Sci. 62: 1766-1773.

Swan, H., Godden, S., Bey, R., Wells, S., Fetrow, J., and H. Chester-Jones. 2007. Passive transfer of immunoglobulin G and preweaning health in Holstein calves fed a commercial colostrum replacer. J. Dairy Sci. 90: 3857-3866.

Tang-Christensen, M. Larsen, P.J., Thulesen, J., Romer, J., and N. Vrang. 2000. The proglucagon-derived peptide, glucagon-like peptide 2, is a neurotransmitter involved in the regulation of food intake. Nature Medicine. 6: 802-807.

Taylor-Edwards, C.C., Burrin, D.G., Holst, J.J., Mcleod, K.R., and D.L. Harmon. 2011. Glucagon-like peptide-2 (GLP-2) increases small intestinal blood flow and mucosal growth in ruminating calves. J. Dairy Sci. 94: 888-898.

Trotz-Williams, L. A., K. E. Leslie, and A. S. Peregrine. 2008. Passive immunity in Ontario dairy calves and investigation of its association with calf management practices. J. Dairy Sci. 91: 3840–3849.

van Goudoever, J.B., Stoll, B., Hartmann, B., Holst, J.J., Reeds, P., and D. Burrin. 2001. Secretion of Trophic gut peptides is not different in bolus- and continuously fed piglets. J. Nutr. 131: 729-732.

Walker, M.P., Evock-Clover, C.M., Elasser, T.H., and E.E. Connor. 2015. Short Communication: Glucagon-like peptide-2 and coccidiosis alter tight junction gene expression in the gastrointestinal tract of dairy calves. J. Dairy Sci. 98: 3432-3437.

Wallace, M. M., B. D. Jarvie, N. R. Perkins, and K. E. Leslie. 2006. A comparison of serum

harvesting methods and type of refractometer for determining total solids to estimate failure of passive transfer in calves. Can. Vet. J. 47: 573–575.

Willms, B., Werner, J., Holst, J.J., Orskov, C., Creutzfeldt, W., and M.A. Nauck. 1996. Gastric emptying, glucose responses and insulin secretion after a liquid test meal: effects of exogenous glucagon-like peptide-1 (GLP-1) - (7-36) amide in Type 2 (noninsulin-dependent) diabetic patients. J. Clin. Endocrinol. and Metabol. 81: 327-332.

Xiao, Q., Boushey, R., Drucker, D.J., and P.L. Brubaker. 1999. Secretion of the intestinotrophic hormone glucagon-like peptide 2 is differentially regulated by nutrients in humans. Gastroenterology. 117: 99-105.

# 2.0 The effect of tube vs. bottle feeding colostrum on IgG absorption, abomasal emptying and plasma hormone concentrations in newborn calves

## 2.1 Introduction

Unlike other mammals, cows do not transfer immunoglobulins to their calves in utero, which results in calves being born agammaglobulinaemic (Godden, 2008). Calves acquire passive transfer of immunity through colostrum, thus proper colostrum management is critical to ensure adequate passive transfer of immunity. Failure of passive transfer of immunity, defined as less than 10 mg/mL of serum IgG between 24 and 48 hours of age, occurs frequently on dairy farms (Godden, 2008). Failure of passive transfer of immunity has been shown to reach 19.2% of calves in the US and between 25-37% of calves in Canada, indicating that colostrum management on farms can be improved (Beam, 2009; Wallace et al., 2006; Trotz-Williams et al., 2008).

Current industry practices feed colostrum through either an esophageal tube or a nipple bottle. Feeding with an esophageal tube is a more time-efficient feeding method while feeding with a nipple bottle is a more natural feeding method. Godden et al. (2009) found that feeding colostrum with a nipple bottle increased serum concentrations of IgG, when calves were fed 1.5 L of colostrum, compared with an esophageal tube, but there was no difference between treatments when 3 L of colostrum was fed. Kaske et al. (2005) found that absorption of IgG was delayed by 3 h when fed with an esophageal tube compared to a nipple bottle. These differences might be due to colostrum entering the rumen, when fed with an esophageal tube, which would delay colostrum reaching the small intestine for IgG absorption (Sharifi et al., 2009). However, previous studies have not measured the quantity of colostrum entering in the rumen when fed with a tube, or abomasal emptying rate to determine if feeding method affects timing of nutrients

reaching the small intestine. In addition, these studies comparing tube vs. bottle feeding only measure serum IgG concentrations and overlook the critical role that colostrum plays in metabolic and gut development in newborn calves (Blum and Hammon, 2000).

Colostrum feeding has been shown to improve glucose metabolism in newborn calves (Hammon and Blum, 1998). In particular, colostrum is necessary for development of the gluconeogenic pathway (Steinhoff-Wagner et al., 2011). Calves that receive colostrum have higher glucose and insulin plasma concentrations in response to milk feedings, indicating a greater glucose response in these calves compared to calves that were not fed colostrum (Hammon and Blum, 1998; Kuhne et al., 2000). Lower abomasal emptying rates have also been associated with lower plasma glucose concentrations, indicating that delaying delivery of colostrum to the small intestine could also decrease blood glucose and insulin concentrations (MacPherson et al., 2016).

Colostrum feeding is also associated with increased gut development in newborn calves (Blum and Hammon, 2000). Gut peptide hormones, GLP-1 and 2, have been demonstrated to have beneficial effects in calves (Taylor-Edwards et al., 2011; Fukumori et al., 2012). Intravenous treatment of GLP-1 was shown to increase plasma insulin while decreasing plasma glucose concentrations both pre- and post-weaning (Fukumori et al., 2012) while treatment of GLP-2 was shown to increase epithelial mass, villus height and crypt depths (Taylor-Edwards et al., 2011). However, these benefits associated with GLP-1 and 2 have yet to be studied in newborn calves with regards to colostrum feeding and since their secretions are stimulated by nutrients reaching the lower gut, feeding method could affect secretion of these hormones (Holst, 2000).

The hypothesis of this study was that feeding colostrum with an esophageal tube compared with a nipple bottle, would decrease abomasal emptying rate, delay colostrum reaching the small intestine, and thus decrease absorption of IgG, plasma concentrations of glucose, insulin, GLP-1 and 2. We also hypothesized that feeding with an esophageal tube would increase stress to these calves and therefore increase serum cortisol concentrations. The objectives of this study were to determine if feeding colostrum with an esophageal tube would affect abomasal emptying rates, serum IgG and cortisol concentrations and plasma concentrations of glucose, insulin, GLP-1 and 2.

## 2.2 Materials and Methods

### *2.2.1 Animals*

Twenty-two Holstein bull calves (birth BW= 44.8 ± 4.13 kg; mean ± SD) from a commercial dairy (Millet, Alberta) were selected for this study; calves used in the present study needed to be single birthed and have a calving ease score of ≤ 3 to enter the trial, using Lombard et al. (2007) 1 to 5 scale with 1= no assistance, 2= assistance by one person without mechanical extraction, 3=assistance by 2 or more people, 4=mechanical extraction used and 5=surgical. They were removed from the dam within 10 min after birth, weighed and housed in individual pens bedded with straw and then dried off for 20 min. This study was approved by the University of Alberta Animal Care and Use Committee for Livestock (AUP 00001890) according to the guidelines of the Canadian Council of Animal Care (Ottawa, ON, Canada, 2009).

*2.2.2 Feeding*

Calves were randomly assigned to a treatment of nipple bottle or esophageal tube and given their colostrum meal through either feeding method at 2 h after birth. All calves were fed 750g of colostrum (HEADSTART®, SCCL, Saskatoon, SK, Canada) mixed with water to reach a final volume of 3L in order to deliver 200g IgG. They were given 30 min to consume the colostrum, and calves with refusals of < 500 ml were tubed the remaining colostrum. One calf was tubed the remainder of its meal and a second calf was removed from the study. Time to consume the colostrum meal was recorded for each calf. The first milk meal was offered at 12 h of life and every 12 h thereafter until the end of the study at 48 hours of life. All calves received 3L of pasteurized milk at each feeding from a pooled source that was pasteurized, frozen and then thawed before feeding. Calves were required to consume at least 50% of the milk meals within 30 min. Any refusals less than 1.5 L were recorded and if refusals were greater than 1.5 L, the calf was removed from the study. One calf was removed due to these criteria.

*2.2.3 Sample Collection*

A 16-gauge jugular catheter (Thermo Fisher Scientific, San Diego, CA, USA) was placed in each calf 1 h after birth in order to collect blood samples frequently. Three attempts on each side of the calf could be made with the catheter before the end point for catheterization was reached. If the catheter could not be inserted within three attempts on each side, the calf was removed from the study. One calf was not used for the study due to this criterion. After each blood collection, catheters were flushed with 6 mL of a saline solution and the extension of the catheter and the catheter itself were filled with 1.5 mL of 2% heparinized saline solution to fill the catheter line, and before each sample collection a small portion (1.5 mL) was discarded to

remove any residual flush in the catheter line. Ten mL of blood were collected at each sampling with 5 mL going into a heparinized tube, inverted 5 times and then placed on ice immediately. Then 2.5 µL of aprotinin (100 µg/mL) was pipetted into the tube before centrifuging the sample at 3000 *g* for 20 min. The plasma was then aliquoted into three 1.5-ml microcentrifuge tubes and immediately frozen at -20°C. The remaining 5 mL of blood was transferred into a blank vacutainer tube to harvest serum, these tubes were kept at room temperature for 3 h before being centrifuged at 3000 *g* for 20 min. The serum was then aliquoted into three 1.5-ml microcentrifuge tubes and immediately frozen at -20°C.

To determine serum IgG and plasma glucose, insulin, GLP-1 and 2 concentrations a total of 22 blood samples were collected at -60, -30, 0, 30, 60, 90, 120, 150, 180, 210, 240, 300, 360, and 480 min relative to feeding of the colostrum meal and at 12, 15, 18, 24, 30, 36, 42, and 48 h of life. To determine serum cortisol concentrations, blood samples at 0 and 30 min relative to the colostrum meal were collected. Acetaminophen was added to the colostrum meal at 150 mg/kg $BW^{0.75}$ to estimate abomasal emptying rate by measuring kinetics of acetaminophen appearance in plasma according to Sen et al. (2006). To determine serum acetaminophen concentrations, blood samples were collected at -30, 30, 60, 90, 120, 150, 180, 210, 240, 300, 360, 480, and 600 min relative to colostrum feeding.

### *2.2.4 Sample Analyses*

Serum samples were analyzed for IgG concentration using the radial immunodiffusion method as described by Chelack et al. (1993) in triplicate and were re-analyzed when the difference between replicates was greater than 1.5 mg/mL. Serum samples were analyzed for acetaminophen concentration in duplicate using the enzymatic Paracetamol Assay Kit-K8002

(Cambridge Life Sciences Ltd, Ely, UK) and were re-analyzed when the difference between replicates was greater than 2 mg/mL. Serum samples were also analyzed for cortisol using the DetectX® Cortisol Enzyme Immunoassay Kit (Arbor Assays®, MI, U.S.A) in triplicate and when inter- and intra-assay CV were higher than 10% samples were re-analyzed . Plasma samples were analyzed in triplicate for glucose concentration using a glucose oxidase/peroxidase enzyme and dianisidine dihydrochloride (Sigma, St. Louis, MO, U.S.A) and absorbance at 450 nm was measured using a SpectraMax 190 plate reader (Molecular Devices Corp., Sunnyvale, CA, U.S.A) and when the inter- and intra-assay CV were higher than 5% samples were re-analyzed. Plasma concentrations of insulin, GLP-1 and 2 were analyzed using the time resolved-fluoroimmunoassay method (competitive solid-phase immunoassay) as described previously by Fukumori et al. (2012). Glucagon-like peptide-1 and 2 analyses were conducted using plates coated with antirabbit gamma-globulin (second antibody) and then rabbit antirat GLP-1 or 2 (first antibody) which bound to the second antibody. Then plasma was added to the plates and the plates were shaken for 8 hours before europium-labeled human GLP-1 or 2 was added, and then plates were shaken an additional 2 hours allowing for competitive binding between GLP-1 or 2 in sample and europium-labeled GLP-1 or 2. After shaking for 2 hours a low pH enhancement solution was added and the plate was shaken for 5 minutes to allow dissociation of europium from the human GLP-1 or 2 and the fluorescence of the europium was measured using a time-resolved fluorometer. Insulin analysis was conducted in a similar manner as GLP-1 and 2, except plates were coated with anti-guinea pig gamma globulin antiserum as the second antibody and after plasma was added the plate was only shaken 3 hours instead of 8. Samples were analyzed in triplicate and when inter- and intra-assay CV were higher than 20% samples were re-analyzed.

*2.2.5 Calculations and Statistics:*

The method used to determine abomasal emptying rate was previously described by Cant et al. (2006). In order to calculate abomasal emptying rate, it was assumed that outflow from the abomasum followed first-order kinetics according to the rate constant $k_{AB}$, elimination from the blood followed first-order kinetics according to the rate constant $k_{el}$, and absorption into the blood from the small intestine was instantaneous. The differential equations describing the mass of acetaminophen in the abomasum (A) and in the blood (B) are

$$\frac{dA}{dt} = -k_{AB}A, \quad [1]$$

and

$$\frac{dB}{dt} = k_{AB}A - k_{el}B, \quad [2]$$

respectively. These equations are integrated and the concentration of acetaminophen in the blood at time t becomes:

$$cB_t = \frac{Dose \cdot k_{AB}}{k_{AB} - k_{el}}\left(e^{-k_{el} \cdot t} - e^{-k_{AB} \cdot t}\right). \quad [3]$$

The statistical program JMP 13 was used to estimate $k_{AB}$ and $k_{el}$ by fitting Equation 3 to the observed blood concentration curves for each calf.

For IgG, glucose, insulin, GLP-1 and 2 maximum concentration ($C_{max}$), time to reach maximum concentration ($T_{max}$) and area under the curve (AUC) was calculated from the raw data in excel. Area under the curve was calculated using the trapezoid rule with the equation Area = (Concentration A + Concentration B)/2 * (Time B – Time A). The area between each time point was calculated and then added together to get total AUC for the entire sampling period. Apparent efficiency of absorption (AEA, %) was calculated using the formula AEA = (Plasma IgG *

Plasma Volume)/ IgG Intake, assuming that plasma volume is 9.9% of birth body weight (Quigley et al., 2002).

A total of 22 calves were initially sampled for this study, one calf was removed due to not consuming more than 2.5 L of the colostrum meal and another calf was removed due to not consuming a milk meal, leaving 20 calves to be used for statistical analysis in this study. Treatment effect for BW, feeding time, milk consumed, Cmax, Tmax, AUC, AEA, $k_{AB}$ and $k_{el}$ was analyzed using one-way ANOVA method of JMP 13. Treatment effect for cortisol at time 0 and 30 minutes was also analyzed using one-way ANOVA method of JMP 13. Overall treatment, time effect and the treatment x time interaction for IgG, glucose, insulin, GLP-1 and 2 was analyzed using the Fit Model method of JMP 13, with the model including time and treatment as fixed effects and calf as a random effect, and time as a repeated measure. Statistical significance was declared when $P \leq 0.05$ and tendencies were declared when $P < 0.10$ but $> 0.05$.

## 2.3 Results

### *2.3.1 Feeding and Body Weight*

Time to consume the colostrum meal was shorter for the tube treatment than the bottle treatment (5.2 ± 1.51 vs. 17.6 ± 1.51 min; $P < 0.0001$; Table 2-1). Calves in the tube treatment consumed significantly more milk during the first milk meal than calves in the bottle treatment (2.97 ± 0.13 L vs. 2.47 ± 0.13 L; $P = 0.01$; Table 2-1). There was no difference between treatments for the remaining meals ($P > 0.20$), and in the last meal all calves consumed the full 3 L (Table 2-1). There was a strong negative correlation between time to consume the colostrum meal and the amount of milk consumed in the first meal (r = -0.81, $P < 0.0001$; Figure 2-1). There was no difference between treatments on BW (BW = 44.8 ± 1.34 kg; $P = 0.98$).

*2.3.2 Immunoglobulin G and Abomasal Emptying*

There was no treatment effect on IgG Cmax, Tmax, AEA or AUC. The maximum serum concentration of IgG was 24.2 and 24.7 ± 0.58 mg/mL ($P = 0.56$), while $T_{max}$ was 786 and 966 ± 161 ($P = 0.44$) min for the bottle and tube treatments, respectively (Table 2-2). The AUC for IgG was also not different between treatments ($P = 0.47$; Table 2-2). The AEA was 52.7 and 53.2 ± 1.63% ($P = 0.84$) for the bottle and tube treatments, respectively (Table 2-2). In addition, there was no treatment effect ($P = 0.72$) or treatment × time interaction ($P = 0.92$) for IgG serum concentrations, but there was a time effect ($P < 0.0001$) (Figure 2-2). Emptying rate was 52.4 and 52.9 ± 7.5%/h for the bottle and tube treatments, respectively, but was not different between treatments ($P = 0.96$; Table 2-2). Elimination rate was 0.66% and -1.68 ± 1.62%/h for the bottle and tube treatments, respectively, and was not different between the treatments ($P = 0.32$). There was a tendency for GLP-1 $T_{max}$ to be negatively correlated with abomasal emptying rate (r = -0.41; $P = 0.08$). There were no other correlations for any other parameters with abomasal emptying rate.

*2.3.3 Glucose and Insulin*

There was no treatment effect ($P = 0.49$; $P = 0.12$) or treatment × time interaction ($P = 0.75$; $P = 0.56$) for plasma concentrations of glucose and insulin, respectively (Figures 2-3 and 2-4). There was a time effect for glucose overall and prior to the first milk meal ($P < 0.0001$), and for insulin when analyzed prior to the first milk meal ($P < 0.0001$) but not for overall ($P = 0.72$). There was a tendency for glucose $C_{max}$ to be higher for tube fed calves, 145.6 ± 7.45 mg/dL, compared with bottle fed calves, 124.6 ± 7.45 mg/dL ($P = 0.06$) (Table 2-3). There was no treatment effect on glucose $T_{max}$ ($P = 0.32$), insulin $C_{max}$ ($P = 0.16$) or insulin $T_{max}$ ($P = 0.91$)

(Table 2-3). When AUC was analyzed across the entire time period for both glucose and insulin, the AUC for glucose was higher in tube fed calves than in bottle fed calves ($P = 0.03$) and the AUC for insulin tended to be higher in tube fed calves than in bottle fed calves ($P = 0.08$) (Table 2-3). When AUC was separated into periods before and after the first milk meal, AUC for both glucose and insulin prior to the first milk meal was higher for tube fed calves ($P = 0.02$; $P = 0.02$; Table 2-3). However, only glucose AUC after the first milk feeding tended to be higher for tube fed calves ($P = 0.06$; Table 2-3). There was no difference between treatments for AUC after the first milk meal for insulin ($P = 0.14$; Table 2-3). Glucose and insulin AUC also tended to be positively correlated (r = 0.40, $P = 0.08$; r = 0.41, $P = 0.07$; r = 0.40, $P = 0.08$) before the first milk meal, after the first milk meal and overall, respectively. In addition, glucose AUC prior to the first milk meal was negatively correlated (r = - 0.60; $P = 0.005$) with time to consume the colostrum meal and positively correlated (r = 0.48; $P = 0.03$) with the amount of milk consumed in the first milk meal (Figure 2-1).

*2.3.4 Glucagon-like Peptide-1 and 2*

There was no treatment effect ($P = 0.26$; $P = 0.43$) or treatment × time interaction ($P = 0.38$; $P = 0.22$) for GLP-1 and 2, respectively (Figures 2-5 and 2-6). There was an overall time effect for both GLP-1 and 2 ($P < 0.0001$; Figures 2-5 and 2-6). There was no treatment effect on $C_{max}$ ($P = 0.50$; $P = 0.74$), $T_{max}$ ($P = 0.99$; $P = 0.20$) or AUC ($P = 0.28$; $P = 0.21$) for GLP-1 or 2, respectively (Table 2-3). The plasma concentrations of GLP-1 and 2 were positively correlated (r = 0.53; $P < 0.0001$). There was also a weak correlation between plasma concentrations of GLP-1 and insulin (r = 0.23; $P < 0.0001$) and there was a tendency for GLP-1 AUC and insulin AUC to be positively correlated (r = 0.39, $P = 0.09$). In addition, GLP-1 and 2 AUC prior to the first milk

meal tended to be negatively correlated with time to consume the colostrum meal (r = - 0.41, $P$ = 0.07; r = - 0.40, $P$ = 0.08) respectively.

### *2.3.6 Cortisol*

There was no difference between treatments for cortisol serum concentrations prior to feeding ($P$ = 0.27), after feeding ($P$ = 0.17) or the change in cortisol concentration ($P$ = 0.51) (Figure 2-7).

## 2.4 Discussion

Previous research has shown conflicting results with regards to passive transfer of immunity between tube and bottle feeding colostrum (Kaske et al., 2005; Godden et al., 2009, Chigerwe et al., 2012). Two studies suggest that colostrum may enter the rumen when fed with an esophageal tube, which would delay colostrum reaching the small intestine, however, emptying rates from the abomasum to the small intestine was not measured to support these speculations (Kaske et al., 2005; Godden et al., 2009). Thus, the objective of this study was to determine if feeding colostrum with an esophageal tube would slow abomasal emptying, delay colostrum reaching the small intestine, and decrease serum concentrations of IgG and plasma concentrations of glucose, insulin, GLP-1 and 2 when compared to feeding with a nipple bottle.

### *2.4.1 Abomasal Emptying and IgG*

The current study measured abomasal emptying using acetaminophen as a marker in accordance with previous studies (Sen et al., 2009, Marshall, 2005), and demonstrated that there was no difference in emptying rates between the two feeding methods. Previous studies report

that electrolyte solutions enter the rumen when fed with an esophageal tube due to failure of the reticular groove reflex, however, these studies used small volumes (Sharifi et al., 2009; Schaer et al., 2005; Nouri and Constable, 2006). The study conducted by Godden et al. (2009) found an increase in serum IgG concentrations in bottle fed calves compared to tube fed calves only when a small volume was fed. The current study fed a larger volume of 3 L, which could explain the lack of difference in emptying rates between treatments. Chapman et al. (1986) demonstrated that the rumen of calves 1-17 d old can hold up to 400 mL of liquid before it flows to the abomasum. It is reasonable to assume that in a newborn calf the rumen would hold less than 400 mL of colostrum, therefore, even if colostrum enters the rumen when feeding with an esophageal tube, less than 400 mL would be remain in the rumen. This means when a small volume is fed with a tube, a greater portion of the meal would remain in the rumen, whereas only a small portion of the meal would remain in the rumen when a large volume is fed. Moreover, a greater proportion of the meal would flow past the rumen to the abomasum. Therefore, colostrum that enters the rumen is likely to affect abomasal emptying rates only when small volumes are fed.

Since there was no difference in abomasal emptying rates, we also observed no difference in serum IgG concentrations or AEA of IgG between the two treatments. As mentioned previously, it is likely that when 3 L is fed most of the meal flows past the rumen to the abomasum, regardless of feeding method, and thus absorption of IgG is similar between treatment groups. Even if a portion of the meal entered the rumen, it is likely the amount is small enough that we would not detect a difference in IgG absorption since a high quality colostrum was fed; calves were fed 200 g of IgG in this study, which is double the recommended 100 g of IgG for adequate passive transfer (Godden, 2009). There is some evidence indicating a possible physiological limit to how much IgG can be absorbed due to a limited number of surface

receptors, for once those receptors are saturated absorption of IgG is limited (Lecce, 1973; Jaster, 2005; Conneely et al., 2004). Therefore, even if colostrum enters the rumen when fed with an esophageal tube, when a large enough amount of IgG is delivered, the colostrum that reaches the small intestine could be sufficient to saturate the receptors and meet maximal absorption of IgG. In this study it is possible that a portion of the meal remained in the rumen because when examining the acetaminophen curve it appears to still be increasing by the end of sampling, indicating the acetaminophen may not be fully recovered by 10 hours after the colostrum feeding. Since the first milk meal was fed at 10 h after the colostrum feeding, samples beyond the first milk meal were not analyzed for acetaminophen concentration. Although it is possible colostrum did enter the rumen, overall it did not negatively affect IgG absorption in these calves.

*2.4.2 Glucose and Insulin*

Previous studies have only measured serum IgG concentrations when comparing feeding colostrum with a tube or a bottle, but colostrum contains many other beneficial components. In particular, colostrum is beneficial to developing the gluconeogenic pathway in newborn calves (Steinhoff-Wagner et al., 2011). Feeding with an esophageal tube could result in colostrum entering the rumen, which would delay lactose absorption from colostrum and thus decrease glucose and insulin concentrations in the blood. Interestingly the opposite was observed in this study; overall AUC for glucose and insulin was higher for calves fed with a tube compared to a bottle.

In order to differentiate between the effect of the colostrum feeding and the effect of the milk feeding on glucose and insulin, both concentrations and AUC were assessed prior to the first milk meal and after the first milk meal. Glucose and insulin concentrations increased after colostrum feeding, but increased considerably more after the first milk meal. The smaller

response in glucose and insulin to colostrum feeding is likely due to colostrum containing only 2.7% lactose compared to milk, which contains 5.0% lactose (Godden, 2008). Other studies have shown a similar effect, where colostrum feeding did not lead to a large response in glucose concentrations, but they observed a large response in glucose after the first milk meal and the response was greater in calves that received colostrum compared to calves that did not (Hammon and Blum, 1998; Kuhne et al., 2000).

Tube feeding increased glucose and insulin AUC prior to the first milk meal, in the current study. Both treatments consumed the same amount of colostrum, and thus lactose, therefore the treatment effect on glucose is not due to different amounts of glucose being consumed. This difference could be attributed to the tube fed calves consuming their colostrum meal faster than the bottle fed calves. Since glucose AUC prior to the first milk feeding was negatively correlated with time to consume the colostrum, it is likely that the faster feeding time resulted in higher glucose AUC for tube fed calves. There are several mechanisms that could explain why feeding time could result in a difference in glucose concentration.

Colostrum intake in newborn calves has been shown to stimulate gluconeogenesis, through supplying AA as gluconeogenic substrates (Girard, 1986). Also, it has been demonstrated that when colostrum feeding is delayed by 24 hours, plasma concentrations of glucose and insulin are lower, possibly due to delayed supply of gluconeogenic substrates from colostrum (Hadorn et al., 1997). This indicates timing of feeding can influence blood glucose concentrations (Hadorn et al., 1997). Although overall emptying (%/hr) rate was not different between treatments, initial time the meal started to empty into the small intestine could have been. Therefore, perhaps the initial time colostrum reached the small intestine was sooner in tube fed calves, due to a shorter feeding time, which stimulated increased gluconeogenesis in the tube

fed calves by supplying gluconeogenic substrates earlier. However, since the difference in feeding time between treatments was only 12 minutes, it seems unlikely that this would have stimulated significantly more glucose production through gluconeogenesis. In addition, since insulin was also higher in tube fed calves, and insulin decreases gluconeogenesis, increased gluconeogenesis does not appear to be the mechanism driving higher glucose AUC in tube fed calves.

It seems more reasonable to assume that less glucose is being utilized before entering the blood in tube fed calves. In beef cattle it has been shown that only 70% of starch digested in the small intestine appears in the blood, some of that missing 30% can be accounted for by glucose utilization in the small intestine (Richards, 1999). It is possible that in the current study, when fed with a tube, initial appearance of colostrum in the small intestine was faster, which would result in glucose entering the bloodstream faster and not allowing as much glucose to be utilized by the gastrointestinal tract compared with bottle fed calves. In addition, previous research has suggested that suckling increases salivary flow and the subsequent swallowing of pregastric enterases (de Passille et al., 1993). This could result in more break down of lactose into glucose, which could be utilized by the gut and could also explain why bottle fed calves would utilize more glucose in the small intestine.

As mentioned previously, tube-fed calves consumed their colostrum faster than bottle fed calves and had higher glucose AUC. They also consumed half a litre more of milk than the bottle fed calves during the first milk meal. Glucose AUC prior to the milk feeding was also positively correlated with the amount of milk consumed and the amount of milk consumed was negatively correlated with time to consume the colostrum meal. This indicates that calves who consumed their colostrum faster had higher glucose AUC and drank more milk in the first meal. This

relationship could provide further evidence that less glucose was utilized in the GIT in tube fed calves than bottle fed calves. If less nutrients are being utilized in the GIT, perhaps demand for nutrients in the gut is higher in tube fed calves and they would consume more milk at the first milk feeding than bottle fed calves. It is unclear from the current study what the mechanism behind higher glucose AUC in tube fed calves is, although less glucose being utilized is a possible explanation.

As mentioned previously, insulin AUC prior to the milk feeding was also higher for tube fed calves. Since glucose and insulin were positively correlated, it is likely the treatment difference in insulin prior to the first milk meal is due to increased plasma glucose concentrations. However, another factor that could be contributing to the treatment difference in insulin is absorption of insulin from colostrum. Colostrum contains large amounts of insulin, with the first colostrum milking containing 65 μg/L compared to mature milk that only contains 1 μg/L (Blum and Hammon, 2000). The colostrum powder product used in this study was made from bovine colostrum, so it could also contain high levels of insulin. However, insulin content has not been measured in powdered colostrum. A study conducted in neonatal piglets demonstrated that insulin was absorbed throughout the small intestine, but as the piglets aged this could only be seen in the distal small intestine, thus as the gut closes the ability to absorb insulin decreases (Shen and Xu, 2000). Although this has not been measured in newborn calves, it is possible that insulin is absorbed early in life prior to gut closure. Any insulin absorbed would occur very early after colostrum feeding, thus the difference in time to consume the colostrum meal is more likely to contribute to absorption of insulin.

Feeding colostrum through an esophageal tube increased AUC for both glucose and insulin prior to the first milk meal, but only tended to increase glucose AUC after the milk

feeding and did not affect insulin AUC after the milk feeding even though tube fed calves consumed more milk in the first milk meal. This indicates that the effect that feeding method has on plasma glucose and insulin concentrations is most pronounced immediately after the colostrum feeding. Feeding colostrum with a tube increased plasma glucose and insulin AUC prior to the first milk feeding, however, future research would need to be conducted to determine which feeding method is better for glucose status in newborn calves.

### *2.4.3 Glucagon-like Peptide-1 and 2*

Although colostrum does not supply large amounts of glucose, it increases gut development in newborns, which increases the absorptive capacity of their gut, thus they are able to absorb glucose from milk more efficiently (Hammon and Blum, 1997). The gut peptide hormone GLP-1 is associated with insulinotropic effects (Fukumori et al., 2012) while GLP-2 is associated with increased gut development in older animals (Taylor-Edwards et al., 2011), but previous research has not measured these hormones in newborns or in response to colostrum feeding. The current study showed no treatment effect on the plasma concentrations of these hormones but showed a significant time effect for both GLP-1 and GLP-2 after colostrum feeding, indicating that colostrum feeding increases plasma concentrations of GLP-1 and 2. Plasma concentrations of GLP-1 and 2 were positively correlated, which agrees with previous research showing these two hormones are co-secreted (Burrin et al., 2001). There was also a tendency for the AUC for insulin and GLP-1 to be positively correlated, suggesting that GLP-1 may have insulinotropic effects in newborn calves, as seen in older calves (Fukumori et al., 2012). GLP-1 has been shown to increase circulating insulin while decreasing circulating

glucose (Fukumori et al., 2012). This could have occurred in the current study as indicated by the correlation between GLP-1 and insulin.

Glucagon-like peptide-1 has also been reported to slow gastric emptying in addition to having insulinotropic effects (Tong and D'Alessio, 2014). There is some evidence in the current study that GLP-1 could have slowed emptying. There was no correlation between abomasal emptying rate and GLP-2 $T_{max}$, but abomasal emptying rate tended to be negatively correlated with GLP-1 $T_{max}$. This correlation signifies that a slower emptying rate increased time to reach maximum concentration of GLP-1. There is a possibility that nutrients reaching the ileum and large intestine stimulated GLP-1 release which resulted in slower emptying rates. Since secretion of both GLP-1 and 2 is initiated by nutrients, primarily carbohydrates and lipids, reaching the small intestine, a slower emptying rate would delay the time nutrients are supplied to the small intestine and thus increase time to reach maximum GLP-1 concentrations (Burrin et al., 2001). Further evidence that timing of nutrients reaching the small intestine stimulates a GLP response is that in the current study AUC for GLP-1 and 2 prior to the first milk meal were negatively correlated with time to consume the colostrum meal. These results demonstrate that GLP-1 could have both insulinotropic effects and a role in controlling gastric emptying in newborn calves. In addition, plasma concentrations of GLP-1 and 2 increased after colostrum feeding indicating that GLP-1 and 2 are secreted in newborn calves, which could have beneficial implications for both metabolism and gut development in these calves.

### *2.4.4 Cortisol*

Another concern with feeding colostrum through an esophageal tube rather a nipple bottle, besides absorption of IgG, is the stress it may give to the calf. Therefore, in this study we

measured cortisol immediately prior to and after feeding to see if treatment would affect cortisol concentration. Feeding with a tube did not increase cortisol concentrations and feeding with a bottle did not decrease cortisol concentrations. Previous research has measured cortisol plasma concentrations in calves that were either allowed to suckle from the dam or were bucket fed, and found cortisol concentrations in suckling calves decreased 30 min after feeding was initiated (Lupoli et al., 2001). However, those calves were a week old and were consuming milk from the dam, not colostrum from a bottle, so there may be an age and dam effect on cortisol concentrations in that study (Lupoli et al., 2001). Although cortisol concentrations were not decreased by bottle feeding, they were also not increased when fed with a tube which could signify that feeding colostrum through an esophageal tube may not have increased stress in these calves. However, there was large variation in serum cortisol concentrations, most likely due to the additional stress from birth, being removed from the dam and catheterization, that these calves underwent. This variation could have made detecting treatment effects difficult. In addition, the sampling time could have made treatment effect difficult to detect. In the current study cortisol was sampled at 30 min after feeding, however, it has been reported that cortisol reached peak concentration prior to 30 min in calves that were castrated (Robertson, 1994). Perhaps peak cortisol concentration was missed in the current study. Although it is possible that tube feeding could increase stress, from the current studies it did not appear to negatively affect the calves. Therefore, if calves are fed colostrum with an esophageal tube in a safe and appropriate manner, it should not negatively affect calves.

## 2.5 Conclusion

In conclusion, these results demonstrate that feeding colostrum with either an esophageal tube or nipple bottle are acceptable methods to ensure successful passive transfer of immunity when calves are fed 3 L of high quality colostrum. Abomasal emptying rates are not affected by feeding method, when a large volume of colostrum is fed. Feeding colostrum increased plasma concentrations of glucose, insulin, GLP-1 and 2, which have positive implications for increased insulinotropic effects, development of the gluconeogenic pathway and the GIT in these calves. Feeding method, however, did not affect plasma concentrations of GLP-1 or 2. Feeding colostrum with an esophageal tube did increase plasma concentrations of glucose and insulin, but the implication of this is unclear from the current study. Feeding colostrum with a tube also resulted in more milk being consumed in the first milk meal, which has not been shown previously. In addition, feeding colostrum with an esophageal tube did not increase cortisol concentrations, providing no evidence to think that tube-feeding increases stress of calves when used appropriately. Producers may use either feeding method for successful colostrum management, when sufficient amounts of high quality colostrum are fed to calves.

## 2.6 Literature Cited

Beam, A.L., Lombard, J.E., Kopral, C.A., Garber, L.P., Winter, A.L., Hicks, J.A., and J.L. Schlater. 2009. Prevalence of failure of passive transfer of immunity in newborn heifer calves and associated management practices on US dairy operations. J. Dairy Sci. 92: 3973-3980.

Blum, J.W., and H. Hammon. 2000. Colostrum effects on the gastrointestinal tract, and on nutritional, endocrine and metabolic parameters in neonatal calves. Livestock Production Science. 66: 151-159.

Burrin, D.G., Petersen, Y., Stoll, B., and P. Sanglld. 2001. Glucagon-like peptide 2: a nutrient-responsive gut growth factor. J. Nutr. 131: 709-712.

Cant, J. P., Walsh, V. N., and R.J. Geor. 2006. Obtaining information on gastric emptying patterns in horses from appearance of an oral acetaminophen dose in blood plasma. In *Nutrient Digestion and Utilization in Farm Animals: Modelling Approaches.* Pg: 69-83. CABI Publishing.

Chapman, H.W., Butler, D.G., and M. Newell. 1986. The route of liquids administered to calves by esophageal feeder. Can J Vet Res. 50: 84-87.

Chelack, B. J., Morley, P.S., and D.M. Haines. 1993. Evaluation of methods for dehydration of bovine colostrum for total replacement of normal colostrums in calves. Can. Vet. J. 34: 407–412.

Conneely, M., Berry, D.P., Murphy, J.P., Lorenz, I., Doherty, M.L., and E. Kennedy. 2014. Effect of feeding colostrum at different volumes and subsequent number of transition milk feeds on the serum immunoglobulin G concentration and health status of dairy calves. J. Dairy Sci. 97: 6991-7000.

Chigerwe, M., Coons, DM., and J.V. Hagey. 2012. Comparison of colostrum feeding by nipple bottle versus oroesophageal tubing in Holstein dairy bull calves. J. Am. Vet. Med. Assoc. 24: 104-9.

de Passille, A.M.B. Christopherson, R., and J. Rushen. 1993. Nonnutritive sucking by the calf and postprandial secretion of insulin, CCK, and gastrin. Physio. & Behav. 54: 1069-1073.

Fukumori, R., Mita, T., Sugino, T., Obitsu, T., and K. Taniguchi. 2012. Plasma concentrations and effects of glucagon-like peptide-1 (7-36) amide in calves before and after weaning. Dom. Anim. Endo. 43: 299-306.

Fukumori, R., Sugino, T., Shingu, H., Moriya, N., Hasegawa, Y., Kojima, M., Kangawa, K., Obitsu, T., Kushibiki, S., and K. Taniguchi. 2012. Effects of calcium salts of long-chain fatty acids and rumen-protected methionine on plasma concentrations of ghrelin, glucagon-like peptide-1 (7 to 36) amide and pancreatic hormones in lactating cows. Dom. Anim. Endo. 42: 74–82.

Girard, J. (1986) Gluconeogenesis in late fetal and early neonatal life. Biol. Neonate 50: 237–258.

Godden, S. 2008. Colostrum Management for Dairy Calves. Veterinary Clinic of North America*:* Food Animal Practice*.* 24: 19-39.

Godden, S.M., Haines, D.M., Konkol, K., and J. Peterson. 2009. Improving passive transfer of immunoglobulins in calves. II: Interaction between feeding method and volume of colostrum fed. J. Dairy Sci. 92: 1758-1764

Hadorn, U., Hammon, H., Bruckmaier, R.M., and J.W. Blum. 1997. Delaying colostrum intake by one day has important effects on metabolic traits and on gastrointestinal and metabolic hormones in neonatal calves. J. Nutr. 127: 2011-2023.

Hammon, H.M., and J.W. Blum. 1997. Prolonged colostrum feeding enhances xylose absorption in neonatal calves. J. Anim. Sci. 75: 2915-2919.

Hammon, H.M., and J.W. Blum. 1998. Metabolic and endocrine traits of neonatal calves are influenced by feeding colostrum for different durations or only milk replacer. J. Nutri. 128: 624-632.

Holst, J.J. 2000. Gut hormones as pharmaceuticals from enteroglucagon to GLP-1 and GLP-2. Regul. Pept. 93: 45-51.

Jaster, E.H. 2005. Evaluation of quality, quantity, and timing of colostrum feeding on immunoglobulin G absorption in Jersey calves. J. Dairy Sci. 88: 296-302.

Kaske, M., Werner, A., Schberth, H.J., Rehage, J., and W. Kehler. 2005. Colostrum management in calves: effects of drenching vs. bottle feeding. J. Anim. Phys. and Anim. Nutr. 89: 151-157.

Kuhne, S., Hammon, H.M., Bruckmaier, R.M., Morel, C., Zbinden, Y., and J.W. Blum. 2000. Growth, performance, metabolic and endocrine traits, and absorptive capacity in neonatal calves fed either colostrum or milk replacer at two levels. J. Anim. Sci. 78: 609-620.

Lecce, J. G. 1973. Effect of dietary regimen in cessation of uptake of macromolecules by piglet intestinal epithelium (closure) and transport into blood. J. Nutr. 103: 751.

Lombard, J. E., Garry, F.B., Tomlinson, S.M., and L.P. Garber. 2007. Impacts of dystocia on health and survival of dairy calves. J. Dairy Sci. 90: 1751- 1760.

Lupoli, B., Johansson, B., Uvnas-Moberg, K., and K. Svennersten-Sjaunja. 2001. Effect of suckling on the release of oxytocin, prolactin, cortisol, gastrin, cholecystokinin, somatostatin and insulin in dairy cows and calves. Journal of Dairy Research. 68: 175-187.

MacPherson, J.A., Berends, H., Leal, L.N., Cant, J.P., Martin-Tereso, J., and M.A. Steele. 2016. Effect of plane of milk replacer intake and age on glucose and insulin kinetics and abomasal emptying in female Holstein Friesian dairy calves fed twice daily. J. Dairy Sci. 10: 8007-8017.

Marshall, T.S., Constable, P.D., Crochik, S.S., and T. Wittek. 2005. Determination of abomasal emptying rate in suckling calves by use of nuclear scintigraphy and acetaminophen absorption. Am J Vet Res. 66: 364-374.

Nouri, M., and P. D. Constable. 2006. Comparison of two oral electrolyte solutions and route of administration on the abomasal emptying rate of Holstein-Friesian calves. J. Vet. Intern. Med. 20: 620–626.

Quigley, J.D. 3rd, Kost, C.J., and T.M. Wolfe. 2002. Absorption of protein and IgG in calves fed colostrum supplement or replacer. J. Dairy Sci. 85: 1243-1248.

Richards, C. J. 1999. Influence of small intestinal protein on carbohydrate assimilation and metabolism in beef cattle. Ph.D. Diss. Univ. Kentucky.

Schaer, S., Herrli-Gygi, M., Kosmeas, N., Boschung, H., and A. Steiner. 2005. Characteristics of acetaminophen absorption in healthy unweaned calves as an indirect measurement of the oroduodenal transit rate of liquid meals. J. Vet. Med. 52: 325-332.

Sharifi, K., Grunberg, W., Soroori, S., Mohri, M., and M.S. Ahrari-Khafi. 2009. Assessment of the acetaminophen absorption test as a diagnostic tool for the evaluation of the reticular groove reflex in lambs. Am. J. Vet. Res. 70: 820-825.

Shen, W., and R. Xu. 2000. Gastrointestinal stability and absorption of insulin in suckling pigs. Comp. Biochem, and Phys. 125: 389-401.

Sen, I., Constable, P.D., and T.S. Marshall. 2006. Effect of suckling isotonic or hypertonic solutions of sodium bicarbonate or glucose on abomasal emptying rate in calves. Am. J. Vet. Res. 67: 1377-1384.

Steinhoff-Wagner, J., Gors, S., Junghans, P., Bruckmaier, R.M., Kanitz, E., and C.C. Metges. 2011. Maturation of endogenous glucose production in preterm and term calves. J. Dairy Sci. 94: 5111-5123.

Taylor-Edwards, C.C., Burrin, D.G., Holst, J.J., Mcleod, K.R., and D.L. Harmon. 2011. Glucagon-like peptide-2 (GLP-2) increases small intestinal blood flow and mucosal growth in ruminating calves. J. Dairy Sci. 94: 888-898.

Tong, J., and D. D'Alessio. 2014. Give the receptor a brake: Slowing gastric emptying by GLP-1. Diabetes. 63: 407–409.

Trotz-Williams, L. A., K. E. Leslie, and A. S. Peregrine. 2008. Passive immunity in Ontario dairy calves and investigation of its association with calf management practices. J. Dairy Sci. 91: 3840–3849.

Wallace, M. M., B. D. Jarvie, N. R. Perkins, and K. E. Leslie. 2006. A comparison of serum harvesting methods and type of refractometer for determining total solids to estimate failure of passive transfer in calves. Can. Vet. J. 47: 573–57

## 2.7 Tables and Figures

**Table 2 - 1.** Effect of tube vs. bottle feeding colostrum on time to consume colostrum and amount of milk consumed in newborn calves.

| Item[1] | Treatments | | SEM | *P* – value |
|---|---|---|---|---|
| | Bottle | Tube | | |
| $Colostrum_{Time}$ (min) | 17.6 | 5.2 | 1.51 | < 0.0001 |
| $Milk^{1}_{amount}$ (L) | 2.47 | 2.96 | 0.130 | 0.01 |
| $Milk^{2}_{amount}$ (L) | 2.96 | 2.97 | 0.035 | 0.84 |
| $Milk^{3}_{amount}$ (L) | 2.90 | 3.00 | 0.05 | 0.19 |
| $Milk^{4}_{amount}$ (L) | 3.00 | 3.00 | 0.00 | NA |

[1]$Colostrum_{Time}$ = time to consume the colostrum meal, $Milk^{1}_{amount}$ = amount of milk consumed in first meal, $Milk^{2}_{amount}$ = amount of milk consumed in second meal, $Milk^{3}_{amount}$ = amount of milk consumed in third meal, $Milk^{4}_{amount}$ = amount of milk consumed in fourth meal, n = 10 per group.

**Table 2 - 2.** Effect of tube vs. bottle feeding colostrum on serum IgG concentrations and abomasal emptying rate in newborn calves.

| | Treatments | | | |
|---|---|---|---|---|
| Item[1] | Bottle | Tube | SEM | *P* - value |
| IgG $C_{max}$ (mg/mL) | 24.2 | 24.7 | 0.58 | 0.56 |
| IgG $T_{max}$ (min) | 786 | 966 | 161.0 | 0.44 |
| IgG AUC mg/mL x 2760 min | 54488 | 51982 | 1666.4 | 0.47 |
| AEA (%) | 52.7 | 53.2 | 1.63 | 0.84 |
| Abomasal Emptying Rate (%/h) | 52.4 | 52.9 | 7.50 | 0.96 |

[1]$C_{max}$ = maximum serum concentration, $T_{max}$ = time of maximum concentration observed, AUC = area under the concentration-time curve, AEA = apparent efficiency of absorption, n = 10 per group.

**Table 2 - 3.** Effect of tube vs bottle feeding colostrum on plasma glucose, insulin, GLP-1 and 2 concentrations in newborn calves.

| | Treatments | | | |
|---|---|---|---|---|
| Item[1] | Bottle | Tube | SEM | *P* - value |
| Glucose $C_{max}$ (mg/dL) | 125 | 146 | 7.5 | 0.06 |
| Glucose $T_{max}$ (min) | 1350 | 1032 | 219.2 | 0.32 |
| Glucose $AUC_{overall}$ mg/dL x 2760 min | 245162 | 272965 | 8493.1 | 0.03 |
| Glucose $AUC_{600}$ mg/dL x 600 min | 42857 | 50016 | 1884.5 | 0.02 |
| Glucose $AUC_{2760}$ mg/dL x 2760 – 600 min | 194425 | 222950 | 9877.8 | 0.06 |
| Insulin $C_{max}$ (ng/mL) | 8.3 | 11.0 | 1.28 | 0.16 |
| Insulin $T_{max}$ (min) | 546 | 561 | 94.3 | 0.91 |
| Insulin $AUC_{overall}$ ng/mL x 2760 min | 8074 | 10768 | 1025.0 | 0.08 |
| Insulin $AUC_{600}$ ng/mL x 600 min | 1945 | 2700 | 215.9 | 0.02 |
| Insulin $AUC_{2760}$ ng/mL x 2760 – 600 min | 6122 | 8033 | 886.0 | 0.14 |
| GLP-1 $C_{max}$ (ng/mL) | 6.2 | 7.9 | 1.76 | 0.50 |
| GLP-1 $T_{max}$ (min) | 1194 | 594 | 244.5 | 0.99 |
| GLP-1 $AUC_{overall}$ ng/mL x 2760 min | 6010 | 11114 | 3215.5 | 0.28 |
| GLP-2 $C_{max}$ (ng/mL) | 3.9 | 4.3 | 0.66 | 0.74 |
| GLP-2 $T_{max}$ (min) | 564 | 672 | 58.0 | 0.20 |
| GLP-2 $AUC_{overall}$ ng/mL x 2760 min | 5115 | 7179 | 1134.4 | 0.21 |

[1]$C_{max}$ = maximum serum concentration, $T_{max}$ = time of maximum concentration observed, $AUC_{overall}$ = area under the concentration-time curve over the entire time period, $AUC_{600}$ = area under the concentration-time curve until 600 minutes $AUC_{2760}$ = area under the concentration-time curve from 600 minutes to 2760 minutes, n = 10 per group.

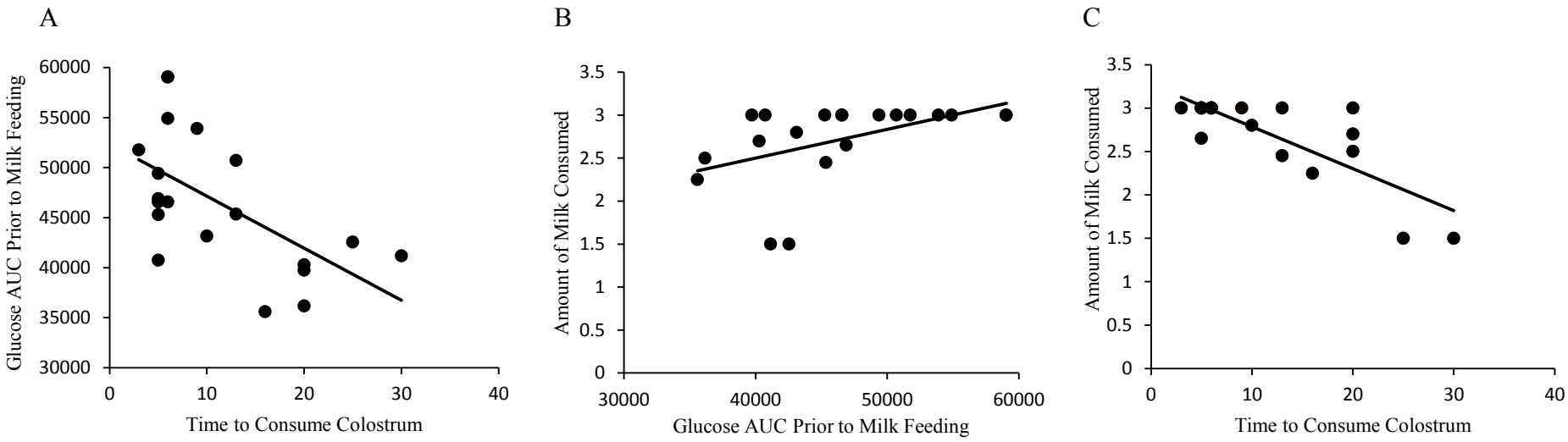


**Figure 2 - 1.** Correlation between A) time to consume the colostrum meal and glucose area under the curve (AUC) prior to the first milk feeding (r = -0.60; $P = 0.005$), B) amount of milk consumed in the first milk meal and glucose AUC prior to the first milk feeding (r = 0.48; $P = 0.03$) and C) time to consume the colostrum meal and amount of milk consumed in the first milk meal (r = -0.81; $P < 0.0001$).

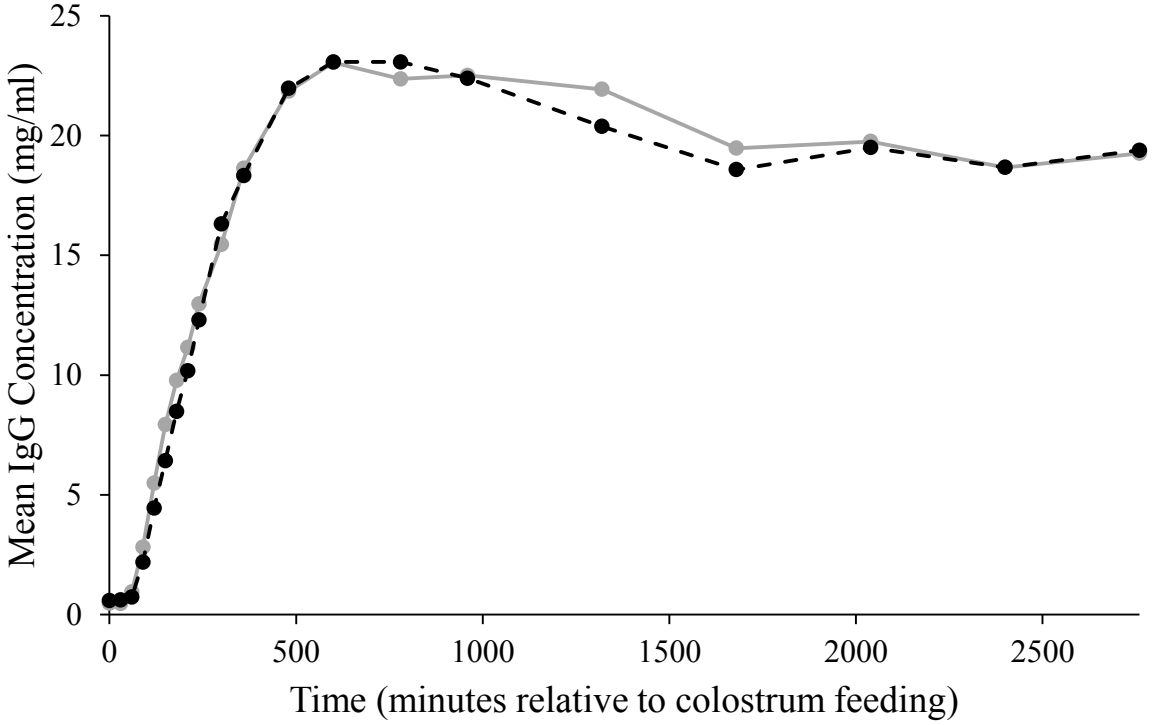


**Figure 2 - 2.** Effect of tube vs. bottle feeding colostrum on serum IgG concentrations in newborn calves. The dotted line indicates calves in the tube treatment while the solid line represents calves in the bottle treatment. Data are least squares mean ± SEM, n = 10 per group. Treatment effect ($P = 0.71$), time effect ($P < 0.0001$) and treatment x time interaction ($P = 0.92$).

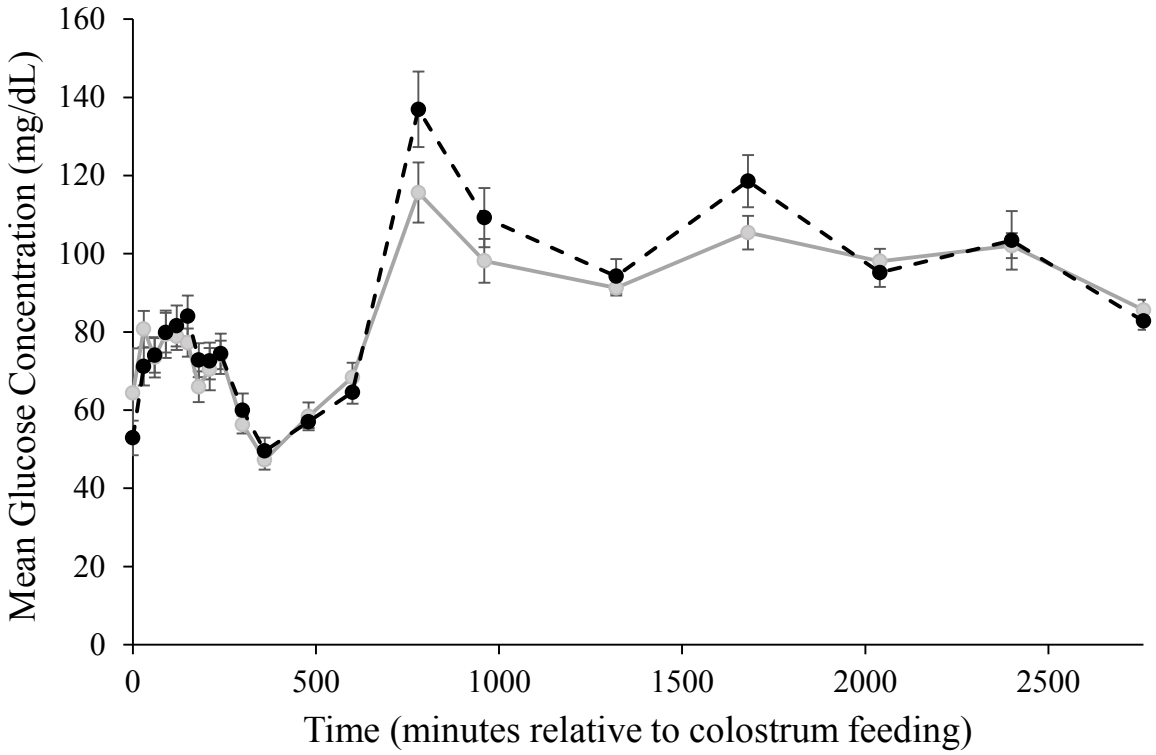


**Figure 2 - 3.** Effect of tube vs bottle feeding colostrum on plasma glucose concentrations in newborn calves. The dotted line indicates calves in the tube treatment while the solid line represents calves in the bottle treatment. Data are least squares mean ± SEM, n= 10 per group. Treatment effect ($P = 0.49$), time effect ($P < 0.0001$) and treatment x time interaction ($P = 0.75$).

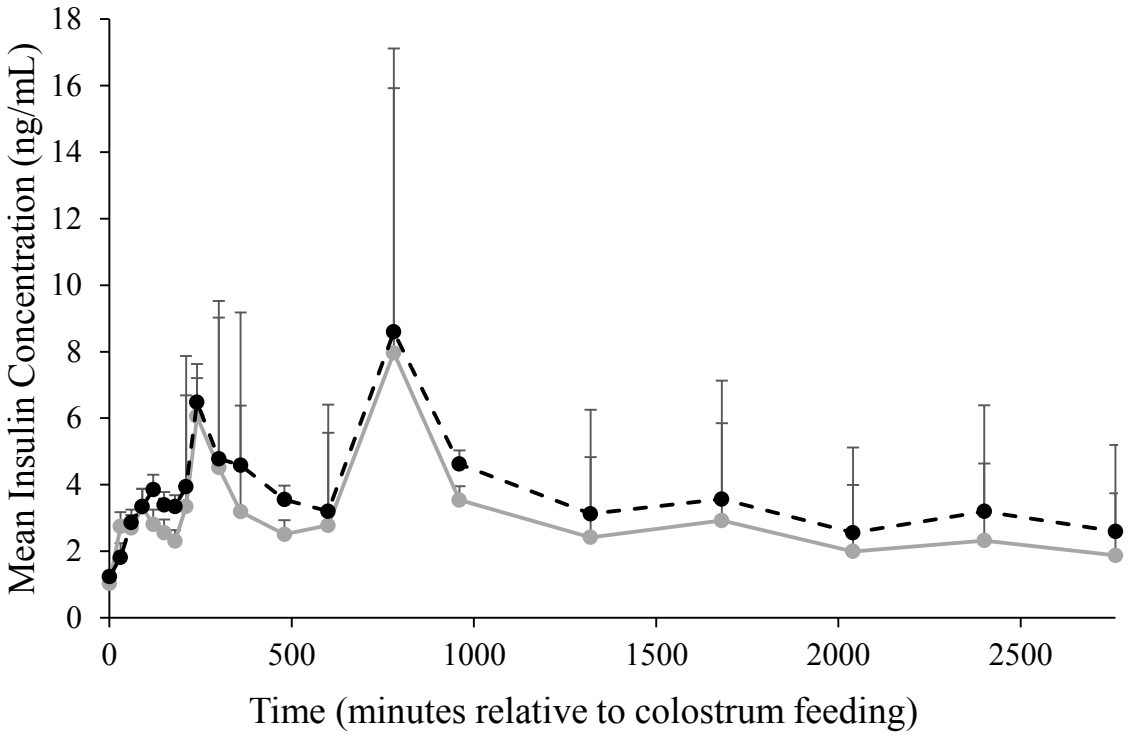


**Figure 2 - 4.** Effect of tube vs bottle feeding colostrum on plasma insulin concentrations in newborn calves. The dotted line indicates calves in the tube treatment while the solid line represents calves in the bottle treatment. Data are least squares mean ± SEM, n = 10 per group. Treatment effect ($P = 0.12$), time effect ($P = 0.68$) and treatment x time interaction ($P = 0.58$).

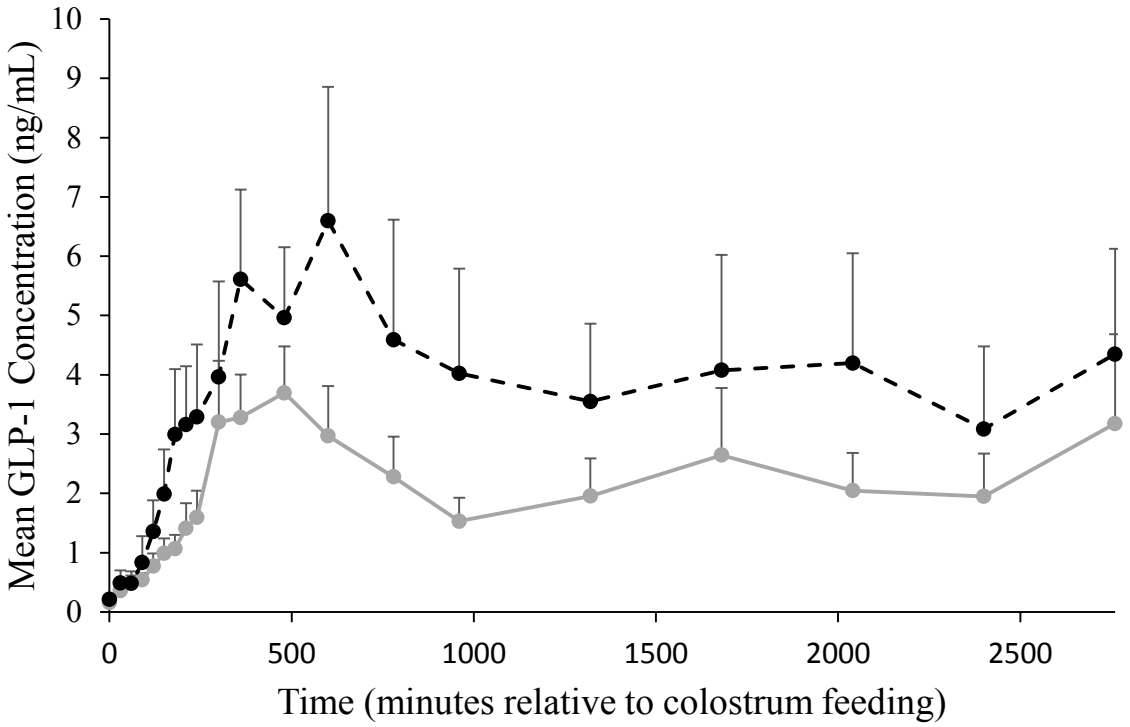


**Figure 2 - 5.** Effect of tube vs bottle feeding colostrum on plasma GLP-1 concentrations in newborn calves. The dotted line indicates calves in the tube treatment while the solid line represents calves in the bottle treatment. Data are least squares mean ± SEM, n = 10 per group. Treatment effect ($P = 0.26$), time effect ($P < 0.0001$) and treatment x time interaction ($P = 0.38$).

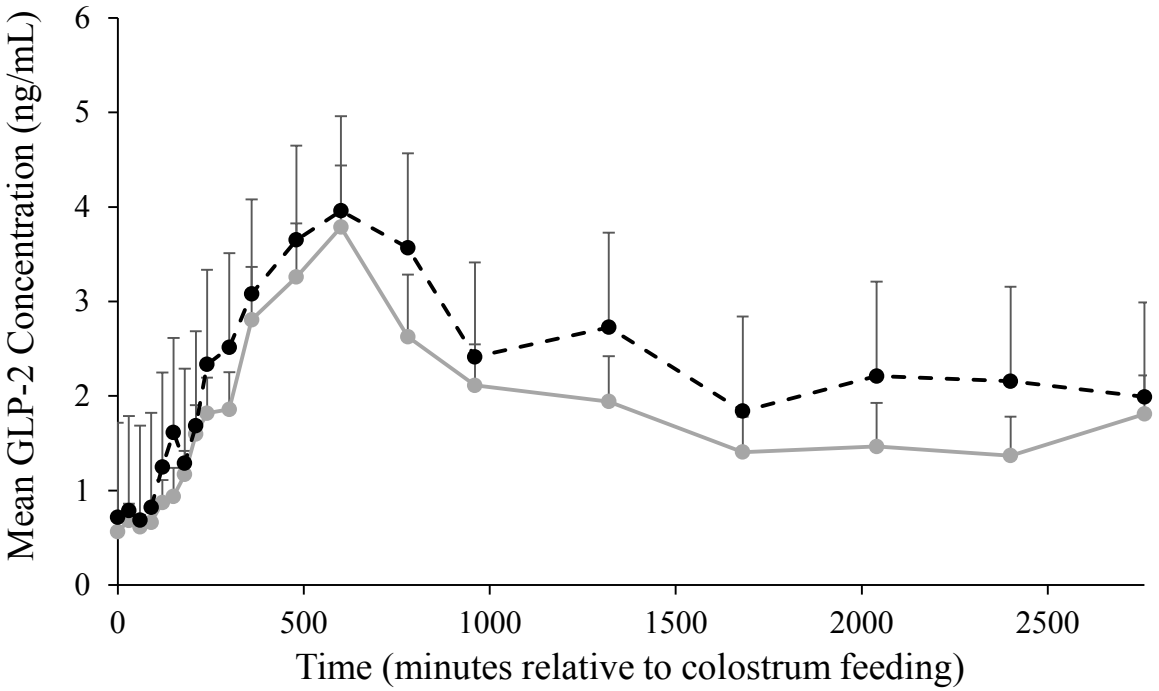


**Figure 2 - 6.** Effect of tube vs bottle feeding colostrum on plasma GLP-2 concentrations in newborn calves. The dotted line indicates calves in the tube treatment while the solid line represents calves in the bottle treatment. Data are least squares mean ± SEM, n = 10 per group. Treatment effect ($P = 0.43$), time effect ($P < 0.0001$) and treatment x time interaction ($P = 0.22$).

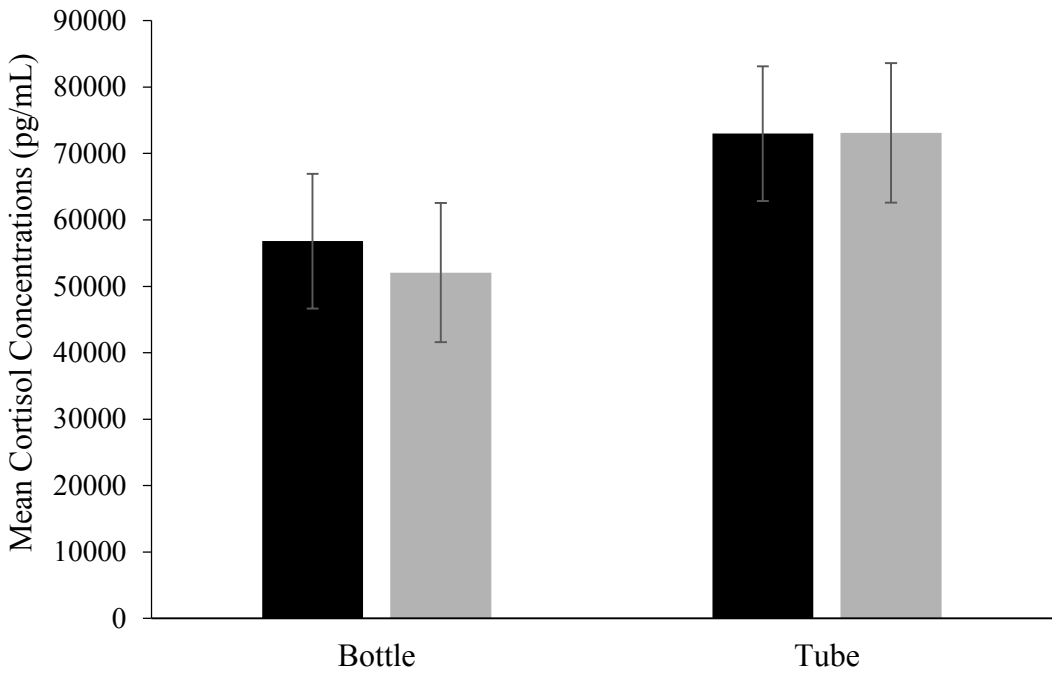


**Figure 2 - 7.** Effect of tube vs bottle feeding colostrum on serum cortisol concentrations in newborn calves. The black bars represent serum cortisol concentrations at time 0 and the grey bars represent serum cortisol concentrations at time 30 minutes. Data are least squares mean ± SEM, n = 10 per group. Treatment effect prior to feeding at time 0 ($P = 0.27$) and treatment effect after feeding at time 30 min ($P = 0.17$).

# 3.0 General Discussion

## 3.1 Importance of the Current Study

Good colostrum management on farm is key to the successful development and productivity of dairy calves. Failure of passive transfer (FPT) of immunity can reach up to 37% on Canadian farms (Trotz-Williams et al., 2008) and approximately 31% of preweaning mortality can be attributed to FPT of immunity (Margerison and Downey, 2005). Therefore, there is a need for research to address colostrum management practices and how they can be improved.

Producers feed colostrum to their calves through two different feeding methods: a nipple bottle or an esophageal tube. A recent study conducted in Canada showed that 33% of farms fed colostrum through an esophageal tube and 66% fed through a nipple bottle (Atkinson et al., 2017). There is conflicting data with regards to which feeding method is better for passive transfer of immunity (Kaske et al., 2005; Godden et al., 2009, Chigerwe et al., 2012). Also previous research did not address if feeding method would affect plasma hormone concentrations. The current study is the first to measure abomasal emptying and plasma hormone concentrations in addition to serum IgG concentrations to determine effects of feeding colostrum through an esophageal tube or nipple bottle.

The current study found that when feeding a large volume of high quality colostrum through an esophageal tube or nipple bottle calves acquire successful passive transfer of immunity. In addition, we found that there was no significant difference in abomasal emptying rate between treatments, suggesting that, even if colostrum entered the rumen, it was not enough to slow abomasal emptying. Feeding method also had no effect on GLP-1 and 2 plasma concentrations. Tube-fed calves had higher glucose and insulin AUC than bottle-fed calves,

which could be due to increased net absorption of glucose in the small intestine when calves are bottle fed, thus more glucose appearing in the blood or increased gluconeogenesis when calves are fed with a tube. It is unclear from this study if there is a negative or positive implication on glucose metabolism due to either feeding method. Tube fed calves also drank more milk in the first milk meal than bottle fed calves. This could be a positive implication for tube feeding calves, and further research should be conducted to see if this relationship always exists. Overall the results of this study demonstrate that when feeding 3 L of high quality colostrum producers can use either a nipple bottle or an esophageal tube to feed their calves.

### 3.2 Limitations

Previous studies have demonstrated that milk enters the rumen when fed with an esophageal tube (Sharifi et al., 2009; Schaer et al., 2005; Nouri and Constable, 2006). Other studies have suggested that colostrum may enter the rumen when fed with an esophageal tube but did not measure this directly (Godden et al., 2009; Kaske et al., 2005). The limitation in the current study was that acetaminophen was used as a marker to estimate emptying rate of colostrum from the abomasum to the small intestine. If colostrum entered the rumen when feeding with an esophageal tube, it would increase the time to reach the small intestine and thus would have a slower emptying rate, as measured by appearance of acetaminophen in the blood. We found no significant difference in emptying rate between bottle and tube feeding, which could indicate that no colostrum entered the rumen, a similar amount of colostrum entered the rumen for both treatments or the amount of colostrum that entered the rumen was not sufficient enough to affect abomasal emptying rate. However, since acetaminophen does not allow us to measure colostrum in the rumen directly, it is unclear which is occurring.

Acetaminophen has been used previously to determine if the meal entered the rumen by comparing time to maximum acetaminophen concentration (Schaer et al., 2005; Nouri and Constable, 2006). These studies found that the time to maximum acetaminophen concentration was greater in tube fed calves, indicating that the meal might have entered the rumen. These studies did not feed colostrum, but fed an electrolyte solution at a volume less than 2 L. Emptying rate is influenced by both meal size and the nutrient content of the meal (Sen et al., 2006). Meals that are larger and are more calorically dense empty at a slower rate, providing a steady supply of nutrients to the small intestine for absorption (Sen et al., 2006). Therefore, since Schaer et al. (2005) and Nouri and Constable (2006) fed smaller volumes of an electrolyte solution, it might have taken less time for the meal to empty completely from the abomasum to the small intestine than in the current study. This is indicated by the shorter time to reach maximum plasma acetaminophen concentration observed in their studies, where $T_{max}$ was 150 min compared to the 600 min demonstrated in the current study. Perhaps when feeding large volumes of colostrum, detecting treatment effects on abomasal emptying within 10 h after the colostrum feeding is less likely. In order to accurately assess if colostrum entered the rumen when fed with an esophageal tube, an ultrasound or radiograph method would be necessary. A study conducted in lambs assessed failure of the esophageal groove reflex with tube-feeding using a radiograph (Sharifi et al., 2009). This visual assessment allowed them to determine if and how much of the meal had entered the rumen (Sharifi et al., 2009). Future studies comparing tube and bottle feeding colostrum should consider using an ultrasound or radiograph to visually assess whether colostrum enters the rumen.

Another limitation of the current study was that only one large volume was evaluated when comparing tube and bottle feeding. Godden et al. (2009) compared tube vs. bottle feeding

colostrum in calves that were fed one meal of either 1.5 L or 3 L. They found that bottle fed calves had higher serum concentrations of IgG than those fed with a tube when 1.5 L was fed, but when calves were fed 3 L of colostrum there was no difference between bottle and tube feeding (Godden et al., 2009). Chapman et al. (1986) administered barium sulfate through an esophageal tube to calves of varying ages, with the youngest being 1 d old and the oldest being 30 d old. They found that when fed with an esophageal tube the solution entered the rumen before flowing to the abomasum and as the calves aged a larger volume was required for the solution to begin flowing to the abomasum (Chapman et al., 1986). The younger calves of 1-17 d old required only 400 mL before the solution flowed out of the rumen, while the older calves of 25-30 d old required 2 L before the solution began flowing out of the rumen (Chapman et al., 1986). Thus, in newborn calves it is reasonable to assume less than 400 mL is required for the meal to start flowing out of the rumen to the abomasum when feeding with a tube. Therefore, differences between tube and bottle-feeding would only be observed at smaller volumes, where a larger proportion of the meal would remain in the rumen.

As mentioned previously, a large volume of high quality colostrum was fed in the current study. Two hundred grams of IgG was delivered in this study, which may have exceeded the physiological limit of how much IgG can be absorbed in the small intestine. In nature, calves would consume frequent but small meals from their dam, and a study that allowed ad libitum access to colostrum through an automated feeder found that calves would make 5 to 10 visits per day (Hammon et al., 2002). Therefore, perhaps calves are only physiologically able to absorb only a certain amount of IgG at one time. In piglets, it has been shown that there is a limited number of surface receptors to absorb IgG and once those receptors are saturated no more IgG is absorbed (Lecce, 1973). There is some evidence that this can hold true in calves too, since calves

have higher AEA when they are fed two smaller volumes of high quality colostrum than when fed one large volume of high quality colostrum (Jaster, 2005). Another study found that calves fed 8.5% of BW of colostrum, compared to 7 or 10% of BW, had greater serum IgG concentrations, indicating that an optimal amount of IgG to feed could exist (Conneely et al., 2014). Since the current study fed a high quality colostrum, it is possible that the maximum amount of IgG was absorbed in calves from either treatment, and beyond this maximum, any difference between the two treatments in IgG concentrations might not be detected.

Another concern with feeding calves through an esophageal tube is possible stress imposed to the calf. In the current study, serum cortisol concentration was measured as an indicator of stress 30 minutes after feeding and was compared to the concentration just before the feeding to determine if feeding method affected cortisol concentrations. Cortisol concentrations were highly variable in both treatments, however, treatment differences were difficult to detect. In addition, since calves were restrained for catheterization prior to feeding, they were already in state of stress from the catheter and birth. Furthermore, the sample was taken 30 min after the feeding because previous studies have shown cortisol concentrations reach peak concentration prior to 30 min and return to baseline 60 min after a stressful event, such as castration in beef calves (Robertson, 1994). Since we wanted to determine if tube feeding caused stress in the calves, the first sample taken after feeding (30 min) was analyzed for cortisol concentration. However, samples taken less than 30 min after feeding may be a better indicator of stress, if any, in calves fed with an esophageal tube. Another study compared cortisol concentrations in one week old calves that were either allowed to suckle from the dam or were bucket fed and found that cortisol concentrations were decreased 30 minutes after suckling (Lupoli et al., 2001). This could be a positive implication for bottle fed calves, but this was not shown in the current study.

This could be because calves in the Lupoli study (2001) were older and suckling from their dam. In order to assess if tube feeding increases cortisol concentrations, samples immediately following feeding would need to be taken.

A possible positive implication for tube fed calves is that they drank more milk in their first milk meal. Tube-fed calves drank 2.97 L in the first milk meal compared to bottle-fed calves that only drank 2.47 L. In the current study there was also a strong negative correlation between the time it took to complete the colostrum meal and the amount of milk consumed in the subsequent first milk meal, meaning that the longer a calf took to drink their colostrum the less milk they would consume 10 h later. This has positive implications for tube-fed calves who consumed their colostrum meal in 5.2 min compared to bottle-fed calves that took 17.6 min. However, since the calves in this study underwent so much additional stress from catheterization and frequent blood sampling, it is possible that the additional time spent with the bottle fed calves, might have exhausted them, which affected their ability to stand and consume all of their milk later and this correlation may not exist in a regular farm setting. A study would need to be conducted on more calves, without intensive sampling, to simply measure the time to consume the subsequent first milk meal and amount of voluntary milk consumed between tube- and bottle-fed calves.

### 3.3 Future Research

Future research regarding tube vs. bottle feeding should consider two factors: colostrum quantity and quality. The current study demonstrated that when feeding a large volume of high quality colostrum, feeding method did not affect serum IgG concentrations. Thus, perhaps

quantity and quality of colostrum is more important than feeding method, but feeding method could affect IgG absorption when smaller volumes are fed. It has been shown that when 1.5 L is fed through an esophageal tube, serum IgG concentrations were lower, than if fed through a nipple bottle (Godden et al., 2009). It has also been shown that feeding two 2 L meals of high quality colostrum, compared with feeding one 4 L meal of high quality colostrum, increased serum IgG concentrations (Jaster, 2005). It appears that absorption of IgG can be optimized through feeding 2 smaller meals compared to one large one (Jaster, 2005) and when feeding a small meal with a nipple bottle compared to a tube (Godden et al., 2009), so perhaps feeding 2 small volumes with a nipple bottle would result in the maximum IgG absorption. Future studies should compare feeding multiple smaller meals compared to one large meal and compare these feeding schemes using either a nipple bottle or esophageal tube.

In addition, this study fed one meal of 200 g of IgG, but quality of colostrum on farm is not always as high as the colostrum used in the current study. It is important to consider on-farm situations when designing colostrum studies. Perhaps when feeding poor quality colostrum, a difference between feeding methods might be observed. Assuming that colostrum enters the rumen when fed through an esophageal tube, this colostrum in the rumen may decrease IgG absorption. When high quality colostrum is fed it is likely any colostrum in the rumen would not affect overall IgG absorption. However, when poor quality colostrum is fed with a tube, the amount that enters the rumen could negatively affect IgG absorption. Future studies should feed colostrum of different qualities to reflect what may be occurring on farm.

Other considerations for future studies comparing tube and bottle feeding would be to use ultrasound or radiograph to measure abomasal emptying rather than acetaminophen. These methods would allow for a visual assessment of whether colostrum enters the rumen when fed

with an esophageal tube (Sharifi et al., 2009). These methods can also be used to measure how much colostrum is in the rumen and how long it takes for that colostrum to flow out of the rumen, which would give a better picture of whether feeding colostrum with an esophageal tube is going to negatively affect the calf.

Previous studies have always focused on absorption of IgG when feeding colostrum through either a nipple bottle or esophageal tube. However, the current study found an interesting result in regards to glucose and insulin, finding that AUC for both parameters were higher in tube fed calves than bottle fed calves. This could be either a positive implication for tube fed calves, indicating increased gluconeogenesis in these calves, or a negative implication for tube fed calves, indicating that bottle fed calves are more efficient at utilizing glucose in the GIT than tube fed calves. The current study only measured peripheral glucose and insulin in plasma, which does not indicate how much glucose is being absorbed from colostrum, utilized by the GIT or produced through gluconeogenesis. It is most likely, however, the shorter time to consume the colostrum meal was driving less glucose to be utilized in the GIT and this resulted in higher glucose and insulin AUC in tube fed calves. Future studies need to address if different feeding times affect glucose and insulin concentrations and if the reason feeding time affects these parameters is due to more glucose being utilized. In order to answer that question a study could be conducted infusing glucose for different durations of time to simulate the difference in feeding time between tube and bottle feeding but remove the factor of actual feeding method. Glucose could be infused directly into the abomasum, delivering glucose to the GIT, and into the jugular vein, bypassing the GIT. Then blood samples could be taken from the artery and the portal vein to measure glucose. Calculating blood flow and knowing the concentration in the artery and the portal vein can indicate how much glucose was utilized in the GIT. Also,

comparing blood glucose concentrations between the two infusion sites would give an indication of how much of the glucose that is infused is in circulation when it bypasses the GIT and how much is in circulation when it goes through the GIT first. Tube fed calves also drank more milk and glucose AUC was also correlated with more milk being consumed in the first milk meal, which could be a positive implication for tube fed calves. Future studies should be conducted on several farms, to account for variation in genetics, feeding calves colostrum through either a tube or a bottle and then recording how much milk they consume in subsequent meals. Perhaps feeding with an esophageal tube has a positive implication for calves, besides passive transfer of immunity.

### 3.4 Conclusion

The current study aimed to determine if feeding colostrum with an esophageal tube would result in a slower emptying rate and thus have lower serum concentrations of IgG and lower plasma concentrations of glucose, insulin, GLP-1 and 2. No difference was observed in abomasal emptying, IgG, GLP-1 or 2, which was likely due to the large volume fed. Glucose and insulin AUC was higher for tube-fed calves, but it is unclear from this study if this is a negative or positive implication. Tube-fed calves also consumed more milk in the first milk meal, which was correlated with the time it took them to complete their colostrum meal. This could be a positive implication for tube-fed calves to consume more milk. Another objective of this study was to determine if feeding colostrum with an esophageal tube would increase cortisol concentrations as an indicator of stress, however, the late sampling time and high variability within calves did not allow us to detect any differences. The results from this study demonstrated that when feeding a large volume of high quality colostrum, successful passive transfer is

achieved with either feeding method and producers can use either an esophageal tube or nipple bottle to feed colostrum.

### 3.5 Literature Cited

Atkinson, D.J., von Keyserlingk, M.A.G., and D.M. Weary. 2017. Benchmarking passive transfer of immunity and growth in dairy calves. J. Dairy Sci. 100: 1-10.

Bickerstaffe, R., and E.F. Annison. 1974. The metabolism of glucose, acetate, lipids and amino acids in lactating dairy cows. J. Agric. Sci. 82: 71-85.

Chapman, H.W., Butler, D.G., and M. Newell. 1986. The route of liquids administered to calves by esophageal feeder. Can J Vet Res. 50: 84-87.

Conneely, M., Berry, D.P., Murphy, J.P., Lorenz, I., Doherty, M.L., and E. Kennedy. 2014. Effect of feeding colostrum at different volumes and subsequent number of transition milk feeds on the serum immunoglobulin G concentration and health status of dairy calves. J. Dairy Sci. 97: 6991-7000.

Chigerwe, M., Coons, DM., and J.V. Hagey. 2012. Comparison of colostrum feeding by nipple bottle versus oroesophageal tubing in Holstein dairy bull calves. J. Am. Vet. Med. Assoc. 241: 104-9.

Godden, S.M., Haines, D.M., Konkol, K., and J. Peterson. 2009. Improving passive transfer of immunoglobulins in calves. II: Interaction between feeding method and volume of colostrum fed. J. Dairy Sci. 92: 1758-1764

Hadorn, U., Hammon, H., Bruckmaier, R.M., and J.W. Blum. 1997. Delaying colostrum intake by one day has important effects on metabolic traits and on gastrointestinal and metabolic hormones in neonatal calves. J. Nutr. 127: 2011-2023.

Hammon, H.M., and J.W. Blum. 1998. Metabolic and endocrine traits of neonatal calves are influenced by feeding colostrum for different durations or only milk replacer. J. Nutri. 128: 624-632.

Hammon, H.M., Schiessler, G., Nussbaum, A., and J.W. Blum. 2002. Feed intake patterns, growth performance, and metabolic and endocrine traits in calves fed unlimited amounts of colostrum and milk by automate, starting in the neonatal period. J. Dairy Sci. 85: 3352-3362.

Jaster, E.H. 2005. Evaluation of quality, quantity, and timing of colostrum feeding on immunoglobulin G absorption in Jersey calves. J. Dairy Sci. 88: 296-302.

Kaske, M., Werner, A., Schberth, H.J., Rehage, J., and W. Kehler. 2005. Colostrum management in calves: effects of drenching vs. bottle feeding. Journal of Animal Physiology and Animal Nutrition. 89: 151-157.

Lecce, J. G. 1973. Effect of dietary regimen in cessation of uptake of macromolecules by piglet intestinal epithelium (closure) and transport into blood. J. Nutr. 103: 751.

Lupoli, B., Johansson, B., Uvnas-Moberg, K., and K. Svennersten-Sjaunja. 2001. Effect of suckling on the release of oxytocin, prolactin, cortisol, gastrin, cholecystokinin, somatostatin and insulin in dairy cows and calves. Journal of Dairy Research. 68: 175-187.

Margerison, J. and N. Downey. 2005. Guidelines for Optimal Dairy Heifer Rearing and Herd Performance. In: Garnsworthy, P.C., Ed., Calf and Heifer Rearing: Principles of Rearing the Modern Dairy Heifer from Calf to Calving, Nottingham University Press, Nottingham. 307-338.

Nouri, M., and P. D. Constable. 2006. Comparison of two oral electrolyte solutions and route of administration on the abomasal emptying rate of Holstein-Friesian calves. J. Vet. Intern. Med. 20: 620–626.

Robertson, I.S., Kent, J.E., and V. Molony. 1994. Effect of different methods of castration on behavior and plasma cortisol in calves of three ages. Research in Veterinary Science. 56: 8-17.

Sen, I., Constable, P.D., and T.S. Marshall. 2006. Effect of suckling isotonic or hypertonic solutions of sodium bicarbonate or glucose on abomasal emptying rate in calves. Am. J. Vet. Res. 67: 1377-1384.

Schaer, S., Herrli-Gygi, M., Kosmeas, N., Boschung, H., and A. Steiner. Characteristics of acetaminophen absorption in healthy unweaned calves as an indirect measurement of the oroduodenal transit rate of liquid meals. J. Vet. Med. 52: 325-332.

Sharifi, K., Grunberg, W., Soroori, S., Mohri, M., and M.S. Ahrari-Khafi. 2009. Assessemtn of the acetaminophen absorption test as a diagnostic tool for the evaluation of the reticular groove reflex in lambs. Am. J. Vet. Res. 70: 820-825.

Trotz-Williams, L. A., K. E. Leslie, and A. S. Peregrine. 2008. Passive immunity in Ontario dairy calves and investigation of its association with calf management practices. J. Dairy Sci. 91: 3840–3849.

**Bibliography**

Afshari, G., M. Nouri, E. B. Hassan, M. R. Mokhber-Dezfooli, and P.D. Constable. 2009. Effect of parenteral administration of ivermectin and erythromycin on abomasal emptying rate in suckling calves. Am. J. Vet. Res. 70: 527–531.

Atkinson, D.J., von Keyserlingk, M.A.G., and D.M. Weary. 2017. Benchmarking passive transfer of immunity and growth in dairy calves. J. Dairy Sci. 100: 1-10.

Baggio, L.L., and D.J. Drucker. 2007. Biology of incretins: GLP-1 and GIP. Gastroenterology. 132: 2131- 2157.

Barrington, G.M., McFadden, T.B., Huyler, M.T., and T.E. Besser. 2001. Regulation of colostrogenesis in cattle. Livestock Production Science. 70: 95-104.

Baumrucker, C.R., Hadsell, D.L., and J.W. Blum. 1994. Effects of dietary insulin-like growth factor I on growth and insulin-like growth factor receptors in neonatal calf intestine. J. Anim. Sci. 72: 428-433.

Beam, A.L., Lombard, J.E., Kopral, C.A., Garber, L.P., Winter, A.L., Hicks, J.A., and J.L. Schlater. 2009. Prevalence of failure of passive transfer of immunity in newborn heifer calves and associated management practices on US dairy operations. J. Dairy Sci. 92: 3973-3980.

Bickerstaffe, R., and E.F. Annison. 1974. The metabolism of glucose, acetate, lipids and amino acids in lactating dairy cows. J. Agric. Sci. 82: 71-85.

Blum, J.W., and H.M. Hammon. 2000. Colostrum effects on the gastrointestinal tract, and on nutritional, endocrine and metabolic parameters in neonatal calves. Livestock Production Science. 66: 151-159.

Burrin, D.G., Petersen, Y., Stoll, B., and P. Sanglld. 2001. Glucagon-like peptide 2: a nutrient-responsive gut growth factor. J. Nutr. 131: 709-712.

Burrin, D., Guan, X., Stoll, B., Petersen, Y., and P.T. Sanglld. 2003. Glucagon-like peptide 2: a key link between nutrition and intestinal adaptation in neonates. J. Nutr. 133: 3712-3716.

Cant, J. P., Walsh, V. N., and R.J. Geor. 2006. Obtaining information on gastric emptying patterns in horses from appearance of an oral acetaminophen dose in blood plasma. In *Nutrient Digestion and Utilization in Farm Animals: Modelling Approaches*. Pg: 69-83. CABI Publishing.

Chapman, H.W., Butler, D.G., and M. Newell. 1986. The route of liquids administered to calves by esophageal feeder. Can J Vet Res. 50: 84-87.

Chelack, B. J., Morley, P.S., and D.M. Haines. 1993. Evaluation of methods for dehydration of bovine colostrum for total replacement of normal colostrum in calves. Can. Vet. J. 34: 407–412.

Conneely, M., Berry, D.P., Murphy, J.P., Lorenz, I., Doherty, M.L., and E. Kennedy. 2014. Effect of feeding colostrum at different volumes and subsequent number of transition milk feeds on the serum immunoglobulin G concentration and health status of dairy calves. J. Dairy Sci. 97: 6991-7000.

Chigerwe, M., Coons, DM., and J.V. Hagey. 2012. Comparison of colostrum feeding by nipple bottle versus oroesophageal tubing in Holstein dairy bull calves. J. Am. Vet. Med. Assoc. 241: 104-9.

Connor, E.E., Baldwin, R.L., Capuco, A.V., Evock-Clover, C.M., Ellis, S.E., and K.S. Sciabica. 2010. Characterization of glucagon-like peptide 2 pathway member expression in bovine gastrointestinal tract. J. Dairy Sci. 93: 5167-5178.

Connor, E.E., Kahl, S., Elsasser, T.H., Baldwin, R.L., Fayer, R., Santin-Duran, M., Sample, G.L., and C.M. Evock-Clover. 2013. Glucagon-like peptide 2 therapy reduces negative effects of diarrhea on calf gut. J. Dairy Sci. 96: 1793-1802.

Constable, P. D., W. Grünberg, and L. Carstensen. 2009. Comparative effects of two oral rehydration solutions on milk clotting, abomasal luminal pH, and abomasal emptying rate in suckling calves. J. Dairy Sci. 92: 296–312.

de Passille, A.M.B. Christopherson, R., and J. Rushen. 1993. Nonnutritive sucking by the calf and postprandial secretion of insulin, CCK, and gastrin. Physiology & Behaviour. 54: 1069-1073.

Donkin, S.S., and L.E. Armentano. 1995. Insulin and glucagon regulation of gluconeogenesis in preruminating and ruminating bovine. J. Anim. Sci. 73: 546-551.

Dube, P., Forse, C.L., Bahrami, J., and P.L. Brubaker. 2006. The essential role of insulin-like growth factor-1 in the intestinal tropic effects of glucagon-like peptide-2 in mice. Gastroenterology. 131: 589-605.

Edwards, C.M.B., Edwards, A.V., and S.R. Bloom. Cardiovascular and pancreatic endocrine responses to glucagon-like peptide-1 (7-36) amide in the conscious calf. Experimental Physiology. 82: 709-716.

Elizondo-Salazar, J.J., and A.J. Heinrichs. 2009. Feeding heat-treated colostrum or unheated colostrum with two different bacterial concentrations to neonatal dairy calves. J. Dairy Sci. 92: 4565-4571.

Fecteau, G., Ballargeon, P., Higgins, R., Pare, J., and M. Fortin. 2002. Bacterial contamination of colostrum fed to newborn calves in Quebec dairy herds. Can. Vet. J. 43: 523-527.

Fukumori, R., Mita, T., Sugino, T., Obitsu, T., and K. Taniguchi. 2012. Plasma concentrations and effects of glucagon-like peptide-1 (7-36) amide in calves before and after weaning. Domestic Animal Endocrinology. 43: 299-306.

Fukumori, R., Sugino, T., Shingu, H., Moriya, N., Hasegawa, Y., Kojima, M., Kangawa, K., Obitsu, T., Kushibiki, S., and K. Taniguchi. 2012. Effects of calcium salts of long-chain fatty acids and rumen-protected methionine on plasma concentrations of ghrelin, glucagon-like peptide-1 (7 to 36) amide and pancreatic hormones in lactating cows. Domestic Animal Endocrinology**.** 42: 74–82.

Georgiev, I.P., Georgieva, T.M., Pfaffl, M., and H.M. Hammon. 2003. Insulin-like growth factor and insulin receptors in intestinal mucosa of neonatal calves. Journal of Endocrinology. 176: 121-132.

Girard, J. 1986. Gluconeogenesis in late fetal and early neonatal life. Biol. Neonate 50: 237–258.

Girard, J., Ferre, P., Pegorier, J.P., and P.H. Duee. 1992. Adaptations of glucose and fatty acid metabolism during perinatal period and suckling-weaning transition. Physiological Reviews. 72: 507-562.

Godden, S. 2008. Colostrum Management for Dairy Calves. Veterinary Clinic of North America*:* Food Animal Practice. 24: 19-39.

Godden, S.M., Haines, D.M., Konkol, K., and J. Peterson. 2009. Improving passive transfer of immunoglobulins in calves. II: Interaction between feeding method and volume of colostrum fed. J. Dairy Sci. 92: 1758-1764.

Grutter, R., and J.W. Blum. 1991. Insulin and glucose in neonatal calves after peroral insulin and intravenous glucose administration. Reprod. Nutr. Dev. 31: 389-397.

Hadorn, U., Hammon, H., Bruckmaier, R.M., and J.W. Blum. 1997. Delaying colostrum intake by one day has important effects on metabolic traits and on gastrointestinal and metabolic hormones in neonatal calves. J. Nutr. 127: 2011-2023.

Hammon, H.M., and J.W. Blum. 1997. Prolonged colostrum feeding enhances xylose absorption in neonatal calves. J. Anim. Sci. 75: 2915-2919.

Hammon, H.M., and J.W. Blum. 1998. Metabolic and endocrine traits of neonatal calves are influenced by feeding colostrum for different durations or only milk replacer. J. Nutri. 128: 624-632.

Hammon, H.M., and J.W. Blum. 2002. Feeding different amounts of colostrum or only milk replacer modify receptors of intestinal insulin-like growth factors and insulin in neonatal calves. Domestic Animal Endocrinology. 22: 155-168.

Hammon, H.M., Schiessler, G., Nussbaum, A., and J.W. Blum. 2002. Feed intake patterns, growth performance, and metabolic and endocrine traits in calves fed unlimited amounts of colostrum and milk by automate, starting in the neonatal period. J. Dairy Sci. 85: 3352-3362.

Hammon, H.M., Steinhoff-Wagner, J., Schonhusen, U., Metges, C.C., and J.W. Blum. 2012. Energy metabolism in the newborn farm animal with emphasis on the calf: endocrine changes and responses to milk-born and systemic hormones. Domestic Animal Endocrinology. 43: 171-185.

Holst, J.J. 2000. Gut hormones as pharmaceuticals from enteroglucagon to GLP-1 and GLP-2. Regul. Pept. 93: 45-51.

Jaster, E.H. 2005. Evaluation of quality, quantity, and timing of colostrum feeding on immunoglobulin G absorption in Jersey calves. J. Dairy Sci. 88: 296-302.

Jehle, P.M, Fussgaenger, R.D, Blum, W.F, Angelus, N.K, Hoeflich. A.,Wolf, E., and R.J. Jungwirth. 1999. Differential autocrine regulation of intestinal epithelial cell proliferation and differentiation by insulin-like growth factor (IGF) system component. Hormone Metabolic Research. 31: 97–102.

Johnson, J.L., Godden, S.M., Molltor, T., Ames, T., and D. Hagman. 2007. Effects of feeding heat-treated colostrum on passive transfer of immune and nutritional parameters in neonatal dairy calves. J. Dairy Sci. 90: 5189-5198.

Jones, C.M., James, R.E., Quigley, J.D., and M.L. McGllllard. 2004. Influence of pooled colostrum or colostrum replacement on IgG and evaluation of animal plasma in milk replacer. J. Dairy Sci. 87: 1806-1814.

Kaske, M., Werner, A., Schberth, H.J., Rehage, J., and W. Kehler. 2005. Colostrum management in calves: effects of drenching vs. bottle feeding. Journal of Animal Physiology and Animal Nutrition. 89: 151-157.

Khan, A.H., and J.E. Pessin. 2002. Insulin regulation of glucose uptake: a complex interplay of intracellular signalling pathways. Diabetologia. 45: 1475-1483.

Kuhne, S., Hammon, H.M., Bruckmaier, R.M., Morel, C., Zbinden, Y., and J.W. Blum. 2000. Growth, performance, metabolic and endocrine traits, and absorptive capacity in neonatal calves fed either colostrum or milk replacer at two levels. J. Anim. Sci. 78: 609-620.

Larson, B.L., Heary, H.L., and J.E. Devery. 1980. Immunoglobulin production and transport by the mammary gland. J. Dairy Sci. 63: 665–71.

Lecce, J. G. 1973. Effect of dietary regimen in cessation of uptake of macromolecules by piglet intestinal epithelium (closure) and transport into blood. J. Nutr. 103: 751.

Lecce, J. G., and D. O. Morgan. 1962. Effect of dietary regimen on cessation of intestinal

absorption of large molecules (closure) in neonatal pigs and lamb. J. Nutr. 78: 265.

Lombard, J. E., Garry, F.B., Tomlinson, S.M., and L.P. Garber. 2007. Impacts of dystocia on health and survival of dairy calves. J. Dairy Sci. 90: 1751- 1760.

Lupoli, B., Johansson, B., Uvnas-Moberg, K., and K. Svennersten-Sjaunja. 2001. Effect of suckling on the release of oxytocin, prolactin, cortisol, gastrin, cholecystokinin, somatostatin and insulin in dairy cows and calves. Journal of Dairy Research. 68: 175-187.

MacPherson, J.A., Berends, H., Leal, L.N., Cant, J.P., Martin-Tereso, J., and M.A. Steele. 2016. Effect of plane of milk replacer intake and age on glucose and insulin kinetics and abomasal emptying in female Holstein Friesian dairy calves fed twice daily. J. Dairy Sci. 10: 8007-8017.

Margerison, J. and N. Downey. 2005. Guidelines for Optimal Dairy Heifer Rearing and Herd Performance. In: Garnsworthy, P.C., Ed., Calf and Heifer Rearing: Principles of Rearing the Modern Dairy Heifer from Calf to Calving, Nottingham University Press, Nottingham. 307-338.

Marshall, T.S., Constable, P.D., Crochik, S.S., and T. Wittek. 2005. Determination of abomasal emptying rate in suckling calves by use of nuclear scintigraphy and acetaminophen absorption. Am J Vet Res. 66: 364-374.

Marshall, T. S., P. D. Constable, S. S. Crochik, T. Wittek, D. E. Freeman, and D. E. Morin. 2008. Effect of suckling an isotonic solution of sodium acetate, sodium bicarbonate, or sodium chloride on abomasal emptying rate and luminal pH in calves. Am. J. Vet. Res. 69: 824–831.

Mayer, B., Doleschall, M., Bender, B., Bartyik, J., Bosze, Z., Frenyo, L., and I. Kacskovics.

2005. Expression of the neonatal Fc receptor (FcRn) in the bovine mammary gland. Journal of Dairy Research. 72: 107-112.

Mokhber-Dezfooli, M.R., Nouri, M., Rasekh, M., and P.D. Constable. 2012. Effect of abomasal emptying rate on the apparent efficiency of colostral immunoglobulin G absorption in neonatal Holstein-Friesian calves. J. Dairy Sci. 95: 6740-6749.

Morrill, K.M., Conrad, E., Lago, A., Campbell, J., Quigley, J., and H. Tyler. 2012. Nationwide evaluation of quality and composition of colostrum on dairy farms in the United States. J. Dairy Sci. 95: 3997-4005.

Muller L.D, and D.K. Ellinger. 1981. Colostral immunoglobulin concentrations among breeds of dairy cattle. J. Dairy Sci. 64: 1727 – 1730.

Nouri, M., and P. D. Constable. 2006. Comparison of two oral electrolyte solutions and route of administration on the abomasal emptying rate of Holstein-Friesian calves. J. Vet. Intern. Med. 20: 620–626.

Nouri, M., and P.D. Constable. 2007. Effect of parenteral administration of erythromycin, tilmicosin, and tylosin on abomasal emptying rate in suckling calves. Am. J. Vet. Res. 68: 1392-1398.

Nouri, M., Hajikolace, M.R., Constable, P.D., and A. Omidi. 2008. Effect of erythromycin and gentamicin on abomasal emptying rate in suckling calves. J. Vet. Intern. Med. 22: 196-201.

Odle, J., Zijlstra, R. T., and S.M. Donovan. 1996. Intestinal effects of milkborne growth factors in neonates of agricultural importance. J. Anim. Sci. 74: 2509-2522.

Paris, M., Fuller, P.J., Carstensen, B., Nagy, E., Taylor, R.G., Sourial, M., Holst, J., Hartmann, B., and J. Binesm. 2004. Digestive Diseases and Sciences. 49: 1688-1695.

Pfaffl M.W., Georgieva T.M., Georgiev I.P., Ontsouka E., Hageleit M., and J.W. Blum. 2002. Real-time RT-PCR quantification of insulin-like growth factor (IGF)-I, IGF-I receptor, IGF-II, IGF-II receptor, insulin receptor, growth hormone receptor, IGF-binding proteins 1, 2 and 3 in the bovine species. Domestic Animal Endocrinology. 22: 91–102.

Petersen, Y.M., Hartmann, B., Holst, J., Huerou-Luron, I., Bjornvad, C.R., and P.T. Sangild. 2003. Introduction of enteral food increases plasma GLP-2 and decreases GLP-2 receptor mRNA abundance during pig development. J. Nutr. 133: 1781-1786.

Quigley, J.D. 3rd, Kost, C.J., and T.M. Wolfe. 2002. Absorption of protein and IgG in calves fed colostrum supplement or replacer. J. Dairy Sci. 85: 1243-1248.

Richards, C. J. 1999. Influence of small intestinal protein on carbohydrate assimilation and metabolism in beef cattle. Ph.D. Diss. Univ. Kentucky.

Robertson, I.S., Kent, J.E., and V. Molony. 1994. Effect of different methods of castration on behavior and plasma cortisol in calves of three ages. Research in Veterinary Science. 56: 8-17.

Roffler, B., Fah, A., Sauter, S.N., Hammon, H.M., Gallmann, P., Brem, G., and J.W. Blum. 2003. Intestinal morphology, epithelial cell proliferation, and absorptive capacity in neonatal calves fed milk-born insulin-like growth factor-I or a colostrum extract. J. Dairy Sci. 86: 1797-1806.

Saltiel, A.R. 2001. New perspectives into the molecular pathogenesis and treatment of type 2 diabetes. Cell. 104: 517-529.

Schaer, S., Herrli-Gygi, M., Kosmeas, N., Boschung, H., and A. Steiner. 2005. Characteristics of acetaminophen absorption in healthy unweaned calves as an indirect measurement of the oroduodenal transit rate of liquid meals. J. Vet. Med. 52: 325-332.

Sharifi, K., Grunberg, W., Soroori, S., Mohri, M., and M.S. Ahrari-Khafi. 2009. Assessemtn of the acetaminophen absorption test as a diagnostic tool for the evaluation of the reticular groove reflex in lambs. Am. J. Vet. Res. 70: 820-825.

Shen, W., and R. Xu. 2000. Gastrointestinal stability and absorption of insulin in suckling pigs. Comparative Biochemistry and Physiology. 125: 389-401.

Sen, I., Constable, P.D., and T.S. Marshall. 2006. Effect of suckling isotonic or hypertonic solutions of sodium bicarbonate or glucose on abomasal emptying rate in calves. Am. J. Vet. Res. 67: 1377-1384.

Steinhoff-Wagner, J., Gors, S., Junghans, P., Bruckmaier, R.M., Kanitz, E., and C.C. Metges. 2011. Maturation of endogenous glucose production in preterm and term calves. J. Dairy Sci. 94: 5111-5123.

Stelwagen, K., Carpenter, E., Haigh, B., Hodgkinson, A., and T.T. Wheeler. 2014. Immune components of bovine colostrum and milk. J. Anim. Sci. 87: 3-9.

Stott, G.H., Marx, D.B., Menefee, B.E., and G.T. Nightengale. 1997a. Colostral immunoglobulin transfer in calves I: period of absorption. J. Dairy Sci. 62: 1632-1638.

Stott, G.H., Marx, D.B., Menefee, B.E., and G.T. Nightengale. 1997b. Colostral immunoglobulin transfer in calves II: the rate of absorption. J. Dairy Sci. 62: 1766-1773.

Swan, H., Godden, S., Bey, R., Wells, S., Fetrow, J., and H. Chester-Jones. 2007. Passive transfer of immunoglobulin G and preweaning health in Holstein calves fed a commercial colostrum replacer. J. Dairy Sci. 90: 3857-3866.

Tang-Christensen, M. Larsen, P.J., Thulesen, J., Romer, J., and N. Vrang. 2000. The proglucagon-derived peptide, glucagon-like peptide 2, is a neurotransmitter involved in the regulation of food intake. Nature Medicine. 6: 802-807.

Taylor-Edwards, C.C., Burrin, D.G., Holst, J.J., Mcleod, K.R., and D.L. Harmon. 2011. Glucagon-like peptide-2 (GLP-2) increases small intestinal blood flow and mucosal growth in ruminating calves. J. Dairy Sci. 94: 888-898.

Tong, J., and D. D'Alessio. 2014. Give the receptor a brake: Slowing gastric emptying by GLP-1. Diabetes. 63: 407–409.

Trotz-Williams, L. A., K. E. Leslie, and A. S. Peregrine. 2008. Passive immunity in Ontario dairy calves and investigation of its association with calf management practices. J. Dairy Sci. 91: 3840–3849.

van Goudoever, J.B., Stoll, B., Hartmann, B., Holst, J.J., Reeds, P., and D. Burrin. 2001. Secretion of Trophic gut peptides is not different in bolus- and continuously fed piglets. J. Nutr. 131: 729-732.

Walker, M.P., Evock-Clover, C.M., Elasser, T.H., and E.E. Connor. 2015. Short Communication: Glucagon-like peptide-2 and coccidiosis alter tight junction gene expression in the gastrointestinal tract of dairy calves. J. Dairy Sci. 98: 3432-3437.

Wallace, M. M., B. D. Jarvie, N. R. Perkins, and K. E. Leslie. 2006. A comparison of serum harvesting methods and type of refractometer for determining total solids to estimate failure of passive transfer in calves. Can. Vet. J. 47: 573–575.

Willms, B., Werner, J., Holst, J.J., Orskov, C., Creutzfeldt, W., and M.A. Nauck. 1996. Gastric emptying, glucose responses and insulin secretion after a liquid test meal: effects of exogenous glucagon-like peptide-1 (GLP-1)-(7-36) amide in Type 2 (noninsulin-dependent) diabetic patients. J. Clin. Endocrinol. and Metabol. 81: 327-332.

Xiao, Q., Boushey, R., Drucker, D.J., and P.L. Brubaker. 1999. Secretion of the intestinotrophic hormone glucagon-like peptide 2 is differentially regulated by nutrients in humans. Gastroenterology. 117: 99-105.