THE IMPACT OF DIRECT-FED MICROBIALS AND ENZYMES ON THE HEALTH AND PERFORMANCE OF HOLSTEIN CATTLE WITH EMPHASIS ON COLOSTRUM QUALITY AND SERUM IMMUNOGLOBULINS IN THE CALF

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THE IMPACT OF DIRECT-FED MICROBIALS AND ENZYMES ON THE HEALTH AND PERFORMANCE OF HOLSTEIN CATTLE WITH EMPHASIS ON COLOSTRUM QUALITY AND SERUM IMMUNOGLOBULINS IN THE CALF

By

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B.S., Cornell University, January 2013

THESIS

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ABSTRACT

THE IMPACT OF DIRECT-FED MICROBIALS AND ENZYMES ON THE HEALTH AND PERFORMANCE OF HOLSTEIN CATTLE WITH EMPHASIS ON COLOSTRUM QUALITY AND SERUM IMMUNOGLOBULINS IN THE CALF

by

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University of New Hampshire, September, 2016

Research has shown that direct-fed microbials (DFM) and enzyme supplementation can impact the dry matter intake (DMI), milk production, and milk composition. However, limited research has evaluated the impact of DFM and enzyme supplementation on colostrum quality and the uptake of the Immunoglobulins A and G (IgA and IgG) by calves. In this study, 36 multiparous Holstein cows were blocked by expected calving date and randomly assigned to 1 of 3 treatments 3 weeks prior to calving and remained on these treatments until week 8 postpartum. These treatments were: 1) 0 g of DFM and enzyme (control), 2) 45.40 g/d of Tri-Lution® (Tri), or 3) 45.40 g/d of Tri-Lution® and 18.16 g/d of Zy-mend® (Tri + Zy). The amount of total mixed ration (TMR) fed and orts refused were measured each day to determine DMI. Blood samples were taken on the cows every Monday, Wednesday, and Friday at 1100 h from the coccygeal veins and arteries to be analyzed for β-hydroxybutyrate (BHBA), glucose, and nonesterified fatty acids (NEFA). Cows were also weighed once a week throughout the course of the study. Colostrum was harvested and weighed at parturition and later analyzed for IgA and IgG concentration via radial immunoassay. Calves were fed 4 L of maternal colostrum within 2 h after calving. Blood samples were also taken from the calves at 0 and 24 h in order to be
analyzed for IgA and IgG concentrations and to determine apparent efficiency of absorption of IgA and IgG. Finally, milk yields were taken daily for 8 wk postpartum and samples were taken once a week and sent to DairyOne (Ithaca, NY) to be analyzed for quality. Prepartum body weight (BW), BW, efficiency of gain, DMI, BHBA, NEFA, and glucose concentrations were not impacted by treatment. There was also no impact of treatment on colostrum yield, IgA and IgG content, and composition with the exception of IgA yield and ash percentage. The ash percentage of colostrum tended ($P = 0.07$) to increase with the Tri and Tri + Zy treatments while the IgA yield ($P = 0.05$) decreased with the Tri treatment. Treatments did not impact BW, serum IgA and IgG concentrations or apparent efficiency of absorption of IgA and IgG of the calves. Postpartum BW, DMI, blood metabolites, milk production and composition, with the exception of BW gain and somatic cell score (SCS), of the cows were not impacted by treatment. Cows on the Tri treatment gained more BW ($P = 0.03$) and tended to have a greater efficiency of gain ($P = 0.09$) in comparison to those on the Tri + Zy treatment, but both treatments did not differ from the control. This suggests that there is a negative effect of applying the Tri-Lution® and Zy-mend® together which might be due to negative interactions among ingredients and microorganisms. An increase in SCS ($P = 0.04$) was also observed with the Tri treatment. All these results indicate that the supplementation of DFM and enzymes is not beneficial in improving the health and performance of dairy cattle during the transition period and early lactation.
In mammals the first mammary secretion produced after parturition is called colostrum. It provides all the essential nutrients such as water, fat, protein, lactose, minerals, and vitamins which can also be found in milk. However, colostrum also contains a higher concentration of immunoglobulins (Ig) than milk. These antibodies along with the nutrients in colostrum are essential for the survival and growth of the neonate especially in the case of ruminants. With dairy calves it has been noted that the failure to provide an adequate amount of high quality colostrum leads to lesser chance of survival and productivity as an adult animal (Beam et al., 2009).

Dairy cattle, like all ruminants, are unable to transfer antibodies such as Ig to their offspring in utero (Akers, 2002). This is because ruminants have synepithelialchorial placentae which prevent this transfer of Ig from the dam to the offspring (Akers, 2002). As a result, ruminants have evolved to transfer their Ig to their offspring via colostrum (Baumrucker et al., 2010).

Colostrogenesis is defined as a particular phase of mammary development in which maternal Ig are transferred into the mammary gland prior to parturition (Barrington et al., 2001). The transfer of Ig starts several weeks prior to parturition, but ceases the day of it (Brandon et al., 1971). Over the course of colostrogenesis, up to 500 g of the IgG can be transferred into the mammary gland (Brandon et al., 1971). It is the higher level of IgG, in particular IgG₁, which is the major difference between colostrum and milk (Brandon et al., 1971).

The transfer of IgG₁ into the mammary gland is facilitated by presence of specific IgG receptors of basal side of the secretory cells (Barrington et al., 2001). Mammary epithelial cells
then internalize and release IgG$_1$ into the lumen of the mammary gland where it becomes incorporated in the mammary secretion (Barrington et al., 2001).

Hormones appear to be the main mechanism in which colostrogenesis is controlled (Barrington et al., 2001). However, the exact mechanism for the onset of colostrogenesis is rather complex and yet to be fully determined (Barrington et al., 2001). It is thought that the decrease in progesterone near parturition might be the cause of less IgG transfer into the mammary gland, however this has yet to be verified (Barrington et al., 2001). Glucocorticoids, which play a major role in lactogenesis, along with prostaglandin F2α are thought to also inhibit colostrogenesis (Barrington et al., 2001). The lactogenic hormone prolactin has also been shown to decrease the expression of the receptor and secretion of IgG$_1$ within the mammary gland (Barrington et al., 2001).

In addition to hormonal regulation of colostrogenesis, there is also the suggestion of local regulation within the mammary gland. This is illustrated by differences in Ig content among glands (Barrington et al., 2001). Different development rate among the glands is thought to be the cause for this but has yet to be proven (Baumrucker et al., 2014).

**Colostrum Composition:**

Although colostrum quality is based upon IgG content (≥ 50 g/L), colostrum contains an array of other nutrients. These components include all those found in milk such as protein, fat, lactose, water, vitamins, and minerals. However, the concentration of protein, fat, minerals, and vitamins in bovine colostrum is often greater than that of milk (Akers, 2002). The reasoning behind this is because the neonatal calf is in a greater need of these nutrients (Akers, 2002). In fact, it is estimated that the adipose and glycogen reserves of a neonate would be depleted within 18 h of birth without supplementation of colostrum (Akers, 2002). As a result, the supplementation of colostrum is essential to nourish the calf as well as providing its immunity.
**Protein:**

Protein is the major nutrient component of colostrum after water. In a nationwide study in the US it was found that the protein content of colostrum averaged $12.7 \pm 3.3\%$ (Morrill et al., 2012). A study performed in Pennsylvania found that protein content of colostrum averaged $14.9 \pm 3.32\%$ (Kehoe et al., 2007).

The protein in both colostrum and milk can be divided into 2 main categories; whey and casein (Akers, 2002). Casein proteins usually contain proline and asparagine and are hydrophobic in milk which causes them to form micelles that help to transport calcium and phosphorus (Akers, 2002). The common forms of caseins are $\alpha$, $\beta$, $\kappa$, and $\gamma$-casein (Akers, 2002). As for the whey portion, these are defined as the proteins which remain suspended when milk is acidified to a pH of 4.6 (Akers, 2002). The proteins of the whey fraction include $\alpha$-lactalbumin and $\beta$ lactoglobulin, Ig, lactoferrin, transferrin, and serum albumin (Akers, 2002).

**Immunoglobulins:**

Butler (1983) reported that Ig make up 95% of the whey proteins in colostrum. However, Ig concentration in the whey protein fraction of milk is less than 7% (Butler, 1983).

Immunoglobulins are proteins with high molecular weights which possess similar antigenic determinants and physio-chemical characteristics (Butler, 1969). It is the physio-chemical characteristics which divides Ig into different classes (Butler, 1969). All Ig share a similar antibody structure (Figure 1). This structure is composed of four chains of polypeptides (Butler 1969). The two heavy polypeptide chains of the antibody are made up of about 400 amino acids and bonded together by sulfur bonds (Akers, 2002). Two light polypeptide chains are then attached to the outside of each of the heavy chains by sulfur bonds (Akers, 2002). It is the combination of these two types of polypeptide chains which create the binding sites for antigens (Akers, 2002).
The stem region of the antibody which is made up of the heavy chains also contains a complement and macrophage binding site (Akers, 2002). Immunoglobulins are not able to destroy antigens on their own. Instead they mark them for destruction by two different means. In the complement system, this means allowing the binding of another complement protein which sets off a reaction with other proteins that leads to the enhancement of inflammatory response or the direct destruction of the antigen (Akers, 2002). The other way is by the binding of macrophages which eventually engulf and destroy the antigen.

**Figure 1: Structure of an immunoglobulin.**

![Diagram of an immunoglobulin](http://med.mui.ac.ir/slide/immunu/igg_5.gif)

The main Ig in bovine colostrum is IgG and comes in two forms IgG\(_1\) and IgG\(_2\). Both IgG\(_1\) and IgG\(_2\) are found in equal concentrations within serum (Barrington et al., 2001). However, IgG\(_1\) concentration in colostrum can be five to 10 times greater than IgG\(_2\) (Barrington et al., 2001). Butler (1983) reported that IgG\(_1\) was 80% of the total amount of Ig found in colostrum. Yet, levels of both Ig are present and decrease drastically with the transition to milk (Barrington et al., 2001). As a result, it has been suggested that there is a selective mechanism for transport of IgG\(_1\) from the maternal blood to colostrum (Barrington et al., 2001). However,
more research is needed to define and understand this particular mechanism for IgG\textsubscript{1} and how it differs from IgG\textsubscript{2} and other Ig.

Colostrum also contains other Ig such as IgA, IgM, IgD, and IgE. In addition to IgG, IgA and IgM are the other main two Ig in bovine milk. Both IgA and IgM are said to be synthesized in the plasma cells of the mammary gland in many species (Hurley and Theil, 2011).

The concentration of IgA in colostrum is higher than that of serum (Butler, 1969). Hence, this provides further evidence that IgA is locally produced in or around the mammary gland as opposed to being sourced from serum (Butler, 1969). Immunoglobulin A is primarily found in the mucosal secretions of the body and is responsible for protecting against mucosal infections (Hurley and Theil, 2011).

Concentration of IgM in colostrum and milk is often higher than that of IgA (Butler, 1983). However, IgM is suggested to make up less than 10\% of the Ig found in colostrum and serum (Butler, 1969). Immunoglobulin M is involved primarily in the general immune response by playing a role in agglutination and complement fixation (Butler, 1969).

**Fat:**

Fat is the next major component of colostrum. Most fat in milk and colostrum of cattle is triglycerides (Akers, 2002). The amount of triglycerides in colostrum does not vary between day 1 and 2 postpartum (Contarini et al., 2014). However, triglycerides concentrations during the first 2 days postpartum are often higher than the rest of the lactation (Contarini et al., 2014). The fatty acids which make up these triglycerides come from 3 main sources: fat stores, diet, and de novo synthesis within the mammary glands (Akers, 2002). About half of the fat within milk is sourced from the diet (Akers, 2002).

A Pennsylvanian study found that colostral fat averaged 6.7 ± 4.16 \% (Kehoe et al. 2007). Morrill et al., (2012) found that fat content averaged 5.6 ± 3.2 \% in a nationwide study.
Another study found a similar average fat content of 5.3% at 24 h after calving (Contarini et al., 2014). This same study also found that fat content was not different from 24 to 120 h after birth.

**Lactose:**

Kehoe et al. (2007) found that lactose of bovine colostrum was 2.49% ± 0.65 while another study of colostrum found the lactose content to be 2.9 ± 0.5% (Morill et al., 2012). The major carbohydrate in milk and colostrum is lactose (Akers, 2002). It is derived from a molecule of galactose and glucose via three general steps (Akers, 2002). The synthesis of lactose is dependent on the presence of α-lactalbulmin within mammary cells (Akers, 2002). When this protein is present it causes the activation of galactosyl transferase which is one of the major enzymes of the lactose synthesis pathway (Akers, 2002). Hence, as α-lactalbulmin gene expression and protein production increases with lactogenesis so does lactose production (Akers, 2002). Kehoe et al. (2007) commented that lactose concentrations are often lower in colostrum and increase with length of lactation. A recent study also reported that lactose content in colostrum 24 h after calving was less than that 48, 72, 96, and 120 h after calving (Contarini et al., 2014). However, 48 h after calving there was no difference among lactose content from those taken at 72, 96, or 120 h (Contarini et al., 2014).

**Minerals:**

Colostrum is the only source of minerals essential for the neonate calf (Kume and Tanabe, 1993). The main minerals of interest found in colostrum are Ca, Cu, Fe, K, Mg, Mn, Na, P, S, and Zn. The values each of these was reported to be 4716, 0.3, 5.3, 2845, 733, 0.1, 1053, 4452, 2595, and 38 mg/kg (Kehoe et al., 2007). These values reported here were higher for all minerals except Cu and Mn that were reported in previous studies (Kehoe et al., 2007). This is likely due to better pre-partum supplementation of minerals on dairies.
Kume and Tanbe (1993) noted that by 24 h after parturition the amount of Ca, Cu, Fe, Mg, Mn, Na, and P declined rapidly in colostrum. Hence, colostrum should be harvested as soon after parturition as possible to provide these essential minerals to the neonate. It was also noted that multiparous cows had lower levels of Ca, Mg, and P in their colostrum and their calves had lower serum levels of Ca, P, and Mg as well (Kume and Tanabe, 1993).

**Vitamins:**

There are an array of both water and fat soluble vitamins provided by colostrum to the neonate calf. Vitamins A, D, and E are known to not be transferred through the placenta to fetus (Quigley and Drewry, 1998). As a result, the consumption of colostrum is the only way to provide these vitamins to the neonate calf (Quigley and Drewry, 1998). The supplementation of these vitamins to the cow during the dry period helps to ensure adequate amounts within the colostrum (Quigley and Drewry, 1998).

Kehoe et al. (2007) reported the concentration of vitamin E in colostrum was 77.2 µg/g of fat which was lower than the 84 µg/g of fat reported by Foley and Otterby (1978). Vitamin D was reported to range from 0.89 to 1.81 IU/g of fat (Foley and Otterby 1978). Finally, the concentration of vitamin A was reported to be 2.95 µg/mL (Foley and Otterby 1978). Kehoe et al. (2007) did not evaluate vitamin A directly but analyzed for its precursors β carotene and retinol and found them to be 0.68 and 4.90 µg/mL, respectfully in colostrum.

In terms of water soluble vitamins, colostrum provides the neonate with thiamin, riboflavin, niacin, B12, folic acid, and pantothenic acid. However, it was noted by Kehoe et al. (2007) that water soluble vitamins are not extensively analyzed in colostrum. This is probably due to a major focus being on IgG and other components like fat. Kehoe et al. (2007) evaluated several of these water soluble vitamins and found them to be 0.34, 0.90, 4.55, 0.60 µg/mL for niacin, thiamin, riboflavin and vitamin B12, respectively.
Water:
Colostrum and milk are both essential in providing water to the calf in order to prevent dehydration. Diarrhea causes death due to dehydration. So, it is crucial that calves are provided with sufficient water free choice even in addition to colostrum and milk.

Factors Affecting Colostrum Quality:
Colostrum quality is defined by the concentration of Ig within the colostrum (NAHMS, 2007). Ideally, the dairy industry aims for cows to produce colostrum with a quality of $\geq 50$ g/L of IgG (NAHMS, 2007). A variety of factors can impact the quality of colostrum and these are explained below.

Breed:
The amount of IgG in colostrum is different among beef and dairy breeds. Guy et al. (1994) found that 2.5 times the amount of IgG$_1$ was removed from the serum in dairy cattle than that of beef cattle during colostrogenesis. However, the concentration of IgG$_1$ in the colostrum of beef cattle was greater than that of dairy cattle (Guy et al., 1994). The reason for this is thought to be because there is a greater effect of IgG$_1$ dilution in dairy cattle (Guy et al., 1994).

In terms of dairy breeds there are some noted differences in IgG concentration. Morin et al. (2001) analyzed the specific gravity of colostrum from four different breeds in order to determine colostrum quality. In that study, they found that Brown Swiss and Ayrshire cows had colostrum with lower specific gravity than that of Holsteins and Jerseys. Hence, concluding that the colostrum of Brown Swiss and Ayrshire was of poorer quality. However, Morin et al. (2001) indicated that the specific gravity of the colostrum might be impacted by various non Ig solids which may vary among breeds.

Morin et al. (2001) and Morrill et al. (2012) suggested that there was also no significant difference between the colostrum quality of Holstein and Jersey cows. However, Muller and
Ellinger (1981) reported that Jersey cattle tended to produce more IgG in comparison to Holstein, Guernsey, Ayrshire, and Brown Swiss. Also, Jerseys and Ayrshires tended to produce more total Ig than other breeds (Muller and Ellinger 1981). However, the number of Jersey cattle sampled in their study was less than for each of the other breeds (Muller and Ellinger 1981).

**Climate/Environment:**
Morrill et al. (2012) found that the concentration of IgG in colostrum was lower from farms in the Southwest in comparison to those from farms in the Northeast and Midwest US. However, there was no difference in IgG concentration between colostrum from the Southwest and Southeast (Morrill et al., 2012). There was no difference in total protein content of colostrum samples from farms in the Southwest and Southeast as well (Morrill et al., 2012). Also, protein content was similar between farms in the Northeast and Midwest (Morrill et al., 2012). However, samples from the Northeast and Midwest had higher protein content than those from the Southeast and Southwest (Morrill et al., 2015).

Morin et al. (2001) found that cows which calved in fall had highest quality colostrum and those that calved during the summer had the lowest. Gulliksen et al. (2008) found that cows which calved during late summer and fall had higher colostrum quality. However, in another study it was found that colostrum produced during late summer/early fall and winter was of the highest quality (Conneely et al., 2013). The lowest quality was produced late spring/early summer and fall (Conneely et al., 2013).

Cabral et al. (2016) reported that IgG concentration was negatively correlated to the number of days above 23°C during the 21 d prepartum period. This was likely due to an increase in colostrum yield with the higher temperature which diluted the concentration of IgG 23°C (Cabral et al., 2016). It was also found in this study that IgG concentration tended to be positively correlated with temperatures between 5° and 23°C. In other words, as the number of
days in the thermal neutral zone (5°C-23°C) during the prepartum period increases, it tends to increase the IgG concentration in the colostrum (Cabral et al., 2016).

**Parity:**

It has been found that as parity increased so did the concentration of IgG in colostrum (Morrill et al., 2012). These results are supported by another study which found that cows with a parity of 3 or greater had better colostrum quality (MacFarlane et al., 2015). This is likely to be a result of more exposure to antigens which causes a greater amount of antibodies in the circulation and mammary secretions of older cows (Cabral et al., 2016). It is also thought to be a result of a greater leakage of blood constitutes into the milk due to a breakdown of barriers from persistent but low grade inflammation (Liua et al., 2009).

Primiparous cows are reported to have the lowest quality colostrum (Morrill et al., 2012; Conneely et al., 2013). In many cases it is recommend to producers that colostrum from heifers be discarded because of it being of lower quality. However, in one study it was found that only 10% of heifers had colostrum quality below the recommended 50 g/L (Conneely et al., 2013). Another study found that it was 2nd parity cows that had the lowest quality colostrum (Gulliksen et al., 2008). As a result, it is suggested that producers test colostrum for IgG.

**Maternal Diet:**

Cows fed a diet deficient in protein were found to have no difference in colostral IgG1, IgG2, IgA, and IgM in comparison to cows fed diets with adequate protein (Burton et al., 1984). In another study, it was found that cows fed a high energy diet, 150% of their requirement, had lower colostral IgG than cows fed a diet at 100% of their dietary requirement (Mann et al., 2016).

A recent study also found the supplementation of nicotinic acid increased the concentration of colostral IgG (Aragona et al., 2016). Although the exact mechanism is not fully
understood, it is thought to be either through a greater transport of IgG into the mammary gland by vasodilation or an increase in microbial protein (Aragona et al., 2016).

**Maternal Dry Period:**

Cows which receive no dry period but are continuously milked have lower concentrations of IgG, IgA, and IgM in their colostrum in comparison to cows which receive a dry period (Verweij et al., 2014). Another study found lower IgG concentration in the colostrum of primiparous cows that did not receive a dry period (Annen et al., 2004). However, it was also noted that there is no difference in colostrum quality between primiparous cows that receive 30 and 60 d dry periods (Annen et al., 2004). In addition, there was no impact of length of dry period on the IgG concentration in colostrum from multiparous cows (Annen et al., 2004).

Cabral et al. (2016) also found that the number of days dry was positively correlated with colostral IgG concentration. In addition, the length of the previous lactation and number of days open were also positively correlated with the quality of colostrum (Cabral et al., 2016). It is thought that a greater length in lactation and open period provides more time for the cow to become metabolically stable before the next pregnancy and lactation (Cabral et al., 2016).

**Time of Milking:**

Time of milking is suggested to play a role in the quality of colostrum. The quality of colostrum measured via refractometer in one study was found to decrease by 0.28 units each hour after parturition (MacFarlane et al., 2015). Connelly et al. (2013) only found a decrease in colostrum quality when it was harvested 9 to 12 h after parturition. Cabral et al. (2016) found no effect of time of harvest on colostrum quality. However, in their study colostrum was harvested about 4.75 h after calving (Cabral et al., 2016).

On large farms (≥ 200 cows) about 68% were milked 2 to 6 hours after calving (Kehoe et al., 2007). In fact, no large farms in that study reported milking cows within the first 1-2 h after
calving (Kehoe et al., 2007). Kehoe et al., (2007) also found that small (≤ 100 cows) and medium (101–200 cows) farms milked their cows sooner after calving than those on large farms. This difference, as well as others, in milking time is attributed to differences in management methods (Kehoe et al., 2007).

**Storage Method:**
A recent study reported that refrigeration does not affect IgG or fat content (Morrill et al., 2012). However, colostrum samples that were refrigerated were reported to have higher protein content in comparison to those that were frozen or kept fresh (Morrill et al., 2012). The exact reason for higher protein contents with refrigerator is not completely understood.

**Pasteurization:**
Studies have found that heat treating colostrum to 60°C for 60 min does not affect IgG levels of colostrum while eliminating harmful bacteria (Donahue et al., 2012; Godden et al., 2012). McMartin et al. (2006) reported that colostrum could be heat treated at 60° for 120 min without altering IgG concentration while destroying bacteria.

   However, Donahue et al. (2012) reported when high quality colostrum was heated to 60°C for 60 min decreased IgG concentrations. In another study it was found that a heat treatment at 60°C for just 30 min decreased IgG concentration (Gelsinger et al., 2014). McMartin et al. (2006) found that heat treating colostrum to 63°C reduced IgG concentration by 34%. Elizondo-Salazar et al. (2010) reported that heat treatments of colostrum ≥ 60°C will denature IgG₁ as well. In addition, it was reported in this study that a heat treatment 63°C caused the greatest reduction in colostral IgG concentration (Elizondo-Salazar et al., 2010).

**Methods for Testing Colostrum Quality:**
There are different methods for testing colostrum quality. The first of these methods is the brix refractometer. Normally, the brix refractometer is used to measure sucrose
concentrations but when no sucrose is present in a solution it can be used to measure the total solids (Quigley et al., 2013). A recent study has determined that the use of the brix refractometer is an acceptable method for measuring colostrum quality (Quigley et al., 2013). This is in part due to the fact that it gives quick results on colostrum quality and can be used easily on farms. Good quality colostrum (>50 g/L) is classified as having a brix reading of ≥ 21% (Quigley et al., 2013). If colostrum is fed and has a brix reading of < 8.4% then it is very likely that there will be a failure of passive transfer (Deelan et al., 2014).

Another method for measuring colostrum quality is via a colostrometer. In this method, colostrum quality is estimated via specific gravity. Fleenor and Stott (1980) showed that there was a linear relationship between colostral IgG concentrations and specific gravity. However, factors such as breed and colostrum temperature can impact the accuracy of reading the specific gravity (Quigley et al., 2013). A specific gravity of > 1.046 is considered good quality colostrum (Fleenor and Stott, 1980). Poor quality colostrum has a specific gravity value of < 1.035 and moderate quality is from 1.035 to 1.046 (Fleenor and Stott, 1980).

Finally, the last method for evaluating colostrum quality is via radial immunoassay. In this method colostrum or blood samples are placed into wells on an agar plate and then allowed to sit for at least 36 h. Over this period the antibodies diffuse out into the agar gel and the diameter of the diffusion ring is measured (Fleenor and Stott, 1981). These values are then calculated using the slope and intercept from the linear equation generated from the standards. This method is said to be statically reliable (Fleenor and Stott, 1981). As a result, radial immunoassay is often used in research and by laboratories. However, because producers need immediate results when determining to feed maternal colostrum, it is not practical on farms.
Passive Transfer:
As mentioned earlier, dairy cattle are unable to transfer antibodies such as Ig to their offspring in utero (Akers, 2002). As a result, calves are born agammaglobulinemic and must receive their immunity through the consumption of the mammary secretions. This process is more commonly referred to as passive transfer (Baumrucker, 2010).

In the dairy industry passive transfer is defined as the transfer of IgG from the colostrum to the calf. Ideally, producers strive to reach 15 g/L of IgG in the serum of the calf by 48 h (Akers, 2002). However, it is suggested that 10 g/L of IgG in the serum of the calf by 48 h is adequate (Akers, 2002).

It is essential that calves receive colostrum as soon after birth as possible in order to achieve passive transfer. This is because by 24 h of age calves are said to no longer be able to absorb IgG (Conneely et al., 2014). The reason for this is because the tight junctions between the epithelial cells within the gastrointestinal tract of a calf begin to closes. This closure of the tight junctions in turn prevents the passage of larger molecules consumed, such as immunoglobulins, through the intestinal lining into the blood stream of the calf.

As a result, age at which the calf consumes colostrum plays a very important role in determining passive transfer. The quality of colostrum also plays another role in determining the passive transfer of IgG as well. However, there are other factors that effect passive transfer and the apparent efficiency of absorption (AEA) for IgG.

Factors Affecting Passive Transfer and Rate ofAbsorption:
Breed:
Roy (1980) noted that there appears to be differences among dairy breeds in terms of Ig absorption. Although the exact reason for this has not been fully explained, it is thought to be result of blood plasma volume. Quigley et al. (1998) found that breed impacted blood plasma
volume. In this study, it was found that the plasma volume of Jersey and Holstein calves was 9.71 and 9.94 % respectively (Quigely et al., 1998).

However, it was body weight of the calf that had the main impact on blood plasma volume (Quigley et al., 1998). As a result, differences in serum IgG levels may be attributed to differences in body weight. This means smaller calves may exhibit higher serum IgG concentrations because of a lesser blood plasma volume and dilution effect. Jones et al. (2014) found that Jersey calves had higher serum IgG levels at 24 h than Holstein calves (16.47 ± 0.71 and 11.12 ± 0.60 g /L respectively) and higher AEA for IgG (21.9 ± 0.9% and 17.0 ± 0.7 % respectively)

**Sex:**

It was determined that sex does not affect the likelihood of passive transfer of IgG (MacFarlane et al., 2015). However, sex is often referred to as a factor that influences passive transfer. In particular, it has been noted that heifer calves have higher serum IgG levels than bull calves (Quigley and Drewry, 1998). The exact reason that heifer calves have higher serum IgG levels is still unknown. It is thought that maybe the larger size of bull calves may influence their serum levels and dilution of IgG as well as the absorption of it (Quigley and Drewry, 1998).

**Dystocia:**

A study performed on dairy farms in the United Kingdom found that calves which experienced dystocia had a lower passive transfer of IgG (MacFarlane et al., 2015). This was also confirmed by a study performed with beef cattle (Waldner and Rosengren, 2009). This reduction in passive transfer is said to be caused by acidosis as a result of a prolonged calving process. In turn, acidosis weakens the calf and makes it less likely to nurse (Waldner and Rosengren, 2009). However, the method of feeding colostrum such as in the case of stomach
tubing can overcome this by depositing colostrum directly into the rumen without the calf needing to suckle.

**Maternal Diet:**

Calves had lower absorption of IgG when fed colostrum from cows fed a diet low in energy and CP (Quigley and Drewry, 1998). Another study also documented that calves born from dams fed a diet with restricted protein had lower absorption of all Ig; IgA, IgG1, IgG2, and IgM (Burton et al, 1984).

A study performed in Oregon found increased serum IgG levels in calves from cows fed 105 mg of Se-yeast/d (Hall et al., 2014). The absorption of IgG of these calves at 48 h was about 62% and higher than control calves as well (Hall et al., 2014).

Gao et al. (2012) reported that calves from dams fed low energy (5.25 MJ/kg of DM) diet 21 d prior to parturition had reduced immunity, antioxidant capacity, growth, and development. In particular, interleukin-2 and 4 (IL-2 and IL-4), were decreased in comparison to calves from cows fed an energy adequate diet (Gao et al., 2012). Interleukin-2 is involved in T cell proliferation and functioning whereas IL-4 plays a role in the synthesis of IgE, IgG1 and IgG2 (Gao et al., 2012). As a result, the immune system of these calves was likely compromised.

**Environment:**

In a study performed by Stott et al. (1976) it was found that newborn calves exposed to hotter temperatures had lower IgG1 absorption. It is thought that the higher rate of corticosteroid produced as a result of heat stress was responsible for this by decreasing the permeability of the intestinal lining (Stott et al., 1976).

Tao et al. (2012) found that calves born from cows that were heat stressed had lower plasma protein levels, AEA of IgG, and serum IgG levels. It was thought that higher cortisol levels as a result of stress would have been the result of these decreased parameters. However, in
this study there was no difference in the circulating cortisol levels of the cow during the prepartum period. As a result, it was unlikely that the cortisol levels of the cow played any role in reducing the passive transfer of IgG of those calves.

Olson et al. (1980) also reported that calves which experienced cold stress had decreased rate of absorption for IgG₁, IgG₂, and IgM within the first 15 h after parturition. Overall, net absorption of IgG₁, IgG₂, and IgM was not different between treatments (Olson et al., 1980). The exact mechanism in which cold stress impacted Ig absorption was not determined in this study (Olson et al., 1980).

**Pasteurization:**

Tyler et al. (2000) found that calves fed colostrum heat treated at 73°C had reduced serum IgG concentrations. It was suggested in this study that the IgG in colostrum was either destroyed or altered in a form which prevented absorption. Gelsinger et al. (2014) confirmed that colostrum that was heated treated to 63°C for 60 min has decreased concentration of IgG.

However, Gelsinger et al. (2014) found that heat treating colostrum to 60°C for 30 min increased serum IgG concentrations by 18.4% and AEAy 21% in calves. Another study reported that calves fed colostrum heated to 60°C for 60 min had higher serum IgG concentrations as well (Godden et al., 2012).

These same calves when followed had a lower rate of pre-weaning illness which is suggested due to lower bacterial contamination of colostrum and higher immunoglobulin concentrations (Godden et al., 2012). Feeding heat treated colostrum has also been shown to promote the colonization of beneficial *Bifidobacterium* while decreasing the colonization of *E. coli* in the small intestine of calves (Malmuthuge et al., 2015). Hence, suggesting that pasteurization of colostrum helps to establish healthy microbial populations within the gastrointestinal tract of calves.
**Feeding Method:**
Godden et al. (2009) found that there was no difference in 24 h serum IgG between calves fed with nipple bottles vs. stomach tubes. Chigerwe et al. (2013) also found that there was no difference in serum IgG, IgA, and IgM concentrations at 24 h between calves fed by either nipple bottle or stomach tube. The AEA of IgG between calves fed via bottle and stomach tube was not different (Godden et al., 2009; Chigerwe et al., 2013). Only when feeding smaller volumes of colostrum was there a higher AEA and serum IgG concentrations were higher for calves fed with nipple bottles (Godden et al., 2009).

Besser et al. (1991) found that 61% of calves allowed to suckle their dams had a failure of passive transfer. This same study found that 19% of calves fed by nipple bottle had failure of passive transfer in comparison to just 11% fed via stomach tube (Besser et al., 1991). However, the three methods were not conducted at each site but instead at three separate sites (Besser et al., 1991). So, management factors at each location may have confounded these results.

A study conducted by Brignole and Stott (1980) found that 42% of calves allowed to suckle on their dams had failure of passive transfer. This was due either to calf not suckling or did not suckle enough colostrum.

**Time of Feeding:**
As mentioned above, it is essential that calves receive colostrum as soon after birth as possible in order to achieve passive transfer. This is because by 24 h of age calves can no longer absorb IgG (Conneely et al., 2014). The reason for this is because the tight junctions between the epithelial cells within the small intestine of a calf begin to close. This closure of the tight junctions in turn prevents the passage of larger molecules consumed, such as Ig, through the intestinal lining into the blood stream of the calf.
In a recent study it was found that peak IgG absorption happened at 24 h of age in calves (Conneely et al., 2014). Calves are usually fed colostrum about 4 h after birth (Kehoe et al., 2007). Kehoe et al. (2007) found that 51% of farms feed colostrum 2-6 h after birth while 43.6% of farms fed it within the first 2 h.

Oskara et al. (2014) showed that AEA of IgG declines slowly up to the 12th h. After 12 h it declines rapidly (Oskara et al., 2014). As a result, these authors suggested two methods in order to obtain the optimal >10 g/L of serum calves at 24 h of age. The first is to feed 120 g of IgG within 1 h of birth and the other is 125 g of IgG from 1-6 h after birth. However, it was also suggested in this study that quality and quantity of colostrum have more to do with IgG uptake by calves than time of feeding (Oskara et al., 2014).

**Quantity:**

In a study performed in Ireland, calves fed at a rate of colostrum of 8.5% of body weight (BW) had greater serum IgG concentrations at 24 h than those fed colostrum at 7% or 10% of BW (Conneely et al., 2014). It was surprising that calves fed colostrum at a rate of 10% of BW had lower serum IgG concentrations. However, this is thought to be due to a greater distension of the abomasum because of the greater volume which slowed the rate emptying and absorption of IgG (Conneely et al., 2014). This higher level of serum IgG concentrations for those calves fed 8.5% was carried through the first 72 h of life (Conneely et al., 2014). Even at 642 h of age these calves fed colostrum at 8.5% their BW had higher serum IgG levels than those fed at 7% of BW (Conneely et al., 2014). However, it was not until 10 wk of age did the serum levels of those calves fed 8.5% become similar to that of those fed 10% (Conneely et al., 2014). As a result, this study suggests that the quantity of colostrum fed can only impact serum IgG levels of the calf to certain point (Conneely et al., 2014).
Previous studies have suggested that larger quantities of colostrum fed to calves do not equal greater serum IgG levels. A study cited by Conneely et al. (2014) reported that calves fed 4 L of colostrum via stomach tube had lower serum IgG levels at 48 h of age than those fed 3 L. It is thought that large quantities of colostrum fed at once might cause distension and reduction of emptying in the abomasum which slows the rate of IgG absorption (Conneely et al., 2014). Conneely et al. (2014) did not find that feeding a lower rate of colostrum at 7% of BW decreased IgG uptake by the calves, but that might have been because colostrum used in that study was of good quality (≥ 50 g/L).

Faber et al. (2005) found that heifer calves fed 4 L of high quality colostrum (50-140 mg/mL of IgG) gained more BW in comparison to those only fed 2 L of colostrum. It was also found that those calves fed 4 L of colostrum had less veterinary costs for disease treatment than those only fed 2 L (Faber et al., 2005). In addition, those heifers fed 4 L of colostrum produced more milk in their first and second lactation (Faber et al., 2005).

**Quality:**

Besser et al. (1985) found that as the concentration of IgG in colostrum increase the AEA of IgG decreased. However, as the concentration of IgG increases in the colostrum so does the concentration in the serum of the calves that consume it (Besser et al., 1985). Morin et al. (1997) also found that calves fed higher quality colostrum had higher serum IgG concentrations at 48 h of age.

**Measuring Passive Transfer:**

The most common way to measure for passive transfer is by looking at the serum IgG concentrations of the calves. This is done via taking blood samples from calves at 24 h of age and analyzing these samples via radial immunoassay for IgG. The concentration of IgG should be ≥15 g/L. If concentration of IgG is < 10 g/L, than there will be failure of passive transfer.
Another method for measuring passive transfer is by looking at serum protein levels. Tyler et al. (1998) suggested that calves with serum protein levels of > 5.0 g/dL had adequate passive transfer and were more likely to survive. Those calves which had serum proteins ≥ 5.5 g/dL had the best survival rate while those who had < 5 g/dL had the highest risk of mortality (Tyler et al., 1998). This indicates that failure of passive transfer occurs at serum protein concentrations of < 5 g/dL.

**Improving Colostrum Quality:**

As explained above factors such as breed, climate, parity, maternal diet and dry period, time of milking, storage method, and pasteurization can influence the quality of colostrum. Although producers strive to lessen the effect of these factors by the best of their means they still cannot completely avoid the production of poor quality colostrum. As a result, producers need to employ other means in order to ensure that calves consume adequate amount of IgG. The industry has developed different methods which producers can use to improve the quality of colostrum or provide a sufficient replacement of it when other factors like disease eliminate the use of maternal colostrum.

**Pooled Maternal Colostrum:**

In the case of pooled colostrum it is basically as the name describes. This is not recommended because it can possibly spread diseases such as Johne’s and Leukosis if not monitored. However, it does provide means of increasing poor quality colostrum by mixing with others of good quality.

It was found by Arthington et al. (2000) that calves that consumed pooled maternal colostrum had greater serum IgG concentrations at 24 h than those calves fed colostrum and bovine serum derived colostrum supplements (Arthington et al., 2000). However, the AEA of
IgG for pooled maternal colostrum was less than that of the bovine serum derived supplement (Arthington et al., 2000).

**Colostrum Supplements:**

Colostrum supplements are primarily derived from colostrum, milk, bovine serum, and chicken egg extracts (Quigley et al., 2001). The role of these supplements is to provide exogenous IgG to the calves (Cabral et al., 2013). Bovine serum supplements are the most promising within the industry due to the fact that they are inexpensive and can be easily obtained from slaughtering facilities (Cabral et al., 2012).

Commercial colostrum supplements provide 25-45 g of IgG per dose (Quigley et al., 2001). It is suggested by veterinarians that calves receive about 100 g of IgG within the first 12 h of life (Quigley et al., 2001). There is concern that calves, especially small breeds like Jerseys, are unable to consume the volume of colostrum required to ensure adequate amounts of IgG (Haines et al., 1990). Hence, confirming that producers cannot completely rely on colostrum supplements to provide all of the IgG needed by the calf (Haines et al., 1990). This means it is important that producers strive to find the best quality colostrum supplement possible in order to ensure the adequate amount of IgG for their calves.

Arthington et al. (2000) found that calves fed colostrum supplemented with bovine serum containing 90 g of IgG/dose had a greater AEA of IgG. These same calves also tended to have fewer treatments for illness than those calves that received colostrum or colostrum replacer (Arthington et al., 2000). Santoro et al. (2004) also reported that calves fed colostrum supplement were medicated less and had better fecal scores during the pre and post-weaning periods. However, this was likely due to the fact that the colostrum supplement had a greater IgG content than the pooled maternal colostrum (10 vs. 7.3%).
A study performed by Morin et al. (1997) found that the addition of colostrum supplement to poor quality colostrum actually decreased the AEA of IgG in calves. It was commented by Morin et al (1997) though that similar results were found in previous studies evaluating colostrum supplements made from colostrum and whey products.

**Colostrum Replacer:**

Colostrum replacers were developed in order to provide full replacement of maternal colostrum when needed (Cabral et al., 2013). Most colostrum replacers are derived from lacteal secretions or serum like colostrum supplements. Cabral et al. (2013) noted that colostrum replacer is an acceptable option to replace maternal colostrum. In fact, majority of colostrum replacers provide > 100 g of IgG per mixed dose, but not all (Cabral et al., 2013).

Those colostrum replacers with <100 g of IgG per mixed dose will not provide passive transfer unless fed at greater quantity (Cabral et al., 2013). Quigley et al. (2001) suggested that a colostrum replacer with > 20% IgG should be fed to calves in order to prevent failure of passive transfer. The method of processing the colostrum replacer and the amount of times fed has a significant impact on the apparent efficiency of IgG absorption (Quigley et al., 2001).

The uptake of IgG by calves fed colostrum replacer is less than that of maternal colostrum (Cabral et al., 2013). Also, calves fed colostrum replacer are more likely to have failure of passive transfer than those fed maternal colostrum (Cabral et al., 2013).

**Colostrum Additives:**

Colostrum additives are products added to colostrum or colostrum replacer to help with IgG absorption in the calf. These products used include components like lactoferrin, sodium bicarbonate, trypsin inhibitor, and selenium (Se).
**Lactoferrin:**
Lactoferrin is a glycoprotein that binds iron in colostrum and milk (Shea et al., 2009). Robblee et al. (2003) found the supplementation of 1 g/d of lactoferrin to calves reduced fecal scores and number of days medicated. Also, calves supplemented with lactoferrin had greater growth rates and feed efficiency (Robblee et al., 2013). Hence, suggesting that lactoferrin has some role to play in improving intestinal health and absorption (Robblee et al., 2013).

However, when lactoferrin was added to colostrum replacer at a rate of 0.5 and 1 g/day it negatively impacted the absorption of IgG of the calves (Shea et al., 2009). Connelly and Erickson (2016) also found that supplementation of lactoferrin at 1 g/d did not impact IgG absorption or intestinal development in calves.

**Sodium bicarbonate:**
Another colostrum additive is sodium bicarbonate (NaHCO$_3$). It is used to buffer colostrum that has been acidified or fermented (Morrill et al., 2010). In one study it was found that addition 29.25 g/dose of NaHCO$_3$ to colostrum replacer increased serum levels and absorption efficiency of IgG (Morill et al., 2010). However, another study found that the addition of 30 g/dose of NaHCO$_3$ to colostrum and milk replacers did not impact serum concentrations or absorption of IgG (Chapman et al., 2012). In addition, Cabral et al. (2014) found that the addition of 30g/dose of NaHCO$_3$ to colostrum replacer actually decreased the AEA and 24 h serum concentrations of IgG in calves.

**Trypsin Inhibitor:**
Trypsin is a pancreatic enzyme that is involved in the digestion of proteins in the small intestine (Hall, 2011). Another substance produced by the pancreas called trypsin inhibitor helps to keep this and other proteolytic enzymes in check (Hall, 2011). In ruminants, this trypsin inhibitor is also present in the colostrum and at a greater level than in other species (Santoro et
al., 2004). This is likely to prevent the breakdown of Ig in colostrum by trypsin in the small intestine of the calves. Quigley et al. (1995) suggested that pancreatic secretions of trypsin might affect the absorption of IgG.

In addition, they also found that the addition of 1 g of trypsin inhibitor to colostrum increased the serum levels of IgG and IgM in the calves (Quigely et al., 1995). However, another study found that the addition of 0.5 g/feeding of trypsin inhibitor to colostrum and colostrum supplement did not impact the absorption or serum concentrations of IgG (Santoro et al., 2004).

**Selenium:**

Selenium is a mineral that play an essential role in the activity of the enzyme glutathione peroxidase which prevents reactive oxygen species from exerting cellular damage (Harvey and Ferrier, 2011). In cattle, Se has also been associated with neutrophil activity, lymphocyte production and activity, and enhancement of the humoral immune response (Spears, 2000). Kamada et al. (2007) found that the addition of 3 mg of Se/1 kg of colostrum increased the serum IgG and Se concentrations in calves. It was also noted in this study that the greatest concentration of IgG was exhibited at supplementation rate of 1 mg/kg (Kamada et al., 2007). However, in another study it was found that the addition of Se at 3 mg/1 kg of colostrum did not impact serum IgG concentrations until 14 d after parturition (Hall et al., 2014).
Part II: Direct-fed Microbials

Direct fed microbials (DFM) and probiotics are often used interchangeably. The U. S. Food and Drug Administration (FDA) define direct-fed microbials as “products that are purported to contain live (viable) microorganisms (bacteria and/or yeast)” (FDA, 2015). Prebiotics are non-living ingredients such as yeast cultures and oligosaccharides which help to promote the growth of naturally occurring microorganisms. Synbiotics are products which contain both probiotic and prebiotic ingredients.

In recent years, there has been a growing concern over the feeding of antibiotics in the livestock industry (Yoon and Stern, 1995). As a result, emphasis has been placed on the use of DFM to replace low dose antibiotic use in the livestock industry (Buntyn et al., 2016). Low dose antibiotics have been used to prevent and treat a wide variety of livestock diseases (Buntyn et al., 2016). The mechanism in which low dose antibiotics do this is by directly impacting the gastrointestinal tract microbes (Buntyn et al., 2016). The alteration of the gastrointestinal tract microbes is then what improves the health and digestion (Buntyn et al., 2015). In turn, it is the better health and digestion in the animal which then promotes better performance (Buntyn et al., 2016).

Direct-fed microbials do the same thing, but by indirectly impacting the gastrointestinal tract microbes (Buntyn et al., 2016). In cattle, the method in which DFM affect the gastrointestinal tract is via increasing microbial growth in the rumen, fermentation and digestibility, and flow of nutrients to the small intestine (Yoon and Stern, 1995). However, other observed benefits of DFM use include antibacterial, antitumor, anticholesterolemic, competitive attachment, and other immune response effects (Yoon and Stern, 1995). Various studies with poultry have showed that DFM increase and enhance many different aspects of immune response
(Lee et al., 2010; McAllister et al., 2011). However, these effects are very dependent on the type of microorganism present in the DFM.

**Bacteria:**

Bacteria are unicellular prokaryotic organisms (Murray et al., 2016). They are in general characterized by the fact that they lack nuclei (Murray et al., 2016). Bacteria often lack organelles like the endoplasmic reticulum, Golgi bodies, and mitochondria (Murray et al., 2016). As for the cell wall, it occurs in one of two forms. The first type of cell wall, which is often found in Gram positive bacteria, is made of a thick, mesh like peptidoglycan layer (Murray et al., 2016). The second type, which is found in the Gram negative bacteria, is made of two layers with one being a thin peptidoglycan layer and the other being a protective outer one (Murray et al., 2016).

Bacteria are classified based on shape, size, and special arrangements (Murray et al., 2016). Specific genotypic and phenotypic characteristics are used to classify them further (Murray et al., 2016). The use of Gram stain is another common and practical method used to distinguish bacteria (Murray et al., 2016).

Most bacteria used as probiotics or DFM are Gram positive (Murray et al., 2016). In total there are 39 different types of bacteria used as DFM (Buntyn et al., 2016). Lactic acid producing bacteria like *Lactobacillus*, *Enterococcus*, and *Bifidobacterium* are often used as DFM (Yoon and Stern, 1995).

Those bacteria classified as lactic acid producing such as *Lactobacillus* and *Streptococcus* are associated with several particular effects in ruminants. These effects include: 1) an increased supply of lactic acid within the rumen, 2) adaptation of rumen microorganisms to the increase of lactic acid, 3) increase in lactic acid using microorganisms, and 4) rumen pH stabilization (Seo et al., 2010). The effects of lactic acid using species such as *Megashphaera* and *Propionibacterium*
include lactic acid conversion of lactic acid to volatile fatty acids (VFA) like propionate within the rumen; increased ruminal pH levels by reduction of lactic acid; reduced methane production; and increased feed efficiency (Seo et al., 2010).

**Fungi:**
Fungi are either multicellular or unicellular eukaryotic organisms which are classified in their own kingdom (Murray et al., 2016). The appearance, shape, and size of the fungi structures vary greatly within the kingdom. However, there are several characteristics that are used to distinguish fungi as a whole from other eukaryotic organisms. The first is that they have thread like structures called hyphae which create vegetation like mats (Murray et al., 2016). Another main characteristic is that they reproduce by the production of spores or by a process called budding (Murray et al., 2016). Fungi also have cell walls composed of chitin and glycogen (Murray et al., 2016). In addition, instead of cholesterol in their cell walls they have ergosterol (Murray et al., 2016).

There is said to be hundreds of thousand different kinds of fungi (Murray et al., 2016). Mader (2007) noted that fungi are classified into three main groups which are then further divided. These groups are the Acomycetes, Basidiomycete, and Zygomycota (Mader, 2007). About 50% of all known fungi appear in the Acomycetes group and 80% of those fungi have some sort of medical importance (Murray et al., 2016). The fungi of medical importance within Acomycetes are divided up into four smaller classes; Eurotiomycetes, Pneumocystidomycetes, Saccharomycetes, and Sordaiomycetes (Murray et al., 2016). The classes of Eurotiomycetes and Saccharomycetes contain the most popular types of DFM use in livestock production; *Asperigillus oryzae* (Yoon and Stern, 1995) and *Saccharomyces cerevisiae* (Buntyn et al., 2016). *Saccharomyces cerevisiae* is a species of yeast commonly used in DFM (Buntyn et al., 2016). It appears in the Saccharomycetes class of the the Acomycetes group. The
Saccharomycetes class is made up on not only yeasts genus *Saccharomyces* but also *Candida* (Murray et al., 2016). In terms of yeasts, these unicellular organisms which reproduce by the process of budding or fission (Murray et al., 2016). On agar this reproduction leads to colonies that appear mucoid, pasty, or round (Murray et al., 2016).

In general, the effects of fungal DFM have six main effects within ruminants which include: 1) the reduction of oxygen, 2) reduction of ruminal lactic acid, 3) production of growth factors such as vitamins and other organic acids, 4) increased microbial populations and their activity, 5) improvement of microbial protein and VFA production, and 6) increased digestibility within the rumen (Seo et al., 2010).

**Feeding Direct-Fed Microbials:**

The use of fungi as DFM has been studied more extensively in dairy cattle nutrition than that of bacteria (Raeth-Knight et al., 2007). However, many different studies have been performed on the effects of feeding DFM. Much of these results are often conflicting. Possible reasons for this is because of different types, strains, and density of microbes used in DFM (Raeth-Knight et al., 2007).

**Transition Cows:**

A majority of DFM research in the dairy industry has focused on transition cows. This research has indicated that DFM containing *Saccharomyces cerevisiae* and *Enterococcus faecium* do not impact pre-partum DMI (Nocek et al., 2006; Oetzel et al., 2007). However, DFM supplementation during the transition period does increase postpartum DMI (Nocek et al., 2003; Nocek and Kautz, 2006). Wohlt et al. (1991) also found that commercial yeast (*Saccharomyces cerevisiae*) culture top dressed at 10 g/d increased DMI of primiparous dairy cattle for the first 6 weeks of postpartum.
Nocek et al. (2003) observed that cows fed 90 g/d of a DFM, containing both yeast (*Saccharomyces cerevisiae*) and bacteria (*Enterococcus faecium*), during the transition period had higher serum glucose and insulin as well as lower NEFA concentrations postpartum (Nocek et al., 2003). However, during the pre-partum period the concentrations of NEFA in the blood does not appear to be impacted by 2 g/d of DFM (Oetzel et al., 2007). It was also found that there was no impact on postpartum blood parameters for cows that were fed DFM only during the pre-partum period (Nocek et al., 2003).

Oetzel et al. (2007) found that DFM, containing both yeast (*Saccharomyces cerevisiae*) and bacteria (*Enterococcus faecium*), fed at 2 g/d increased the percentage of milk fat in first lactation cattle. Also, milk protein percentage was increased in cattle of second and greater lactations (Oetzel et al., 2007). No impact was found on milk yield (Oetzel et al., 2007; AlZahad et al., 2014b). However other studies have observed an increased milk yield for transition cows supplemented with 2 to 90 g/d of DFM, containing both yeast (*Saccharomyces cerevisiae*) and bacteria (*Enterococcus faecium*), in both pre and postpartum periods (Nocek et al., 2003; Nocek and Kautz, 2006; Oetzel et al., 2007).

No studies have evaluated colostrum quality from cows supplemented with DFM during the pre-partum period. Studies with poultry have indicated that DFM increase IgG and IgM concentrations and/or cells responsible for IgA, IgG, and IgM production (Lee et al., 2010). One study involving dairy goats looked at plasma IgA, IgG, and IgM concentrations.. No impact on Ig concentrations was observed in the goats supplemented with the DFM (Marakoudakis et al., 2010). However, only one species of bacteria was used in the DFM and goats were already well into their lactation before treatments were applied.
**Lactating Cows:**

Wohlt et al. (1991) found that primiparous cows fed 10 g/day of yeast culture through the first 18 wk of lactation had higher milk yields than those cows fed a control diet. Milk and milk fat yields were also shown to increase with 1g/d of live yeast (*Saccharomyces cerevisiae*) supplementation (Moallem et al., 2009). AlZahal et al. (2014a) found that cows supplemented with 4 g/d of dry active yeast had higher milk yields from wk 7 through 10 but not during the first 6 wk of lactation (AlZahal et al., 2014a). A study with both multiparous and primiparous cows in mid lactation fed 5 g/d of a DFM containing *Lactobacillus acidophilus* and *Propionibacteria freudenreichii* found no impact on milk composition or yield as well (Raeth-Knight et al., 2007).

Moallem et al. (2009) found no impact on milk fat and protein percentages in cattle supplemented with or without live yeast. In a study with dairy goats it was found that supplementing a probiotic containing *Lactobacillus reuteri*, *Lactobacillus alimentarius*, *Enterococcus faecium*, and *Bifidobacterium bifidum* at $10^9$ cfu/mL changed the profile of milk fatty acids (Apãs et al., 2015). In particular, it increased several polyunsaturated fatty acids (PUFA) which increased the overall PUFA concentration in milk (Apãs et al., 2015).

No difference in serum β-hydroxybutyrate concentrations were observed in lactating cows fed DFM (Oetzel et al., 2007; AlZahad et al., 2014b). Boyd et al. (2011) found no impact on blood glucose and urea N concentrations of primipaous and multiparous cows in mid lactation fed DFM containing bacteria as well.

Dry matter intake has been increased by 4% when live yeast was fed (Moallem et al., 2009). Feed efficiency has also been improved by 7% when mid lactation cows were supplemented with 60 g supplementation of yeast culture (Schingoethe et al., 2014). In this same study, DMI was found to not be impacted by yeast culture supplementation (Schingoethe et al.,
2014). It is thought that the higher feed efficiency may be due to a greater digestibility of the diet but this effect was not evaluated in this study (Schingoethe et al., 2014).

No impact was found on rumen fermentation and diet digestibility of mid lactation cows supplemented with a DFM containing bacteria (Raeth-Knight et al., 2007). In another study with lactating dairy cattle fed 1g/d of live yeast there was no impact on the total tract digestibility of dry matter (Moallem et al., 2009). However, Marden et al. (2008) found that supplementation with 5 g/d of live yeast increased the total tract digestibility of organic matter.

In addition, rumen pH was also higher in cows fed live yeast compared to those on the control diet (Marden et al., 2008). Moallem et al. (2009) also found that lactating cows supplemented with live yeast tended to have higher rumen pH and lower ammonia concentration immediately after feeding. Marden et al. (2008) cited that the higher rumen pH was due perhaps to a reduction in lactate.

AlZahal et al. (2014a) also found that the supplementation of cows with dry active yeast reduced subacute ruminal acidosis in lactating cows when switched from high forage to high grain diets. This is attributed to the higher DMI and ruminal pH as a result of the dry active yeast (AlZahal et al., 2014a).

In addition, cows fed 5 g/d of live yeast had higher concentration of VFA in comparison to control cows (Marden et al., 2008). However, the overall VFA concentration was not different from that of cows fed sodium bicarbonate (Marden et al., 2008). The concentration of propionate produced in cattle fed live yeast was greater than that of cows fed a control diet or one with sodium bicarbonate (Marden et al., 2008).

In vitro studies with fungal based DFM have been shown to increase VFA production (Martin and Nisbet, 1992). This is in response to an increase in cellulolytic bacteria and their
activity within the rumen. Yoon and Stern (1996) reported an increase in both cellulolytic and proteolytic bacteria with the supplementation of fungal DFM to lactating cows.

**Heifers:**

In a study with heifers fed different increments of yeast (*Saccharomyces cerevisiae*) culture it was found that dry and organic matter digestibility increased with dose (Lascano et al., 2012). The greatest effect on the digestibility of DM, NDF, and ADF was at the yeast culture supplementation rate of 30 g/d (Lascano et al., 2012).

Serum glucose concentrations were the highest at the supplementation rate of 30 g/d of yeast culture (Lascano et al., 2012). There was a trend \((P \leq 0.10)\) of glucose concentrations increasing with increased dosage of yeast culture (Lascano et al., 2012). However, the impact yeast culture has on serum glucose and triglyceride concentrations appear to be effected by the amount of starch in the diet (Lascano et al., 2012). Heifer calves fed a diet high in starch did not have a change in glucose concentrations with any level of yeast culture supplementation (Lascano et al., 2012). Triglycerides decreased with increasing yeast culture supplementation in heifers fed a low starch diet (Lascano et al., 2012). However, triglycerides increased with an increase in yeast culture supplementation in heifer calves fed a diet high in starch (Lascano et al., 2012). Blood urea N, creatine, and lactate remained unchanged with supplementation of yeast culture at any rate in heifers fed both low and high starch diets (Lascano et al., 2012).

It was found in weaned dairy goats that supplementation of probiotic bacteria increases the integrity of the intestinal villi (Apãs et al., 2014). Also, there were less oocysts of *Eimeria* spp. in the intestinal tract of goats receiving probiotic bacteria which suggests a decreased chance of coccidiosis in these animals in comparison to the control ones (Apãs et al., 2014).
Calves:

Clymer et al. (2015) found that heifer calves fed a DFM of yeast (*Saccharomyces cerevisiae*) and bacteria (*Kluyveromyces* spp.) had greater average daily gain (ADG) than those fed a DFM of just bacteria (*Propionibacterium freudenreichii* and *Lactobacillus acidophilus*). Both DFM used were mixed into the replacer and fed at rate of 1.2 billion cfu/d which was gradually decreased to 400 million cfu/d by the end of the study at d 63.

Another study with heifer calves fed 80 mL/d of live culture containing *Faecalibacterium prausnitzii* reported higher weight gains during the preweaning period (Foditsch et al., 2015). Jatkauskas and Vrontniakiene (2010) found increased ADG and BW in calves supplemented with 2.4 g/d of a probiotic containing *Enterococcus faecium*.

A study performed with bull calves also found that supplementation with a probiotic containing *Lactobacillus plantarum* increased the feed conversion ratio (Zhang et al., 2016). However, there was no impact on ADG or apparent digestibility in this trial which suggest the calves ate less (Zhang et al., 2016). The addition of *Bacillus subtilis* to the probiotic solution produced no additional effects on ADG and apparent digestibly as well.

Jatkauskas and Vronniakiene (2010) reported that calves receiving probiotics had greater DMI. Another study also found that the DMI of calves increased marginally for the first 4 wk when supplemented with yeast at 2% of the DM of the grain (Magalhães et al., 2008). However, after 4 wk of age there was no impact of yeast (*Saccharomyces cerevisiae*) on DMI (Magalhães et al., 2008). These researchers did not find any impact on feed efficiency or BW with the supplementation of yeast. Geiger et al. (2014) reported no impact of a DFM, containing unknown microbes supplemented at 4 g/d on the DMI and feed efficiency of bull and heifer calves.
Blood parameters or rumen pH were not impacted in calves supplemented with DFM as well (Geiger et al., 2014). This is thought perhaps because the DFM was placed in the replacer and not on the starter (Geiger et al., 2014). As a result, the DFM likely bypassed the rumen and eliminated the responses which usually occurs the in adult ruminants. Magalhães et al. (2008) also noted that yeast supplementation on starter grain for calves had no impact on blood glucose and BHBA concentrations as well.

Magalhães et al. (2008) found that the supplementation of yeast at 2% of DM of starter grain positively impacted calf health. In particular, it tended (P < 0.08) to decrease the incidence of diarrhea and fever (Magalhães et al., 2008). As a result, feeding yeast reduced health disorders, treatment with anti-inflammatory and anti-diarrheic drugs, and increased net income by $48/calf for those supplemented with yeast.

A reduction in the occurrence and length of scours as well as reduced mortality was reported in a study with veal calves fed 45 mL of probiotic containing six different strains of *Lactobacillus* (Timmerman et al., 2005). Foditsch et al. (2015) also reported similar results with heifer calves fed *Faecalibacterium prausnitzii* during the preweaning period. In fact, it was reported in one study that supplementation of probiotics to calves can decrease the rates of scours by 30% (Jatauskas and Vrotniakiene, 2010).

This reduction in the occurrence of scours among calves is likely due in part to reduction of the microbial factors that contribute to it. It has been reported that the supplementation of *Lactobacillus* at a rate of $5 \times 10^8$ cfu/L of milk had a reduction in fecal coliform counts over time (Ellinger et al., 1980). Jatauskas and Vrotniakiene (2010) found reduction in both fecal clostridia and enterococci counts with the supplementation of probiotics to calves as well.
Timmerman et al. (2005) also noted that supplementation of probiotics tended to reduce the need of antibiotics for other digestive and respiratory infections. Magalhães et al. (2008) found no impact of yeast supplementation on the occurrence of respiratory infections in calves. However, a study with heifer calves found that those supplemented with a bacteria based DFM had higher need for treatment of respiratory infections than those supplemented with a DFM combination of yeast and bacteria (Clymer et al., 2015).

Kawakami et al. (2010) found that phagocytic activity of leukocytes was increased in Holstein calves that received a probiotic containing *Lactobacillus plantarum* and *Candida* sp. Magalhães et al. (2008) also found that yeast culture supplementation in calves tended to increase the amount of phagocytized bacteria and their removal. However, there was no apparent impact of yeast culture supplementation on the number and activity neutrophils or the humoral immune response (Magalhães et al., 2008).

Quezada-Mendoza et al. (2011) also found no impact lymphocyte count, plasma IgG, and fecal and salivary IgG concentrations in calves fed 20 g/d of a probiotic that contained fermentation products of *Lactobacillus gasseri* and *Propionibacterium freudenreichii*. Al-Saiady (2010) reported that young bull calves supplemented with probiotic containing two types of *Lactobacillus* at a rate of 1.25 g/100 kg of milk had higher serum IgG concentration and white blood cell counts in comparison to those not fed probiotics (Al-Saiady, 2010). However, serum albumin, globulin, and total protein concentrations of these calves were not impacted (Al-Saiady, 2010).
Part III: Enzymes and Ruminant Nutrition

Enzymes are proteins that act to catalyze biological reactions but are not consumed during the reactions (Harvey and Ferrier, 2011). The structure of an enzyme contains a pit called an active site (Harvey and Ferrier, 2011). In this active site there are amino acid (AA) chains which allow the enzyme to bind to substances (Harvey and Ferrier, 2011). Some substances which bind to enzymes are called coenzymes (Harvey and Ferrier, 2011). These coenzymes help to make an active enzyme called a holoenzyme which can then bind and catalyze the reactions with its targeted substrate.

In most cases, enzymes are very specific to the type of substrate in which they bind and reactions which they cause (Harvey and Ferrier, 2011). Once the substrate binds to the active site of an enzyme it causes the initiation of the transition state (Harvey and Ferrier, 2011). After this, the active site then provides other catalytic groups which help to form and facilitate the transition state (Harvey and Ferrier, 2011). At the end of the transition state the substrate is no longer present, but instead there is a product (Mader, 2007). The release of the product completes the reaction and allows the active site on the enzyme to return to its previous state (Mader, 2007).

In similar details, most molecules do not readily react with one another unless activated to do so (Mader, 2007). There are various ways to activate molecules in order for them to react with one another. However, in general, it requires energy and the amount of it needed for a reaction to occur is called the energy of activation. This energy of activation is different depending on what reaction and organism the reaction is occurring in. The presence of an enzyme reduces the amount of energy needed to bring about this reaction (Figure 2).
Many factors can affect the velocity of a reaction catalyzed by enzymes (Figure 3). The main factors affecting it include pH, substrate concentration, and temperature of the environment in which the reaction is being catalyzed (Harvey and Ferrier, 2011). However, there are also other mechanisms which are in place to regulate the activity of enzymes as well. These factors which regulate enzymes include allosteric activation/inhibition, compartmentation, covalent control/modification, and induction/repression of genes. In most cases, enzymes have one or more of these regulations which allows them to be turned on and off depending on the needs and physiological situations.
Rumen Microorganisms and Enzymes:

In nutrition there is a wide variety of critical enzymes. However, when discussing the nutrition of herbivores those include not only the enzymes produced by the animal but those produced by the microorganisms which live in a symbiotic relationship within the animal as well. It is enzymes produced by the microorganisms which are responsible for the reactions which help to facilitate the breakdown of plant material. Herbivores have failed to evolve to produce these particular enzymes required to help breakdown plant sugars. As a result, they are dependent on the microorganisms for the production of these enzymes and the products which they produce for survival.

There are three main types of microorganism responsible for producing these enzymes that breakdown plant matter within the rumen; bacteria, fungi, and protozoa. A majority of rumen bacteria and prozotoa species have no preference of host species (Church, 1988). Geographical location appears to impact the diversity of rumen microorganisms to some degree (Church, 1988). However, it is the particular environmental factors of the rumen which have the
most influence on various populations of microorganisms and their activity. These factors include buffering capacity/pH, diet/nutrient availability, temperature, osmotic pressure, and oxidation reduction potential (Church, 1988).

**Bacteria:**

Bacteria are the largest group of microorganism found within the rumen at concentrations of $10^{10}$ to $10^{11}$ cells/mL of rumen fluid (Church, 1988). Most of these bacteria are obligate anaerobes (Church, 1988). However, the ones that are facultative anaerobes can be found in densities up to $10^7$ and $10^8$ cells/mL of rumen fluid (Church, 1988).

Bacteria are classified based upon the shape, size, and structure. In ruminant nutrition, it is common for bacteria to be classified based on the substrates fermented (Church, 1988). As a result, there are eight different groups of bacteria recognized by ruminant nutritionists. These groups are the cellulolytic, hemicellulolytic, pectinolytic, amylolytic, ureolytic, methane-producing, sugar-utilizing, acid-utilizing, proteolytic, ammonia-producing, and lipid-utilizing (Church, 1988).

The cellulolytic species are classified based on their ability to degrade the major plant cell wall component of cellulose (Church, 1988). These bacteria have the capability to produce the enzyme cellulase. Research has shown that cellulase is actually a complex of several enzymes (Church, 1988) which help to break the β bonds between the glucose molecules that make up cellulose. The major species of cellulolytic bacteria in the rumen are *Fibrobacter succinogenes, Ruminococcus flavefaciens, Ruminococcus albus,* and *Butrivibrio fibrisolvens* (Church, 1988).

Hemicellulolytic species are classified based on their ability to degrade hemicellulose. As in the case with cellulolytic species, the hemicellulolytic species produce hemicellulase in order to breakdown hemicelluloses. Hemicellulolytic bacteria often are classified as cellulolytic or
pectinolytic as well (Church, 1988). The reason for this is because in some species it is essentially the enzyme complex of cellulase which is responsible for breaking down the hemicellulose. In other cases, there is also the presence of pectinolytic enzymes such as exopeptate and endopeptate lyase (Church, 1988). The major species of hemicellulolytic bacteria are *Butrivibrio fibriosolvens*, *Fibrobacter succinogenes*, and *Ruminococcus* spp. (Church, 1988).

The major species of pectinolytic bacteria in the rumen are *Butrivibrio fibriosolvens*, *Prevotella ruminicola*, *Lachnospira multiparous*, *Succinivibrio dextrinosolvens*, *Prevotella bryantii*, and *Streptococcus bovis* (Church, 1988).

Finally, amylolytic species are those responsible for degrading amylose or starch. In most species this degradation of starch is facilitated by the extracellular enzyme α-amylase (Church, 1988). This particular enzyme randomly cleaves the chains of starch (Church, 1988). The level of starch in a diet will influence the presence and density of amylolytic bacteria in the rumen (Church, 1988). Major amylolytic bacteria in the rumen include *Streptococcus bovis*, *Ruminobacter ruminicola*, *Ruminobacter amylophilus*, and *Succinimonas amylolytica* (Church, 1988).

**Fungi:**
Fungi make up a much smaller portion of the rumen microbial community than that of bacteria and protozoa. In addition, rumen fungi are also anaerobic (Gordon and Phillips, 1988). There are five main genera of fungi that have been identified in ruminants. These genera are *Neocallimastix*, *Orpinomyces*, *Anaerimyces*, *Piromyces*, and *Caecommyces* (Dehority, 2003). It is yet to be determined if fungi significantly impact the function of the rumen (Church, 1988).

It has been established that fungi degrade both cellulose and xylan within the rumen environment (Church, 1988). Gordon and Phillips (1998) noted that rumen fungi will also breakdown cellobiose, fructose, gentiobiose, galactose, glucose, and mannose. Also, studies have
shown the rumen fungi produce a wide variety polysaccharide degrading enzymes including cellulases and xylanases (Gordon and Phillips, 1998). These enzymes are secreted externally or directly associated with the rhizomycelium (Gordon and Phillips, 1998). Many factors appear to impact fungi enzyme activity such growth conditions and presence of other biological substrates (Gordon and Phillips, 1998).

Protozoa:

Protozoa are the second largest group of microorganisms in the rumen with a concentration of $10^5$ to $10^6$ cells/mL (Church, 1988). About 60% of the microbial fermentation products in the rumen are a result of protozoa activity (Church, 1988). Most of rumen protozoa are classified as ciliates and all are anaerobic. There are six major genera of protozoa found in ruminants. These genera are *Isotricha*, *Dasytricha*, *Entodinium*, *Diplodinium*, *Epidinium*, and *Ophryoscolex* (Church, 1988).

*Isotricha* species primarily utilizes glucose, pectin, starch, and sucrose (Church, 1988). *Dasytricha* utilizes cellobiose, glucose, maltose, and starch (Church, 1988). *Diplodinium* species primarily utilize cellulose, hemicelluloses, and starch (Church, 1988). However, there are *Diplodinium* species that utilize glucose and sucrose as well (Church, 1988). As for *Dasytricha*, *Entodinium*, *Epidinium*, and *Ophryoscolex*, these genera use a variety of plant sugars which is often dependent on the species (Church, 1988).

In one study it was determined that protozoa species of *Epidinium caudatum*, *Ostracodinium obtusum dilobum*, *Eudiplodinium maggii*, and *Ophryoscolex caudatus* had the highest cellulase activity (Dehority, 2003). However, other protozoa species which have cellulase activity include *Metadinium affine*, *Eudiplodinium bovis*, *Polyplastron multivesiculatum*, *Diplodinium pentacanthum*, *Enoploplastron triloricatum*, *Ophryoscolex tricoronatus*, and *Ostracodinium gracile* (Dehority, 2003).
Enzymes – Animal Agriculture Application:

About 40 to 70% of forage dry matter (DM) is composed of plant cell walls (Beauchemin et al., 2004b). However, Beauchemin et al. (2004b) cited that only about 65% of that cell wall material is actually digested within the gastrointestinal (GI) tract. Other studies noted by Beauchemin et al. (2004b) found about 50% of cell wall material is digested in the GI tract with around 30% of it occurring in the rumen. In other words, there is a lot of plant material that never gets digested and goes to waste. As a result, the agriculture industry has been striving to find ways to increase the digestion of plant cell wall components.

There is an array of methods the industry uses in order to improve the digestion of plant cell wall components. These methods include biological, chemical, and physical methods. Biological methods include, but are not limited to, the use of bacterial and fungal extracts, molecular engineering, and plant breeding and selecting (Adesogan et al., 2014). The chemical methods include treatment of the forages with acids, alkalis, nitrates, and other chemicals which aid in degradation of the cellular components (Adesogan et al., 2014). Finally, the physical methods include the common feed industry practices such as cracking, heating, streaming, rolling, etc. (Adesogan et al., 2014).

The supplementation of exogenous digestive enzymes is a biological method employed by the agriculture industry to improve the digestibility of forages for ruminants. Most exogenous enzymes used in the ruminant nutrition are derived from either bacterial or fungal extracts. These enzyme containing extracts are usually done by batch fermentation (Beauchemin et al., 2004a). Once fermentation is complete, the enzymes are then separated from the microbes and other residues, concentrated, and purified (Beauchemin et al., 2004a).

The activity of enzymes is very dependent on the type and strain of bacteria and fungi (Beauchemin et al., 2004a). Common fungal species used to derive β-glucanase, cellulase,
hemicellulase, and xylanse activity include Aspergillus oryzae and niger, Trichoderma longibrachiatum, and Penicillum funiculosum (Beauchemin et al., 2004a). As for bacteria, many Bacillus species are used to obtain β-glucanase, cellulase, hemicellulase, and xylanse activity. However, there are many bacterial and fungal species used to produce exogenous fibrolytic enzymes for many industries besides that of the feed industry. Those enzymes used in the feed industry come in either two forms; liquid or granular and are applied to forage either prior to ensiling or directly at time of feeding.

**Enzyme Additives for Silage:**

In some cases fresh forages that are ensiled do not have sufficient populations of microorganism to facilitate proper fermentation of carbohydrates (Barnes et al., 2003). As a result, producers can apply silage additives at the time of ensiling in order to promote the fermentation of forages. These silage additives can be composed of an array of components but the primary ones are bacteria, enzymes, organic acids, mold inhibitors, sugars, or a combination of the latter (Barnes et al., 2003).

The goal of enzyme additives for silage is to breakdown the structural and nonstructural carbohydrates in order to release fermentable sugars (Barnes et al., 2003). These fermentable sugars then help to provide a growth medium which stimulates bacteria growth and production of lactic acid (Barnes et al., 2003). However, enzyme additives can also help with improving the quality of the forage such as by reducing the concentration of ADF and NDF (Barnes et al., 2003).

**Silage Fermentation:**

Dehghani et al. (2012) reported that alfalfa silage treated with an enzyme product containing glucanase, β-glucanase, and pectinase activity at a rate of 500 mg/kg DM decreased the ammonia, butyric acid, and pH levels of the silage. In turn, this enzyme treatment increased
the lactic acid concentration and overall fermentation of the alfalfa silage (Dehghani et al., 2012). Dean et al. (2005) found no difference in the lactic acid concentration of bermuda grass silage treated with various fibrolytic enzymes products. However, one particular type of enzyme product applied at a rate of 0.65, 1.3, 2.6 g/kg of DM decreased DM loss, pH, and ammonia concentration of bermuda grass silage in comparison to the control (Dean et al., 2005).

Sheperd and Kung (1996b) reported that an enzyme additive containing cellulase and hemicellulase activity at 0.22, 2.2, and 22 L/ton of corn silage had no impact on fermentation acids or nitrogenous compound within the silage at any stage of maturity. The addition of enzymes decreased ADF, hemicelluloses, and NDF across all the different stages of corn at ensiling (Sheperd and Kung, 1996b). Only in the milk and black stages of maturity did enzyme treatment decrease acid detergent lignin content in corn silage (Sheperd and Kung, 1996b).

Colombatto et al. (2004) found in an in vitro study that an enzyme additive derived from Trichoderma reesei decreased the ADF and NDF content of corn silage. In addition, this same enzyme additive increased the organic matter digestibility of the silage (Colombatto et al., 2004). However, a different enzyme additive derived from Aspergillus niger and Trichoderma spp. reduced the organic matter digestibility of corn silage (Colombatto et al., 2004).

Nadeau et al. (2000) found that the NDF concentration of alfalfa silage decreased with increasing levels of cellulase up to 10 mL/kg. It was also found that immature alfalfa was much more responsive to the addition of cellulase than more mature alfalfa (Nadeau et al., 2000). This is probably attributed to a lesser amount of lignin in the immature alfalfa which may improve the access of cellulase to the cellulose fibers (Nadeau et al., 2000). Holtshausen et al. (2011) observed an increased disappearance of ADF and NDF in alfalfa hay and barley treated with enzyme product containing endoglucanase and xylanase activity at 2 μL/g of substrate DM.
**Animal Production:**

Stokes (1992) found that the application of an enzyme product containing cellobiase, cellulase, glucose oxidase, and xylanase to 2nd crop grass legume silage at harvest increased DMI, milk production, and milk fat percentage of cows. It also was found that this enzyme treatment increased the milk fat percentage and content (Stokes, 1992). In a study with lactating dairy cattle it was found that the addition of an enzyme supplement containing cellulase and xylanase activity improved digestion and milk production (Yang et al., 1999). In fact, milk production was increased by 7% for cows fed both treatment rates at 2 g/kg of hay and 1 g/kg of DM in comparison to the control treatment (Yang et al., 1999).

Sheperd and Kung (1996a) found that the application of enzyme additive containing cellulase and hemicellulase activity at 220 mL/tonne at harvest did not impact DMI, ruminal fermentation, feed efficiency, or milk production and composition in multiparous cows in mid lactation. In a complementary lamb feeding study there were no observed impacts of enzymes on nutrient digestion with the exception that lambs fed silage treated with enzymes had higher N retention (Shepard and Kung, 1996a).

McAllister et al. (1999) found that feedlot steers fed barley ryegrass silage treated with an enzyme product containing cellulase and xylanase activity at different rates (0, 1.25, 3.5 or 5.0 L/t DM) tended to linearly increase DMI ($P = 0.09$) from day 56 to 120 and final weight ($P = 0.08$) after 120 d on a backgrounding ration. In addition, the feed efficiency and feed intake between day 0 and 56 was quadratically increased with enzyme supplementation (McAllister et al., 1999). The carcass characteristics of these steers were not impacted by enzyme supplementation (McAllister et al., 1999).
**Enzyme Feed Additives:**

Beauchemin et al. (1999) found no impact on milk production with the supplementation of an enzyme product which contained cellulase, pectinase, and xylanase activity at 2.5 g/kg of TMR DM. However, 4% fat corrected milk production tended to be greater with enzyme supplementation (Beauchemin et al., 1999). Holtshausen et al. (2011) found no impact of an enzyme product containing endoglucanase and xylanase activity fed at 0.5 or 1 mL/kg of DM to cows in early lactation as well. In addition, Reddish and Kung (2007) found no impact of various rates of dry enzyme supplementation containing cellulase and xylanase activity on the milk production and composition of cows in early to mid lactation.

Holtshausen et al. (2011) did find that cows in early lactation fed an enzyme product had higher milk production efficiency. Beauchemin et al. (1999) also noted that enzyme supplementation numerically increased milk production efficiency. No other impacts on milk production were found with enzyme supplementation by Holtshausen et al. (2011). However, it was noted by Beauchemin et al. (1999) that enzyme supplementation increased milk protein content.

Reddish and Kung (2007) found no impact of enzyme supplementation on DMI. Rode et al. (1999) also found no impact of enzyme supplementation containing amylases, cellulases, and xylanases at rate 1.3 g/kg of TMR DM on DMI on cows in early lactation. Similar responses were observed for DMI of transition cows fed α-amylase at 0.1% of DM (DeFrain et al., 2005) and primiparous cows in mid lactaion fed 10 g/d of amylase (Nozière et al., 2014). In addition, Yang et al. (2000) found no impact on DMI of cows supplemented with an enzyme product containing cellulase and xylanase activity at a rate of 50 mg/kg of DM.

Beauchemin et al. (1999) reported that enzyme supplementation improved the ruminal NDF digestion of the diet containing hull-less barley in comparison to the one containing regular
barley. Rode et al. (1999) reported that enzyme supplementation increased the digestibility of ADF, DM, CP, and NDF. Total tract digestibility was reported to be higher in early lactation cows supplemented with enzymes (Yang et al., 2000). Also, digestibility of starch and true ruminal digestibility of organic matter was higher in cows supplemented with amylase (Nozière et al., 2014). However, Nozière et al. (2014) reported no difference in digestibility of DM, ADF, or NDF with enzyme supplementation.

Chung et al. (2012) found no impact of supplementation of an enzyme product containing endoglucanase and xylanase at a rate of 1 mL/kg of DM on VFA, NH₃, or CH₄ production. In addition, there was no impact of this enzyme treatment on the total population densities of bacteria, methanogens, and protozoa within the rumen (Chung et al., 2012). Enzyme supplementation did increase particular types of bacteria in the rumen such as Ruminobacter amylophilus (Chung et al., 2012). In addition, Fibrobacter succinogenes tended \( (P = 0.06) \) to increase with increasing supplementation of enzymes (Chung et al., 2012). Beauchemin et al. (1999) found that the supplementation of an enzyme product which contained cellulase, pectinase, and xylanase activity at 2.5 g/kg of TMR DM increased microbial N.

The supplementation of amylase tended to increase the total concentration of VFA in the rumen with the proportion of propionate being increased and acetate and butyrate being decreased (Nozière et al., 2014). Supplementation of amylase to transition cows has also been shown to increase the prepartum concentrations of BHBA and NEFA and the postpartum concentrations of glucose (DeFrain et al., 2005).
CHAPTER II
THE IMPACT OF DIRECT-FED MICROBIALS ON THE HEALTH AND PERFORMANCE OF HOLSTEIN CATTLE WITH EMPHASIS ON COLOSTRUM QUALITY AND SERUM IMMUNOGLOBULINS IN THE CALF

Introduction
The production and rearing of calves is an essential component of the dairy industry. Without healthy calves to serve as replacements for older and less productive animals, the industry would not be able to sustain and even increase the production of milk. As a result, attention needs to be given to the health and nutrition of calves.

Attention to the health of the calf needs to be applied even before it is born. As a result, the feeding and management of transition cows become important as well. Tao et al. (2012) found that calves born from cows that were heat stressed during the prepartum period had lower plasma protein concentrations, AEA of IgG, and serum IgG concentrations. Gao et al. (2012) reported that calves from dams fed a low energy (5.25 MJ/kg of DM) diet 21 d prior to parturition had reduced immunity, antioxidant capacity, growth, and development in comparison to calves from cows fed a diet with adequate energy. In particular, interleukin-2 and 4 (IL-2 and IL-4) were decreased. Interleukin-2 is involved in T cell proliferation and functioning whereas IL-4 plays a role in the synthesis of IgE, IgG1 and IgG2 (Gao et al., 2012). As a result, the immune system of these calves was likely compromised. Burton et al., (1984) found that calves born from dams fed a diet with restricted protein had lower absorption of IgA, IgG1, IgG2, and IgM (Burton et al, 1984). This was further supported by another study which documented that calves had lower absorption of IgG when fed colostrum from cows fed a diet low in energy and CP (Quigley and Drewry, 1998).
Calves are born agammaglobulinemic. This is because cattle have synepithelialchorial placentae which prevent transfer of Ig from the dam to the offspring in utero (Akers, 2002). As a result, cattle and other ruminants have evolved to transfer their Ig via the mammary secretion of colostrum. Colostrogenesis is defined as a particular phase of mammary development in which maternal Ig are transferred into the mammary gland prior to parturition (Barrington et al., 2001). The transfer of Ig starts several weeks prior to parturition, but ceases the day of it (Brandon et al., 1971). Over the course of colostrogenesis, up to 500 g of the IgG can be transferred into the mammary gland (Brandon et al., 1971). It is the higher level of IgG, in particular IgG₁, which is the difference between colostrum and milk (Brandon et al., 1971).

Colostrum quality is basically defined by the concentration of Ig (IgA, IgG, ad IgM) within the colostrum (NAHMS, 2007). However, it is common for colostrum quality to be based just on IgG content. Ideally, the dairy industry aims for cows to produce colostrum with a quality of ≥ 50 g/L of IgG (NAHMS, 2007). There are many factors which can impact colostrum quality such as breed, climate/environment, parity, maternal dry period, maternal diet, time of harvest, and pasteurization. In turn, the quality of colostrum also plays an important role in determining the passive transfer of IgG.

In the dairy industry, passive transfer is defined as the transfer of IgG from the colostrum to the calf. Ideally producers strive to reach 15 g/L of IgG in the serum of the calf by 48 h (Akers, 2002). However, it is suggested that 10 g/L of IgG in the serum of the calf by 48 h is adequate (Akers, 2002). It is essential that calves receive colostrum as soon after birth as possible in order to achieve passive transfer. This is because by 24 h of age calves are said to no longer be able to absorb IgG (Conneely et al., 2014) because the tight junctions between the epithelial cells within the gastrointestinal tract of a calf begin to close. This closure of the tight
junctions in turn prevents the passage of larger molecules consumed, such as Ig, through the intestinal lining into the blood stream of the calf. Hence, age at which the calf consumes colostrum plays a very important role in determining passive transfer. However, there are also many other factors such as breed, sex, dystocia, climate/environment, pasteurization of colostrum, and quantity of colostrum fed that can also affect passive transfer and AEA of IgG. As a result of so many factors impacting both passive transfer and colostrum quality, it is essential that there is sufficient research on all factors which producers can consult when making management and nutrition decisions.

Direct-fed microbials (DFM) are becoming increasingly popular in the feeding of ruminants with transition cow nutrition being one of the main areas of interests. Previous studies have indicated that DFM supplementation can increase prepartum DMI (Wohlt et al., 1991) and postpartum DMI (Nocek et al., 2003; Nocek & Kautz, 2006). In addition to higher DMI intakes post-partum, it was found that cows receiving DFM also had higher glucose and lower BHBA and NEFA levels in the blood (Nocek et al., 2003; Nocek and Kautz, 2006), suggesting that DFM supplementation helps to maintain health and performance of cows during the transition period. Prepartum and postpartum supplementation of DFM have been shown to increase milk yields postpartum (Nocek and Kautz, 2003; Nocek et al., 2006; Oetzel et al., 2006). However, impact on milk composition was conflicting. Some studies though have documented that supplementation of cow with DFM can increase the milk fat and protein percentage (Nocek et al., 2003; Oetzel et al., 2007).

No studies have evaluated the effects of prepartum DFM supplementation on colostrum quality in terms of IgG. A study using dairy goats found no impact of DFM supplementation on plasma IgA, IgG, and IgM levels (Marakoudakis et al., 2010). Concentration of Ig in the milk
was not evaluated in this study. Quezada-Mendoza et al. (2011) also found no impact on plasma, fecal or salivary IgG concentrations in calves fed 20 g/d of a probiotic that contained fermentation products of *Lactobacillus gasseri* and *Propionibacterium freudenreichii*. However, a study performed by Al-Saiady (2010) reported that young bull calves supplemented with a probiotic containing 2 types of *Lactobacillus* at a rate of 1.25 g/100 kg of milk had higher serum IgG concentration than those calves that did not receive probiotics. Studies with poultry have also indicated that DFM supplementation can increase IgG and IgM concentrations and/or cells responsible for IgA, IgG, and IgM production (Lee et al., 2010). Hence, it can be concluded that there is potential for DFM to illicit an immune response, such as an increase of IgG, in the cow which could possibly impact its health, colostrum, milk, and calf.

The supplementation of enzymes has also been shown to effect the health and performance of dairy cattle. In a study with lactating dairy cattle, it was found that the addition of an enzyme supplement containing cellulase and xylanase activity improved digestion and milk production (Yang et al., 1999). Milk production was increased by 7% for cows fed both treatment rates at 2 g/kg of hay and 1 g/kg of DM in comparison to the control treatment (Yang et al., 1999). Beauchemin et al. (1999) found that 4% fat corrected milk production tended to be greater and milk protein content was greater in cows supplemented with enzymes than those that were not. Holtshausen et al. (2011) also found that cows in early lactation fed an enzyme product had higher milk production efficiency compared to the control ones. However, no studies with dairy cattle have evaluated the impact of enzyme supplementation on colostrum yield, composition, and quality.

The objectives of this study were to evaluate the impact of feeding DFM alone or in combination with enzymes in comparison to a control on: 1) the quantity and composition of the
colostrum with focus on IgG and IgA; 2) weight of calf and Ig uptake; 3) DMI both pre and postpartum; 4) blood glucose, BHBA, and NEFA concentrations and 5) the quantity and quality of the milk produced during the first 8 weeks of lactation. Our hypotheses was feeding DFM alone or in combination with enzymes during the transition period would: 1) increase colostral IgG and IgA, 2) increase pre and postpartum DMI, 3) increase milk production, 4) increase pre and postpartum serum glucose concentration, 5) decrease pre and postpartum serum BHBA and NEFA concentrations, and 6) increase serum and apparent efficiency of absorption for IgG and IgA in the calf.

Materials & Methods

Experimental and Treatment Design:

This experiment was reviewed and approved by the University of New Hampshire Institutional Animal Care and Use Committee (Protocol #141107).

Thirty-six multiparous Holstein cows were used in this study. They were grouped into 12 blocks based on expected calving date. Within each block cows were randomly assigned to 1 of 3 treatments: 1) 0 g/d Tri-Lution® or Zy-mend® (Control); 2) 45.40g/d of Tri-Lution® (Tri); and 3) 45.40 g/d of Tri-Lution® and 18.16 g/d f Zy-mend® (Tri + Zy). Tri-Lution® is a DFM containing Enterococcus faecium and Saccharomyces cerevisiae at 1323 billion cfu/kg. Zy-mend is a combination of DFM and enzyme supplement that contains 882 billion cfu/kg. The DFM portion of Zy-Mend® is composed of Enterococcus faecium and Saccharomyces cerevisiae. The enzyme portion of Zy-mend® contains both amylase and cellulase activity. The ingredients of Tri-Lution® are calcium carbonate, rice hulls, active dry yeast, mineral oil, fructooligosaccharides, dried Enterococcus faecium fermentation product, sodium silico aluminate, and natural and artificial flavors. Zy-mend® ingredients include rice hulls, corn
distillers dired grains with solubles, active dry yeast, dried *Aspergillus oryae* fermentation extract, sodium silico aluminate, dried *Trichoderma longibrachiatum* fermentation extract, dried *Bacillus subtilis* fermentation extract, dried *Aspergillus niger* fermentation extract, torula dried yeast, dried *Enterococcus faecium* fermentation product, *Yucca schidigera* extract, riboflavin supplement, calcium gluconate, niacin supplement, biotin, pyridoxine hydrochloride, thiamine mononitrate, vitamin B\textsubscript{12} supplement, citric acid, natural and artificial flavors, and mineral oil.

Cows began the study 3 wk prior to expected calving date and continue through 8 wk of lactation. All feed ingredients and nutrient composition can be found in Table 1. Feed ingredients and nutrient composition of the prepartum, postpartum low, and postpartum high TMR are found in Tables 2, 3, 4, and 5 respectively. All cows were fed the prepartum TMR prior to calving and then switched to the postpartum low TMR immediately postpartum which helped to transition them to the postpartum high TMR. After the 2\textsuperscript{nd} wk of lactation, all cows were transferred to the postfresh high TMR. All cows were kept in tie stalls bedded with mattresses and kiln dried sawdust from d -21 to 56. Each cow was fed in separate wooded feed tubs (90 x 90 x 90 cm). Three days prior to the expected calving date cows were moved to individual maternity pens (4.3 x 3.7 m) and were kept there until they had calved and/or cleaned. At all times the cows had access to automated water bowls (Delaval, Tumba, Sweden).

**Cow Measurements:**

Daily DMI were recorded for each cow throughout the study. Samples of TMR and orts were taken each day and then frozen at -20°C until further processing. All samples were then dried at 55°C in a forced air oven for 72 h (Binder Inc., Bohemia, NY and 1380 FM, VWR Scientific, Radnor, PA). Orts were composited by cow by week while TMR samples were pooled by week. Both orts and TMR samples were ground to pass through a 1 mm sieve using a Wiley Mill (Thomas Wiley Laboratory Mill Model 4, Thomas Scientific, Swedesboro, NJ and Wiley
Mill Standard Model 3, Arthur H. Thomas Co., Philadelphia, PA). All samples were then subsampled and shipped to Agri-King, Inc. (Fulton, IL) for a complete nutrient analysis. The samples were analyzed for moisture and DM (method 935.29, AOAC, 1999), ADF (method 973.18, AOAC, 1999), NDF (method 2002.04, AOAC, 1999), CP (method 990.03, AOAC, 1999), soluble protein (SP; Krishnamoorthy et al., 1982), heat damaged protein (HD; method 973.18 & 976.06, AOAC, 1999), neutral detergent insoluble protein (NDIP; method 2002.04 (minus sulfite) and 976.06, AOAC, 1999), ash (method 942.05, AOAC, 1999), fat (method 920.39, AOAC, 1999), starch (Enzymatic method analyzed on RFA using Glucose Trinder; Glucose Reagent Set, Amresco, Solon, OH), and lignin (method 973.18, AOAC, 1999). Samples were also analyzed for Ca, P, Mg, K, Na, Fe, Cu, Zn, Mn (method 985.01) and S (method 923.01, AOAC, 1999), Cl (method 915.01, AOAC, 1999), Co (method 2006.03, AOAC, 1999), Cr; (Binnerts et al., 1968), NH3-N (Univ of Wi Ext. SKU: A3769, MAP 4.3 adapted from USEPA 351.2 and ISO 11732).

Blood samples were taken from the cows every Monday, Wednesday, and Friday at 1100 h starting day -21 through 56. Two samples were taken from the coccygeal vein of each cow using 22 gauge needles and 10 mL vacutainer tubes with and without sodium heparin (Covidien LLC, Mansfield, MA). The serum samples were allowed to clot, centrifuged at 1,388 x g (5430 R Eppendorf, Hauppaue, NY ), 2,325 x g (5810R, Eppendorf, Hauppaue, NY) or 1,704 x g (Servall Lynx 4000, Thermo Fisher Scientific, Chelmsford, MA) at 4ºC for 20 min and frozen at -20ºC. Plasma samples were taken in 10 mL vacutainer tubes with sodium heparin and then centrifuged at 1,388 x g (5430 R Eppendorf, Hauppaue, NY ), 2,325 x g (5810R, Eppendorf, Hauppaue, NY) or 1,704 X g (Servall Lynx 4000, Thermo Fisher Scientific, Chelmsford, MA) at 4ºC for 20 min. Both plasma and serum samples were then stored at -20ºC until shipped to
Agri-King (Fulton, IL) for NEFA, glucose, and BHBA analysis via enzyme linked immunosorbent assay (Wako Chemicals USA, Inc., Richmond, VA). Serum samples from day of parturition were also analyzed for IgG by radial immunoassay (Triple J Farms, Bellingham, WA).

Urine samples were collected every Monday, Wednesday, and Friday at 1100 h starting from day -21 through calving and analyzed for pH within 1 h after collection using either a portable (VWR SP20 SympHony; Thermo Fisher Scientific; Chelmsford, MA) or stationary (Orion Star A214; Thermo Fisher Scientific; Chelmsford, MA) pH meter.

Colostrum was harvested within 60 min of calving. The colostrum was weighed, sampled, and analyzed for quality using either a colostrometer (Precision Hydrometer; Biogenics; Mapleton, OR) or refractometer (Sugar/Brix Refractometer 300001; SPER Scientific; Scottsdale, AZ). One 40 mL sample was then frozen at -20°C until analyzed for IgG and IgA by radial immunoassay (Triple J farms, Bellingham, WA) while the other 40 mL sample was preserved with 2 bromo-2-nitropropan-1, 3 diol and shipped to DairyOne Cooperative, Inc. (Ithaca, NY) for analysis of fat (method 989.05, AOAC 2006), total protein (method 991.20, AOAC, 2006), total solids (method 990.20, AOAC, 2006), ash (method 942.05), and lactose (calculated by the following equation: % total solids - % fat - % total protein - % ash).

All cows were milked twice a day and weights were recorded at each milking until d 56 at 0500 and 1600 h. Milk samples were taken at 1600 h on Tuesdays and 0500 h on Wednesday. These a.m. and p.m. milk samples were then composited by the respective yields for each milking for each cow. All samples were placed into 40 mL vials containing 2 bromo-2-nitropropan-1, 3 diol and stored at 4°C until shipped to DairyOne Cooperative, Inc. (Ithaca, NY) for quality analysis. Samples were analyzed for fat, protein, ash, lactose, milk urea nitrogen...
(MUN), and somatic cell Count (SCC) using mid- infrared reflectance spectroscopy (Foss MilkoScan 4000, Foss Electric, Hillerød, Denmark).

Body weight was also recorded every Friday from day -21 to 56 for each cow using a platform scale (Cardinal Scale Manufacturing Co., Hooksett, NH).

Calving ease was documented at calving with the score of 1) unassisted calving, 2) some assistance easy calving, or 3) assisted difficult calving. There was 37 calves born alive on this study and 32 of those calves were born with a calving score of 1 (10 control, 11 Tri, and 11 Tri + Zy). Three other calves were born with a calving score of 2 (2 control and 1 Tri) and 2 calves were born with a score of 3 (1 control and 1 Tri).

**Calf Measurements**

All calves were removed from the cow prior to nursing as soon after calving as possible. Calves were weighed using a platform scale (Salter Scales, Fairfield, NJ), navel dipped with 7% iodine, and placed into 1 x 2.15 m pens with kiln dried sawdust. Heifer calves received their required Bovine Rota-Coronavirus (Calf Guard; Zoetis Inc.; Kalamazoo, MI) and *Escherichia coli* vaccinations (Bar-Guard-99; Boehringer Ingelheim; St. Joseph, MO). Blood samples were taken from the jugular vein on the calves at 0 and 24 h using 22 gauge needles and 10 mL vacutainer tubes (Covidien LLC, Mansfield, MA). Samples were then clotted, centrifuged at 1,388 x g (5430 R Eppendorf, Hauppauge, NY), 2,325 x g (5810R, Eppendorf, Hauppauge, NY) or 1,704 x g (Servall Lynx 4000, Thermo Fisher Scientific, Chelmsford, MA) at 4ºC for 20 min and then frozen at -20º C until IgG and IgA radial immunoassay was performed (Triple J farms, Bellingham, WA). Apparent efficiency of absorption of IgG was calculated using the following formula: ((Plasma IgG [g/L] * BW [kg]* 0.09)/IgG intake [g]) *100. Also, apparent efficiency of absorption of IgA was calculated using the following formula: ((Plasma IgA [g/L] * BW [kg] *0.090 /IgA intake [g]))*100.
After the 0 h blood samples, 3 to 4 L of maternal colostrum was fed to the calves via bottle or stomach tube.

**Statistical Analysis**

**Prepartum Data:**

Colostrum yield, IgG (g/L), IgA (g/L), total protein yield (kg), total protein content (%), fat yield (kg), fat content (%), lactose yield (kg), lactose content (%), ash yield (kg), and ash content (%) were analyzed using the MIXED procedure of SAS 9.4 (2013) according to the following model:

\[
Y_{ijk} = \mu + B_i + T_j + P_k + E_{ijk}
\]

Where: \(Y_{ijk}\) = the dependent variable; \(\mu\) = the overall mean; \(B_i\) = the random effect of block (I = 1,…12); \(T_j\) = the effect of treatment (j = Control, Tri, Tri + Zy); \(P_k\) = the covariate measure of parity (k = 1,…6); and \(E_{ijk}\) = the residual error.

Differences among treatments were determined using the LSMEANS option for all procedures in SAS 9.4. Significant effects were defined as \(P \leq 0.05\) and trends were defined as \(0.05 \leq P \leq 0.10\). The covariate of expected parity was included in the models (with the exception of pH) only when \(P \leq 0.25\).

Initial urine pH data was first transformed to H\(^+\) ion concentration as suggested by Murphy (1982) and then analyzed using the GLM procedure in SAS 9.4 (2013) according to the following model:

\[
Y_{ij} = \mu + B_i + T_j + E_{ij}
\]

Where: \(Y_{ij}\) = the dependent variable; \(\mu\) = the overall mean; \(B_i\) = the random effect of block (I = 1,…12); \(T_j\) = the effect of treatment (j = Control, Tri, Tri + Zy); and \(E_{ij}\) = the residual error.
Differences among treatments were determined using the LSMEANS option for all procedures in SAS 9.4. Significant effects were defined as $P \leq 0.05$ and trends were defined as $0.05 \leq P \leq 0.10$.

Weekly urine pH data was first transformed to $H^+$ ion concentration as suggested by Murphy (1982) and then analyzed using repeated measures in the GLM procedure in SAS 9.4 (2013) according to the following model:

$$Y_{ijkl} = \mu + B_i + T_j + W_k + TW_{jk} + C_l + E_{ijkl}$$

Where: $Y_{ijkl} =$ the dependent variable; $\mu =$ the overall mean; $B_i =$ the random effect of block ($I = 1, \ldots 12$); $T_j =$ the effect of treatment ($j =$ Control, Tri, Tri + Zy); $W_k =$ the effect of week ($k = -3, -2, -1$); $TW_{jk} =$ the treatment by week interaction; $C_l =$ the covariate measure of initial urine $H^+$; and $E_{ijkl} =$ the residual error.

Differences among treatments were determined using the LSMEANS option for all procedures in SAS 9.4. Significant treatment effects were defined as $P \leq 0.05$ and trends were defined as $0.05 \leq P \leq 0.10$. The covariate of initial urine pH was included in the model only when $P \leq 0.25$.

Body weight, BW gain, efficiency of gain, DMI, BHBA, NEFA, and glucose were analyzed using the REPEATED procedure of SAS 9.4 according to the following model:

$$Y_{ijkl} = \mu + B_i + T_j + W_k + TW_{jk} + P_l + E_{ijkl}$$

Where: $Y_{ijkl} =$ the dependent variable; $\mu =$ the overall mean; $B_i =$ the random effect of block ($I = 1, \ldots 12$); $T_j =$ the effect of treatment ($j =$ Control, Tri, Tri + Zy); $W_k =$ the effect of week ($k = -3, -2, \text{ and } -1$); $TW_{jk} =$ the treatment by week interaction; $P_l =$ the covariate measure of parity ($k = 1, \ldots 6$); and $E_{ijkl} =$ the residual error.
Differences among treatments were determined using the LSMEANS option for all procedures in SAS 9.4. Significant interactions and treatment effects were defined as $P \leq 0.05$ and trends were defined as $0.05 \leq P \leq 0.10$. The covariate of expected parity was included in the models (with the exception of pH) only when $P \leq 0.25$. The covariate structure used was either autoregressive, compound symmetry, or unilateral and was dependent on which had the lowest BIC.

**Calf Data:**
Calf data for serum IgG, IgA, AEA, and BW were analyzed using the MIXED procedure of SAS 9.4 according to the following model:

$$Y_{ijk} = \mu + B_i + T_j + P_k + E_{ijk}$$

Where: $Y_{ijk}$ = the dependent variable; $\mu$ = the overall mean; $B_i$ = the random effect of block ($I = 1, \ldots, 12$); $T_j$ = the effect of treatment ($j =$ Control, Tri, Tri + Zy); $P_k$ = the covariate measure of parity ($k = 1, \ldots, 6$); and $E_{ijk}$ = the residual error.

Differences among treatments were determined using the LSMEANS option for all procedures in SAS 9.4 (2013). Significant interactions and treatment effects were defined as $P \leq 0.05$ and trends were defined as $0.05 \leq P \leq 0.10$. The covariate of expected parity was included in the models only when $P \leq 0.25$.

**Postpartum Data:**

Body weight, BW gain, efficiency of gain, DMI, BHBA, NEFA, and glucose were analyzed using the REPEATED procedure of SAS 9.4 according to the following model:

$$Y_{ijkl} = \mu + B_i + T_j + W_k + TW_{jk} + P_l + E_{ijkl}$$

Where: $Y_{ijkl}$ = the dependent variable; $\mu$ = the overall mean; $B_i$ = the random effect of block ($I = 1, \ldots, 12$); $T_j$ = the effect of treatment ($j =$ Control, Tri, Tri + Zy); $W_k$ = the effect of week ($k = -3, \ldots, 6$); $TW_{jk}$ = the interaction of treatment and week; $P_l$ = the covariate measure of parity ($l = 1, \ldots, 6$); and $E_{ijkl}$ = the residual error.
-2, and -1); $TW_{jk}$ = the treatment by week interaction; $P_l$ = the covariate measure of parity ($k = 1, \ldots , 6$); and $E_{ijkl}$ = the residual error.

Data for post-partum DMI, milk yield, energy corrected milk yield, milk efficiency, total protein yield (kg), total protein content (%), fat yield (kg), fat content (%), lactose yield (kg), lactose content (%), ash yield (kg), and ash content (%), NEFA, BHBA, and glucose were analyzed using the REPEATED procedure of SAS 9.4 according to the following model:

$$Y_{ijkl} = \mu + B_i + T_j + W_k + TW_{jk} + P_l + E_{ijkl}$$

Where: $Y_{ijkl}$ = the dependent variable; $\mu$ = the overall mean; $B_i$ = the random effect of block ($i = 1, \ldots , 12$); $T_j$ = the effect of treatment ($j = \text{Control, Tri, Tri + Zy}$); $W_k$ = the effect of week ($k = 1, \ldots , 8$); $TW_{jk}$ = the treatment by week interaction; $P_l$ = the covariate measure of current parity ($k = 1, \ldots , 6$); and $E_{ijkl}$ = the residual error.

Differences among treatments were determined using the LSMEANS option for all procedures in SAS 9.4 (2013). Significant interactions were defined as $P \leq 0.05$ and trends were defined as $0.05 \leq P \leq 0.10$. The covariate of current parity was included in the models only when $P \leq 0.25$. The covariate structure used was either autoregressive, compound symmetry, or unilateral and was dependent on which had the lowest BIC.

**Results**

**Prepartum Cow Data**

In this study, 1 Tri + Zy cow was omitted from the study because she aborted her calf.

One control cow and another Tri cow were put down a few days after calving due to severe hypocalcaemia. Thirty cows had a calving ease of 1 (10 control, 10 Tri, and 10 Tri + Zy).
Another 3 cows had a calving ease of 2 (2 control and 1 Tri). Finally, 2 cows had a calving ease of 3 (1 control and 1 Tri).

The composition of the prepartum TMR can be found in Tables 2 and 3. Nutrient analysis of prepartum orts for each treatment can be found in Table 6.

All cows had similar initial urine pH (Table 9). There was no impact of treatment fed during the 21 d prior to parturition on the BW, BW gain, efficiency of gain, DMI, urine pH, BHBA, NEFA, glucose, and colostrum yield (Table 9; Figures 4, 5, 6, 7, 8, 9, 10 and 11). There was also no impact on colostrum quality with the exception of ash % and IgA yield (Table 9). Ash percentage (%) tended to increase with Tri and Tri + Zy treatments ($P = 0.07$). However, the Tri treatment decreased IgA yield ($P = 0.05$).

**Calf Data**

There were a total of 38 calves born on this study. One calf was removed from the study because it was stillborn leaving a total of 37 which were sampled. Twenty two of the calves were heifers (9 Control, 10 Tri, and 3 Tri + Zy) and 15 were bulls (3 Control, 4 Tri, and 9 Tri + Zy).

A total of 13 calves did not receive 4 L of maternal colostrum. Six of these 13 calves did not receive the total 4 L because of trouble in bottle feeding and stomach tubing the full amount of colostrum. Four calves received only 3 L of maternal colostrum since that was all the dam produced (2 control, 1 Tri, and 2 Tri + Zy). Three more calves did not receive any maternal colostrum because of dam being leukemia positive or produced no colostrum (2 control and 1 Tri). As a result, these 3 calves did not have any blood samples taken at 24 h of age since they received good quality colostrum from other cows not on the study or colostrum replacer. Also, one control calf did not have a 24 hr blood sample taken because it was accidentally fed more colostrum by the farm staff.
Twenty six of the calves were fed colostrum with a nipple bottle (6 Control, 10 Tri, and 10 Tri + Zy). Six calves were fed colostrum with a nipple bottle and stomach tube (4 Control and 2 Tri). One Tri calf was fed entirely by stomach tube.

Calf weight, serum IgG at 0 and 24 h, serum IgA at 0 and 24 h, AEA of IgG, and AEA of IgA were similar across treatments (Table 10).

**Postpartum Cow Data**

Two cows were treated for hypocalcaemia (1 control and 1 Tri). Three control cows were treated for retained placenta. Another control cow also had udder edema. Finally, 2 cows were treated for mastitis (1 Tri and 1 Tri + Zy).

The nutrient composition of the postpartum TMR can be found in Tables 4 and 5, respectively. Nutrient composition of postpartum low and high orts can be found in Tables 7 and 8, respectively.

There was no effect of treatment on BW (Table 11 and Figure 12). Body weight gain was greater ($P = 0.03$) and efficiency of gain tended to be greater ($P = 0.09$) for those cows on the Tri treatment in comparison to those on the Tri +Zy treatment (Table 10; Figures 13 and 14).

Dry matter intake, BHBA, NEFA, and glucose concentrations were not different among treatments (Table 11; Figures 15, 16, 17, and 18). No effect of treatment was observed for milk yield, energy corrected milk yield, and milk efficiency (Table 11; Figures 19, 20, and 21).

Treatment did not impact milk fat, true protein, lactose, or total solids yield (Table 11; Figures 22, 23, 24, and 25). Percentage of fat, true protein, lactose, total solids, and MUN were not impacted by treatment as well (Table 11; Figures 26, 27, 28, 29, and 30). Treatment impacted somatic cell score (Table 11 and Figure 31). Cows on Tri + Zy treatment had lower somatic cell scores ($P = 0.04$) on average than those on the Tri treatment. However, the control cows were similar to both the Tri and Tri + Zy treatments (Table 11 and Figure 31).
Discussion

Prepartum Data:

No effect of treatments was found on the BW, BW gain, or efficiency of gain during the prepartum period. There was no effect of treatment on the DMI of the cows 21 d prior to parturition. This coincides with previous studies which found that DFM containing *Saccharomyces cerevisiae* and *Enterococcus faecium* fed at either 2 g/d or 90 g/d during the 21 d prepartum period had no impact on DMI (Nocek et al., 2003; Oetzel et al., 2007). Prepartum DMI data in this study do not agree with that of Nocek and Kautz (2006) who found that the supplementation of DFM containing both *Saccharomyces cerevisiae* and *Enterococcus faecium* at a rate of 2 g/d increased DMI during the prepartum period. Also, Dann et al. (2000) found that supplementation of *Saccharomyces cerevisiae* culture at 60 g/d increased DMI of Jersey cows during the 21 d prepartum period.

There was also no effect of treatment on prepartum urine pH. Previous studies have not indicated that prepartum supplementation of DFM and enzymes impacts urine pH. This might be due to the fact that both of these additives do not have enough of an anion capacity to bring about a change in the metabolic pH.

No effect of treatment was observed for serum BHBA, NEFA, or glucose concentrations which suggest that DFM and enzyme supplementation did not have a major impact on digestion and metabolism during the prepartum period. These results for glucose and NEFA are supported by other studies which found no impact of DFM supplementation on prepartum glucose and NEFA (Nocek and Kautz, 2006; Oetzel et al., 2007). However, DFM supplementation has been reported to decrease prepartum BHBA which is in contrast to what was reported in this study (Nocek and
Kautz, 2006; Oetzel et al., 2007). Defrain et al. (2005) also reported increased BHBA concentrations for cows supplemented with enzymes.

Colostrum yield was not altered by treatment. No studies have indicated the impact of Tri and Tri + Zy treatments on colostrum yield. The lack of impact of treatment in this study on colostrum yield is perhaps due to the fact that there was no impact on prepartum DMI. There was also no impact on colostrum composition with the exception of IgA yield and ash %. In the case of IgA yield, the Tri treatment decreased it. Ash percentage also tended to increase with the Tri and Tri + Zy treatments. In both cases, it is not fully understood why these results were observed and further research would need to be performed in order to determine what is causing these results. However, the reduction in IgA yield might be linked to the greater SCS observed postpartum for cows fed the Tri treatment. In other words, there might have been a greater degradation of milk components such as IgG due to a greater enzymatic activity of the somatic cells or the presence of bacteria within the mammary gland.

**Calf Data**

No impact treatment was observed for calf BW. There also was no impact of treatment on the serum IgG and IgA concentrations in calves at 0 h. The serum IgG concentrations at 24 h and AEA of IgG in calves was similar across all treatments. A previous study showed that calves from cows supplemented with a 105 mg of Se-yeast/d had higher serum and absorption of IgG than those from control cows (Hall et al., 2014). In studies where calves were given DFM there were no impacts on serum IgG concentrations (Al-Saiady 2010; Quezada-Mendoza et al., 2011). In both these studies, the calves were over 24 h old and AEA was not evaluated. The serum concentrations of IgA and AEA of IgA was not impacted by DFM and enzyme supplementation.
Postpartum Data

No effect of treatment was observed for overall BW. Body weight gain was different among treatments. In particular, the BW gain of cows on the Tri treatment was greater (less negative) than those on the Tri + Zy treatment \((P = 0.03)\). Both treatments were not different from the control animals. This suggests that there was a greater partitioning of nutrients towards body condition in cows on the Tri treatment in comparison to those on the Tri + Zy treatment. The reason for this greater partitioning of nutrients is not fully understood and further research would need to be performed in order to determine the underlying interactions.

Efficiency of gain tended to also be greater \((P = 0.09)\) for cows on the Tri treatment in comparison to those on the Tri + Zy treatment. This confirms that there were more nutrients being partitioned to the body condition of the cow. In other words, those cows on the Tri treatment lost less body weight during the postpartum period. Also, these data suggest that Zy-mend® should not be fed in addition to Tri-Lution® since it cancels out the efficiency of gain effect. The underlying mechanism responsible for this is not known and further research will need to be performed in order to determine it. However, the effects are likely due to negative interactions between the different ingredients and microorganism in each product.

Dry matter intake during the 8 wk postpartum periods was not different among treatments. In particular, the lack of effect of treatment on DMI is in contrast to that found in previous studies with DFM supplementation. An array of studies with various types and rate of DFM supplementation have been shown to increase DMI during early lactation (Nocek et al., 2003; Nocek and Kautz, 2006; Dann et al., 2007; Oetzel et al., 2007; Moallem et al., 2009). Only Schingoethe et al. (2014) found that supplementation of *Saccharomyces cerevisiae* culture did not effect DMI of lactating cattle. As for studies with enzyme supplementation, several studies
have shown that there was no impact of enzymes supplemented at various rates on DMI of lactating cows (Rode et al., 1999; Yang et al., 2000; Reddish and Kung, 2007).

Treatment did not alter serum glucose, BHBA, and NEFA concentrations. In contrast, Nocek et al. (2003) reported that postpartum concentrations of glucose increased and NEFA decreased with DFM supplementation. DeFrain et al. (2005) also reported that enzyme supplementation increased postpartum glucose concentrations. However, no effect of DFM and enzyme supplementation on postpartum BHBA and NEFA concentrations have been reported (DeFrain et al., 2005; Oetzel et al., 2007). Only Nocek and Kautz (2006) reported that DFM increased postpartum BHBA concentrations in transition cows which is undesirable.

Milk yield was not altered by DFM and enzyme treatments likely due to similar DMI across treatments. Dann et al. (2000) and Oetzel et al. (2007) found no difference in milk yield of cows supplemented with or without DFM. In addition, AlZahal et al. (2014a) found no impact of DFM on milk yield during the first 6 wk of lactation. Only between week 7 and 10 did AlZahal et al. (2014a) observe an increase in milk yield of cows supplemented with DFM in comparison those that were not. Other studies have found increases in milk yield with DFM supplementation during early lactation as well (Wohlt et al., 1991; Nocek et al., 2003; Nocek and Kautz, 2006; Moallem et al., 2009). In addition, it has been shown that enzyme supplementation increased milk production (Yang et al., 1999). However, most studies with enzyme supplementation have indicated that it does not impact milk production (Beauchemin et al., 1999; Reddish and Kung 2007; Holtshausen et al., 2011).

Energy corrected milk yield was not altered by treatment. Schingoethe et al. (2004) found a similar result of no impact on ECM yield with the supplementation of yeast. Boyd et al. (2011) found an increase in ECM with the supplementation of DFM containing Lactobacillus
acidophilus and Propionibacterium freudenreichii which was likely due to an increase in the yield of the components such as fat and protein of the milk. However, both of these bacteria though were not in the DFM supplemented in this study.

Milk efficiency of cows on the Tri and Tri +Zy treatments were similar to those of the control. Beauchemin et al. (1999) and Boyd et al. (2011) found no effect of DFM supplementation on milk efficiency as well. Sheperd and Kung (1996a) found that the application of an enzyme additive containing cellulase and hemicellulase to silage also did not alter milk efficiency of cows. This is all in contrast to the 7% increase in milk efficiency that Schingoethe et al. (2004) found with yeast supplementation. In addition, Holtshausen et al. (2011) found that cows in early lactation fed an enzyme product had higher milk production efficiency. However, in the study by Holtshausen et al. (2011) the enzyme product contained mainly xylanase and endoglucanase activity. Whereas the enzyme product used in this study primarily contained amylase and cellulase activity.

There also was no effect of treatment on milk composition with the exception of SCS. Dann et al. (2000) also found no impact of DFM on the concentrations of fat, protein, lactose, total solids, and MUN. Moallem et al. (2009) found no impact on the milk fat and protein percentages between cattle supplemented with DFM. Nocek and Kautz (2006) found no impact of DFM supplementation on milk fat and protein yields as well as milk protein percentages. A similar response of no effect on milk composition has been observed for enzyme supplementation (Reddish and Kung, 2007). Oetzel et al. (2007) found that DFM supplementation only increased milk fat percentage for cows in their first lactation. In addition, Oetzel et al. (2007) found an increased in milk protein percentage for cows with 2 or more lactations.
In this study there was an effect of treatment on SCS. The SCS of cows on the Tri and Tri + Zy treatments were not different from the control. It was the Tri treatment that was different from the Tri + Zy treatment. Dann et al. (2000) did not find any impact of DFM supplementation on somatic cells count. This suggested that it might have been the addition of enzymes that caused the difference in SCS in the current study. However, Reddish and Kung (2007) reported no impact of enzyme supplementation containing cellulase and xylanase activity on somatic cell counts. As a result, it can be concluded that there is some other underlying factors contributing to the differences between treatments and further research would need to be performed to determine this.

**Conclusion**

Supplementation of DFM and enzymes did not alter prepartum DMI, BW, BW gain, efficiency of gain, glucose, BHBA, NEFA, and urine pH in comparison to the control. There was also no change in colostrum yield and composition. However, Tri and Tri + Zy treatments tended to increase ash percentage in colostrum. Also, the Tri treatment in comparison to the control and Tri + Zy tended to decrease IgA yield of colostrum. This effect on IgA yield is likely linked to the higher SCS observed postpartum for Tri cows. No impact of treatment was observed for BW, serum IgG at 0 and 24hr, serum IgA at 0 and 24 hr, and AEA of IgG and IgA of calves. There was also no effect of DFM and enzyme supplementation on the postpartum DMI, BW, BHBA, NEFA, and glucose concentrations. Postpartum BW gain was greater ($P = 0.03$) and efficiency of gain tended ($P = 0.09$) to be greater for cow on the Tri treatment in comparison to those on Tri +Zy. This indicates that cows lose less weight postpartum on the Tri treatment. However, the addition of Zy-mend® (enzymes) eliminated this effect of the Tri treatment due to some conflicting mechanism possibly among the different ingredients and microorganism within each product. No effect of treatment was observed for milk yield and composition. Only cows on the
Tri treatment had increased SCS scores in comparison to those on the control and Tri +Zy treatments. As a result, it can be concluded from this research that DFM and enzyme supplementation is not beneficial for improving the health and performance of dairy cattle during the transition period and early lactation.
Table 1: Feed Ingredients and Nutrient Composition

<table>
<thead>
<tr>
<th>Nutrient Composition</th>
<th>Corn Silage</th>
<th>Grass Silage</th>
<th>RUP Mix (Provaal Elite)</th>
<th>Soy/Urea Mix</th>
<th>Energy Mix</th>
<th>Bergafat F-100</th>
<th>Dry Cow Mix</th>
<th>Prefresh Mineral Mix</th>
<th>Lactation Mineral</th>
</tr>
</thead>
<tbody>
<tr>
<td>DM, % ± SD</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DM %</td>
<td>32.5 ± 4.7</td>
<td>35.4 ± 4.7</td>
<td>90.0</td>
<td>88.6</td>
<td>84.3</td>
<td>.</td>
<td>89.7</td>
<td>94.9</td>
<td>93.3</td>
</tr>
<tr>
<td>CP %</td>
<td>7.5 ± 0.4</td>
<td>16.8 ± 1.0</td>
<td>93.0</td>
<td>47.1</td>
<td>7.8</td>
<td>.</td>
<td>19.9</td>
<td>14.3</td>
<td>3.9</td>
</tr>
<tr>
<td>RUP %</td>
<td>6.0 ± 0.3</td>
<td>13.6 ± 0.9</td>
<td>.</td>
<td>.</td>
<td>3.9</td>
<td>.</td>
<td>7.4</td>
<td>.</td>
<td></td>
</tr>
<tr>
<td>ADF %</td>
<td>24.5 ± 3.0</td>
<td>35.1 ± 4.7</td>
<td>.</td>
<td>8.3</td>
<td>12.5</td>
<td>.</td>
<td>13.9</td>
<td>.</td>
<td></td>
</tr>
<tr>
<td>NDF %</td>
<td>40.1 ± 5.3</td>
<td>50.2 ± 5.1</td>
<td>.</td>
<td>5.8</td>
<td>21.8</td>
<td>.</td>
<td>23.7</td>
<td>.</td>
<td></td>
</tr>
<tr>
<td>NFC %</td>
<td>.</td>
<td>.</td>
<td>25.4</td>
<td>63.9</td>
<td>.</td>
<td>33.4</td>
<td>.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Starch %</td>
<td>35.0 ± 5.9</td>
<td>2.4 ± 0.7</td>
<td>.</td>
<td>5.8</td>
<td>47.3</td>
<td>.</td>
<td>16.3</td>
<td>.</td>
<td></td>
</tr>
<tr>
<td>Sugar %</td>
<td>0.7 ± 0.2</td>
<td>4.1 ± 0.7</td>
<td>11.7</td>
<td>8.3</td>
<td>.</td>
<td>5.8</td>
<td>.</td>
<td>.</td>
<td></td>
</tr>
<tr>
<td>Fat %</td>
<td>3.2 ± 0.3</td>
<td>4.2 ± 0.5</td>
<td>2.4</td>
<td>2.6</td>
<td>3.5</td>
<td>99</td>
<td>2.3</td>
<td>.</td>
<td></td>
</tr>
<tr>
<td>Lignin %</td>
<td>2.8 ± 0.4</td>
<td>5.9 ± 1.3</td>
<td>.</td>
<td>.</td>
<td>2.1</td>
<td>.</td>
<td>.</td>
<td>.</td>
<td></td>
</tr>
<tr>
<td>Ash %</td>
<td>3.5 ± 0.7</td>
<td>9.9 ± 0.8</td>
<td>7.1</td>
<td>2.6</td>
<td>3.0</td>
<td>.</td>
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<td></td>
</tr>
<tr>
<td>Ca %</td>
<td>0.2 ± 0.03</td>
<td>0.7 ± 0.10</td>
<td>0.7</td>
<td>0.5</td>
<td>0.4</td>
<td>.</td>
<td>1.8</td>
<td>15.3</td>
<td>13.0</td>
</tr>
<tr>
<td>P %</td>
<td>0.2 ± 0.02</td>
<td>0.3 ± 0.02</td>
<td>0.1</td>
<td>0.7</td>
<td>0.2</td>
<td>.</td>
<td>0.3</td>
<td>1.6</td>
<td>2.7</td>
</tr>
<tr>
<td>Mg %</td>
<td>0.1 ± 0.02</td>
<td>0.2 ± 0.02</td>
<td>0.2</td>
<td>0.4</td>
<td>0.2</td>
<td>.</td>
<td>1.2</td>
<td>3.3</td>
<td>4.7</td>
</tr>
<tr>
<td>K %</td>
<td>1.0 ± 0.22</td>
<td>2.8 ± 0.26</td>
<td>0.2</td>
<td>1.9</td>
<td>0.6</td>
<td>.</td>
<td>1.1</td>
<td>0.6</td>
<td>5.4</td>
</tr>
<tr>
<td>Na %</td>
<td>.</td>
<td>.</td>
<td>0.2</td>
<td>0.05</td>
<td>0.04</td>
<td>.</td>
<td>0.6</td>
<td>.</td>
<td>8.4</td>
</tr>
<tr>
<td>Cl %</td>
<td>.</td>
<td>.</td>
<td>0.2</td>
<td>0.08</td>
<td>0.2</td>
<td>.</td>
<td>1.6</td>
<td>3.5</td>
<td>9.5</td>
</tr>
<tr>
<td>S %</td>
<td>0.1 ± 0.01</td>
<td>0.3 ± 0.02</td>
<td>1.4</td>
<td>0.4</td>
<td>0.2</td>
<td>.</td>
<td>1.0</td>
<td>2.8</td>
<td>0.6</td>
</tr>
<tr>
<td>Mn (mg/kg)</td>
<td>.</td>
<td>.</td>
<td>8.7</td>
<td>40.7</td>
<td>27.9</td>
<td>.</td>
<td>1388</td>
<td>1665</td>
<td></td>
</tr>
<tr>
<td>Fe (mg/kg)</td>
<td>.</td>
<td>.</td>
<td>2315</td>
<td>174</td>
<td>328.6</td>
<td>.</td>
<td>2815</td>
<td>4024</td>
<td></td>
</tr>
<tr>
<td>Cu (mg/kg)</td>
<td>.</td>
<td>.</td>
<td>8.9</td>
<td>13.5</td>
<td>5.5</td>
<td>.</td>
<td>288</td>
<td>409</td>
<td></td>
</tr>
<tr>
<td>Zn (mg/kg)</td>
<td>.</td>
<td>.</td>
<td>35</td>
<td>53.5</td>
<td>21.7</td>
<td>.</td>
<td>1420</td>
<td>2240</td>
<td></td>
</tr>
</tbody>
</table>

1 RUP mix (Provaal Elite, Perdue, Kings Mountain, NC)
2 Soy urea mix (Poulin Grain, VT) contained 7.3% distillers grain, 69.1% soybean meal, 21.8% canola meal, and 1.8% urea.
3 Energy mix (Poulin Grain, VT) contained 4.1% molasses, 45.8% corn meal, 14.7% stream flaked corn, and 35.3% whole beet pulp.
4 Bergafat F-100 (Berg + Schmidt Functional Lipids, Liberty, IL) contained 100% vegetable oils.
5 Dry Cow mix (Poulin Grain, VT) contained 10.9% beet pulp, 21.9% soy hulls, 3.1% molasses, 14.9% soybean meal, 1.2% salt, 1.9% calcium carbonate, 1.9% magnesium oxide, 2.6% calcium sulfate, 0.7% Vitamin E 20000, 0.2% Poulin Dairy Vitamin Premix, 21.4% corn meal, 0.5% Rumensin 90, 8.1% Amino Plus, 0.5% Dimune Trace Pack, 1.1% magnesium sulfate, and 9.5% Biochar.
6 Prefresh mineral mix (Agri-King, Inc., Fulton, IL) contained 18.1% soybean meal, 0.1% Rumensin, 1.8% magnesium oxide, 1.2% feed bond, 2.6% dicalcium phosphate, 23.4% Soy Chlor, 2.6% calcium sulfate, 6.5% magnesium sulfate, 28.0% calcium (38%), 1.5% salt, and 9.8% Dry Cow Micro Pack.
7 Lactation mineral mix (Agri-King, Inc., Fulton, IL) contained 12.2% corn distillers, 0.1% Rumensin 90, 0.1% magnesium oxide, 1.1% feed bond, 16.7% sodium bicarbonate, 13.9% dical 18.5, 26.4% calcium (38%), 9.1% potassium chloride, 6.8% salt, 1.1% selenium yeast 600, and 4.9% Dairy Max.
Table 2: Prepartum TMR Composition – Diet 1

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>DM (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corn Silage</td>
<td>42.0</td>
</tr>
<tr>
<td>Grass Silage</td>
<td>26.8</td>
</tr>
<tr>
<td>RUP Mix (Provaal Elite)</td>
<td>2.5</td>
</tr>
<tr>
<td>Energy Mix</td>
<td>18.8</td>
</tr>
<tr>
<td>Soy/Urea Mix</td>
<td>3.4</td>
</tr>
<tr>
<td>Prefresh Mineral Mix</td>
<td>6.8</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Nutrient Composition</th>
<th>DM, % ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>CP %</td>
<td>15.8 ± 1.4</td>
</tr>
<tr>
<td>ADF %</td>
<td>26.0 ± 2.3</td>
</tr>
<tr>
<td>NDF %</td>
<td>42.3 ± 3.2</td>
</tr>
<tr>
<td>NFC %</td>
<td>34.7 ± 2.5</td>
</tr>
<tr>
<td>Starch %</td>
<td>18.5 ± 2.7</td>
</tr>
<tr>
<td>Fat %</td>
<td>2.8 ± 0.3</td>
</tr>
<tr>
<td>Lignin %</td>
<td>3.2 ± 0.8</td>
</tr>
<tr>
<td>Ash %</td>
<td>8.1 ± 0.7</td>
</tr>
<tr>
<td>Ca %</td>
<td>0.7 ± 0.16</td>
</tr>
<tr>
<td>P %</td>
<td>0.3 ± 0.04</td>
</tr>
<tr>
<td>Mg %</td>
<td>0.6 ± 0.12</td>
</tr>
<tr>
<td>K %</td>
<td>1.8 ± 0.26</td>
</tr>
<tr>
<td>Na %</td>
<td>0.2 ± 0.07</td>
</tr>
<tr>
<td>Cl %</td>
<td>0.8 ± 0.16</td>
</tr>
<tr>
<td>S %</td>
<td>0.4 ± 0.08</td>
</tr>
<tr>
<td>Mn (mg/kg)</td>
<td>80.4 ± 22.2</td>
</tr>
<tr>
<td>Fe (mg/kg)</td>
<td>313.2 ± 85.5</td>
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<tr>
<td>Cu (mg/kg)</td>
<td>17.3 ± 5.6</td>
</tr>
<tr>
<td>Zn (mg/kg)</td>
<td>90.2 ± 35.9</td>
</tr>
</tbody>
</table>

1The prepartum TMR – diet 1 was fed to the prepartum cows for the first 2 months of the study, but was discounted after several cases of hypocalcemia.
2Perdue, Kings Mountain, NC
3Poulin Grain, VT
4Poulin Grain, VT
5Agri-King, Inc., Fulton, IL
Table 3: Prepartum TMR Composition – Diet 2

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>DM (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corn Silage</td>
<td>40.1</td>
</tr>
<tr>
<td>Grass Silage</td>
<td>25.5</td>
</tr>
<tr>
<td>RUP Mix (Provaal Elite)</td>
<td>2.4</td>
</tr>
<tr>
<td>Energy Mix</td>
<td>1.0</td>
</tr>
<tr>
<td>Dry Cow Mix</td>
<td>30.4</td>
</tr>
<tr>
<td>Soy Urea Mix</td>
<td>1.0</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Nutrient Composition</th>
<th>DM, % ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>CP %</td>
<td>15.8 ± 1.4</td>
</tr>
<tr>
<td>ADF %</td>
<td>26.0 ± 2.3</td>
</tr>
<tr>
<td>NDF %</td>
<td>42.3 ± 3.2</td>
</tr>
<tr>
<td>NFC %</td>
<td>34.7 ± 2.5</td>
</tr>
<tr>
<td>Starch %</td>
<td>18.5 ± 2.7</td>
</tr>
<tr>
<td>Fat %</td>
<td>2.8 ± 0.3</td>
</tr>
<tr>
<td>Lignin %</td>
<td>3.2 ± 0.8</td>
</tr>
<tr>
<td>Ash %</td>
<td>8.1 ± 0.7</td>
</tr>
<tr>
<td>Ca %</td>
<td>0.7 ± 0.16</td>
</tr>
<tr>
<td>P %</td>
<td>0.3 ± 0.04</td>
</tr>
<tr>
<td>Mg %</td>
<td>0.6 ± 0.12</td>
</tr>
<tr>
<td>K %</td>
<td>1.8 ± 0.26</td>
</tr>
<tr>
<td>Na %</td>
<td>0.2 ± 0.07</td>
</tr>
<tr>
<td>Cl %</td>
<td>0.8 ± 0.16</td>
</tr>
<tr>
<td>S %</td>
<td>0.4 ± 0.08</td>
</tr>
<tr>
<td>Mn (mg/kg)</td>
<td>80.4 ± 22.2</td>
</tr>
<tr>
<td>Fe (mg/kg)</td>
<td>313.2 ± 85.5</td>
</tr>
<tr>
<td>Cu (mg/kg)</td>
<td>17.3 ± 5.6</td>
</tr>
<tr>
<td>Zn (mg/kg)</td>
<td>90.2 ± 35.9</td>
</tr>
</tbody>
</table>

1The prepartum TMR – diet 2 was fed to prepartum cows starting month 2 of the study to the end after the previous diet was discontinued due to several cases of hypocalcemia.
2Perdue, Kings Mountain, NC
3Poulin Grain, VT
4Poulin Grain, VT
5Poulin Grain, VT
<table>
<thead>
<tr>
<th>Ingredient</th>
<th>DM (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corn Silage</td>
<td>49.4</td>
</tr>
<tr>
<td>Grass Silage</td>
<td>13.6</td>
</tr>
<tr>
<td>RUP Mix (Provaal Elite)²</td>
<td>1.0</td>
</tr>
<tr>
<td>Lactation Mineral Mix³</td>
<td>3.8</td>
</tr>
<tr>
<td>Soy/Urea Mix⁴</td>
<td>15.5</td>
</tr>
<tr>
<td>Energy Mix⁵</td>
<td>16.7</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Nutrient Composition</th>
<th>DM (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CP %</td>
<td>15.6 ± 1.0</td>
</tr>
<tr>
<td>ADF %</td>
<td>24.9 ± 2.2</td>
</tr>
<tr>
<td>NDF %</td>
<td>40.6 ± 3.0</td>
</tr>
<tr>
<td>NFC %</td>
<td>36.9 ± 2.7</td>
</tr>
<tr>
<td>Starch %</td>
<td>22.5 ± 2.9</td>
</tr>
<tr>
<td>Fat %</td>
<td>2.8 ± 0.3</td>
</tr>
<tr>
<td>Lignin %</td>
<td>3.1 ± 0.6</td>
</tr>
<tr>
<td>Ash %</td>
<td>7.6 ± 0.5</td>
</tr>
<tr>
<td>Ca %</td>
<td>0.6 ± 0.08</td>
</tr>
<tr>
<td>P %</td>
<td>0.4 ± 0.03</td>
</tr>
<tr>
<td>Mg %</td>
<td>0.4 ± 0.04</td>
</tr>
<tr>
<td>K %</td>
<td>1.6 ± 0.12</td>
</tr>
<tr>
<td>Na %</td>
<td>0.4 ± 0.06</td>
</tr>
<tr>
<td>Cl %</td>
<td>0.7 ± 0.10</td>
</tr>
<tr>
<td>S %</td>
<td>0.2 ± 0.02</td>
</tr>
<tr>
<td>Mn (mg/kg)</td>
<td>93.3 ± 17.1</td>
</tr>
<tr>
<td>Fe (mg/kg)</td>
<td>307.5 ± 50.0</td>
</tr>
<tr>
<td>Cu (mg/kg)</td>
<td>21.5 ± 4.6</td>
</tr>
<tr>
<td>Zn (mg/kg)</td>
<td>97.0 ± 14.5</td>
</tr>
</tbody>
</table>

¹Postpartum Low TMR was fed the first 2 weeks after parturition as a transition diet from the higher forage based prepartment TMR to the higher concentrate based postpartum high TMR.
²Perdue, Kings Mountain, NC
³Agri-King, Inc., Fulton, IL
⁴Poulin Grain, VT
⁵Poulin Grain, VT
Table 5: Postpartum High TMR Composition

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>DM (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corn Silage</td>
<td>43.2</td>
</tr>
<tr>
<td>Grass Silage</td>
<td>7.1</td>
</tr>
<tr>
<td>Bergafat 100&lt;sup&gt;2&lt;/sup&gt;</td>
<td>1.0</td>
</tr>
<tr>
<td>RUP Mix (Provaal Elite)&lt;sup&gt;3&lt;/sup&gt;</td>
<td>1.7</td>
</tr>
<tr>
<td>Lactation Mineral Mix&lt;sup&gt;4&lt;/sup&gt;</td>
<td>4.4</td>
</tr>
<tr>
<td>Soy/urea Mix&lt;sup&gt;5&lt;/sup&gt;</td>
<td>16.0</td>
</tr>
<tr>
<td>Energy Mix&lt;sup&gt;6&lt;/sup&gt;</td>
<td>26.7</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Nutrient Composition</th>
<th>DM, % ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>CP %</td>
<td>15.7 ± 1.3</td>
</tr>
<tr>
<td>ADF %</td>
<td>22.5 ± 1.9</td>
</tr>
<tr>
<td>NDF %</td>
<td>37.9 ± 2.8</td>
</tr>
<tr>
<td>NFC %</td>
<td>38.5 ± 2.6</td>
</tr>
<tr>
<td>Starch %</td>
<td>24.6 ± 2.9</td>
</tr>
<tr>
<td>Fat %</td>
<td>3.9 ± 0.6</td>
</tr>
<tr>
<td>Lignin %</td>
<td>2.7 ± 0.5</td>
</tr>
<tr>
<td>Ash %</td>
<td>7.3 ± 0.4</td>
</tr>
<tr>
<td>Ca %</td>
<td>0.6 ± 0.09</td>
</tr>
<tr>
<td>P %</td>
<td>0.4 ± 0.04</td>
</tr>
<tr>
<td>Mg %</td>
<td>0.4 ± 0.04</td>
</tr>
<tr>
<td>K %</td>
<td>1.6 ± 0.21</td>
</tr>
<tr>
<td>Na %</td>
<td>0.4 ± 0.05</td>
</tr>
<tr>
<td>Cl %</td>
<td>0.7 ± 0.05</td>
</tr>
<tr>
<td>S %</td>
<td>0.2 ± 0.02</td>
</tr>
<tr>
<td>Mn (mg/kg)</td>
<td>101.3 ± 11.4</td>
</tr>
<tr>
<td>Fe (mg/kg)</td>
<td>338.6 ± 56.0</td>
</tr>
<tr>
<td>Cu (mg/kg)</td>
<td>24.6 ± 4.1</td>
</tr>
<tr>
<td>Zn (mg/kg)</td>
<td>107.1 ± 12.3</td>
</tr>
</tbody>
</table>

<sup>1</sup>The postpartum high TMR was fed from week 2 through week 8 and had a greater amount of concentrates in order to sustain and maintain milk production.

<sup>2</sup>Berg + Schmidt Functional Lipids, Liberty, IL

<sup>3</sup>Perdue, Kings Mountain, NC

<sup>4</sup>Agri-King, Inc., Fulton, IL

<sup>5</sup>Poulin Grain, VT

<sup>6</sup>Poulin Grain, VT
Table 6: Nutrient composition of prepartum orts from cows fed Control, Tri, or Tri + Zy treatments during the 21 days prior to parturition.

<table>
<thead>
<tr>
<th>Nutrient Composition</th>
<th>Control</th>
<th>Tri</th>
<th>Tri + Zy</th>
</tr>
</thead>
<tbody>
<tr>
<td>CP %</td>
<td>13.1 ± 1.7</td>
<td>13.1 ± 1.2</td>
<td>13.6 ± 1.5</td>
</tr>
<tr>
<td>ADF %</td>
<td>27.6 ± 2.6</td>
<td>28.3 ± 2.0</td>
<td>28.0 ± 2.4</td>
</tr>
<tr>
<td>NDF %</td>
<td>45.2 ± 4.0</td>
<td>46.2 ± 3.8</td>
<td>45.9 ± 3.9</td>
</tr>
<tr>
<td>NFC %</td>
<td>32.5 ± 4.2</td>
<td>31.1 ± 3.6</td>
<td>31.1 ± 3.7</td>
</tr>
<tr>
<td>Starch %</td>
<td>19.4 ± 3.8</td>
<td>18.9 ± 2.2</td>
<td>17.4 ± 3.0</td>
</tr>
<tr>
<td>Fat %</td>
<td>2.4 ± 0.4</td>
<td>2.3 ± 0.5</td>
<td>2.3 ± 0.4</td>
</tr>
<tr>
<td>Lignin %</td>
<td>3.4 ± 0.8</td>
<td>3.2 ± 0.7</td>
<td>3.5 ± 1.0</td>
</tr>
<tr>
<td>Ash %</td>
<td>8.8 ± 1.4</td>
<td>9.6 ± 1.6</td>
<td>9.2 ± 1.4</td>
</tr>
<tr>
<td>Na %</td>
<td>0.2 ± 0.1</td>
<td>0.2 ± 0.06</td>
<td>0.3 ± 0.06</td>
</tr>
<tr>
<td>Mg %</td>
<td>0.5 ± 0.1</td>
<td>0.6 ± 0.11</td>
<td>0.6 ± 0.11</td>
</tr>
<tr>
<td>P %</td>
<td>0.3 ± 0.04</td>
<td>0.3 ± 0.04</td>
<td>0.3 ± 0.04</td>
</tr>
<tr>
<td>S %</td>
<td>0.4 ± 0.10</td>
<td>0.4 ± 0.07</td>
<td>0.4 ± 0.08</td>
</tr>
<tr>
<td>K %</td>
<td>1.7 ± 0.23</td>
<td>1.7 ± 0.13</td>
<td>1.7 ± 0.26</td>
</tr>
<tr>
<td>Ca %</td>
<td>0.6 ± 0.10</td>
<td>0.7 ± 0.10</td>
<td>0.7 ± 0.11</td>
</tr>
<tr>
<td>Cl %</td>
<td>0.7 ± 0.20</td>
<td>0.7 ± 0.26</td>
<td>0.8 ± 0.15</td>
</tr>
<tr>
<td>Mn (mg/kg)</td>
<td>71.8 ± 23.4</td>
<td>69.1 ± 21.2</td>
<td>75.0 ± 23.7</td>
</tr>
<tr>
<td>Fe (mg/kg)</td>
<td>288.4 ± 97.2</td>
<td>307.6 ± 117.9</td>
<td>358.2 ± 164.2</td>
</tr>
<tr>
<td>Cu (mg/kg)</td>
<td>16.1 ± 3.9</td>
<td>16.5 ± 4.1</td>
<td>16.6 ± 3.1</td>
</tr>
<tr>
<td>Zn (mg/kg)</td>
<td>76.3 ± 31.3</td>
<td>75.0 ± 32.2</td>
<td>81.0 ± 27.1</td>
</tr>
</tbody>
</table>
Table 7: Nutrient composition of postpartum low ors from cows fed Control, Tri, or Tri + Zy treatments.

<table>
<thead>
<tr>
<th>Nutrient Composition</th>
<th>Control</th>
<th>Tri</th>
<th>Tri + Zy</th>
</tr>
</thead>
<tbody>
<tr>
<td>DM, % ± SD</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CP %</td>
<td>13.3 ± 1.8</td>
<td>12.8 ± 1.2</td>
<td>13.3 ± 1.0</td>
</tr>
<tr>
<td>ADF %</td>
<td>26.0 ± 2.7</td>
<td>26.3 ± 2.3</td>
<td>26.5 ± 1.6</td>
</tr>
<tr>
<td>NDF %</td>
<td>43.4 ± 4.1</td>
<td>43.9 ± 4.1</td>
<td>44.0 ± 2.2</td>
</tr>
<tr>
<td>NFC %</td>
<td>34.6 ± 4.1</td>
<td>34.4 ± 3.8</td>
<td>33.9 ± 2.0</td>
</tr>
<tr>
<td>Starch %</td>
<td>22.4 ± 3.5</td>
<td>22.1 ± 3.2</td>
<td>21.7 ± 2.1</td>
</tr>
<tr>
<td>Fat %</td>
<td>2.3 ± 0.4</td>
<td>2.3 ± 0.3</td>
<td>2.3 ± 0.3</td>
</tr>
<tr>
<td>Lignin %</td>
<td>3.0 ± 0.9</td>
<td>2.8 ± 0.4</td>
<td>3.1 ± 0.7</td>
</tr>
<tr>
<td>Ash %</td>
<td>8.4 ± 1.4</td>
<td>8.5 ± 1.1</td>
<td>8.5 ± 0.9</td>
</tr>
<tr>
<td>Na %</td>
<td>0.4 ± 0.07</td>
<td>0.3 ± 0.06</td>
<td>0.4 ± 0.09</td>
</tr>
<tr>
<td>Mg %</td>
<td>0.3 ± 0.05</td>
<td>0.3 ± 0.03</td>
<td>0.3 ± 0.05</td>
</tr>
<tr>
<td>P %</td>
<td>0.4 ± 0.04</td>
<td>0.4 ± 0.04</td>
<td>0.4 ± 0.04</td>
</tr>
<tr>
<td>S %</td>
<td>0.2 ± 0.03</td>
<td>0.2 ± 0.02</td>
<td>0.2 ± 0.02</td>
</tr>
<tr>
<td>K %</td>
<td>1.6 ± 0.18</td>
<td>1.5 ± 0.13</td>
<td>1.7 ± 0.15</td>
</tr>
<tr>
<td>Ca %</td>
<td>0.6 ± 0.11</td>
<td>0.5 ± 0.09</td>
<td>0.6 ± 0.11</td>
</tr>
<tr>
<td>Cl %</td>
<td>0.6 ± 0.22</td>
<td>0.6 ± 0.08</td>
<td>0.6 ± 0.24</td>
</tr>
<tr>
<td>Mn (mg/kg)</td>
<td>83.0 ± 16.6</td>
<td>83.0 ± 13.3</td>
<td>91.4 ± 18.5</td>
</tr>
<tr>
<td>Fe (mg/kg)</td>
<td>288.9 ± 94.1</td>
<td>310.7 ± 56.4</td>
<td>392.1 ± 179.1</td>
</tr>
<tr>
<td>Cu (mg/kg)</td>
<td>21.3 ± 4.7</td>
<td>21.0 ± 3.3</td>
<td>22.7 ± 5.3</td>
</tr>
<tr>
<td>Zn (mg/kg)</td>
<td>85.8 ± 14.7</td>
<td>84.8 ± 14.1</td>
<td>93.8 ± 18.6</td>
</tr>
</tbody>
</table>
Table 8: Nutrient composition of postpartum high orts from cows fed Control, Tri, or Tri + Zy treatments.

<table>
<thead>
<tr>
<th>Nutrient Composition</th>
<th>Control</th>
<th>Tri</th>
<th>Tri + Zy</th>
</tr>
</thead>
<tbody>
<tr>
<td>DM, % ± SD</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CP %</td>
<td>12.8 ± 1.5</td>
<td>12.5 ± 1.8</td>
<td>12.3 ± 1.5</td>
</tr>
<tr>
<td>ADF %</td>
<td>24.2 ± 3.1</td>
<td>24.3 ± 2.8</td>
<td>25.9 ± 3.1</td>
</tr>
<tr>
<td>NDF %</td>
<td>40.9 ± 4.5</td>
<td>41.8 ± 4.7</td>
<td>44.5 ± 4.8</td>
</tr>
<tr>
<td>NFC %</td>
<td>36.9 ± 3.9</td>
<td>36.4 ± 3.6</td>
<td>34.7 ± 4.0</td>
</tr>
<tr>
<td>Starch %</td>
<td>24.2 ± 3.7</td>
<td>24.3 ± 3.6</td>
<td>22.5 ± 4.0</td>
</tr>
<tr>
<td>Fat %</td>
<td>3.2 ± 0.8</td>
<td>3.1 ± 0.8</td>
<td>2.5 ± 0.7</td>
</tr>
<tr>
<td>Lignin %</td>
<td>2.7 ± 0.9</td>
<td>2.7 ± 0.7</td>
<td>3.1 ± 1.2</td>
</tr>
<tr>
<td>Ash %</td>
<td>7.9 ± 1.2</td>
<td>8.0 ± 1.1</td>
<td>8.0 ± 1.6</td>
</tr>
<tr>
<td>Na %</td>
<td>0.4 ± 0.10</td>
<td>0.4 ± 0.08</td>
<td>0.3 ± 0.05</td>
</tr>
<tr>
<td>Mg %</td>
<td>0.3 ± 0.05</td>
<td>0.3 ± 0.06</td>
<td>0.3 ± 0.06</td>
</tr>
<tr>
<td>P %</td>
<td>0.4 ± 0.06</td>
<td>0.4 ± 0.05</td>
<td>0.4 ± 0.04</td>
</tr>
<tr>
<td>S %</td>
<td>0.2 ± 0.04</td>
<td>0.2 ± 0.03</td>
<td>0.2 ± 0.05</td>
</tr>
<tr>
<td>K %</td>
<td>1.5 ± 0.26</td>
<td>1.4 ± 0.13</td>
<td>1.4 ± 0.15</td>
</tr>
<tr>
<td>Ca %</td>
<td>0.6 ± 0.12</td>
<td>0.5 ± 0.13</td>
<td>0.6 ± 0.12</td>
</tr>
<tr>
<td>Cl %</td>
<td>0.6 ± 0.08</td>
<td>0.6 ± 0.18</td>
<td>0.6 ± 0.15</td>
</tr>
<tr>
<td>Mn (mg/kg)</td>
<td>87.6 ± 16.1</td>
<td>90.6 ± 18.8</td>
<td>84.9 ± 11.4</td>
</tr>
<tr>
<td>Fe (mg/kg)</td>
<td>334.0 ± 99.6</td>
<td>345.5 ± 119.7</td>
<td>333.3 ± 87.1</td>
</tr>
<tr>
<td>Cu (mg/kg)</td>
<td>21.5 ± 4.0</td>
<td>23.2 ± 5.8</td>
<td>24.0 ± 21.8</td>
</tr>
<tr>
<td>Zn (mg/kg)</td>
<td>91.9 ± 17.7</td>
<td>93.2 ± 19.3</td>
<td>88.1 ± 16.2</td>
</tr>
</tbody>
</table>
Table 9: Body weight, DMI, urine pH, blood metabolites, colostrum yield, and colostrum composition for cows fed Control, Tri, or Tri + Zy treatment during the 21 d prior to parturition.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control</th>
<th>Tri</th>
<th>Tri + Zy</th>
<th>SE</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>BW (kg)</td>
<td>814.9</td>
<td>780.7</td>
<td>808.0</td>
<td>19.7</td>
<td>0.62</td>
</tr>
<tr>
<td>BW Gain (kg/wk)</td>
<td>13.9</td>
<td>55.6</td>
<td>39.6</td>
<td>40.4</td>
<td>0.74</td>
</tr>
<tr>
<td>Efficiency of Gain (BWG/DMI)²</td>
<td>0.12</td>
<td>0.16</td>
<td>0.13</td>
<td>0.09</td>
<td>0.92</td>
</tr>
<tr>
<td>DMI (kg)</td>
<td>14.7</td>
<td>14.4</td>
<td>15.2</td>
<td>0.7</td>
<td>0.60</td>
</tr>
<tr>
<td>Initial Urine pH³</td>
<td>8.19</td>
<td>8.24</td>
<td>8.20</td>
<td>3.66 x 10⁻¹⁰</td>
<td>0.29</td>
</tr>
<tr>
<td>Urine pH³</td>
<td>7.42</td>
<td>7.87</td>
<td>7.52</td>
<td>9.49 x 10⁻⁹</td>
<td>0.17</td>
</tr>
<tr>
<td>BHBA (mmol/L)</td>
<td>0.62</td>
<td>0.64</td>
<td>0.55</td>
<td>0.08</td>
<td>0.64</td>
</tr>
<tr>
<td>Glucose (mg/dL)</td>
<td>63.9</td>
<td>64.3</td>
<td>68.0</td>
<td>2.1</td>
<td>0.32</td>
</tr>
<tr>
<td>NEFA (μmol/L)</td>
<td>96.6</td>
<td>94.2</td>
<td>80.5</td>
<td>32.9</td>
<td>0.92</td>
</tr>
<tr>
<td>Colostrum Yield (kg)</td>
<td>10.7</td>
<td>6.6</td>
<td>9.4</td>
<td>1.6</td>
<td>0.16</td>
</tr>
<tr>
<td>IgG Content (mg/mL)</td>
<td>79.1</td>
<td>88.2</td>
<td>91.1</td>
<td>6.0</td>
<td>0.34</td>
</tr>
<tr>
<td>IgG Yield (g)</td>
<td>836.8</td>
<td>562.0</td>
<td>782.3</td>
<td>116.5</td>
<td>0.19</td>
</tr>
<tr>
<td>IgA Content (mg/mL)</td>
<td>6.3</td>
<td>6.1</td>
<td>7.0</td>
<td>0.6</td>
<td>0.48</td>
</tr>
<tr>
<td>IgA Yield (g)(^5)</td>
<td>63.3(^x)</td>
<td>37.2(^y)</td>
<td>62.8(^y)</td>
<td>8.8</td>
<td>0.05</td>
</tr>
<tr>
<td>Fat Percentage (%)</td>
<td>5.8</td>
<td>5.1</td>
<td>4.9</td>
<td>0.9</td>
<td>0.75</td>
</tr>
<tr>
<td>Fat Yield (kg)</td>
<td>0.66</td>
<td>0.37</td>
<td>0.52</td>
<td>0.13</td>
<td>0.24</td>
</tr>
<tr>
<td>TP(^6) Percentage (%)</td>
<td>13.7</td>
<td>16.1</td>
<td>16.1</td>
<td>1.0</td>
<td>0.13</td>
</tr>
<tr>
<td>TP(^6) Yield (kg)</td>
<td>1.4</td>
<td>1.1</td>
<td>1.33</td>
<td>0.2</td>
<td>0.41</td>
</tr>
<tr>
<td>TS(^6) Percentage (%)</td>
<td>26.0</td>
<td>25.3</td>
<td>24.9</td>
<td>2.2</td>
<td>0.93</td>
</tr>
<tr>
<td>TS(^6) Yield (kg)</td>
<td>2.7</td>
<td>1.7</td>
<td>2.2</td>
<td>0.4</td>
<td>0.13</td>
</tr>
<tr>
<td>Ash Percentage (%)²</td>
<td>1.07b</td>
<td>1.16ab</td>
<td>1.25a</td>
<td>0.05</td>
<td>0.07</td>
</tr>
<tr>
<td>Ash Yield (kg)</td>
<td>0.11</td>
<td>0.06</td>
<td>0.08</td>
<td>0.02</td>
<td>0.25</td>
</tr>
<tr>
<td>Lactose Percentage (%)</td>
<td>5.5</td>
<td>2.9</td>
<td>2.6</td>
<td>1.6</td>
<td>0.35</td>
</tr>
<tr>
<td>Lactose Yield (kg)</td>
<td>0.47</td>
<td>0.19</td>
<td>0.26</td>
<td>0.12</td>
<td>0.18</td>
</tr>
</tbody>
</table>

¹Treatments: Control = No Tri-Lution® or Zy-mend®; Tri = 27.24 g/d of Tri-Lution®; Tri + Zy = 27.24 g/d of Tri-Lution® and 18.16g/d f Zy-mend®.
²Efficiency of gain = body weight gain (kg)/ dry matter intake (kg)
³The standard error for the urine pH values is in H⁺ ion concentration.
⁴Means in same row with superscripts x, y differ (p ≤ 0.10).
⁵TP = True Protein.
⁶TS = Total Solids.
⁷Means in subscript significantly differ (p ≤ 0.05).
Table 10: Calf weight, serum IgG, serum IgA, IgG apparent efficiency of absorption, and IgA apparent efficiency of absorption of calves from cows fed a Control, Tri or Tri + Zy treatment 21 d prior to parturition.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control</th>
<th>Tri</th>
<th>Tri + Zy</th>
<th>SE</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calf Weight (kg)</td>
<td>44.1</td>
<td>44.7</td>
<td>45.8</td>
<td>2.0</td>
<td>0.83</td>
</tr>
<tr>
<td>Serum IgG 0 h (mg/mL)</td>
<td>1.7</td>
<td>0.3</td>
<td>1.2</td>
<td>1.0</td>
<td>0.58</td>
</tr>
<tr>
<td>Serum IgG 24 h (mg/mL)</td>
<td>24.6</td>
<td>25.9</td>
<td>26.3</td>
<td>3.4</td>
<td>0.92</td>
</tr>
<tr>
<td>Serum IgA 0 h (mg/mL)</td>
<td>0.03</td>
<td>0.00</td>
<td>0.00</td>
<td>0.02</td>
<td>0.44</td>
</tr>
<tr>
<td>Serum IgA 24 h (mg/mL)</td>
<td>1.43</td>
<td>1.54</td>
<td>1.49</td>
<td>0.15</td>
<td>0.51</td>
</tr>
<tr>
<td>IgG AEA^2 (%)</td>
<td>34.1</td>
<td>31.1</td>
<td>33.5</td>
<td>3.4</td>
<td>0.80</td>
</tr>
<tr>
<td>IgA AEA^3 (%)</td>
<td>29.3</td>
<td>28.1</td>
<td>29.5</td>
<td>2.9</td>
<td>0.93</td>
</tr>
</tbody>
</table>

^1Treatments: Control = No Tri-Lution® or Zy-mend®; Tri = 27.24 g/d of Tri-Lution®; Tri + Zy = 27.24 g/d of Tri-Lution® and 18.16g/d f Zy-mend®.

^2Apparent Efficiency of Absorption of IgG = ((Plasma IgG [g/L] * BW [kg] * 0.09)/IgG Intake[g])*100

^3Apparent Efficiency of Absorption of IgA = ((Plasma IgA [g/L] * BW [kg] * 0.090/IgA Intake[g]))*100
Table 11: Postpartum BW, DMI, blood metabolites, milk yield and composition for cows fed Control, Tri, or Tri + Zy treatments during the first 8 weeks of lactation.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control</th>
<th>Tri</th>
<th>Tri + Zy</th>
<th>SE</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>BW(kg)</td>
<td>739.4</td>
<td>723.9</td>
<td>742.1</td>
<td>19.0</td>
<td>0.74</td>
</tr>
<tr>
<td>BW Gain (kg/wk)&lt;sup&gt;2&lt;/sup&gt;</td>
<td></td>
<td>-5.7&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>-1.3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>-7.9&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.8</td>
</tr>
<tr>
<td>Efficiency of Gain (BWG/DMI)&lt;sup&gt;3&lt;/sup&gt;</td>
<td>-0.04&lt;sup&gt;xy&lt;/sup&gt;</td>
<td>-0.02&lt;sup&gt;x&lt;/sup&gt;</td>
<td>-0.05&lt;sup&gt;y&lt;/sup&gt;</td>
<td>0.01</td>
<td>0.09</td>
</tr>
<tr>
<td>DMI (kg)</td>
<td>24.7</td>
<td>24.3</td>
<td>24.0</td>
<td>0.8</td>
<td>0.78</td>
</tr>
<tr>
<td>BHBA (mmol/L)</td>
<td>0.82</td>
<td>0.70</td>
<td>0.75</td>
<td>0.07</td>
<td>0.43</td>
</tr>
<tr>
<td>Glucose (mg/dL)</td>
<td>53.1</td>
<td>56.3</td>
<td>52.7</td>
<td>2.0</td>
<td>0.33</td>
</tr>
<tr>
<td>NEFA (μmol/L)</td>
<td>231.0</td>
<td>185.1</td>
<td>210.5</td>
<td>25.6</td>
<td>0.39</td>
</tr>
</tbody>
</table>

**Milk**

| Yield (kg)                       | 48.4    | 43.4    | 47.7     | 2.1  | 0.17    |
| ECM Yield (kg)<sup>4</sup>       | 45.2    | 41.4    | 43.7     | 2.0  | 0.34    |
| Milk Efficiency<sup>5</sup>      | 1.87    | 1.75    | 1.83     | 0.08 | 0.50    |
| Fat Content (%)                  | 3.03    | 3.21    | 2.76     | 0.17 | 0.17    |
| Fat Yield (kg)                   | 1.44    | 1.34    | 1.33     | 0.08 | 0.49    |
| True Protein Content (%)         | 2.96    | 3.07    | 3.03     | 0.09 | 0.64    |
| True Protein Yield (kg)          | 1.41    | 1.30    | 1.42     | 0.05 | 0.19    |
| Lactose Content (%)              | 4.81    | 4.78    | 4.84     | 0.04 | 0.56    |
| Lactose Yield (kg)               | 2.3     | 2.1     | 2.3      | 0.1  | 0.13    |
| Total Solid Content (%)          | 11.7    | 12.0    | 11.6     | 0.3  | 0.43    |
| Total Solid Yield (kg)           | 5.6     | 5.1     | 5.5      | 0.2  | 0.23    |
| MUN(mg/dL)                       | 12.3    | 12.9    | 13.2     | 1.1  | 0.79    |
| Somatic Cell Score<sup>6</sup>   | 1.7ab   | 2.7a    | 0.9b     | 0.5  | 0.04    |

<sup>1</sup>Treatments: Control = No Tri-Lution® or Zy-mend®; Tri = 27.24 g/d of Tri-Lution®; Tri + Zy = 27.24 g/d of Tri-Lution® and 18.16g/d f Zy-mend®.

<sup>2</sup>Means in same row with superscripts a, b significantly differ (P < 0.05).

<sup>3</sup>Efficiency of gain = body weight gain (kg)/ dry matter intake (kg); Means in same row with superscripts x, y differ (P < 0.10).

<sup>4</sup>ECM Yield = energy corrected milk yield; ECM Yield = ((12.82 × fat lbs) + (7.13 × protein lbs) + (0.323 × milk lbs)) / 2.2046.

<sup>5</sup>Milk efficiency = energy corrected milk yield (kg)/ dry matter intake (kg).

<sup>6</sup>Means in same row with subscripts a, b significantly differ (P < 0.05).
Figures

Figure 4: Prepartum Body Weight by Week

![Graph showing body weight changes over weeks for different groups. The graph plots body weight (kg) against week, with error bars indicating variability. The groups compared are Control, Tri, and Tri+Zy.](image-url)
Figure 5: Prepartum Body Weight Gain by Week
Figure 6: Prepartum Efficiency of Gain by Week
Figure 7: Prepartum Daily Dry Matter Intake by Week

Dry Matter Intake (kg)

Week

Control
Tri
Tri + Zy
**Figure 8: Prepartum Urine pH by Week**

Prepartum urine pH standard error is $9.49 \times 10^{-9}$ and is in $\text{H}^+$ concentration.
Figure 9: Prepartum Serum β-Hydroxybutyrate Concentration by Week
Figure 10: Prepartum Serum Non-esterified Fatty Acid Concentration by Week
Figure 11: Prepartum Serum Glucose Concentration by Week
Figure 12: Postpartum Body Weight by Week
Figure 13: Postpartum Body Weight Gain by Week
Figure 14: Postpartum Efficiency of Gain by Week
Figure 15: Postpartum Daily Dry Matter Intake by Week

Dry Matter Intake (kg)

Week

Control
Tri
Tri + Zy
Figure 16: Postpartum Serum β-Hydroxybutyrate Concentration by Week
Figure 17: Postpartum Serum Non-esterified Fatty Acid Concentration by Week
Figure 18: Postpartum Serum Glucose Concentration by Week

![Graph showing postpartum serum glucose concentration by week. The graph compares the glucose levels over eight weeks for three groups: Control, Tri, and Tri + Zy. The x-axis represents the week, ranging from 1 to 8, and the y-axis represents glucose concentration in mg/dL, ranging from 40 to 70. Each group is represented by a different line color: blue for Control, red for Tri, and green for Tri + Zy.]
Figure 19: Milk Yield by Week

Milk Yield (kg)

Week

Control
Tri
Tri + Zy
Figure 20: Energy Corrected Milk Yield by Week

<table>
<thead>
<tr>
<th>Week</th>
<th>Control</th>
<th>Tri</th>
<th>Tri + Zy</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Figure 21: Milk Efficiency by Week

Milk Efficiency = Energy Corrected Milk Yield/Dry Matter Intake

1Milk Efficiency = Energy Corrected Milk Yield/Dry Matter Intake
Figure 22: Milk Fat Yield by Week

Milk Fat Yield (kg)

Week

Control
Tri
Tri + Zy
Figure 23: Milk True Protein Yield by Week

![Graph showing milk true protein yield by week for Control, Tri, and Tri + Zy groups.](image-url)
Figure 24: Lactose Yield by Week

![Lactose Yield by Week](image)

- **Lactose Yield (kg)**
- **Week**
  - Control
  - Tri
  - Tri + Zy
Figure 25: Total Solids Yield by Week

![Graph showing Total Solids Yield by Week with different curves for Control, Tri, and Tri + Zy groups.](image-url)
Figure 26: Milk Fat Percentage by Week
Figure 27: Milk True Protein Percentage by Week

- **Control**
- **Tri**
- **Tri + Zy**

![Graph showing milk true protein percentage by week](image-url)
Figure 28: Lactose Percentage by Week

Lactose Percentage (%)

Week

1 2 3 4 5 6 7 8

Control
Tri
Tri + Zy
Figure 29: Total Solids Percentage by Week

![Graph showing Total Solids Percentage by Week with different treatments: Control, Tri, and Tri + Zy. The graph displays the percentage of total solids for each week from 1 to 8, with error bars indicating variability.](image-url)
Figure 30: Milk Urea Nitrogen by Week

Milk Urea Nitrogen (mg/dL)

Week

Control
Tri
Tri + Zy
Figure 31: Somatic Cell Score by Week

- Somatic Cell Score
- Week
- Control
- Tri
- Tri + Zy

1 2 3 4 5 6 7 8

-2.0 -1.0 0.0 1.0 2.0 3.0 4.0 5.0 6.0
LIST OF REFERENCES


Arthington, J. D., M. B. Cattell, J. D. Quigley III. Effect of Dietary IgG Source (Colostrum, Serum, or Milk-Derived Supplement) on the Efficiency of Ig Absorption in Newborn Holstein Calves. J. Dairy Sci. 83(7):1463–1467.


Appendices
Appendix A

University of New Hampshire
P.O. Box 5
Durham, NH 03827

19 Dec 2014

Elecckson, Peter S
Biological Sciences, Dairy Nutrition Research Center
30 O'Hare Road
Danham, NH 03827

IACUC # 14-31637
Protocol: The Impact of Direct Feed Microbial Supplementation (TF, Lactic and Zy-Mono) on Health and Performance of Transition Cows with Emphasis on GI Content in Coliform and Gi5
Category: C
Approval Date: 11 Dec 2014

The Institutional Animal Care and Use Committee (IACUC) reviewed and approved the protocol entitled for this study under Category C on Page 8 of the Application for the Review of Numbers, Animal Use in Research or Instruction - the Research Protocol Checklist. Animal Identification, Policy and Procedures or disease which will be treated with appropriate medications, diagnostics or other assessments.

Approval is granted for a period of three years from the approval date above. Continued approval throughout the three year period is contingent upon completion of annual reports on the use of animals. At the end of the three year approval period you must submit a new application and request for extension to continue this project. Requests for extension must be filed prior to the expiration of the original approval.

Please Note:
1. All cage, pen, or other animal identification records must include your IACUC number above.
2. Use of animals in research or instruction is approved contingent upon participation in the University Occupational Health Program for persons handling animals. Participation is mandatory for all principal investigators and other personnel employed by the University and students also. Information about the program, including forms, is available at: http://health.unh.edu/applications/health-care-assurance-handling.

If you have any questions, please contact me at 862-4525 or Julie Simpson at 862-2560.

Pet the IACUC
Rick Secor
Vice Chair

cc: FAE