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COLOSTRUM COMPONENTS AND THEIR IMPACT ON DIGESTIVE FUNCTION AND GROWTH OF DAIRY CALVES

A Thesis in

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by

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ABSTRACT

Colostrum is the first important step in raising healthy dairy calves. Feeding of adequate volumes of colostrum within the first 2 hours after birth improves calf health, immunity and productive lifespan. Two experiments were conducted, the first was to evaluate and record colostrum management and nutrient composition to determine any improved or deleterious changes. The second was to evaluate the effects of a particular component of colostrum, nucleotides, on enhancing absorptive capacity and decreasing diarrhea in neonatal dairy calves.

For the first experiment, data and samples were obtained from 55 farms around the state of Pennsylvania. Samples were analyzed for fat, protein, lactose, total solids, ash, immunoglobulins, lactoferrin, water- and fat-soluble vitamins and minerals. Results of means of fat, protein, lactose and lactoferrin were as follows: 6.7%, 14.92%, 2.49% and 0.82 mg/ml, respectively. Immunoglobulin G1, G2, A and M were 35.1 ± 11.9, 5.6 ± 1.9, 1.7 ± 0.9 and 4.3 ± 2.8 g/L, respectively. Fat-soluble vitamins included retinol, tocopherol and B-carotene and were 4.9, 2.92 and 0.68 ug/g, respectively. Means of water-soluble vitamins were 0.34, 0.90, 4.55, 0.60, 0.15, 0.21 and 0.04 for niacinamide, thiamine, riboflavin, cyanocobalamin, pyridoxal, pyridoxamine and pyridoxine, respectively. Means of minerals (mg/kg) consisted of calcium 4716.08; phosphorus 4452.12; magnesium 733.24; sodium 1058.93; potassium 2845.89; zinc 38.10; iron 5.33; copper 0.34 and manganese 0.10.

In comparison to previous reviews of bovine colostrum, most nutrient levels have increased. Management practices have also improved in areas of colostrum storage and feeding. Calves were fed by bottle in 85% of farms and only 1 farm allowed calves to nurse the dam. Calves were fed first colostrum within 6 hours of birth in 95% of farms and 38% of farms had a supply of frozen colostrum readily available for use.

For the second experiment, to analyze for effects of feeding purified or yeast derived nucleotides, calves were fed milk replacer at 10% of BW daily and treatments consisted of no nucleotide supplementation (C), purified nucleotide supplementation (N) and yeast supplementation (P; Alltech, Inc.). Growth, health, fecal dry matters and fecal bacteria were monitored and blood was analyzed for hematocrits, glucose, blood urea

nitrogen and creatinine. After the second day of scouring, xylose (0.5 g/kg of BW) was administered orally once and blood was taken before administration for a baseline as well as 1, 2, 3 and 4 h post-dosing. Concentrations of plasma xylose increased with time post-dosing, but did not vary by treatment. Hematocrits were significantly higher for treatment N for weeks 2 and 5. Growth parameters and plasma concentrations of glucose, BUN, creatinine and IgG were not different between treatments. There were also no treatment effects on fecal dry matters and concentrations of fecal bacteria. Intestinal parameters, including morphology, enzyme activities, DNA and protein concentrations were not significantly affected by nucleotide supplementation. Calves supplemented with purified nucleotides had higher hematocrits, higher bacterial concentrations of Clostridium in feces and similar xylose absorption to control calves. Calves supplemented with yeast-derived nucleotides had increased xylose uptake 4 hours after dosing, highest Lactobacilli and Bifidobacteria concentrations in feces but a higher ratio of BUN to creatinine indicating higher dehydration. Dietary supplementation of nucleotides did not appear to enhance any growth, health or metabolic parameters in this model. More research needs to be conducted to investigate optimal concentrations of supplemented nucleotides as well as method and time of administration.

Neonatal calf diarrhea is a persistent problem on dairy farms. Education for improved colostrum management and feeding needs to be developed and consistently presented to producers to improve calf health and management. More research also needs to be conducted to determine optimal levels and methods of feeding of nucleotides in order to decrease calf morbidity and mortality in the first weeks of life.

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Chapter 1

INTRODUCTION

Neonatal calf diarrhea is a major cause of death and economic loss in the dairy industry. According to NAHMS (2002), calf mortality averages 8.7% annually of which 62.1% is due to scours. In 1991, mortality due to scours averaged 52.5% and 60.5% in 1996 indicating an expanding problem in the dairy industry. Surveyed farms indicated that 30.5% of producers still allow calves to first nurse off the dam and 16% of calves are not fed first colostrum until 12 h after birth.

Colostrum feeding and management is the first important step in raising healthy calves. Feeding colostrum in adequate amounts and quickly after birth can improve calf immunity, health and productive lifespan. The most recent review of bovine colostrum composition was compiled in 1978 by Foley and Otterby; most recent surveys of colostrum management and calf care were completed 20 years ago (Goodger, 1986; Heinrichs, 1987). Since then, management practices, nutrition and nutrient analysis techniques have changed warranting a recent review (Kehoe, 2006).

According to NAHMS (2002), 12.5% of farms in the U. S. feed milk replacer and 87.5% feed waste milk. Both of these feed sources are low in nucleotides and may be inadequate during times of intestinal infection as well as periods of rapid growth. All species investigated have shown an improvement in intestinal health and immunity when provided with dietary nucleotides during periods of stress. This is an indication that calves would experience similar positive effects from nucleotide supplementation which may even replace the use of medicated milk replacers.

Delayed and inadequate colostrum feeding can result in increased morbidity and mortality. Treatment of sick calves is not only expensive, but is often difficult due to numerous causative pathogens (Constable, 1996; Torres-Medina, 1985). Therefore, the first objective of this study was to record methods used for the colostrum management and to analyze colostrum nutrient composition in comparison with other reviews. The second objective was to evaluate whether supplemental nucleotides, important

components of colostrum, would affect intestinal health and calf growth and result in a decrease in calf scouring and morbidity.

Chapter 2

REVIEW OF COLOSTRUM NUTRIENT COMPOSITION

Colostrum, defined as the secretions of the mammary gland prior to parturition, is extremely important for all mammals. Ingestion of colostrum is most important in ruminants with a cotyledenary placenta that inhibits transfer of immunoglobulins in utero. Post parturition, many neonates must ingest colostrum to attain passive transfer of immunity. Regardless of placental structure and transfer of immunity in utero, ingestion of colostrum after birth also confers additional protection by binding to intestinal receptors which could otherwise be bound by bacteria.

For the bovine neonate adequate colostrum management and feeding reduce neonatal mortality, strengthen immunity and increase animal life span (Besser et al., 1988; Besser and Gay, 1994; Quigley and Drewry, 1998). Delaying intake of colostrum in dairy calves not only decreases transport of immunoglobulins but also fat-soluble vitamins (Zanker, 2000). Dairy calves that receive colostrum 12 to 25 h after birth have lower plasma concentrations of beta-carotene, retinol and alpha-tocopherol for almost a month after birth compared to calves that receive colostrum within 7 h (Zanker, 2000). These vitamins play a role in immunity, and their absence may predispose neonates to enteric infections, thereby re-enforcing the importance of proper colostrum feeding.

The composition of colostrum is important in satisfying nutritional requirements for neonates. Colostrum provides immunity as well as necessary nutrients which only minimally cross the placenta, such as fat-soluble vitamins (Spielman et al., 1946). Neonates also require fat and protein for energy and muscle development in the first days of life as well as growth factors, hormones and many other biologically important components found in colostrum (Roy, 1990).

Placental structure

Different placental structures allow different amounts of immunoglobulins to reach the fetus from the maternal blood supply. The fetal part of the placenta, or the chorion, contains chorionic villi which vary in distribution depending on the species. The

degree of intimacy these villi allow with the maternal endothelium plays a role in nutrient transport between maternal and fetal blood. Transport of large molecular weight nutrients depends on how many membranes separate the fetal and maternal blood supplies (Senger, 1999).

Ruminants have a cotyledonary placenta where the chorionic villi are concentrated in cotyledons which originate from the chorion and attach to the caruncle of the uterus. This is also known as a synepitheliochorial placenta. This type of placenta is non-invasive due to 8 membranes separating the fetal and maternal blood circulations (Fowden, 2006). The epitheliochorial placenta, which is found in pigs and horses, is described as diffuse and contains chorionic villi that are closely spaced and distributed evenly all over the placenta. Zonary placentas are found in dogs and cats and consist of a band of chorionic villi that form a broad zone near the middle of the fetus. Rodents and primates have a discoid placenta which concentrates chorionic villi in one or two discs, in one region of the chorion, that interface with the endometrium (Senger, 1999). Although rodents have the same discoid placenta as primates and humans, they have additional trophoblast layers which contribute to more of a maternal-fetal exchange barrier (Fowden, 2006). Primates and humans develop a placenta that is fully in contact with maternal circulation (Fowden, 2006). Although little immunoglobulin is transported in the first trimester, receptors for immunoglobulin transport develop and the fetus becomes fully protected by its mother's immune system before birth (Simister, 2003).

Ruminant colostrum composition

Fat

Fat content in bovine colostrum is extremely variable, ranging anywhere from 0.3 to 18%; however, most cows produce colostrum with much higher levels of fat compared to milk (Parrish, 1950). Reported colostrum values range from 6.7% (Parrish, 1950) to 7.78% (Weiss et al., 1992) and 9.2% (Lona-D, 2001).

Research has been conducted to try and increase fat in colostrum using higher dietary fat. However, supplementing beef or Holstein cows with higher dietary fat during the last part of gestation has shown no effect on levels of fat in colostrum (Weiss *et al.*, 1992; Weiss et al., 1990). Effects of fat supplementation during the latter part of

gestation on early lactation milk result in a numerical increase of fat composition (Davenport, 1969; Nocek et al., 1986). This may be due to ruminants using energy during gestation to increase fat reserves and improve body condition. Once lactation begins, animals with previous supplementation are able to secrete more fat into milk due to a larger established fat reserve to draw upon. Colostrum is most likely unaffected due to the fact that it is secreted before the beginning of lactation when the body reserves are still unused.

Although dietary fat levels do not produce changes in the fat content of bovine colostrum, composition of fatty acids may change. Linoleic acid is the main fuel for brown adipose tissue heat production, and when rats are fed high levels of linoleic acid, heat production from the metabolism of brown adipose tissue is increased in rat pups (Nedergaard, 1983). Feeding beef heifers diets with safflower oil, which contains high concentrations of linoleic acid, increases the ability of calves to maintain their body temperature (Dietz, 2003; Lammoglia et al., 1999). Although effect of dietary linoleic acid on fatty acid composition of colostrum has not been investigated, calves drinking colostrum with higher concentrations of linoleic acid may have increased metabolism of brown adipose tissue.

Fatty acid composition of colostrum has been compared between species and breeds. Colostrum from Holstein cows has lower concentrations of octanoic and decanoic fatty acids compared with Nubian and Alpine goats (Attaie et al., 1993). The fatty acid composition of bovine milk has also been compared in similarity to butter fat, which is high in oleic acid and has lower concentrations of low molecular weight fatty acids (Anantakrishnan, 1946; Baldwin, 1944). These differences in fatty acid composition are most likely due to differences in the needs of the neonates and have developed evolutionarily.

Protein

Neonates need high amounts of protein in the first days of life to sustain energy levels and growth. Protein levels are high in bovine colostrum, averaging 14% as reported in a review by Foley and Otterby (1978), which is much higher than mature milk at 3.9% (Akers, 2002). However, manipulation of protein in the diet does not affect

colostral protein content. Researchers have reported that although beef heifers restricted in protein intake gain less during the last trimester, there is no effect on colostrum energy and protein content (Carstens, 1987). Hook *et al.* (1989) fed Holstein heifers either 9.9% or 13% dry matter crude protein and reported that although total protein was higher in serum for heifers fed higher crude protein diets, concentrations of IgG and IgM in colostrum were not different. Holstein heifers fed 12.7% and 14.7% also did not differ in concentrations of protein or IgG in colostrum (Santos, 2001). The reason for this may be due to a similar mechanism to fat supplementation, where protein is used for body condition and colostrum remains unaffected.

Colostral proteins consist of many different proteins, including immunoglobulins, which are either produced in the mammary gland or transported from the circulatory system of the dam. Two major proteins found in the colostral whey of ruminants are alpha-lactalbumin and beta-lactoglobulin. These proteins are synthesized in the mammary gland at higher concentrations during colostrum production than during normal milk production.

Beta-lactoglobulin concentrations in colostrum are 18.9 and 17.3 mg/ml for cows and sheep, respectively. In colostral secretions, beta-lactoglobulin is about 5 times higher than in milk and decreases rapidly and drastically as colostrum transitions into milk. The function of beta-lactoglobulin is not known completely, but due to its structure it has been hypothesized to bind retinol-binding protein and be involved in the uptake of vitamin A in the intestine of newborn calves (Papiz et al., 1986). Beta-lactoglobulin also has the ability to bind long chain fatty acids and triglycerides in vitro and may be involved in the transport of lipid either in the mammary gland or calf intestine (Diaz de Villegas et al., 1987).

Alpha-lactalbumin is the functional subunit of the lactase enzyme and is needed for lactose synthesis in the mammary gland (Brodbeck et al., 1967). Alpha-lactalbumin concentrations in cow and sheep colostrum are 2 and 2.3 mg/ml, respectively, which are only about 1.5 times higher than whole milk (Perez et al., 1990).

Another important protein found in colostrum is lactoferrin, which is a glycoprotein that binds iron. Supplementation of lactoferrin in milk replacer fed to young dairy calves is reported to reduce morbidity and improve growth before weaning

(Robblee, 2003). Although differences in quantifying lactoferrin levels may exist due to laboratory techniques, bovine colostrum contains lower lactoferrin concentrations than other species (Masson, 1971). Yoshida *et al.* (2000) used chromatography and reported an average of 0.34 ± 0.23 mg/ ml for 6 cows. In contrast, Tsuji *et al.* (1990) used 45 Holstein cows and reported an average of 1.96 ± 0.27 mg/ ml using radial immunodiffusion (RID). Sanchez *et al.* (1988), using DEAE-Sephadex chromatography, reported concentrations of 0.83 ± 0.58 mg/ ml. In comparison, human colostrum contains 10.58 mg/ ml lactoferrin (Fransson et al., 1980).

Other proteins found in colostrum are albumin and transferrin. Transferrin is a glycoprotein similar to lactoferrin and also functions in transporting and binding iron. Transferrin concentration in first milking colostrum is 1.07 ± 0.45 mg/ ml and decreases with increased lactation (Sanchez *et al.*, 1988). Albumin is most commonly found in plasma as a transport protein for many biologically active proteins (Peters, 1985). The amino acid sequence of bovine plasma albumin is identical to milk albumin and, therefore, was commonly believed to be a marker for the disruption of tight junctions in the mammary gland. However, Shamay *et al.* (2005) reported that along with many other tissues that secrete albumin, bovine mammary gland explants also express albumin mRNA and secrete albumin that is synthesized directly from the tissue into the medium. Although mastitic and dry mammary glands secrete higher levels of mRNA, healthy tissue also secretes an average of 1 ng/ mg of tissue. This indicates that using albumin as an indicator of infection is still applicable; however, the function of tight junctions may not be affected. Albumin in first milking colostrum is reported to average 2.63 ± 0.86 mg/ ml (Sanchez *et al.*, 1988).

Immunoglobulins

Immunoglobulins from ruminants generally have the same features as other mammalian immunoglobulins. Established classes of immunoglobulins are immunoglobulin G1 (IgG1), immunoglobulin G2 (IgG2), immunoglobulin M (IgM) and immunoglobulin A (IgA); all are secreted at high concentrations into colostrum (Micusan, 1977). A secretory component of IgA (sIgA) has also been classified (Pahud,

1970) indicating the small intestine of calves secretes sIgA which can aid in further protection.

Past research has indicated that colostrum from first-calf heifers may be lower in concentration of IgG when compared with colostrum of older cows. This may be due to multiple causes however a major factor may be due to age which would limit the time on the farm for exposure to pathogens and vaccinations. Less exposure and vaccinations may decrease the concentration of circulating anitobdies which would decrease the antibody content transferred into colostrum. Muller and Ellinger (1981) used a university herd to compare immunoglobulin levels across breeds including Holstein, Brown Swiss, Guernsey, Ayrshire and Jersey. Differences between breeds ranged from Holstein colostrum containing lowest concentrations of IgG and Jersey containing the highest. First parity colostrum averaged 2.64% of IgG as a percentage of total colostrum as compared with 5.52% for second parity, 7.69% and 6.9% for third and fourth parities.

Oyeniyi and Hunter (1978) reported higher concentrations of IgG in fourth through seventh lactation colostrum compared to younger lactations (41.6 and 31.4 mg/ ml, respectively). Similarly, Tyler *et al.* (1999) collected colostrum samples from 77 Holstein cows at a university herd and found higher concentrations in third and greater lactations (97 mg/ ml IgG), but no differences between first and second lactation colostrum (66 and 75 mg/ ml, respectively). Although younger animals tend to produce less immunoglobulin in colostrum, it is not recommended to throw out this colostrum but to test all colostrum for quality since older animals may also produce colostrum with lower immunoglobulin content.

Few groups researching colostral immunoglobulin concentrations have specified colostrum volumes produced, which significantly affect immunoglobulin concentration. Devery-Pocius and Larson (1983) reported significantly lower volumes produced by first-lactation heifers (23.1 L) than by other lactation groups (ranging from 32.9 to 36.4 L), however, values reported were composites of the first four milkings. Larson and Kendall (1957) reported significantly lower volumes produced by first-lactation heifers that averaged 10 kg, but only 8 animals were used. Other research reported differences in colostral immunoglobulin concentrations while maintaining all animals on similar diets. In a study utilizing 919 cows from one farm sampled over 4 years, Pritchett *et al.* (1991)

reported IgG1 was lower in first and second lactation cows than older lactations and that volume (which averaged 8.5 kg) was a significant factor in dictating the concentration of immunoglobulins present in colostrum.

Previous research indicates that nutrient availability prepartum does not significantly affect colostrum IgG content. Halliday *et al.* (1978) reported no differences in colostrum IgG content and total protein when beef cows were fed 12 weeks before parturition on diets ranging from 65 to 125% of maintenance for 2 consecutive years. Santos *et al.* (2001) fed Holstein cows different amounts of rumen undegradable protein, which did not result in significant differences in colostrum IgG content. Beef cows fed diets nutritionally restricted at 57 or 100% of NRC requirements for energy and protein also did not produce colostrum differing in IgG concentration (Hough et al., 1990), further indicating lack of nutritional effect on colostrum quality.

Concentrations of IgA in bovine colostrum are found in lower concentrations than IgG and research results are dependent on the assay used to be specific enough for such small concentrations. Stott *et al.* (1981) reported a concentration of 17.8 mg/ ml for IgA using microdiffusion discs as described by Masseyeff and Zisswiller (1969); however, most other researchers use RID assays and report values between 3 and 4 mg/ ml (Husband, 1972; Mach, 1971; Pahud, 1970; Porter, 1972). These differences may be due to the sensitivity of the microdiffusion discs compared to the radial immunodiffusion assay used by the majority of other labs.

Concentrations of IgM are lower in colostrum than IgG but higher than IgA and. Stott *et al.* (1981) reported a concentration of 2 mg/ ml for IgM. Other work was variable, reporting values anywhere from 3 to 12 mg/ ml (Husband, 1972; Klaus, 1969; Mach, 1971; Pahud, 1970; Porter, 1972). Muller and Ellinger (1981) reported IgA tended to be lower for first parity cows, and Rzedzicki *et al.* (1982) reported no effect of first through third parities on IgM concentrations in colostrum. These subtypes of immunoglobulins should be affected similarly to IgG; the variation of IgM and IgA may also be influenced by parity, breed, vaccinations, health status and other factors, however this needs further research.

There is also currently a need for more reliable and repeatable assays that can handle the high solids content of colostrum. Immunodiffusion, as described by

Ouchterlony (1958), is the most common method for immunoglobulin analysis; however, methods differ between research groups and there is a need for standardization. Many labs use whey from colostrum rather than whole colostrum, which has been shown to elevate immunoglobulin concentrations (Fleenor et al., 1981). When evaluating serum concentrations of IgG, some studies use zinc sulphate turbidity (ZST) units to determine total IgG levels; these may not be comparable to other assays that use other assays to determine total IgG (Reid, 1972). Comparisons between assays have been published using serum and have shown successful results with RID, serum electrophoresis and zinc sulfate turbidity but poor results with sodium sulfite precipitation and using a refractometer (Pfeiffer, 1977; Rumbaugh, 1978). Colostrum is even more difficult to work with than serum, and no comparison of assays has been published as of yet.

Lactose

Lactose concentrations in colostrum average 2.5% (Eales, 1982; Parrish, 1950; Praphulla, 1959). Lactose is low in colostrum and acts inversely of other constituents, such as solids, protein and ash, which are all found in high concentrations and decrease over time. Lactose is an osmotically active component of milk which is a reason for the high concentration of water found in mature milk. Electrolytes, which are also osmotically active, are unable to compensate for the low amounts of lactose which causes colostrum to be thicker with a lower concentration of water (Ontsouka et al., 2003). In the newborn, lactase is found in low concentrations at birth and increases over time, which coincides well with lactose concentrations in colostrum and milk (Zabielski, 1999).

Fat-soluble vitamins

Fat-soluble vitamins are an important component in colostrum. The onset of deficiency in the newborn is based on the diet of the dam, which affects the body stores of the young through both low colostrum concentrations and reduced placental transfer (Loosli, 1949). Although tocopherols pass across placental membranes and are stored by the fetus, neonates are still born with low levels and rely on colostrum to replenish their body stores (Hidiroglou, 1993; Zanker, 2000). In newborn lambs, kids, and piglets,

plasma tocopherol increases 4-fold before colostrum feeding if dams are supplemented with tocopherol during the last stages of pregnancy compared to those not supplemented. Colostrum from supplemented animals also contains 2 to 3 times more tocopherol, which further increases tocopherol storage in the neonate after colostrum ingestion. Selenium may also play a large role in transporting vitamin E across the placental membrane and should be considered when formulating gestational diets (Malm, 1976).

Concentrations of fat-soluble vitamins are highly variable between individuals and are also dependent on maternal reserve status, diet, and season. Stewart and McCallum (1938) reported variations of retinol from 35 to 1181 IU/ 100 mL of colostrum between individual cows on the same management system; this highlights inter-sample variation of fat-soluble vitamins. Weiss *et al.* (1990) also found a significant correlation between fat in colostrum and fat-soluble vitamin concentrations. Due to the close connection of fat-soluble vitamin absorption with the presence of fat in the diet, fat needs to be accounted for when analyzing fat-soluble vitamin concentrations in colostrum and other fluids. Concentrations of alpha-tocopherol in colostrum reported for cows fed without supplemental alpha-tocopherol and cows fed 1000 IU alpha-tocopherol were 95.6 and 125.4 ug/ g fat, respectively. Colostrum concentrations of beta-carotene from these same cows were 49.6 and 23.6 ug/ g fat, although the reason for the increase in beta-carotene concentrations in unsupplemented animals is unknown (Weiss *et al.*, 1990).

The concentration of vitamin A found in colostrum is related to dietary concentrations of vitamin A, which impacts the concentration of vitamin A in the blood of cows before parturition. Plasma vitamin A in newborn calves is four-fold greater when dams are supplemented with vitamin A prepartum and two-fold greater when supplemented with carotene in the prepartum diet compared to unsupplemented cows (Spielman et al., 1947). Although ruminant placental structure is non-invasive and fat-soluble vitamins are large molecular weight substances there must be some transport of vitamin A because cows supplemented with dietary vitamin A birth calves with higher plasma and liver stores of vitamin A than unsupplemented cows.

Spielman *et al.* (1947) reported vitamin A concentrations in colostrum for cows not supplemented with vitamin A to be 1245 to 1425 IU/ 100 ml. The colostrum of vitamin A supplemented cows contained 5850 IU of vitamin A/ 100 ml, assuming 1 IU

was 0.25 micrograms of vitamin A (Spielman et al., 1947). Other research reports similar values in cows without extra vitamin A supplementation, ranging from 280 to 1920 IU/ 100 ml in colostrum. There is also an effect of parity, where first parity cows produced colostrum with more than two times the amount of vitamin A, per 100 ml of milk, compared with the production of colostrum in their second parity (Hansen et al., 1946b).

Water soluble vitamins

Water soluble vitamins have not been extensively analyzed in colostrum. Previous to high performance liquid chromatography (HPLC), researchers attempting analysis of water soluble vitamins used methods such as rat growth, microbiologic assays, chemical and fluorometric assays. Many of these methods resulted in variability between assays and technicians. For example, Roderuck *et al.* (1945) reported a 20% increase in riboflavin content using microbiological assays compared with fluorometric analysis due to the difficulty of replicating microbiological methods. Many researchers also combined different methods to measure different vitamins which may increase variation.

There is also variability in concentrations of water-soluble vitamins due to diet, season and individual animals. Lawrence *et al.* (1946) reported a high variation between seasons for niacin. Riboflavin content in milk can be affected by diet as well as breed. Holstein milk contains an average of 34% more riboflavin than Jersey milk (Theophilus and Stamberg, 1945). Other research, however, has indicated Jersey milk to be highest in riboflavin content with Guernsey, Brown Swiss, Ayrshire and Holstein following in respectively lower concentrations (Hand and Sharp, 1939). Holstein colostrum is 3.6 times higher in riboflavin than milk, although it rapidly loses this high concentration of riboflavin within the first 24 hours (Theophilus and Stamberg, 1945). There is an inverse relation between milk yield per day and riboflavin content and this may extend to colostrum where higher volumes of colostrum produced may contain lower amounts of riboflavin (Hand and Sharp, 1939; Johnson et al., 1941; Theophilus and Stamberg, 1945).

Dietary riboflavin may not play a large role in final colostrum concentrations. Cows that were fed low amounts of riboflavin in the diet still had milk concentrations of riboflavin only 25 percent less than cows that were fed riboflavin adequate diets,

indicating that riboflavin that is produced in the rumen is transferred into milk to maintain riboflavin levels (Johnson et al., 1941). Research has shown that supplementation of B-vitamins causes huge ruminal losses, ranging from 40 to 99% depending on the vitamin, and intestinal absorption is unaffected by supplementation (Santschi et al., 2005). Since B vitamins are also synthesized by microbes in the rumen, concentrations of B vitamins are less prone to dietary influences than other vitamins and this may extend to concentrations in colostrum; this needs to be further researched.

Quantification of water-soluble vitamins in bovine colostrum has not been researched in recent years. The main source of information for B vitamin concentrations in colostrum is from a review by Foley and Otterby (1978) which compiled data up through 1978. Although research has shown that supplementation of dairy cows with certain B vitamins, such as thiamin, niacin and folic acid, can improve milk production and components, the effect of B vitamin supplementation on colostrum was not analyzed (Girard et al., 1997; Girard et al., 1998; Shaver et al., 1988).

Minerals

Most minerals are water-soluble and therefore easily pass through the placental membrane. The fetus is able to store minerals in utero and is born with sufficient stores. Only in areas with dietary deficiencies of minerals do neonates have problems with deficiencies. However, colostrum is also a good source of minerals for newborn calves. Mineral content is high in colostrum, and concentrations decrease over the following week to concentrations found in mature bovine milk. Average zinc content of cow colostrum is 13.57 mg/ L, similar to sheep and human colostrum (Sato and Murata, 1932b). Calcium, phosphorus, copper and iron are all high with concentrations at 0.16 g/ dl, 0.17 g/ dl, 0.39 ug/ ml and 1.9 ug/ ml, respectively, but drop to levels found in mature milk within 25 hours. Conversely, manganese, which has been reported at 0.09 ug/ ml (Kincaid et al., 1992) and 0.16 mg/ L (Sato and Murata 1932b), is not found in higher concentrations in colostrum than in mature milk.

Feeding cows high levels of zinc at 2,000 ppm results in higher plasma zinc concentrations but lower milk concentrations, indicating that the udder may discriminate in zinc transport (Miller et al., 1965). Supplementation of manganese in the diet

increases manganese concentration in the milk, although concentrations of manganese in the diet approaching 2 ounces daily may cause feed refusal (Archibald and Lindquist, 1943). Other mineral interactions may also cause problems. Addition of zinc to the diet in high concentrations (5,000 ppm) depresses copper, calcium, magnesium and phosphorus concentrations in both milk and colostrum (Hill et al., 1983). Zinc has the ability to chelate other minerals and decreases intestinal absorption.

Selenium and iodine are two minerals that can vary widely according to geographic location and the geochemical environment. Reviews have been published correlating concentrations of selenium and iodine in colostrum and milk according to geographic location in both human and animal milks (Casey et al., 1995). Much research has reported effects of supplementing gestating animals with selenium and the increase of selenium in colostrum (Mahan, 2000; Weiss et al., 2005). Austin *et al.* (1980) reported that supplementing cows with iodine increased colostrum concentrations of iodine; however, calves received most of the iodine found in plasma from placental transfer in utero rather than colostrum ingestion.

Growth factors

Colostrum contains many bioactive compounds, such as insulin-like growth factors and transforming growth factors. Prolonged feeding of colostrum to calves has been reported to have an effect on small intestinal development due to these growth factors compared with calves fed milk replacer (Buhler et al., 1998). Feeding colostrum causes hyperplasia of the intestinal epithelium, resulting in a decrease in crypt depth: villus height ratios in calves (Buhler *et al.*, 1998) and rats (Berseth et al., 1983), indicating an increase in differentiated cells. Absorption of xylose, a marker used to evaluate enterocyte function, is higher in calves fed colostrum for 6 feedings than in calves fed colostrum only once or only milk replacer, suggesting an increase in absorptive capacity due to increased numbers of differentiated enterocytes and greater intestinal surface area (Buhler *et al.*, 1998).

Insulin-like growth factors (IGF) are part of the insulin family of hormones and growth factors, and include IGF-I, IGF-II and relaxin. Concentrations of IGF-I are reported in first milking colostrum from 289 to 902 ug/ L (Sparks et al., 2003; Vega et

al., 1991). Insulin-like growth factors are heat and acid stable allowing them to be ingested and to reach the small intestine intact (Baumrucker et al., 1993). Schober *et al.* (1990) reported high numbers of IGF-I receptors present in the neonatal pig intestine, which enhances uptake of IGF-I into systemic circulation. Sparks *et al.* (2003) also reported increases of IGF-I in the serum of calves after colostrum feeding indicating that absorption of IGF-I occurs.

Transforming growth factor-βs (TGF-β) are found in 3 forms, including TGF-β1, TGF-β2, and TGF-β3. Most mature cells are able to produce one of the isoforms of TGF-βs during tissue repair and inflammation (Urashima et al., 2000). Concentrations of TGF-β1 in first milking colostrum are 12.4 to 42.6 ng/ ml. Cells have three receptor types (I, II, and III) that are capable of binding all three TGF-βs in mammals (Letterio, 1998) and increase circulating levels of TGF-βs in calves after colostrum consumption.

Nucleotides

Nucleotides are part of the non-protein nitrogen fraction of colostrum and consist of a purine or pyrimidine base, a pentose ring and one to three phosphate groups. Pyrimidine nucleotides include orotate monophosphate (OMP) and uridine monophosphate (UMP) and purine nucleotides include adenosine monophosphate (AMP), uridine monophosphate (IMP) and guanosine monophosphate (GMP).

Nucleotides are found in high levels in colostrum compared with mature milk but exhibit different patterns depending on the stage of lactation. Colostrum has high levels of pyrimidine derivatives such as UDP-glucose, UDP-galactose and UMP but low levels of guanosine derivatives, AMP and CMP (Gil, 1995; Kobata et al., 1962). Levels of UMP and GMP decrease as colostrum transitions to milk while OMP increases to higher levels in mature milk than are found in colostrum (Johnke et al., 1962). Both colostrum and milk also contain 3'-5'-cyclic AMP (Kobata *et al.*, 1962).

Bacterial contamination

The cleanliness of colostrum is extremely important since it is being fed to animals without fully developed immune systems. The intestinal absorption of newborn calves is phagocytic and receptors exist for binding of immunoglobulins to allow for

whole protein transport. It has been reported that bacteria fed with colostrum compete for intestinal receptors as well as exfoliate the brush border membrane, reducing numbers of available receptors (Staley et al., 1985). Calf intestinal segments inoculated with live bacteria culture had lowest uptake of [I^{125}] gamma-globulins compared with calves inoculated with sterile bacteria broth (James et al., 1981). This is also supported by results of James and Polan (1978), who showed that calves inoculated with duodenal fluid either before or after colostrum feeding had depressed concentrations of serum gamma-globulins.

Some research has reported that colostrum contamination is high due to poor management and storage. Surveyed farms in Canada showed that 94.4% of colostrum samples from participating farms contained at least one microorganism cultured from the sample. Contamination in some samples reached as high as 3×10^6 bacteria/mL and was higher during summer months compared with winter months (Fecteau et al., 2002).

Stewart *et al.* (2005) reported that significant bacterial contamination does not occur until the colostrum is harvested into a container and stored for calf feeding. Therefore, most bacterial contamination is not from the udder or the dam, it is due to storage without refrigeration, equipment not properly sanitized and warmer ambient temperatures. When pasteurized colostrum and waste milk are fed until weaning, calves gain weight and mortality decreases due to lower incidence of scours and pneumonia. Once mature, these calves have been estimated to be worth an extra $8.13 in gross margin per calf compared with calves fed raw colostrum and waste milk (Jamaluddin et al., 1996). This indicates that feeding colostrum that is clean and uncontaminated may provide an advantage over calves fed poorly stored colostrum. The long term effects of feeding contaminated colostrum to calves still need to be researched.

Colostrum of other species

Substituting colostrum

Neonates that do not acquire maternal immunity in utero require colostrum after birth for passive transfer of immunoglobulins. During the first 24 hours after birth, neonates, which need colostrum for passive transfer of antibodies, have a nonselective macromolecular transport mechanism in their small intestine which allows

immunoglobulins to pass into the blood undigested. Research has shown that using other species' colostrum to feed bovine neonates may allow for some immune protection as compared with feeding nothing at all.

The ability to compare the nutrient content of colostrum between species may be valuable when colostrum is not available to feed to the newborn. For example, ewe colostrum is many times unavailable and cow colostrum may be used as a substitute (Eales, 1982).

Crawford (2003) fed purified equine IgG to newborn kittens and reported an increase in kitten plasma IgG. However, when kittens were infected with *Staphylococcus aureus*, bacterial phagocytosis was low even though the equine IgG contained specific *S. aureus* antibodies. Similarly, foals fed bovine IgG attained the same concentrations of IgG and IgM as foals fed with equine IgG; however, the bovine IgG was unable to prevent septicemia after foals were challenged with *Actinobacillus equuli* (Crawford, 2003; Lavoie, 1989). Using other species' colostrum needs to be evaluated carefully because even though concentration of IgG is increased, the functionality of the absorbed IgG may not be compatible with other species.

Sow colostrum

The piglet does not attain transfer of immunity *in utero* due to the epitheliochorial structure of the placenta. The sow mammary glands are located throughout the inguinal, abdominal and thoracic regions of the body and contain 14 separate glands with 2 cisterns per teat (Colville et al., 2002). Similar to ruminant colostrum, the main immunoglobulin in sow colostrum is IgG (Porter, 1969).

Sow colostrum is heavily influenced by diet fed prepartum where higher dietary fat and altering fat type both affect the amount of fat as well as the fatty acid profile of colostrum (Newcomb et al., 1991). Although this has been unsuccessful in ruminants, supplementing pigs with fat during gestation increases fat content of sow colostrum. Because fat in colostrum can contribute up to 60% of energy for piglets, this can decrease piglet mortality from hypothermia in the first hours of life (Hartmann et al., 1984). Supplementing sows with 6% of their diet as either animal fat or corn oil consistently

increases milk fat 7 days postpartum as well as milk volume produced (Pettigrew, 1981). This can have many beneficial effects on increasing piglet survivability.

Calcium levels in gestational diets also affect sow colostrum composition by affecting serum levels of 1, 25 Dihydroxycholecalciferol (1, 25$(OH)_2$ D_3), the active form of vitamin D. Sows fed high levels of calcium had low levels of plasma 1, 25$(OH)_2$ D_3 which in turn decreases concentrations of calcium in colostrum (Wuryastuti et al., 1991). Vitamin E in the diet also affects tocopherols in sow colostrum. As dietary alpha- and gamma-tocopherols increase, colostrum concentrations of alpha- and gamma-tocopherols increase through the third parity then begin to decline for later parities. This explains why piglets from older sows are more likely to be vitamin E deficient than those from younger sows (Mahan, 1994). The concentration of zinc in sow milk may also be changed through dietary manipulation; however, colostrum is reported to be unaffected (Hill et al., 1983).

Ascorbic acid is found in high levels in sow colostrum and decreases as lactation progresses; however, supplementation of the diet with ascorbate does not affect colostrum concentrations. Piglets do not synthesize ascorbate until around one week after farrowing, and therefore may be more susceptible to infections and ascorbate deficiency if colostrum levels are low (Wegger et al., 1984). Because ascorbate is a water-soluble vitamin, supplementation of sows during gestations may help increase fetal reserves of ascorbate as well as decrease oxidative stress in utero and during parturition.

Equine, llama and alpaca colostrum

The placentation in the equine species is epitheliochorial and non-invasive which does not allow immunoglobulins to pass to the fetus (Jeffcott, 1972). Two equine mammary glands are found in the inguinal region and each of the 2 glands contains 2 cisterns per teat (Colville *et al.*, 2002).

The main immunoglobulin in the colostrum of equine species is IgA among other smaller concentrations of IgGa, IgGb, IgM and IgE. IgE is found in mare colostrum in very low levels, an average of 773 ug/ ml during the first milking, and is absorbed in corresponding levels in neonatal foals (Wagner et al., 2006).

There is much variation between Ig concentrations in mare colostrum; however, the concentration of Ig in colostrum is not due to size or weight of the horse indicated by similar Ig levels between heavy draft horses and ponies (Pearson, 1984). There could be many various reasons of the large variation in Ig content of colostrum. These could include age of mare, number of lactations, yield of colostrum and equine management; more research needs to be done to determine the causes of variability.

Camel colostrum

Camel milk is important in Middle Eastern countries and is typically consumed by nomadic cultures. Colostrum management is still a problem and pre-weaning calf mortality ranges around 20 to 30% on camel farms (Kamber, 2001). Colostrum, although hard to acquire, has been reported to contain different subclasses and immunoglobulin structures from other ruminants. Camel colostrum was reported to contain IgG_1, IgG_2 and IgG_3 as the dominant immunoglobulins. Smaller amounts of IgA and IgM were detected (Azawi, 1996).

A review of colostrum antibodies between different species, including camels, is shown in Tables 1 and 2, which are adapted from a review by Wernery (2001). Table 1 reviews different subclasses of Ig, and Table 2 reports Ig concentrations in colostrum of alpaca and camels.

Table 1-1. Immunoglobulin G, IgM and IgA subclasses in new and old world camelids.

Species	IgG types	IgM	IgA	Reference
Alpaca	G	M	ND	Garmendia and McGuire (1987)
Llama	G_{1a}, G_{1b}			Ghahroudi (1997)
	G_{2a}, G_{2b}			
	G_{2a}, G_{2b}, G_{2c}, G_3			Woolven et al. (1999)
Camel	G_1, G_2	M	A	Grover et al. (1983)
	G_1, G_2, G_3	M	ND	Azwai et al. (1993)
	G_1, G_2, G_3	ND	ND	Hamers-Casterman et al. (1993)

ND – not determined.

The serum immunoglobulins of both old and new world camels do not contain light chains, only heavy chain dimers, but still retain the ability of extensive antigen binding (Hamers-Casterman, 1993). Although not determined as of yet, these heavy chain type of antibodies may also be expressed in the colostrum, which should prevent producers from interchanging the use of camel colostrum and other species found in the same areas.

Table 2-2. Colostrum immunoglobulin G concentrations in camelids.

Species	IgG (mg/ml)	Reference
Alpaca	10-280	Garmendia et al (1987)
Camel	70-220	Ungar-Waron et al. (1987)
	58.6	Kamber (2001)

Dog and cat colostrum

The endotheliochorial placental structure of dogs and cats does not allow complete transfer of immunoglobulins in utero; only about 10 to 20% traverse the number of placental layers to reach the fetus (Harding, 1961). Other animals such as humans, rats, rabbits and guinea pigs acquire a significant amount of passive immunity in utero. Most dog colostrum and milk literature is in beagles however there may be slight differences between breeds.

Dog colostrum has high levels of iron which are concentrated to over 10 times serum levels. This may indicate that the transfer of iron into mammary secretions is a different mechanism from other species where iron concentration in mammary secretions is equal to or lower than serum (Lonnerdal, 1981).

Degradation of passively acquired immunoglobulins in kittens occurs around 4 to 6 weeks of age, although can be earlier depending on lower absorption of Ig concentrations after birth (Harding, 1961). Endogenous Ig production begins around 2 weeks of age and increases through 4 weeks of age indicating that kittens with poor passive Ig transfer are at most risk before 4 weeks of age (Levy, 2001).

Rodent colostrum

Rat immunoglobulin classes have been characterized to contain 7 classes in the serum; these include IgG1, IgG2a, IgG2b, IgG2c, IgA, IgE and IgM. However, some work has shown no IgM present in rat colostrum, milk or saliva but only in serum (McGhee, 1975; Michalek, 1975). Unlike ruminants, the predominant immunoglobulin class found in colostrum is IgA. Protein malnourished rats have similar concentrations of IgA compared to normal nourished rats (0.48 ± 0.12 vs. 1.15 ± 0.24 mg/ ml, respectively) in colostrum but two-fold lower concentrations of IgG2a (Michalek, 1975). This is similar to ruminants that have been shown to produce comparable nutrient quality of colostrum independent of diet. Both species may have adapted analogously in order to provide good quality colostrum to their young even in times of malnourishment.

Primate colostrum

Human milk and colostrum contains over 80 different oligosaccharides but many are present in low concentrations (Newburg, 1995). Carbohydrate content of colostrum is similar to other species (6.3% w/v) and the oligosaccharide content is 6.5% which is equivalent to 4 g/ L of colostrum. The concentration of carbohydrate in human colostrum is over 20 g/ L (Newburg, 1995). Rhesus monkey milk was evaluated and found to contain a similar composition of complex oligosaccharides as human milk (Kunz, 1993). Because most colostrum is higher than mature milk, it is safe to say that the oligosaccharide content is even higher than values mentioned for mature milk.

Bear colostrum

Small amounts of lactose make up oligosaccharides in species including marsupials, monotremes and bears. These animals birth altricial young which have higher needs for electrolytes such as sodium, potassium and calcium salts. The low lactose content of colostrum allows higher electrolyte content with an overall lower osmolarity (Urashima *et al.*, 2000). This allows colostrum and milk to stay iso-osmotic to maternal plasma but at the same time contain high levels of carbohydrates (Green, 1984).

The milk from bears (Ursidae) and coati (Procyonidae) contains small amounts of lactose relative to other saccharides (Urashima, 1997) which may also extend to concentrations of saccharides found in colostrum. In the polar bear (Ursus maritimus), milk contains 0.3 to 3% carbohydrate but lactose is only found in trace amounts which is similar to milk of the Ezo brown bear and Japanese black bear. In both the polar and Japanese black bear, the dominant saccharide was alpha-3'-galactosyllactose. The polar bear and Japanese black bear also contain oligosaccharides with the presence of human A and B antigen indicating the homology of oligosaccharide units in humans and bears (Urashima *et al.*, 2000).

Free glucose and galactose are undetectable but lactose and myo-inositol are present. However, although free lactose only constitutes 10% of the total carbohydrates, trisaccharides make up over 50% of brown bear milk (Urashima, 1997). The same trend may exist for colostrum.

Marsupial and monotreme colostrum

Free lactose is generally a minor component of marsupial milk. The milk differs by having low lactose and high fucose content (Messer, 1973). Tammar wallaby milk showed the concentration of total hexoses to be 3% at the beginning of lactation (Messer, 1979). The main carbohydrate identified in echidna and platypus milk was fucosyllactose, a trisaccharide (Messer, 1973).

Pinniped colostrum

Milk of pinnipeds contains low concentrations of carbohydrate however, lactose is the major carbohydrate (Jennes, 1964). In contrast, Messer *et al.* (1988) found that free lactose only makes up about 0.01% (w/ v) of milk. Analysis of carbohydrate content of this milk also reported a 6 to 1 ratio of galactose to glucose and that 50% or more of milk was fat. The California sea lion has no lactose due to the absence of alpha-lactalbumin (Johnson, 1972) and pups also have no lactase activity (Sunshine, 1964). This must also extend to the composition of colostrum and knowledge of this can avoid situations in captivity such as when an infant walrus pup developed severe diarrhea when fed bovine milk as its diet (Schroeder, 1933).

Other nutrient analysis on sea lions has only been done on milk (Pilson, 1962) and resulted in high concentrations of total fat, protein, solids and ash (34.9%, 13.6%, 52.7% and 0.64%, respectively). This indicates that colostrum should be similar or even higher in composition than the milk concentrations mentioned

Conclusion

Colostrum in all mammals is classified as the secretions of the mammary gland before and within the first few days of parturition. Although composition differs between species of mammals, colostrum is a benefit to all neonates whether or not they require passive transfer of immunoglobulins. For each species, the composition of colostrum was designed to provide the nutritional requirements for the respective neonate.

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REVIEW OF INTESTINAL FUNCTION AND NUCLEOTIDE SUPPLEMENTATION

Small intestinal morphology

The small intestine regulates digestion and absorption through a large surface area. The surface area of the small intestine is greatly amplified by longitudinal and circular concentric folds (Barnwal, 1975; Trier, 1981). These folds are mainly located in the distal duodenum and proximal jejunum and are less visible in the ileum (Trier, 1981).

The small intestine is comprised of three tissue layers; muscularis mucosa, lamina propria, and epithelium. The muscularis mucosa is a continuous sheet of smooth muscle 3 to 10 cells thick that separates the mucosa from the submucosa. Contractions of the muscularis may add to the movement of the villi, mixing the contents of the small intestinal lumen and increasing absorptive function. This may also facilitate emptying of the crypt luminal contents by causing compression of the lumen (Trier, 1981).

The lamina propria lies between the epithelium and the muscularis mucosa. It surrounds the crypt epithelium and contains cells that are important for immune function as well as blood vessels that provide a nutrient supply (Trier, 1981). The third layer is a single cell layer of epithelial cells that line the villi and the crypts. The crypt epithelium contains undifferentiated cells in the process of mitosis and proliferation (Weiser, 1973). Although the villus epithelium absorbs many substances, it also secretes numerous substances that act to maintain homeostasis. The brush border includes long, thin microvilli that line columnar epithelial cells and increase the absorptive surface 7- to14-fold (Trier, 1981). These villi are the functional units of the intestine and are comprised of vasculature and lymph vessels (Phillips, 1985).

Differentiation of enterocytes occurs along the villus, starting out as undifferentiated cells at the crypt base and migrating into specified cell types (Sassier, 1978). The replacement of cells maintains a steady state in the population of epithelial cells and migration from crypt to villous tip is approximately 72 hours in the rat (Leblond, 1948). Dividing cells that are labeled in the crypts either migrate up the villus or remain in the crypts to divide again (Zile, 1977). The enterocytes are where absorption and secretion occurs, however they must be fully mature to perform these

functions to their fullest capacity. Mature cells are located towards the tips of the villi which is also where the most contact occurs with intestinal contents (Phillips, 1985).

Causes of enteric disease

Enteric bacteria most commonly disrupt the absorptive and secretory mechanisms of the intestinal tract. Three different methods exist for intestinal function to be disrupted that result in diarrhea. One is bacterial enterotoxins from pathogens such as Escherichia coli and salmonella which cause hypersecretion. Enterotoxins attach to intestinal mucosa and activate intracellular secondary messengers such as cyclic AMP and cyclic GMP. These secondary messengers activate protein kinases which release calcium from intracellular stores. Increased calcium ions cause chloride secretion by activating calcium-dependent regulator protein (CDR) or calmodulin (Argenzio, 1985).

A second disruption of intestinal function occurs through villous atrophy. This is usually associated with viruses such as coronavirus and rotavirus. Villous atrophy causes both a decreased secretion of disaccharidases as well as lack of absorption. Glucose stimulated sodium and water absorption are also disrupted (Keljo, 1981).

A third disruption of intestinal function is by protozoa such as cryptosporidium or coccidia. These cause villous atrophy as well as inflammation which decreases microvillar disaccharidase concentrations and causes malabsorption (Tzipori, 1983).

Effects of pathogens on intestinal functions

Normal small intestinal villous function includes secretion of disaccharidases which locate in the brush border and digest carbohydrates. Solutes, including sodium, chloride and glucose, are transported by carriers or passive diffusion through the villous cell membrane (Reifen, 1998; Torres-Medina, 1985). Crypt cells, which are too immature to either digest or absorb, secrete bicarbonate and chloride ions into the lumen of the small intestine, directing water movement through passive diffusion. During normal conditions, absorption of substances through the villi outweighs the crypt secretion, resulting in net absorption of water, substrates, and ions (Moon, 1978). When these enzymes or transport processes are negatively affected, hypermotility occurs which

causes substrates, ions and water to be excreted out of the digestive tract at a faster rate than absorption can occur (Torres-Medina, 1985).

Diseases that affect mucosal function of the intestine destroy villus architecture and decrease absorptive surface area of the small intestine (Bridger et al., 1978; Clark, 1993; Holland, 1992; Saif et al., 1986). Infection frequently causes lesions to appear on the intestinal surface, decreasing the villus to crypt ratio. Villi become thick and lined with cuboidal epithelial cells which are unable to secrete normal levels of disaccharidases (Bridger *et al.*, 1978; Clark, 1993; Mebus, 1975; Pensaert, 1994; Saif *et al.*, 1986). Some villi become ragged at the luminal surface causing the lamina propria to be exposed at the villous tips (Bridger *et al.*, 1978; Mebus, 1975).

This loss of epithelial function results in malabsorption of water, sodium, and chloride (Torres-Medina, 1985). These poorly absorbed solutes, in addition to undigested lactose remaining in the small intestinal lumen, can cause osmotic retention of fluid which further draws water from the enterocytes (Clark, 1993; Menzies, 1990). As excretion of electrolytes and fluids continues, loss of electrolytes from extracellular fluid (ECF) causes water to move out of the hypo-osmotic ECF (Constable, 1996; Nappert, 2000). Continued diarrhea increases the risk of dehydration, lactic academia, hypoglycemia and may eventually lead to death (Clark, 1993; Pensaert, 1994).

Calf Mortality

Neonatal calf diarrhea is a major cause of death and economic loss in the dairy industry. According to the National Animal Health Monitoring Service (NAHMS), calf mortality averages 8.7% annually of which 62.1% is due to scours (USDA, 2002). In 1991, mortality due to scours averaged 52.5% and 60.5% in 1996 indicating an expanding problem in the dairy industry. Treatment of scouring calves is often difficult to diagnose because of numerous causative pathogens (Constable, 1996; Torres-Medina, 1985). Typical infections in dairy calves usually result in multiple infections with rotavirus, coronavirus, E. coli enterotoxin and cryptosporidia (Moon, 1978). Alterations in cellular potassium levels and hypoglycemia cause calves to become lethargic. Water loss decreases total circulatory fluid volume stimulating vasoconstriction to maintain blood pressure. Blood flow to extremities is decreased, causing them to cool and atrophy

(Torres-Medina, 1985). Lack of blood flow to the tips of the intestinal villi decreases absorption of ingested nutrients and impairs villous function (Nappert, 2000). Calves become depressed, anorexic and their feces may include mucus and milk curds. If diarrhea is not treated, calves can become too weak to stand after 2-4 days and death may occur (Pensaert, 1994; Torres-Medina, 1985).

Economic losses from scouring calves are high for producers. Although there is no research on economic impact of treating calves with oral rehydration solutions, treatment of scouring calves for 3 days with an oral rehydration solution may be estimated generally about 7 dollars per calf. However, extra labor and time spent treating calves can add up to over 50 dollars per calf. Additionally, if a veterinarian has to be called, costs incurred can total over 150 dollars spent per calf. Considering most calves scour at 1-2 weeks of age, treatment costs can escalate exponentially. Reducing the number of calves that scour and need treatment can save hundreds of dollars per month for producers. Supplementation of nucleotides to milk replacer may help alleviate the incidence of calf scouring and associated costs.

Nucleotide sources

Nucleotides are non-protein, nitrogenous compounds found in many foods such as seafood, legumes and organ meats. Nucleotides are known as "semi-essential" nutrients because, although the body is able to endogenously synthesize them, situations such as rapid growth, disease states and limited nutrient intake may require dietary supplementation of exogenous sources of nucleotides. Nucleotides are made up of 3 components which include a nitrogenous base, a pentose and one or more phosphate groups. Nucleosides consist of a nitrogenous base linked to a pentose which is either D-ribose for ribonucleic acid (RNA) or 2-deoxyribose for deoxyribonucleic acid (DNA) (Gil, 1995).

Dietary nucleotides are mainly contained in deoxyribonucleic acid (DNA) and ribonucleic acid (RNA) in cells. Nucleic acids are able to survive stomach acids and are degraded to nucleotides in the small intestine by pancreatic nucleases and intestinal phophodiesterases. Nucleotides are then hydrolyzed further to their component nucleosides which can be hydrolyzed by nucleosidases and nucleoside phosphorylases to

purine and pyrimidine bases and riboses. Nucleotides are not absorbed well due to the high negative charge of their phosphate group and the absence of a transport system therefore the main form absorbed are nucleosides (Mathews and Van Holde, 1996; Sanderson and He, 1994; Uauy, 1989).

Nucleotide synthesis

The body utilizes nucleotides from two sources, an exogenous source through the diet and an endogenous source through de novo synthesis. De novo synthesis of purine nucleotides utilizes carbon and nitrogen from glycine, nitrogen from aspartate, nitrogen from glutamine, carbon from tetrahydrofolate derivatives and carbon from carbon dioxide. The first committed step of purine synthesis utilizes glutamine and phosphoribosylpyrophosphate (PRPP) to form phosphoribosylamine which, through ten subsequent steps, leads to the formation of inosinate (IMP). From IMP, adenosine monophosphate (AMP) and guanosine monophosphate (GMP) are formed (see Figure 1) (Voet and Voet, 1990).

Synthesis of purine nucleotides is tightly regulated through negative feedback inhibition. High levels of purine nucleotides inhibit formation of PRPP. Itakura et al. (1981) demonstrated that depletion of purine nucleotides in the liver causes a 2.3-fold increase of hepatic PRPP. Other inhibitors include high levels of both AMP and GMP which inhibit the formation of PRPP as well as the conversion of IMP to precursors of AMP and GMP (Itakura et al., 1981; Stryer, 1995). High levels of hypoxanthine usually found in bone marrow and gastrointestinal tissue also inhibit purine de novo synthesis (King et al., 1983).

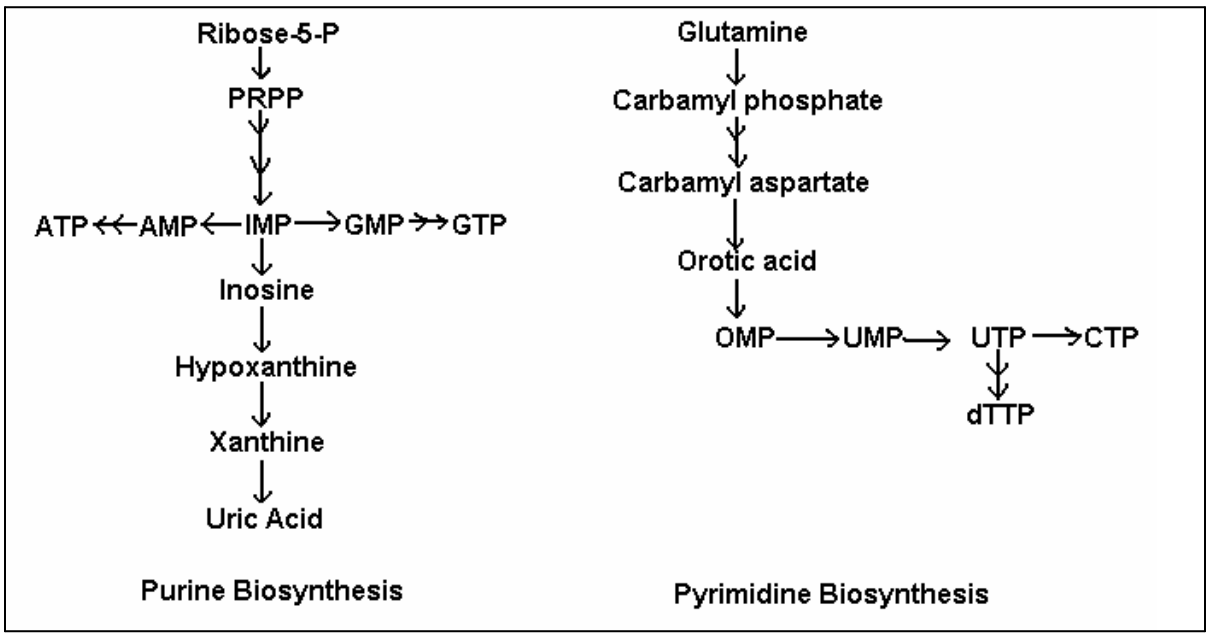

Figure 1. De novo synthesis of purine and pyrimidine based nucleotides. PRPP= phosphoribosylpyrophosphate. OMP = orotidine-5'-monophosphate. UMP = uridine-5'-monophosphate. CTP = cytidine-5'-monophosphate. dTTP = deoxythymidine-5;-triphosphate.

The salvage pathway for purine nucleotides utilizes precursors already available in the body from many sources such as dietary nucleic acid digestion, DNA repair pathways or even cell death metabolites. Nucleic acids and nucleotides that are degraded provide free purine bases for the formation of new purine nucleotides by the salvage pathway which is more efficient and conserves energy. Purine bases are attached to the ribose phosphate moiety of PRPP with specific enzymes for salvage reactions. Adenine phosphoribosyl transferase (APRT) attaches adenine to PRPP to form AMP and hypoxanthine-guanine phosphoribosyl transferase (HGPRT) catalyzes the formation of IMP and GMP attaching PRPP to hypoxanthine and guanine, respectively (Salati et al., 1984; Stryer, 1995). Salvage is limited by the rate limiting enzyme HGPRT which decreases in activity as purines are limited, increasing de novo purine synthesis (Leleiko et al., 1987).

Pyrimidine de novo synthesis is similar to purine de novo synthesis (see Figure 1). The first reaction committed solely to pyrimidine synthesis is the formation of carbamoyl aspartate from carbamoyl phosphate and aspartate catalyzed by aspartate transcarbamoylase (ATCase). This is a rate-limiting enzyme and is inhibited by high

levels of the end product, cytosine monophosphate (CTP). As the availability of substrates increases, salvage pathway activity also increases, decreasing de novo synthesis (Sonoda and Tatibana, 1978).

Salvage of pyrimidine nucleotides utilizes pyrimidine phosphoribosyltransferase which catalyzes a pyrimidine base attachment to PRPP converting it to a pyrimidine nucleoside monophosphate (Carver, 1995).

Absorption of nucleotides

Absorption of nucleosides is either by passive diffusion using a concentrative nucleoside transporter (CNT1) or a sodium-dependent purine nucleoside transporter (SPNT). Dietary supply of nucleotides affects nucleoside absorption, in that when dietary nucleotides are low, genes for CNT1 are down-regulated in the liver but up-regulated in the small intestine (Felipe et al., 1997; Sanderson and He, 1994; Valdés et al., 2000). This is explained by the high capacity of the liver for de novo nucleotide synthesis which does not require absorption of dietary nucleosides whereas the gastrointestinal tract has a higher need for dietary nucleosides due to its low capacity for de novo synthesis and reliance on salvage pathway synthesis (Leleiko et al., 1987).

Sonoda and Tatibana (1978) carbon labeled dietary nucleotides to study the metabolic fate of purines and pyrimidines in mice. They reported that 8 hours post-feeding, 95% of bases are absorbed and utilized by gastrointestinal tissues and liver and only 5% are retained in the tissues (Sonoda and Tatibana, 1978). They found that absorption of pyrimidines occurs as nucleosides rather than free bases and no radioactivity is detected in the feces for fed nucleotides. Other work reports that both purines and pyrimidines are not transported intact but are converted to their respective metabolites, uric acid and hypoxanthine for purines, and uracil and thymine for pyrimidines, which are forms that can be readily salvaged by other cells (Sanderson and He, 1994).

Adenine is handled differently from other purines by the body and may be the most reutilized purine (Ho et al., 1979). It is toxic when fed at more than 0.2% of the diet whereas other purines are not (Savaiano and Clifford, 1978). Adenine is preferentially taken up by tissues, unaltered structurally, and retained at higher concentrations than

guanine, xanthine and hypoxanthine (Ho et al., 1979; Salati et al., 1984; Savaiano et al., 1980; Savaiano and Clifford, 1978). Unlike other purines which are phosphorylated first before absorption, adenine is more commonly unphosphorylated. This may be due to the high rate of saturation of APRT, which phosphoribosylates adenine to adenosine monophosphate but is at much lower concentrations in enterocytes. This may enable intestinal cells to salvage more purine nucleotides (Salati et al., 1984). Other investigators report that adenine is rapidly absorbed and converted to inosine and 85% is excreted by 8 hours post-feeding in the main excretory form of allantoin (Ho et al., 1979; Savaiano et al., 1980; Savaiano and Clifford, 1978; Sonoda and Tatibana, 1978).

Nucleotide metabolism

Nucleotides are a part of almost every cell and metabolic process in the body. Adenine triphosphate (ATP) is a nucleotide that consists of high energy phosphate bonds used as energy for cellular processes. Nucleic acids such as DNA and RNA are composed of the nucleotides adenine, thymine, guanine, cytosine and uracil. Nucleotides play a role in metabolism as part of coenzymes and vitamins. Adenine derivatives include coenzyme A, nicotinamide-adenine dinucleotide (NAD), nicotinamide-adenine dinucleotide phosphate (NADP) and flavin adenine dinucleotide (FAD). These all play important roles in lipid, carbohydrate and protein synthesis. Nucleotides are also carriers of electrons in cellular oxidative processes (Uauy, 1989).

Adenosine is different from other nucleotides in metabolism. Adenosine increases blood flow to intestinal tissue (Sawmiller and Chou, 1990). In the gut, adenosine has been shown to decrease inflammatory changes due to reperfusion of the gut after postprandial and reactive hyperemia (Kaminski and Proctor, 1992). These roles may have positive effects in gastrointestinal function in animals. Mice fed chow supplemented with AMP develop greater intestinal wall protein content and gut wall thickness when compared to mice fed a nucleotide free diet or mice fed only chow diet with minimal nucleotide levels (Kulkarni et al., 1986b).

The main excretory pathway of all nucleotides fed is through the urine, except for thymine which is exhaled via lungs at higher concentrations. As the dose of nucleotides

increases, utilization by tissues is also greater indicating nucleotide contribution to tissue syntheses may be significant (Sonoda and Tatibana, 1978).

Intestinal tissue synthesis of nucleotides

Most tissues have the capability to synthesize purines and pyrimidines de novo, however, some tissues such as bone marrow, lymphocytes and gastrointestinal mucosa are limited in their capacity of de novo synthesis. Highly proliferating cells contain higher concentrations of nucleotides and therefore require higher concentrations of nucleotides. To compensate for the lack of de novo synthesis, highly proliferating cells have much higher levels of salvage pathway purines and pyrimidines (Marijnen et al., 1989).

MacKinnon and Deller (1973) reported that the intestinal mucosa is incapable of de novo synthesis of nucleotides. They demonstrated lack of de novo synthesis using everted sacs of guinea pig proximal jejunum incubated with labeled precursors needed for purine de novo synthesis. This tissue did not take up carbon labeled glycine, instead taking up labeled adenine for salvage synthesis of purines (Mackinnon and Deller, 1973). However, later work by LeLeiko et al. (1987) reported that although intestinal tissue does not favor de novo synthesis of nucleotides, animals fed a nucleotide deficient diet can utilize de novo purine synthesis in their small intestine (Leleiko et al., 1983; Leleiko et al., 1987). During normal states of nutrition, gastrointestinal tissue contains high levels of hypoxanthine which inhibits de novo synthesis of purines. These high levels are hypothesized to come from metabolism of adenine in the liver and erythrocytes (King et al., 1983). However, when dietary sources are poor, hypoxanthine levels decrease, in turn decreasing inhibition of de novo synthesis and allowing intestinal tissue to perform de novo synthesis.

Developing and growing intestinal tissue has a high requirement for nucleotides (Uauy et al., 1990). Rats fed nucleotide supplemented or nucleoside free diets had no significant differences in gut length or gut and mucosal wet weight. However, the nucleoside fed group had 50% more protein and 77% more DNA (mg per mucosal wet weight) in the proximal small intestine (middle and distal segments were not significantly different). Villus height in the proximal segment was 25% greater with 87% higher

maltase activity in nucleoside-fed rats compared to rats not supplemented with nucleosides.

Lopez-Navarro et al. (1996) found that rats fed a nucleotide-free diet have a significant decrease in RNA concentrations and decreased number of ribosomes associated with the endoplasmic reticulum. Protein synthesis is also decreased as is the size of the nucleolus. This is consistent with other work which shows nucleotide deficiency to decrease immunoglobulin secretion (Navarro et al., 1996). Calves fed milk replacer supplemented with nucleotides tend to have higher concentrations of IgG compared to calves fed unsupplemented milk replacer (Oliver et al., 2002; Oliver et al., 2003; Oliver et al., 2004). These variables increase with a proportional increase in dietary nucleotides. The only cellular stores of nucleotides exist as RNA and DNA and until de novo synthesis can provide nucleotides, RNA may act as protection for the cell from nucleotide depletion (Leleiko et al., 1987; López-Navarro et al., 1996b; López-Navarro et al., 1996a).

Infants fed formulas supplemented with nucleotides have significantly decreased incidence of diarrhea, however other infections conditions such as eye, skin, urinary tract and upper and lower respiratory infections are comparable between infants supplemented with nucleotides and control infants not supplemented with nucleotides (Brunser et al., 1994; Pickering et al., 1998). The metabolism of purines and pyrimidines is conducted primarily in the intestine and liver which have first access, and most nucleosides are utilized and removed before reaching other tissues. This may be one of the reasons why susceptibility of infants to infections outside the gastrointestinal tract was not affected by nucleotide supplementation (Pickering et al., 1998; Sonoda and Tatibana, 1978).

Immune benefits

Dietary nucleotides have been shown to affect the immune system, especially the cell-mediated response of the adaptive immunity branch. Mice fed a nucleotide free diet and later supplemented with dietary nucleotides demonstrated a restored T-cell dependent response reflected in increases of antibodies (Navarro et al., 1996). Infants fed cow milk-based formula, which is lower in nucleotides than human breast milk, that was supplemented with nucleotides have higher antibody responses to Hib conjugate vaccine

(HibTITER) and DTP (Diphtheria and Tetanus Toxoids and Pertussis Vaccine Adsorbed) which is consistent with an effect on responses dependent on T helper cells (Pickering et al., 1998).

Nucleotide supplementation increases concentrations of interleukin-2 (IL-2) which are released by T-helper cells when they are activated to induce further naïve T-cell proliferation and differentiation (Goldsby et al., 2003). Mice supplemented with nucleotides had significantly increased natural killer cell cytotoxocity at 2 months and increased IL-2 production when compared with unsupplemented mice which may indicate enhanced immunity (Carver et al., 1990).

The influence of nucleotides in maintaining the immune system is most likely on antigen processing and lymphocyte proliferation. Mice with heart tissue allografted into their ears have a delayed rejection when fed a nucleotide free diet which may be due to the suppression of uncommitted T lymphocyte cells indicating the role of nucleotides in T lymphocyte differentiation and maturation. Addition of RNA to the diet increases allograft rejection response time as well as a primary lymphocyte response. However, challenging mice with lipopolysaccharide (LPS) does not result in any response differences between treatment groups indicating that nucleotides have no effect on B cells (Van Buren et al., 1983).

Mice inoculated with Staphylococcus aureus and fed a nucleotide free diet with added yeast RNA or uracil show increased resistance, yet, addition of adenine to the diet has no effect on resistance to Staphylococcus aureus. Mice fed a nucleotide free diet without nucleotide supplementation and inoculated with the same concentrations of Staphylococcus aureus experience 100% mortality. Inoculated mice benefit from nucleotide supplementation beginning 3 or 4 days post challenge when cellular immunity begins to respond. Once the host begins to mount specific T cell immunity, survivability increases dramatically (Kulkarni et al., 1986a). This may be due to the low capacity of lymphocytes to synthesize nucleotides de novo and the increased need for nucleotides in rapidly proliferating lymphocytes (Marijnen et al., 1989). Other work has shown mice fed a nucleotide free diet have increased susceptibility to murine candidiasis, a fungal infection, which the authors hypothesize may be caused by decreased T helper cell

activity, decreased T effector function or decreased macrophage function (Fanslow et al., 1988).

Neonatal calves

Calves are most often susceptible to enteric infections before weaning. Many infections which are most common in young calves, such as coronavirus, cryptosporidia, and E. coli, result in endotoxemia and sepsis. Recovery of the small intestine after chronic diarrhea is slow and incomplete yet, Bueno et al. (1994) showed that weanling rats suffering from diarrhea fully recover with nucleotide supplementation to the same extent as rats not challenged with diarrhea. Rats supplemented with nucleotides have significantly lower numbers of intraepithelial lymphocytes, higher microvillous surface area, no cytoplasmic vesiculation and improved mitochondrial function (Bueno et al., 1994). This indicates an improvement in intestinal health post-challenge and may have the same effect in neonatal calves infected with environmental pathogens.

Calves infected with an enteric pathogen usually suffer from endotoxemia caused by endotoxins found in the cell wall of bacteria (Torres-Medina et al., 1985). Rats challenged with lipopolysaccharides (LPS), used to mimic endotoxemia, have a 100% survival rate when treated with an intraperitoneal injection of nucleotides compared to rats injected with saline which experienced 40% mortality (Haji et al., 1995). Positive responses have also been reported in neonatal bull calves challenged with LPS. Calves supplemented with nucleotides tend to have higher IgG concentrations than calves not supplemented with nucleotides (Oliver et al., 2003). In contrast, Mateo et al. (2003) supplemented piglets with nucleotides post-weaning and found no differences in serum IgG. Piglets supplemented with nucleotides did have higher levels of fecal L. acidophilus and Bifidobacterium spp. when compared with control diets. Control piglets had higher levels of Cl. perfringens throughout 2 weeks post-weaning than supplemented piglets indicating that nucleotide supplementation during post-weaning in piglets has a positive influence on gastrointestinal microflora (Mateo et al., 2004).

A constant supply of nucleotides provided in the diet also decreases incidence of diarrhea until weaning. In human infants, a decrease in diarrhea was reported when infants were fed with cow milk-based formula that was supplemented with additional

nucleotides (Kulkarni et al., 1986a). Similarly, calves supplemented with a yeast derived product had significantly lower fecal scores with higher IgG concentrations compared to control calves during the first week of trial (Oliver et al., 2002). These preliminary data indicate that nucleotide supplementation in neonatal calf diets may enhance intestinal health and immunity.

Nucleotide concentrations for feeding

Cow's milk is similar in nucleotide content compared to other species (Table 1). Gil and Sanchez-Medina (1981) found that nucleotide content in cow milk reached a maximum at 1 to 2 d after parturition rather than in first milking colostrum. Bovine milk contains substantially high amounts of orotate and is the highest of all nucleotides for nearly all sampling time points compared to other species. In sows, nucleotide content peaks at 7 d lactation except for UMP which is highest in first milking colostrum at 555.6 umol/ 100 ml, and throughout lactation, represents 86 to 90% of nucleotides (Mateo, 2004).

Cow's milk is lower in nucleotide concentrations than human breast milk. Pyridine nucleotides in cow milk range from 0.92 to 4.42 compared to 4.78 to 9.14 umol/ dl in human milk. Purine nucleotides range from 0.40 to 2.35 in cow milk and 0.54 to 4.89 umol/ dl in human milk (Gil, 1981; Gil, 1982a). This is an important distinction that needs to be recognized when formulating infant milk replacers. Most infant formulas are based on cow milk and is also centrifuged (which discards cells that contain most of the DNA and RNA) and then thermally processed, further lowering content. Currently, one U. S. infant formula company (Wyeth, Inc.) supplements with nucleotides at 3.8 to 4.2 mg/ 100 kcal. Other infant replacer companies in Japan and Europe also supplement 2.2 to 2.84 mg/ 100 kcal (Gil, 1995). The nutritional significance of dietary nucleotides has been found to be beneficial for human infant nutrition.

Table 2-4. Nucleotide content of milk from different species and infant milk replacer.

Variable	Cow	Goat	Sheep	Sow	Human	Similac
AMP	6.18	4.70	29.73	12.80	3.34	0.01
CMP	5.25	6.45	32.75	7.10	5.51	0.27
GMP	--	--	3.46	14.00	0.33	0.01
UMP	39.00	53.77	113.30	263.10	1.77	0.01
IMP	--	--	--	2.60	--	--
Orotate	16.67	6.29	3.02	--	--	--
Time of lactation	1 – 2d	1 - 2d	1 – 2d	7d	2d	--
Number of animals	4 to 7	4 to 7	4 to 7	12	--	--

Adapted from Gil (1995).

Thermal processing of milk also plays a role in resulting nucleotide content of dairy products. Standard sterilization of milk consists of heating at 135 ºC for 12 s in a glass tube-heat exchanger, ultra high temperature (UHT) sterilization heats at 150 ºC for 2.4 s and high temperature short time (HTST) heats in a plate-heat exchanger at 75 ºC for 15 s. Both the standard sterilization and UHT significantly lower AMP, CMP and orotate concentrations however HTST only causes a slight decrease in AMP and CMP concentrations and orotate remains unchanged (Gil, 1982b). Therefore it is important to take sterilization into account when formulating infant and calf milk replacers in order to enhance nucleotide retention as much as possible. Heating of dairy products affects nucleotides depending on the time spent at high temperatures; shorter times affect nucleotide content less even at higher temperatures.

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Chapter 3

A SURVEY OF COLOSTRUM NUTRITIONAL COMPOSITION AND MANAGEMENT PRACTICES ON DAIRY FARMS IN PENNSYLVANIA

ABSTRACT

A survey of colostrum management and nutrient composition was performed on dairy farms in Pennsylvania. Data and samples were obtained from 55 farms around Pennsylvania. Samples were analyzed for fat, protein, lactose, total solids, ash, immunoglobulins, lactoferrin, water- and fat-soluble vitamins and minerals. Mean percent of fat, protein, and lactose in colostrum were 6.7, 14.92, and 2.49% and respectively. Immunoglobulin G1, G2, A, M and lactoferrin were 35.1, 5.6, 1.7, 4.3 and 0.82 mg/ml, mg/ml, respectively. Mean concentration of fat-soluble vitamins including retinol, tocopherol and B-carotene and were 4.9, 2.92 and 0.68 ug/g, respectively. Mean concentration of water-soluble vitamins were 0.34, 0.90, 4.55, 0.60, 0.15, 0.21 and 0.04 for niacinamide, thiamine, riboflavin, cyanocobalamin, pyridoxal, pyridoxamine and pyridoxine, respectively. Mean concentration of select minerals (mg/kg) in colostrum were also determined (calcium 4716.08; phosphorus 4452.12; magnesium 733.24; sodium 1058.93; potassium 2845.89; zinc 38.10; iron 5.33; copper 0.34 and manganese 0.10). The findings of this study showed that mean concentrations of most nutrient components in colostrum have increased when compared to values previously reported in literature. The results of the study also showed that management practices had improved over time particularly with regard to colostrum storage and feeding. It was also observed that the herd size had an influence on the management and colostrum quality. Based on the findings of the study it can be inferred that although improvements have been made with regard to colostrum management and quality, there is still a need to educate producers on issues related to storage and timely feeding of colostrum to increase passive transfer and decrease the rate of calf morbidity and mortality.

INTRODUCTION

Proper colostrum management and feeding reduces calf mortality, strengthens immunity and increases animal life span (Besser and Gay, 1994; Besser, 1988; Quigley and Drewry, 1998b). Delaying intake of colostrum not only decreases passive transfer of immunoglobulins but also fat-soluble vitamins (Zanker, 2000). In fact, calves that receive colostrum 12 to 25 h after birth, have lower plasma concentrations of beta-carotene, retinol and alpha-tocopherol for almost a month after birth than calves that receive colostrum within 7 h (Zanker, 2000). These vitamins play a role in immunity which may predispose calves to enteric infections thereby re-enforcing the importance of proper timing and amount of colostrum feeding.

According to a U. S. farm survey conducted in 2002 by the National Animal Health Monitoring System (Heinrichs et al., 1994b), 33.7% of farms still allow calves to nurse their dam. This shows significant improvement from Michigan dairies surveyed in 1973 when 77% of dairies allowed calves to nurse their dam (Speicher and Hepp, 1973). In 1994, a survey of Swedish farms reported that a higher risk of developing diarrhea in calves is associated when calves receive colostrum by suckling the dam (Svensson, 2003).

Farms that separate calves and feed colostrum manually, either by bottle or tube, reported an average of 2.7 hours until calves were fed first colostrum after birth in Pennsylvania. Swedish farmers reported first colostrum feeding within an average of 3 hours after birth (Svensson et al., 2003) whereas only 60% of Alberta farms fed calves within 4 hours of birth (Spicer and Goonwardene, 1994). Calves need to be fed colostrum in a timely manner due to the loss of passive transfer of immunoglobulins within 12 hours of calving.

The composition of colostrum is important in satisfying nutritional requirements for neonatal dairy calves. Colostrum provides immunity as well as necessary nutrients which only minimally cross the placenta, such as fat-soluble vitamins (Spielman et al., 1946). Calves also require fat and protein for energy and muscle development in the first days of life as well as growth factors and many other nutrients which are concentrated in the first lacteal secretions of the dam post calving (Roy, 1990).

Foley and Otterby (1978) presented a comprehensive review on the composition of bovine quality. Since their last report in 1978, no exhaustive study had been done on determining the composition of bovine quality. Similarly surveys done on colostrum management and calf care date to early to mid 1980s (Foley and Otterby, 1978; Goodger and Theodore, 1986; Heinrichs, 1987). Since the last two decades, management practices, nutrition and nutrient analysis techniques have changed considerably. This necessitates that composition of bovine quality and colostrum management practices be evaluated in light of recent changes in the dairy industry. A study was conducted to examine composition of bovine quality and management practices associated with handling, storage and feeding of colostrum. The first objective of this study was to record methods used for the handling, storage, feeding and management of colostrum. The second objective was to analyze colostrum nutrient composition and contents in comparison with other research. The analysis of colostrum management and nutrient content will enable identification of areas in need of improvement to allow for increased survival of dairy calves.

MATERIALS AND METHODS

Questionnaire:

A questionnaire was developed to ascertain information about management of colostrum which included milking procedures, storage of colostrum and treatment protocols. Calf feeding questions included time of feeding after birth, volume fed and method of feeding. The prototype questionnaire was field tested after which redundancies and unclear questions were modified. The refined questionnaire consisted of 33 questions and was administered to the producer by a single interviewer. The questionnaire survey took about 15 minutes for completion. All procedures were approved by the Human Subjects Review Committee at the Pennsylvania State University and all subjects gave informed written consent.

Dairy herds

A total of 55 dairy herds in 15 counties in Pennsylvania participated in the study. Farms were initially contacted by the authors or a county extension educator with instructions. Colostrum samples were collected and surveys administered to 55 Holstein dairy farms in Pennsylvania from July 2004 through March 2005. A convenience sample of farms was chosen from the four major dairy regions within Pennsylvania. Farms were sampled within these areas roughly proportional to the number of operating dairy farms within the region.

Determination of nutritional composition of colostrum

Processing of colostrum for analysis

Prior to arrival on the farm, producers were asked to retain a sample of colostrum within 3 d of the interview. One cow per farm was fully milked out and colostrum was mixed thoroughly, sampled into a container and then refrigerated. On large farms, samples were usually taken from refrigerated buckets or 2 quart nipple bottles which would have been fed to calves. Samples were then transported on ice to the laboratory where eleven 25 ml aliquots of each sample were taken and stored at -20 ˚C for later analyses. For nutrient composition, 55 samples were analyzed except for fat where 1 sample was not recorded; only 23 mineral samples of manganese were quantified. Fifty four samples were analyzed for water-soluble vitamins; only 5 samples were quantifiable for cyanocobalamin and pyridoxine.

Determination of G_1, G_2, M and A immunoglobulins and lactoferrin

Immunoglobulin G_1, G_2, M and A were analyzed using radial immunodiffusion assays (Bethyl Laboratories, Montgomery, TX) following kit protocols provided. Dilutions were made using saline and consisted of a four-fold dilution for IgA and IgM, 10 fold dilution for IgG1 and 2 fold dilution for IgG2. All samples and kits were brought to room temperature and samples were thoroughly shaken before use. In each assay, a standard of bovine serum albumin was used as well as an aliquot of bovine colostrum from the same cow to ensure kits were within the appropriate range of the standards.

Lactoferrin was analyzed using an enzyme linked immunosorbent assay (ELISA) and assay instructions were followed (Bethyl Laboratories, Montgomery, TX).

Determination of vitamin concentrations

Beta-carotene, retinol and alpha-tocopherol were extracted by methods from Vitamin Analysis for the Health and Food Sciences (1999) and analyzed following methodology of Arnaud *et al.* (1991) using high performance liquid chromatography (HPLC). Water-soluble vitamins, including nicotinamide, pyridoxal, pyridoxine, pyridoxamine, folic acid, riboflavin, cyanocobalamin and thiamin, were analyzed using the HPLC method as described by Albala-Hurtado et al. (1997) at the Proteomics Facility of the Hucks Institute of Life Sciences at the Pennsylvania State University.

Determination of minerals

One aliquot of colostrum was freeze-dried and analyzed for calcium, phosphorus, sodium, potassium, magnesium, manganese, iron, copper, zinc and sulfur using EPA methodology (USEPA, 1986). The EPA Method 3051 was used to microwave digest samples using nitric acid. After digestion, samples were analyzed using Inductively Coupled Plasma Spectrometry (EPA method 6010).

Estimation of fat, lactose and protein

Fat was analyzed using the Babcock fat procedure as described in Standard Methods for the Examination of Dairy Products (2004) and lactose was analyzed using methods by Feitosa Teles *et al.* (1978). Crude protein was analyzed using Kjeldahl analysis and corrected using a factor of 6.38. Total solids and ash were performed based on AOAC (1975).

Statistical analysis

The General Linear Model procedure of SAS 8.2 (2002) was used to analyze for differences between nutrients and survey data. Questions, nutrients and correlations were also analyzed by herd size where herds were grouped < 100 cows (n = 17), 101 – 200 cows (n = 10) and > 201 cows (n = 28). Each nutrient analysis was correlated to all 33

survey questions using the General Linear Model procedure and significance was declared at $P < 0.05$ and trends at $P < 0.15$.

RESULTS AND DISCUSSION

This farm survey covers not only management of colostrum but also aspects of dry cow management. Knowing these statistics can help managers and consultants focus on areas within colostrum management and feeding that need improvement. A previous survey reported improvements in colostrum management and feeding when producers were provided with information regarding calf health (Heinrichs and Kiernan, 1994a). Following extension and consultant recommendations, producers increased colostrum feeding volume from 6 to 7.7 lbs and decreased time of feeding after birth from 2.6 h to 2.4 h.

Colostrum composition

Composition of colostrum ingredients obtained during this survey was compared to concentrations found in Foley and Otterby's review (Table 3-1). Components such as lactoferrin and individual IgG subclasses were analyzed in the current study but not reported in Foley and Otterby's review due to a lack of adequate methodology for analyzing these components at that time.

Fat was measured at an average of 6.7% which is similar to the other literature reviews. Parrish et al. (1950) measured fat using the same procedure as the current review, the Babcock procedure, and reported 6.7 ± 2.7%. Values may be similar because researchers have reported that feeding different levels of dietary fat during gestation does not affect fat content of colostrum (Dietz, 2003).

Protein was also found to be similar between reviews illustrating the lack of influence of dietary factors. Researchers have reported that beef heifers restricted in protein intake gain less weight during the last trimester with no effect on colostrum energy and protein content (Carstens, 1987).

Lactose means were 2.5 ± 0.7% which were similar to what others reported (Eales, 1982; Parrish, 1950; Praphulla and Anantakrishnan, 1959). Lactose is low in colostrum and acts inversely of other constituents, such as solids, protein and ash, which

are all found in high concentrations and decrease over time. However, in the neonate calf, lactase is found in low concentrations at birth and increases over time which coincides well with lactose in milk (Zabielski, 1999).

Concentration of IgG1 was 34.9 ± 11.9 mg/ml which is higher than the review of Foley and Otterby that reported 32 mg/ml total IgG of which IgG1 is approximately 85%. Previous research indicates that nutrient availability prepartum does not significantly affect colostrum IgG content. Halliday et al. (1978) reported no differences in colostrum IgG content and total protein when beef cows were fed 12 wk before parturition on diets ranging from 65 to 125% of maintenance for 2 consecutive years. Santos *et al.* (2001) fed Holstein cows different amounts of rumen undegradable protein, which did not result in significant differences in colostrum IgG content.

Other factors do play a role in immunoglobulin content of colostrum. The volume of colostrum produced, parity of the dam, dry period length, vaccination and many other factors have been reviewed and reported to affect IgG content (Quigley and Drewry, 1998a; Weaver, 2000). Any of these factors may have played a role in accounting for the low IgG content of some samples in the current survey, but without knowing which factors were different, they cannot be accounted for with this study.

Concentrations of IgA in the current study were much lower than in previous research. In the current survey, IgA was obtained using RID, however in other studies, microdiffusion discs were used as described by Masseyeff and Zisswiller (1969). Because of the low concentrations of IgA found in bovine colostrum, a more specific and sensitive assay may have produced greater concentrations in the current survey. Stott et al. (1981) reported a concentration of 17.8 mg/ml for IgA using microdiffusion discs however most other work reported values between 3 and 4 mg/ml (Mach and Pahud, 1971; Pahud and Mach, 1970; Porter, 1972).

Stott et al. (1981) reported a concentration of 2 mg/ml for IgM which is lower than the current survey. Other work was variable, reporting values anywhere from 3 to 12 mg/ml (Mach and Pahud, 1971; Pahud and Mach, 1970; Porter, 1972). The variation of IgM as well as IgA may also be influenced by parity, breed, vaccinations, health status and other factors similar to IgG. There is currently a need for more reliable and repeatable assays which can take into account the high solids content of colostrum.

Fat-soluble vitamins are an important component in colostrum. Although tocopherols pass placental membranes and are stored by the fetus, neonates are still born with low levels and rely on colostrum (Hidiroglou and Butler, 1993; Zanker, 2000). Results of fat-soluble vitamin concentrations in colostrum are similar between reviews however retinol concentrations are numerically higher in the current survey compared with Foley and Otterby's review. This may be due to the high variation in concentrations of fat soluble vitamins between individuals as well as dependence on maternal reserve status, diet, and season. Recently more emphasis has been placed on dry cow nutrition therefore this may also result in higher fat-soluble concentrations in colostrum. Stewart and McCallum (1938) reported variations of retinol from 35 to 1181 IU/100 mL of colostrum between individual cows on the same management system; this highlights inter-sample variation. Weiss et al. (1990) also found a significant correlation between fat in colostrum and fat-soluble vitamin concentrations. Many studies did not take fat into account which could affect the results.

Water soluble vitamins have not been extensively analyzed. Previous to HPLC, researchers attempting analysis used methods such as rat growth, microbiologic assays, chemical and fluorometric assays. Many of these methods resulted in variability between assays and technicians. For example, Roderuck et al. (1945) reported a 20% increase in riboflavin content using microbiological assays compared with fluorometric analysis due to the difficulty of replicating microbiological methods. Means of water-soluble vitamins for the current study analyzed by HPLC were 0.34, 0.90, 4.55, 0.60, 0.15, 0.21 and 0.04 ug/ml for niacinamide, thiamine, riboflavin, cyanocobalamin, pyridoxal, pyridoxamine and pyridoxine, respectively. However cyanocobalamin and pyridoxine were only quantifiable in 5 farm samples. The review of data before 1978 by Foley and Otterby included pantothenic acid and folate which the current study did not analyze. Data from the past review was much lower for concentrations of thiamin and cyanocobalamin (vitamin B12), higher for niacin and similar for riboflavin. Some research used values of thiamine, niacin and riboflavin from pooled colostrum of Holsteins and Jerseys with methods ranging from microbiological and fluorometric to enzymatic, depending on the study. Lawrence et al. (1946) reported a high variation between seasons for niacin.

Lactoferrin is a glycoprotein that binds iron and has been shown to reduce morbidity and improve growth in neonatal dairy calves (Robblee, 2003). Using an ELISA in the current study, lactoferrin averaged 0.82 ± 0.54 mg/ml. Yoshida et al. (2000) used chromatography and reported an average of 0.34 ± 0.23 mg/ml for 6 cows. In contrast, Tsuji et al. (1990) used 45 Holstein cows and reported an average of 1.96 ± 0.27 mg/ml using RID. Differences may exist due to laboratory techniques, however, bovine colostrum does contain lower lactoferrin concentrations than other species (Masson and Heremans, 1971).

Analyses of colostral samples in the current survey resulted in values that were higher than reviewed by Foley and Otterby for all minerals except copper and manganese. Kume and Tanabe (1993) reported that minerals are highest in colostrum to provide calves with minerals after birth. Although most minerals are found in sufficient amounts in colostrum, concentrations are dependent on diet and parity of the dam and this can result in mineral deficiencies in calves if supplementation is not provided (Kume and Tanabe, 1993).

Survey results

Management of dry cows (Table 3-2) included vaccinations, housing and supplemental vitamins, among other things. Straw was the predominant bedding material used in maternity pens (54%) which is similar to previous survey results in which 57.2% of farms also used straw (Heinrichs et al., 1987). This is likely due to the cost and availability factors of straw in the Northeast at the time of the survey. All farms surveyed vaccinated dry cows of which 29% used J-5 and Scourguard®. Vaccinations increase the transfer of immunoglobulins specific for diseases and enhance protection in calves through passive transfer (Moon et al., 1993) thereby improving the quality of the colostrum.

A mineral/vitamin premix was also added to dry cow feed in 90.7% of farms however only 3.7% of farms administered any type of vitamin injections. Heinrichs et al. (1987) indicated 30.7% of farms using injectable vitamins and one possible reason for this dramatic decrease in the current survey may be in part due to an increase in popularity and availability of supplementing vitamins and minerals in feed.

Amount, timing and delivery of colostrum to heifer calves were surveyed (Table 3-3). Calves were fed by bottle in 85% of farms and only 1 farm allowed calves to nurse the dam. This is an improvement from previous surveys which reported 16% and 22% of calves nursing off the dam unassisted (Goodger and Theodore, 1986; Heinrichs et al., 1987). In 1990, surveyed farms in France reported that 15% allowed calves to nurse off their dam and 32% of farms in Alberta, Canada, left the calf to nurse the dam (Faye, 1991; Spicer and Goonwardene, 1994).

Calves need to be fed colostrum in a timely manner due to the loss of passive transfer of immunoglobulins within 12 hours of calving (Stott et al., 1979). Previous surveys reported that most farms provided calves with colostrum within 4 hours of birth. In Pennsylvania, calves received colostrum an average of 2.7 hours after birth and in Alberta, 60% of calves received colostrum within 4 hours (Heinrichs et al., 1987; Spicer and Goonwardene, 1994). In the current study, 43.6% of farms fed calves colostrum within 2 hours and 51% fed between 2 and 6 hours after birth. A different survey done on large California dairies reported that 45.3% of farms only checked on calves once per day which could prolong colostrum feeding for up to 24 hours after birth (Goodger and Theodore, 1986).

In the current survey, calves were fed an average of 2-4 liters of first milking colostrum for the first feeding in 57% of farms, however 37% fed 2 quarts or less for the first feeding. Previous survey results are similar with an average of 2.8 kg fed for the first feeding (Heinrichs et al., 1987). Additionally, only 74% of farms fed a second feeding of first milking colostrum in the current survey. The amount that calves were fed for the first feeding and whether a second feeding was fed were significantly correlated ($P < 0.05$) and 9% of farms that fed 2 quarts or less for the first feeding did not feed a second feeding of first milking colostrum. This dramatically increases the chance for failure of passive transfer which increases calf mortality (Weaver, 2000).

Handling, storage and milking of colostrum was also reported (Table 3-4). Use of frozen colostrum has increased from 22% in a survey conducted by Heinrichs et al. (1987) to 38%, currently, indicating increased awareness of the importance of timely colostrum feeding. However, this is sub par to 68% of dairies in Canada which maintain a supply of frozen colostrum (Spicer and Goonwardene, 1994).

Correlations between nutrients

Farms with SCC < 200,000 had colostrum with significantly higher vitamin E, vitamin A, potassium, IgG2 and total solids (6.38 ± 0.82, 3.61 ± 0.42, 1722.56 ± 183.55, 7.27 ± 0.61 and 30.29 ± 1.25, respectively) than farms with SCC > 200,000 (4.22 ± 0.61, 2.63 ± 0.31, 5787.86 ± 203.38, 5.15 ± 0.46 and 25.75 ±0.95, respectively). The colostrum of cows without mastitis has been reported to contain numerically higher concentrations of vitamin A and beta-carotene than colostrum with mastitis (Johnston and Chew, 1984). This indicates that cows housed at dairies with lower SCC counts may produce colostrum that is higher in nutrient quality. In contrast, niacinamide was found in significantly higher concentrations ($P < 0.02$) for farms with SCC >200,000 (0.45 ug/ml) compared to farms with SCC < 200,000 (0.23 ug/ml).

Least squares means of lactose were higher for farms that did not give vitamin injections than for farms that did use vitamin injections during the dry period (3.29 ± 0.28 and 2.41 ± 0.09, respectively; $P < 0.004$). The reason for this correlation is unknown.

Correlations by farm size

Farm size plays a large role in the management of farms which may reflect on colostrum quality and management choices. Lower number of farms < 100 cows fed a vitamin/mineral premix to dry cows compared with farms > 201 and 101 - 200 cows ($P < 0.04$). A colostrometer was used in 43% of farms > 201 cows compared with 10% of farms 101 – 200 cows and 12% of farms < 100 cows. Eight percent of large farms reported using a pasteurizer whereas no farms < 200 cows used a pasteurizer ($P < 0.09$). This is due to the availability of resources and the use for larger equipment on farm. Farms that used a pasteurizer on farm also used a colostrometer ($P < 0.01$) and did not feed colostrum to calves from their respective dams ($P< 0.02$).

Larger farms also stored more colostrum. Of farms < 100 cows, 59% reported not storing any colostrum compared with 21% of farms > 201 cows. This is most likely due to the fact that smaller farms have fewer calves and directly feed colostrum to calves from their respective dams. Eighty nine percent of farms < 100 and 101 – 200 cows reported feeding colostrum to calves from their respective dams compared with only 43% of farms > 201 cows. This is supported by findings of the NAHMS survey (2002) which

showed that 65.4% of farms under 100 cows did not store colostrum compared with 44.6% of farms 100 to 499 cows. This may lead to higher calf mortality although the few surveys that analyzed calf mortality by herd size found that both small (<61 milking cows) and large (> 180 milking cows) herds have higher calf mortality (James et al., 1984). In contrast, Jenny et al. (1981) and the NAHMS (2002) survey reported that calf mortality decreased as herd size increased.

Farms > 201 cows tended to milk cows later after calving than farms 101 – 200 and < 100 cows ($P < 0.12$). No farms > 201 cows reported milking cows within 1-2 hours after calving compared with 10-12% of farms < 200 cows. Sixty eight percent of cows on farms > 201 cows were milked between 2 to 6 hours after calving compared to 60% of farms 101 - 200 cows and 35% of farms < 100 cows. However, 67% of farms > 201 cows reported that this was similar whether cows calved during the day or at night unlike 35% of farms < 100 cows and 40% of farms 101 – 200 cows that reported this was longer if cows calved at night. This is an example of the difference in resources where small farms, although efficient during the day, usually have less labor for the night and therefore cows must wait to be milked when the day crew begins work.

Analyzing farms by different size categories showed some differences however farms < 100 cows and 101 – 200 cows were more similar compared with farms > 201 cows. In summary, it can be generalized that smaller farms under 100 or 200 cows are managed differently compared with their larger counterparts. Differences in resources, including the availability of workers, finances and feed, result in significant differences of management and colostrum quality.

IMPLICATIONS

The current survey reveals certain improvements in the management and feeding of colostrum. The number of farms currently storing frozen colostrum has increased compared with previous surveys. More farms are separating calves from their dams and manually feeding colostrum which enhances passive transfer. Nutrient concentrations in colostrum have also increased indicating that dry cows may be receiving diets that more adequately meet their nutrient requirements.

Differences exist in management and nutrient quality between small and large farms. Larger farms report longer times of milking colostrum after calving, higher somatic cell counts and lower nutrient content of colostrum compared with small farms. However, more large farms store colostrum, feed a mineral/vitamin supplement and use a colostrometer compared with small farms. Improvement in colostrum management and feeding can be better focused by considering farm size.

Nevertheless, there is still room for improvement as indicated by a steadily increasing calf mortality rate as reported by the NAHMS survey (2002). Education in colostrum management and feeding needs to be emphasized and continued to keep improving the care of dairy calves.

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Table 3-1. Comparison of colostrum composition as reported in the current survey and review by Foley and Otterby (1978). Mean, standard error and minimum to maximum levels are all for the current survey.

Item	Sample number	Mean	SE	Min-Max	Foley&Otterby
Fat (%)	54	6.70	4.16	2.0-26.5	6.7
Protein	55	14.92	3.32	7.1-22.6	14
Lactose	55	2.49	0.65	1.2-5.2	2.7
Total Solids	55	.	.	18.3-43.3	23.9
Ash	55	0.05	0.00	0.02-0.07	
IgG (mg/ml)	.	.	.		32
IgG1	55	34.96	12.23	11.8-74.2	.
IgG2	55	6.00	2.82	2.7-20.6	.
IgA	55	1.66	0.99	0.5-4.4	.
IgM	55	4.32	2.84	1.1-21.0	.
Lactoferrin	55	0.82	0.54	0.1-2.2	.
Retinol (ug/g)	55	4.90	1.82	1.4-19.3	2.79
Tocopherol	55	2.92	3.65	0.6-10.4	.
Vit E/g fat	55	.	.	.	84
B-Carotene	55	0.68	0.63	0.1-3.4	.
Thiamin (ug/ml)	54	0.90	0.28	0.3-2.1	0.58
Riboflavin	54	4.55	0.31	2.4-9.2	4.83
Niacin	54	0.34	1.57	0.0-1.6	0.96
Vitamin B12	5	0.60	0.35	0.2-1.1	0.05
Folic acid	.	.	.		0.01
Pyridoxal	54	0.15	0.07	0.1-0.3	.
Pyridoxamine	54	0.21	0.07	0.1-0.5	.
Pyridoxine	5	0.04	0.07	0.0-0.2	.
Pantothenic Acid	.	.	.	.	1.73
Ca (mg/kg)	55	4716.10	1898.00	1775.1-8593.5	2599.99
P	55	4452.10	1706.29	1792.4-8593.5	.
Mg	55	733.24	286.07	230.3-1399.6	399.99
Na	55	1058.93	526.02	329.7-2967.8	699.99
K	55	2845.89	1159.89	983.2-5511.4	1399.99
Zn	55	38.10	15.90	11.2-83.6	11.55
Fe	55	5.33	3.09	1.7-17.5	1.89
Cu	55	0.34	0.14	0.13-0.64	0.57
S	55	2595.67	904.97	889.4-4143.7	.
Mn	23	0.10	0.11	0.0-0.36	0.19

[1]Part of samples were quantified < 0.05 and therefore not included in averages.

Table 3-2. Management of dry cows and heifers before calving.

	Farms % of the herd			Total %
	< 100	101 - 200	> 200	
What is the somatic cell count from the previous month?				
< 200,000	50	50	25	36
> 200,000	50	50	75	64
Do you add a vitamin/mineral premix to the dry cow diet?				
No	24	0	4	9
Yes	76	100	96	91
What vaccinations are given to dry cows?				
J-5	18	0	18	16
Scourguard	24	30	14	22
J-5 and BVD	24	40	25	29
J-5, BVD, Scourguard	12	0	14	12
Other	24	30	29	22
Are cows given any vitamin injections during the dry period?				
No	65	50	50	59
Vitamin E and Selenium	29	20	0	28
Other	6	30	50	13
What type of bedding are dry cows housed on in the maternity barn at calving?				
Straw	59	60	50	55
Hay	18	0	0	5
Shavings and straw	6	10	11	7
Other	18	30	39	33
How many cows are grouped in maternity pens?				
Individually	24	20	25	24
Groups of 2-4	24	20	7	15
Groups of 5 or more	52	60	68	62
Are prefresh heifers housed in the same maternity pens near or with older cows?				
Yes	100	90	93	96
No	0	10	7	4
How long are heifers housed with older cows before calving?				
>2 month	24	40	14	23
1-2 months	35	10	29	28
<1 month	35	40	50	45
Not housed together	0	10	7	4
In the last year, have you tested for ...?				
Johne's	12	20	14	15
Johne's and BVD	35	50	46	45
Johne's, BVD and Leukosis	12	10	18	15
None	35	0	0	11
Other	6	20	21	13

Table 3-3. Feeding of colostrum to newborn heifer calves.

	Farms % of the herd			
	< 100	101 - 200	> 200	Total %
How much colostrum do heifer calves receive for the first feeding?				
2 quarts or less	29	50	36	37
2-4 quarts	59	40	61	57
4 quarts or more	12	.	4	6
Do calves receive (2 feedings)?				
Yes	82	50	71	74
No second feeding	18	50	29	26
How is colostrum fed to calves?				
Allowed to nurse off the dam	0	0	4	2
Hand-fed from bucket or bottle	100	80	82	86
Hand-fed using esophageal feeder	0	20	14	11
When are heifer calves fed colostrum?				
Within the first two hours	41	40	46	44
Within 2-6 hours	47	60	50	51
After 6 hours	12	0	4	6
When are heifer calves fed the second feeding of colostrum?				
6-8 hours later	24	0	21	20
8-12 hours later	41	50	50	53
12-24 hours later	6	0	0	2
Not fed second feeding	29	50	29	26

Table 3-4. Management of colostrum and storage.

	Farms % of the herd			Total %
	< 100	101 - 200	> 201	
On average how long after calving is a cow milked for first colostrum?				
Immediately, <1 hour	12	10	0	6
1-2 hours	29	10	11	16
2-6 hours	35	60	68	56
>6 hours	24	20	21	22
If colostrum is stored, what kind of container is it stored in?				
Open top container	6	0	11	7
Closed container	35	50	68	55
Not stored	59	50	21	38
How is colostrum stored after milking?				
Frozen	35	40	39	38
Refrigerator	6	10	36	22
Stored without refrigeration	0	0	4	2
Not stored	59	50	21	38
Are calves fed colostrum from their own dam?				
Yes	88	80	43	65
No	12	20	57	35
Is colostrum used from first calf heifers?				
Yes	88	70	75	78
No--is there a source of stored colostrum (fresh or frozen)?				
Yes	0	10	11	7
No	12	20	14	15
Is first milking colostrum pooled?				
Yes	18	0	21	16
No	82	100	79	84
Is a colostrum supplement used in addition to colostrum?				
Yes	6	20	25	18
No	94	80	75	82
Do you use a colostrometer?				
Yes	12	10	43	27
No	88	90	57	73
Is a pasteurizer used on the farm?				
Yes	0	0	11	6
No	100	100	88	95

Chapter 4

BACTERIAL CONTAMINATION OF COLOSTRUM

ABSTRACT

High quality colostrum is defined as that which has high immunoglobulin content, low bacterial counts (cfu/ml) and is pathogen free. A study was conducted to determine bacteriological quality of colostrum and identify management factors that influenced the bacteriological quality of colostrum. Colostrum samples from representative dairy herds in Pennsylvania (n = 55) were analyzed for a variety of bacteriological counts. Information on farm management practices was collected through an administered questionnaire survey. Samples were analyzed for standard plate count (SPC), preliminary incubation count (PIC), laboratory pasteurization count (LPC), S*taphylococcus aureus* (SA), coagulase negative staphylococci (CNS), streptococci (SS), coliforms (CC) and non-coliforms (NC). Bacterial counts were log transformed and general linear model was used to identify management practices that influenced bacteriological quality of colostrum ($P < 0.1$). Based on the indicators of milk quality, it was observed that the SPC (<10,000 cfu/ml), PIC (<10,000 cfu/ml), LPC (< 100 cfu/ml), and CC (<100 cfu ml) were within acceptable levels in 40, 17, 67, and 31% of colostrum samples. Counts of mastitis pathogens including *Staphylococcus aureus* (0 cfu/ml), Coagulase negative staphylococci (<1000 cfu/ml), and streptococci (<1000 cfu/ml) were within acceptable limits in 58, 76, and 38% of colostrum samples. This observation indicates that bacteriological quality of colostrum needs to be considerably improved. The findings of the study suggest that high counts of CNS in colostrum were associated with herds that had > 101 cows and SCC > 200,000. Farms that refrigerated colostrum samples had higher PIC and CC than farms that used frozen colostrum or colostrum that was used for immediate feeding. Farms < 100 cows that fed calves with colostrum from their own dam had higher counts of SPC, PIC and CC than those that stored colostrum. The findings of the study show that although some colostrum samples have acceptable bacterial counts, other farms with elevated counts should improve their colostrum

collection, handling and storage practices. It is recommended that educational programs be developed to improve colostrum quality.

INTRODUCTION

Colostrum that is low in bacterial counts and free of pathogens is extremely important because it is being fed to animals that do not possess a developed immune system. The intestinal absorption of newborn calves is phagocytic and receptors exist for binding of immunoglobulins to allow for whole protein transport. It has been reported that bacteria fed with colostrum compete for intestinal receptors as well as exfoliate the brush border membrane, reducing numbers of available receptors (Staley et al., 1985). Calf intestinal segments inoculated with live bacteria culture had lowest uptake of [I^{125}]-gamma-globulin compared with calves inoculated with sterile bacteria broth (James et al., 1981). This was also supported by James and Polan (1978), who reported that calves inoculated with duodenal fluid either before or after colostrum feeding, had lower concentrations of serum gamma-globulin.

The long term effects of feeding contaminated colostrum to calves have not been researched. However, failure of passive transfer has been reported to decrease productivity and life span of heifers later in life (DeNise et al., 1989). When pasteurized colostrum and waste milk are fed until weaning, calves gain weight and mortality decreases due to lower incidence of scours and pneumonia. Once mature, these calves have been estimated to be worth an extra $8.13 in gross margin per calf compared with calves fed raw colostrum and waste milk (Jamaluddin et al., 1996). These results suggest that feeding colostrum with low bacterial counts would provide an advantage over feeding colostrum containing high bacteria counts.

Some research has reported that bacterial contamination of colostrum is due to poor management and storage. Surveyed farms in Canada showed that 94.4% of colostrum samples contained at least one species of microorganism cultured from the sample. Contamination in some samples reached as high as 3×10^6 cfu/ ml and was higher during summer months compared with winter months (Fecteau et al., 2002). Stewart et al. (2005) reported that significant bacterial contamination did not occur until colostrum was harvested into a container and stored for calf feeding. Therefore, most

bacterial contamination of colostrum was not from the udder or the dam, it was due to failure to properly sanitize equipment, storage without refrigeration, and warmer ambient temperatures.

Due to the importance of colostrum handling and the need for farms to be aware of any ongoing problems, the objective of this survey was to determine levels of bacteria found in colostrum and to investigate any correlations with management, storage or handling of colostrum on dairy farms in Pennsylvania.

MATERIALS AND METHODS

Questionnaire:

A questionnaire was developed to ascertain information about management of colostrum which included milking procedures, storage of colostrum and treatment protocols. Calf feeding questions included time of feeding after birth, volume fed and method of feeding. The prototype questionnaire was field tested after which redundancies and unclear questions were modified. The refined questionnaire consisted of 33 questions and was administered to the producer by a single interviewer. The questionnaire survey took about 15 minutes for completion. All procedures were approved by the Human Subjects Review Committee at the Pennsylvania State University and all subjects gave informed written consent.

Dairy herds

A total of 55 dairy herds in 15 counties in Pennsylvania participated in the study. Farms were initially contacted by the authors or a county extension educator with instructions. Colostrum samples were collected and surveys administered to dairy producers (n= 55 Holstein dairy farms) in Pennsylvania from July 2004 through March 2005. A convenience sample of farms was chosen from the four major dairy regions within Pennsylvania. Farms sampled within these areas were comparative to the number of operating dairy farms within the region.

Determination of Bacterial Contamination of Colostrum

Processing of colostrum for analysis

Prior to arrival on the farm, producers were asked to retain a sample of colostrum within 3 d of the interview. One cow per farm was fully milked out and colostrum was mixed thoroughly, sampled into a container and then refrigerated. On large farms, samples were usually taken from refrigerated buckets or 2 quart nipple bottles which would have been fed to calves.

Samples were then transported on ice to the laboratory where samples were analyzed for standard plate count (SPC), preliminary incubation count (PIC), laboratory pasteurization count (LPC), *Staphylococcus aureus* (SA), Staphylococcus agalactiae (SAG), coagulase negative staphylococci (CNS), streptococcus (SS), coliform (CC) and non-coliform (NC) as described by Jayarao et al (2004).

Samples were thoroughly mixed and 1 ml was transferred to a sterile tube containing 9 ml of quarter-strength Ringer's solution (Oxoid, Unipath Ltd., UK). Following centrifugation for 15 s at 3,000 *x* g speed, 50 uL was plated on selective and nonselective media. Plate count agar was used for enumeration of SPC, PIC and LPC and samples were incubated at 32 ºC for 48 h. Edward's agar supplemented with colistin sulfate and oxolinic acid was used to estimate numbers of SS and SAG (Sawant et al., 2002). To determine CC and NC, MacConkey's agar no. 3 (Oxoid) was used and Baird Parker's agar (Difco) was used for CNS and SA; these were incubated at 37 ºC for 48 h. The Autoplate 4000 user guide (Spiral Biotech) was used to enumerate bacterial counts.

Statistical Analysis

The General Linear Model procedure of SAS 8.2 (2002) was used to analyze for differences between bacterial counts and survey data. Data were log transformed to normalize residuals and obtain p-values but are presented as untransformed means in the table. Log transformed means are presented when trends or significance was obtained in correlation with questions. Bacterial contamination of colostrum was also analyzed by farm size. Questions, nutrients and correlations were also analyzed by herd size where herds were grouped < 100 cows (n = 17), 101 – 200 cows (n = 10) and > 201 cows (n = 28). Each nutrient analysis was correlated to all 33 survey questions using the General Linear Model procedure and significance was declared at $P < 0.10$ due to the high variability of bacterial counts.

RESULTS AND DISCUSSION

It is well documented that colostrum and milk differ in composition, physiological and biological characteristics, however they are exposed to same environmental and hygiene practices used for collection of milk. Based on this it can be assumed that bacteriological quality of colostrum should not differ considerably in the number and type bacteria present in raw milk. Recently, Jayarao et al (2004) provided guidelines for evaluating bacteriological quality of raw milk. Although these guidelines are primarily for bulk tank milk, the same guidelines have been applied to draw inferences on the bacteriological quality of colostrum.

The SPC provides an estimate of the total number of aerobic bacteria present in colostrum. In our study, the SPC of colostrum ranged from $1.4x10^2$ to $9.0x10^6$ (Table5-2) Nearly 40% of the colostrum samples had a SPC of < 10,000 cfu/ml (Table 5-3). A SPC of >10,000 cfu/ml in raw milk correlates well with unsanitary conditions associated with unclean udders before milking, poor teat and teat end sanitation, cleaning and sanitation of milking equipment, and cooling of milk (Chambers, 2002). Perhaps the same set of observations could be applied to colostrum.

The PIC is used as an indicator of the number of psychrotrophic bacteria in raw milk. Milk with high PIC can influence the keeping quality of raw milk and reflect on the sanitation practices followed on farm (Jones and Sumner, 1999; Jayarao et al., 2001). In our study only 17% of the colostrum samples had a PIC count of <10,000 cfu/ml. (Table 5-3). As observed with SPC, the PIC varied considerably between colostrum samples, the mean PIC count was observed to be $12x\ 10^7$ cfu/ml (Table 5-2). This suggests that hygiene practices followed during collection and storage of colostrum samples need urgent attention.

The LPC determines the number of thermoduric bacteria present in raw milk. In our study 67% of the colostrum samples had a LPC count of <100 cfu/ml (Table 5-3). High counts of thermoduric bacteria (>200 cfu/ml) have been associated with herds with poor milking hygiene, unclean equipment, and improper sanitizing practices of the buckets and milking equipment (Murphy, 1997). Thermoduric bacteria have been shown to release proteolytic and lipolytic enzymes that breakdown proteins and milk fat. This

could result in the breakdown of essential peptides and protective factors in milk which could decrease its nutritional value. Furthermore, a mastitic udder can be a source of proteolytic enzymes released by the cow (plasmin and other cellular proteases) that can also break down milk proteins and fat resulting in lower milk quality (Straley, 2005). It is felt the same observations are applicable colostrum.

The primary habitat of *Staphylococcus aureus* is the infected udder. It readily colonizes the skin of the teats and teat ends when there is damage to the skin surface (chapping, frostbite, cuts, scabs and warts). *S. aureus* infections are usually chronic or subclinical, occasionally showing mild clinical signs (Hogan et al., 1989). The type of herd (closed or open) and milking practices followed on the farm influence the persistence and spread of *S. aureus* in the herd. In our study, nearly 58% of the colostrum samples were free of *S. aureus*, while colostrum samples with S. aureus had counts ranging from 0-12,000 cfu/ml (Tables 5-2 and 5-3).

Unlike other Streptococcal organisms, *S. agalactiae* is a contagious mastitis pathogen and its primary habitat is the infected udder. *S. agalactiae* are shed in large numbers from infected quarters and can be easily cultured from milk. The most frequent method of introducing *S. agalactiae* into a clean herd is by purchasing adult cows without prior testing. Herds with cows that have *S. agalactiae* infections should be considered as possibly having poor mastitis prevention and control practices. In our study, 1000 cfu/ml of *S. agalactiae* was isolated from one colostrum sample. Streptococci consist of a large heterogeneous group of organisms which are a common cause of mastitis in cows during early lactation and dry period. This observation supports the finding of the current study that reported only 38% of cows with Streptococcal counts of < 1,000 cfu/ml. Cows are usually infected with Streptococci from environmental sources and spread it during milking or by the environment. In herds with persistently high (>1000 cfu/ml) counts of Streptococci in milk, a higher incidence of clinical and subclinical mastitis due to *S. uberis* or *S. dysgalactiae* can be expected.

The CNS are normal residents of the skin surface. The CNS are an opportunistic group of bacteria, which gain access into the teat canal and the gland from skin sources. The infection is usually mild and transient in nature however clinical mastitis due to CNS has been widely reported in literature. In our study, 76% of the colostrum samples had <

1,000 cfu/ml with a mean of 620 cfu/ml.

Coliform organisms include *Escherichia coli*, Klebsiella spp., Enterobacter spp. and Citrobacter spp. A high proportion of new infections with coliforms occur approximately 2 weeks before and 2 weeks after drying off. In milking herds, susceptibility to infection is highest at calving and decreases considerably as lactation progresses. During hot and humid weather conditions, cows are at higher risk of developing coliform mastitis (Hogan et al., 1999). Gram-negative non-coliform organisms, such as Pseudomonas and Serratia, can cause severe mastitis, including outbreaks of clinical mastitis (Erskine et al., 1987; Kamarudin et al., 1996). It can be speculated that the most frequently observed problems related to elevated bacterial counts in colostrum could be attributed to 1) absence of an established milking protocol, 2) poor udder condition and health, and 3) deficient management practices related to general farm hygiene and stall maintenance.

Since farm management practices may differ by farm size and this may impact colostrum quality, survey question results are presented by size for farms < 100, 101 – 200 and > 200 cows (Table 4-1). Calves were fed by bottle in 85% of farms, and only 1 farm, which had > 201 cows, allowed calves to nurse off the dam. This is an improvement from previous surveys that reported 16% and 22% of calves nursing off the dam unassisted (Goodger, 1986; Heinrichs, 1987). Stewart et al. (2005) reported that colostrum was least contaminated by bacteria when it was collected aseptically from the teat and only increased in bacterial contamination due to storage, handling and other human factors.

All bacteria counts were numerically higher for farms with higher somatic cell counts compared with farms with somatic cell counts < 200,000. Farms with SCC > 200,000 that were < 100 cows had significantly lower counts of CNS compared to farms > 201 ($P < 0.07$). This is supported by calf mortality trends, which increase as farm size increases, indicating that bacterial contamination of colostrum may be one of many factors that play a role in neonatal survival (Jenny et al., 1981; USDA, 2002)

Colostrum was not stored on 59% of farms < 100 cows compared with 50% of farms 101 – 200 cows and 21% > 201 cows; this was also significantly correlated with 88% of farms < 100 cows feeding calves from their own dam compared with 89% of

farms 101 – 200 cows and only 43% > 201 cows. Smaller farms tended to feed calves directly after milking the respective dams and therefore not as many small farms stored colostrum. Also, colostrum was frozen in 35% of farms < 100 cows and 40% of farms 101 – 200 cows and 39% > 201 cows, indicating that the use of frozen colostrum in Pennsylvania has increased from 22% in a previous survey conducted by Heinrichs et al. (1987). This is presumably due to increased awareness of the importance of having colostrum available to increase passive transfer in calves.

Concentrations for all bacterial counts were significantly higher for samples of colostrum from farms that reported storing colostrum in the refrigerator compared with farms that reported storing frozen colostrum. Also, farm size had an effect on bacterial counts of SPC, PIC and CC when different storage methods were used. Counts were lower in samples taken from farms < 100 cows whether colostrum was frozen or not stored compared with farms 101 – 200 cows; farms > 201 cows were also numerically higher but not different than the other farm sizes. Farms < 100 cows that froze colostrum resulted in values of 80,958 ± 616,538, 294,294 ± 8,145,274 and 35,194 ± 195,224 cfu/ml for SPC, PIC and CC, respectively, compared with farms 101 – 200 cows which resulted in 9,070,000 ± 1,631,207, 52,240,000 ± 21,550,371 and 1,980,000 ± 516,515 cfu/ml, respectively.

Storing colostrum in a closed-top container compared with not storing colostrum concluded with lower bacterial counts of LPC and CC. Storage of colostrum in closed-top containers resulted in counts of LPC and CC which were 145 ± 1,005 and 118,934 ± 216,384 cfu/ml, respectively. Farms where colostrum was not stored in any manner had higher counts of LPC and CC, which were 698 ± 740 and 279,598 ± 159,301 cfu/ml, respectively. This is most likely due to lower bacterial contamination in colostrum that is quickly stored in a closed container compared to colostrum that most likely sits waiting to be fed directly to calves.

Colostrum from farms 101 – 200 and > 201 cows that reported feeding calves from their respective dams had lower counts of SPC, PIC, LPC, CNS, SS and CC compared with farms that did not feed calves with colostrum from their own dam. This may be due to the availability of labor to quickly feed the colostrum which would not allow high levels of bacterial contamination. Bacterial counts from farms < 100 cows

were actually the opposite where farms that reported not feeding colostrum from their own dams had significantly lower counts of CNS and SS and tended to have lower counts of LPC and PIC compared with farms that reported feeding colostrum to calves from their own dam. On small farms with limited labor, colostrum may not be fed quickly enough to calves and instead may sit in the bucket where bacterial contamination increases rapidly. Colostrum that is saved for other calves may be stored quickly thereby preventing high bacterial contamination.

Colostrum samples from farms > 201 cows that used a colostrum replacer had higher counts of PIC than farms < 100 and 101 – 200 cows (30,668,500 ± 11,964,503, 18,000 ± 13,815,418, and 26,100 ± 16,920,363 cfu/ml, respectively) and also higher counts of CNS (238,600 ± 314,828, 346 ± 363,532 and 6,120 ± 445,234, respectively). However, all counts were numerically higher for farms that did not use a colostrum replacer compared with farms that did. Farms using a colostrum replacer may be trying to improve passive transfer in calves thereby improving colostrum management which may result in lower bacterial counts.

The ranges of bacterial counts reported for colostrum samples were extremely large and variable (Table 4-2). The means were high however the mode which describes the most prevalent value for a dataset was zero for most of the bacterial counts. For SPC, the mode was 2,800 cfu/ml which is well under the standard for grade A quality milk. All other bacterial counts had modes of 0 except for PIC with a mode of 1,400 cfu/ml.

Although there were samples which were highly contaminated with bacteria, the cumulative distribution of bacterial counts shows that most samples were within reasonable limits (Table 4-3). Of all samples, 62% resulted in SPC values of less than 100,000 cfu/ml compared with 18% of samples that were less than 1 million and 20% less than one billion cfu/ml. One hundred percent of samples analyzed for LPC and SA resulted in less than 100,000 cfu/ml; also, 94% of samples analyzed for CNS resulted in less than 100,000 cfu/ml, 80% of SS and 73% of CC. This represents how misleading the dataset may be due to the skewed nature of the bacterial counts. Most samples were relatively low in bacterial counts taking into consideration that colostrum is naturally higher in bacteria compared with normal milk.

IMPLICATIONS

Farm size affected colostrum quality where farms < 100 cows tended to have lower bacterial counts compared with farms > 201 cows. Although more of the larger farms stored frozen colostrum, more of these farms also stored colostrum by refrigeration. This may have caused higher bacterial counts compared with smaller farms where the majority fed calves directly from their own dam without any storage. Many of the management practices and bacterial counts were similar between farms < 100 and 101 – 200 cows indicating that differences in management begin with farms > 201 cows. Therefore, farm size must be taken into account when discussing changes in colostrum management. Overall results of this survey reveal that colostrum fed to calves on Pennsylvania farms often contains more bacterial contamination compared with milk independent of farm size. While calf health was not investigated, the level of bacteria found in colostrum suggests the potential for improved calf health through closer attention to colostrum collection, storage, and handling practices.

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Table 4-1. Various aspects of colostrum management practices in farms containing < 100, 101 – 200 and > 201 cows.

	Number of cows		
	< 100	101-200	>201
Item	% Herds	% Herds	% Herds
Number of herds	17	10	28
Prior month's SCC			
< 200,000	50	50	22
> 200,000	50	50	78
Colostrum collection method			
Pre-dip, machine milk, post-dip	88	100	86
Wash, dip, machine milk, dip	0	0	7
Machine milk, post-dip	12	0	4
Other	0	0	3
Colostrum storage container			
Open container	6	0	10
Closed container	35	50	68
Not stored	59	50	21
Colostrum storage method			
Frozen	35	40	39
Refrigerated	6	10	36
Stored without refrigeration	0	0	4
Not stored	59	50	21
Calves fed colostrum from own dam			
Yes	88	89	43
No	12	11	57
Use a colostrum replacer			
Yes	6[a]	20[b]	25[b]
No	94	80	75

[a,b] indicates a significant difference between farm sizes for the respective answer ($P < 0.10$).

Table 4-2. Description of bacteria counts found in 55 colostrum samples from surveyed dairy farms.

Bacterial Counts[1,2]	Cfu/ml				Standard Error
	Mean	Range	Median	Mode	
SPC	997,540	140-9070000	15,300	2,800	290,326
PIC	12,094,750	240-90700000	324,000	1,400	3,307,111
LPC	620	0-18,000	30	0	339
SA	310	0-12,000	0	0	220
CNS	164,960	0-3980000	2,260	0	81,248
SS	256,720	0-5,6000,000	2,140	0	114,233
CC	323,370	0-3,950,000	600	0	103,622
NC	111,540	0-3,000,000	360	0	57,495

[1]Least squares means for standard plate count (SPC), preliminary incubation count (PIC), laboratory pasteurization count (LPC), Staphylococcus aureus (SA), coagulase negative Staphylococci (CNS), Streptococci (SS), Coliforms (CC) and non-coliforms (NC).
[2]Only one farm sample had bacterial counts of Staphylococcus agalactiae (SAG) and was not included in the table.

Table 4-3. Distribution of bacterial counts in colostrum samples.

Bacterial Counts (cfu/ml)[1]	Cumulative Distribution of Bacterial Counts							
	SPC	PIC	LPC	SA	CNS	SS	CC	NC
0	0	0	22	58	4	5	16	40
< 100	0	0	67	76	40	16	31	47
<1,000	11	2	91	96	76	38	53	65
<10,000	40	17	98	98	85	67	68	74
<100,000	62	41	100	100	94	80	73	85
< 1,000,000	80	63			100	93	89	98
<10,000,000	100	78				100	100	100
< 100,000,000		100						

[1]Least squares means for standard plate count (SPC), preliminary incubation count (PIC), laboratory pasteurization count (LPC), Staphylococcus aureus (SA), coagulase negative Staphylococci (CNS), Streptococci (SS), Coliforms (CC) and non-coliforms (NC).

Table 4-4. Bacteria counts that varied by size of < 100, 101 – 200 and > 201 cows in the milking herd.

	Number of cows					
	< 100		101-200		>201	
Item	Means	SE	Means	SE	Mean	SE
SPC[1]	620,899	510,919	1,582,394	685,470	1,032,019	417,164
PIC	7,753,498	5,742,962	23,197,750	7,704,992	10,876,707	4,689,109
LPC	211[a]	596	263[b]	800	1016[ab]	487
SA	711	385	54	517	130	315
CNS	226,768[a]	143,341	275,798[b]	192,312	82,710[b]	117,037
SS	329,538[a]	200,539	487,639[b]	269,052	122,652[b]	163,740
CC	145,216	179,512	663,329	240,840	316,232	146,571
NC	45,038	101,654	99,262	136,383	160,430	83,000

[1]Least squares means for standard plate count (SPC), preliminary incubation count (PIC), laboratory pasteurization count (LPC), Staphylococcus aureus (SA), coagulase negative Staphylococci (CNS), Streptococci (SS), Coliforms (CC) and non-coliforms (NC).
[a,b] indicate significant differences between farm sizes ($P < 0.10$).

Chapter 5

EFFECTS OF NUCLEOTIDE SUPPLEMENTATION IN MILK REPLACER ON SMALL INTESTINAL ABSORPTIVE CAPACITY IN DAIRY CALVES

ABSTRACT

Prior to weaning young calves often become infected with viruses and bacteria that may cause damage to the intestinal lining. To examine the potential for enhancement of recovery of small intestinal function during scours, milk replacer was supplemented with nucleotides from birth through weaning. Calves were fed milk replacer at 10% of BW daily and treatments consisted of no nucleotide supplementation (C), purified nucleotide supplementation (N) and yeast cell content supplementation (S; Alltech, Inc.). Average daily gain, health scores, fecal dry matter and fecal bacteria were monitored and blood was analyzed for hematocrit, glucose, blood urea nitrogen (BUN) and creatinine. Calves were monitored twice daily for scouring; 48 h after scours was recorded, xylose (0.5 g/kg of BW) was administered orally as a marker of intestinal function. Blood was taken once pre-dosing and 1, 2, 3 and 4 h post-dosing. Concentrations of plasma xylose increased with time post-dosing, but did not vary by treatment. Blood hematocrit was significantly higher for treatment N for weeks 2 and 5 compared with other treatment groups. Twelve calves were slaughtered and intestinal tissue was evaluated for morphology, enzyme activities and mRNA expression of the nucleoside transporter. Although there were few significant differences between treatments, treatment S calves had higher expression of nucleoside transporter mRNA, numerically longer villi and lower alkaline phosphatase. Growth measurements and plasma concentrations of glucose, BUN, creatinine and IgG were not different between treatments; however the BUN to creatinine ratio was higher for treatment N indicating possible decreased kidney function. There were also no treatment effects on fecal dry matter and concentration of fecal bacteria; however treatment N had highest detrimental and lowest beneficial bacteria overall indicating an unfavorable intestinal environment. The supplementation of purified nucleotides did not appear to improve intestinal morphology or function and resulted in higher fecal water loss and dehydration. Supplementation of nucleotides with

yeast cell contents tended to increase intestinal function, provide a more beneficial intestinal environment and improve intestinal morphology. More research needs to be conducted to determine if higher concentrations of supplemented yeast nucleotides and better timing of administration can further improve growth, health and metabolic measurements.

INTRODUCTION

Neonatal diarrhea is the cause of 62% of annual calf mortality which represents a large economic loss to the dairy industry (Torres-Medina, 1985; USDA, 2002). Viral pathogens that affect mucosal function of the intestinal tissues destroy villus architecture and decrease absorptive surface area of the small intestine (Bridger et al., 1978; Holland, 1992; Saif et al., 1986). Loss of epithelial function results in malabsorption of water, sodium, and chloride (Torres-Medina, 1985). Continued scouring increases the risk of dehydration and hypoglycemia and, if not treated, can lead to death (Clark, 1993; Pensaert, 1994).

Nucleotides are non-protein nitrogenous compounds found in many foods such as seafood, legumes and organ meats. Nucleotides are known as "semi-essential" nutrients because, although the body is able to endogenously synthesize them, intestinal tissue that is developing or diseased requires supplemental nucleotides (Uauy et al., 1990).

Both nucleosides, which are nucleotides without attached phosphate groups, as well as nucleotides, have been shown in different species to enhance intestinal maturation (Uauy *et al.*, 1990) as well as aid in recovery from diarrhea (Brunser et al., 1994; Bueno et al., 1994; Kulkarni et al., 1986). Recovery of the small intestine after chronic diarrhea is slow and incomplete, yet Bueno *et al.* (1994) showed that weanling rats suffering from diarrhea fully recovered after nucleotide supplementation to the same extent as rats not challenged with diarrhea. Rats supplemented with nucleotides had significantly lower numbers of intraepithelial lymphocytes, higher microvillous surface area, no cytoplasmic vesiculation and improved mitochondrial function (Bueno *et al.*, 1994).

Research with veal calf nucleoside transport using brush border membrane vesicles (Theisinger et al., 2002; Theisinger et al., 2003) showed the nucleotide transporters, N1 and N2 (Ngo et al., 2001) were present at an early age and the authors

hypothesized that the predominant function was to absorb nucleosides released from desquamated enterocytes. Dietary nucleosides also have been reported to upregulate the nucleoside transporter protein in a variety of tissues, including the small intestine. As nucleotide concentrations in the diet increase, the ability of the enterocytes to absorb nucleosides increases (Valdes et al., 2000).

Nucleic acids include the nucleotides adenine, thymine, guanine, cytosine and uracil. Cow colostrum contains 3.97 ± 0.75 µmol/100ml adenosine monophosphate, 3.19 ± 0.41 µmol/100ml cytosine monophosphate and 18.63 ± 5.01 µmol/100ml uridine monophosphate; guanosine monophosphate was not observed (Gil and Sanchez-Medina, 1981). Dried milk replacer ingredients such as whey, whey protein concentrate and skim milk usually have very low levels of nucleotides which necessitates nucleotide supplementation by many human milk replacer companies. Currently, supplementation with nucleotides is not common in the dairy milk replacer industry.

Treatment of calves is necessary once scouring begins. The ability to decrease the duration and/or intensity of scours would allow faster recovery thereby saving time and labor for producers. We hypothesized that increased provision of dietary nucleosides would result in the improvement in intestinal health post-challenge in scouring dairy calves. We further hypothesized that the addition of nucleotides to the diet of neonatal calves would increase expression of the nucleoside transporter enabling an increase in absorption of nucleosides. This would lead to decreased cellular energy expenditure and allow improved enterocyte regeneration during scours resulting in enhanced intestinal function and absorption. The objective of this study was to evaluate supplementation of milk replacer with nucleotides on intestinal absorptive function and animal health in pre-weaned calves.

MATERIALS AND METHODS

Animals, Housing and Diet

The trial utilized 69 Holstein heifer and bull calves born from September 2005 to April 2006 at the Pennsylvania State University dairy facility. Treatments were randomly assigned in blocks and calves were housed in individual pens inside a ventilated barn. All calves received 4 L of colostrum within 6 h of birth followed by 4

feedings of waste milk before feeding of milk replacer. Milk replacer fed to calves contained 22% CP (all milk protein), 15% fat (porcine lard) (Akey, Inc., Lewisburg, OH), and contained no additional additives or medication. Fresh calf starter was offered ad libitum beginning at 3 d and intake was recorded daily. Water was changed daily and offered free choice continuously. Calves were monitored in individual pens from birth until 1 wk after weaning and then moved to a group pen at 6 wk of age. Calf starter and milk replacer samples were collected twice monthly and analyzed for nutrient composition (Table 5-1).

Treatments included a control (C) group fed milk replacer only with no supplementation of nucleotides, a nucleotide group (N) receiving milk replacer supplemented with purified nucleotides (Lalilab, Inc., Raleigh, NC) and a group (S) fed milk replacer supplemented with nucleotides contained in cell contents from the yeast species *Saccharomyces cerevisiae* (NuPro; Alltech, Inc., Lewisburg, OH). Treatments were weighed into scintillation vials and distilled water was added the night before feeding. Control calves also received a vial with only distilled water and calf barn personnel were not aware of treatment assignments. Nucleotide treatment consisted of 80 µmol/L AMP, 64 µmol/L CMP and 374 µmol/L UMP. TheSaccharomyces cerevisiae treatment was calibrated to equal concentrations of purified AMP, CMP and UMP and was fed at 0.094 g/L which resulted in 79.5 µmol/L AMP, 60.6 µmol/L CMP and 379.4 µmol/L UMP.

Blood, Health and Growth Measurements

Calves were weighed immediately after birth before colostrum feeding and then measured for heart girth, hip height and withers height. These measurements were taken on a weekly basis after birth. One blood sample, 6 ml, was taken at 48 h after birth for total immunoglobulin G (IgG) concentration to determine status of passive transfer and then weekly for 3 wk. Blood was taken from the jugular vein thereafter once a week at 4 h after AM milk feeding. Vacutainers (Becton, Dickinson and Co., Franklin Lakes, NJ) containing sodium heparin were used for blood urea nitrogen (BUN) and hematocrit. Vacutainers containing sodium fluoride/potassium oxalate were used to determine

glucose and vacutainers containing a clot enhancer were used for creatinine. Before processing, capillary tubes were spun for hematocrit values after which blood was centrifuged for 15 min at 3600 x *g*. Plasma and serum were stored at -20 ˚C until further analysis. Samples were analyzed for BUN using Stanbio Urea Nitrogen Kit 580 (Stanbio Laboratory, San Antonio, TX), plasma glucose using Sigma Glucose Kit 510 (Sigma Chemical Co., St. Louis, MO) and serum creatinine (Stanbio Laboratory, San Antonio, TX). Concentrations of IgG were analyzed using a radial immunodiffusion (RID) assay (Bethyl, Montgomery, TX). Health scores were conducted daily on each calf to evaluate scores of fecal, respiration and general appearance (Lesmeister et al., 2004). Fecal scores were based on a 1-5 scale where 1 was firm, 5 was watery with no consistency and a score of 3 was considered scours. Calves were checked twice daily to monitor the onset of scours.

Fecal Sampling and Xylose Analysis

Fecal samples were obtained manually from the rectum from calves (n = 18 calves/treatment) every other day during the first part of the study and analyzed daily for dry matter content by drying in an oven at 100 ºC for 48 h. Fecal samples were taken weekly from 7 calves per treatment during the last portion of the experiment and cultures analyzed for *Clostridium perfringens, Lactobacillus acidophilus* and *Bifidobacterium spp*. Samples were diluted in saline to achieve a workable dilution and incubated overnight anaerobically at 37 ºC. Agars used for culture were Remel CDC PEA agar (01048), Anaerobe Systems LMRS agar (AS-6429) and Anaerobe Systems Bifidobacterium species agar (AS-6423) based on the methods of Rada and Petr (2002).

Calves were monitored daily for scour scores and 48 h after the first scour score of >3, xylose, a commonly used marker for intestinal absorptive function, was fed at 0.5 g/kg of BW (n = 8 calves/treatment). Jugular blood samples were obtained in heparinized vacutainers prior to xylose feeding to provide baseline values at time 0 and then after xylose feeding at 1, 2, 3 and 4 h. Serum was separated from the sample by centrifugation at 3600 *x* g for 15 minutes, collected, and frozen at -20 ˚C for later analysis

of xylose concentrations. Xylose concentrations were determined spectrophotometrically by the method described by Merritt and Duelly (1983).

Intestinal Sampling

After the sampling for xylose absorption was performed, twelve bull calves (n = 4 calves/treatment) were taken to the PSU Meats Laboratory and slaughtered using captive bolt gun and exsanguination. Immediately following exsanguination, the entire small and large intestines were removed from the body cavity. Within 5 minutes, two 1 cm sections were rinsed in saline and cut from each of the duodenum (within 10 cm of pylorus), jejunum (estimated midpoint of small intestine) and ileum (within 10 cm of cecum) and placed in 10% formalin for morphometric analysis. Sections from these same areas were also rinsed with saline and scraped with a razor blade, weighed to 3 g and frozen in liquid nitrogen for enzyme analysis. One gram was then scraped and placed in 5 ml of RNALater (Ambion, AMS Technology, Cambridgeshire, UK) for examination of nucleoside transporter (SLC28A1; bovine sodium-coupled nucleoside transporter, member 1) mRNA abundance by real-time reverse transcription polymerase chain reaction (RT-PCR). The liver and spleen were extracted and weighed and the small intestine was separated from the colon and both were weighed and measured.

Intestinal analyses

Intestinal tissue was embedded in paraffin blocks, sectioned at 3-6 µm and stained with hematoxylin and eosin. Villus lengths and crypt depths were measured for each intestinal section using Scion Image (NIH) and means of twenty measurements were calculated per segment for each calf. Only whole villus/crypt units were used for measurement, which included whole rounded villus tips visibly connected to complete crypts which ended near the muscularis mucosal layer. To measure villus length and crypt depth, the villus/crypt junction was first determined. This included visibly determining where the villus came out of the intestinal wall and where the crypt receded into the intestinal wall (Figure 5-1).

Intestinal scrapings were analyzed for enzyme activity. Three grams of scrapings were thawed and homogenized in 12 ml of distilled water, while samples were on ice. Alkaline phosphatase was measured as a marker of enterocyte maturation in the upper villus zone (Weiser, 1973) and lactase and maltase were measured as brush border markers (Dahlquist, 1964). Protein was measured using a Pierce BCA assay with bovine serum albumin as a standard (Rockford, IL) and DNA was quantified following methods described by Burton (1956). All experimental procedures were approved by The Pennsylvania State University Animal Care and Use Committee.

Polymerase Chain Reaction

Total RNA was extracted from duodenum, jejunum and ileum samples using Versagene RNA Tissue kits (Gentra Systems, Minneapolis, MN). Concentrations of RNA were measured spectrophotometrically in a Shimadzu Bio-mini and accepted ratios of 260: 280 nm between 1.8 and 2.1. Due to low RNA concentrations in the orginal extractions, samples were concentrated further by Speed-Vac (Savant) and concentration measurements repeated. One microgram of RNA was reverse transcribed using Protoscript reverse transcriptase (New England BioLabs; Ipswich, MA). Primers used were designed with Primer Premier 5 software (Premier Biosoft International, Palo Alto, CA) using a predicted bovine sequence for the intestinal nucleoside transporter (SLC28A1) found by BLAST (Basic Local Alignment Search Tool; Altschul et al., 1990). Bovine cyclophilin (Table 5-7) was used as a housekeeping gene to correct for nonspecific changes in mRNA abundance (Greger et al., 2006).

The RT-PCR procedure was performed using DyNamo HS SYBR Green (New England BioLabs; Ipswich, MA) and quantified on an Opticon Monitor System (Genetic Technologies, Miami, FL). Amplification and denaturation conditions used were 95 ºC for 15 min and 35 repeated cycles of denaturation at 94 ºC for 10 seconds, annealing at 62 ºC for 30 seconds and extension at 72 ºC for 30 seconds. The melting curve began at 72 to 95 ºC with a heating rate of 0.2 ºC per second and a final extension step at 72 ºC for 10 minutes. The PCR product was visualized by agarose gel electrophoresis and the presence of a single product at the expected molecular weight was confirmed. The

product was extracted from the gel and was sequenced at the Penn State Nucleic Acid Facility (http://www.huck.psu.edu/stf/naf/home.html). A BLAST search was performed to confirm that the sequence was identical with the bovine nucleoside transporter sequence (Solute carrier protein; SLC28A1, member 1; GenBank Accession No. BC108181).

Statistical Analysis

Least squares means were analyzed using a repeated measures analysis of the Mixed procedure of SAS 8.2 (2001) with week as a repeated effect. Growth measurements taken at birth were used as covariates for growth analysis, hour 0 was used as a covariate for xylose analysis and day 0 was used as a covariate for fecal bacteria analysis. The slopes between time points of xylose concentrations were also analyzed. The statistical model used for analysis was:

$$Y_{ijkl} = \mu + T_i + W_j + B_k + (TW)_{ij} + (TB)_{ik} + (WB)_{jk} + (TWB)_{ijk} + calf_l + e_{ijkl},$$

where:

Y_{ijk} = dependent variables,

μ = overall mean,

T_i = fixed effect of treatment i, where i = C, N or S supplementation,

W_j = repeated measure of week j,

B_k = block effect of treatment,

$(TW)_{ij}$ = effect of treatment by week interaction,

$(TB)_{ik}$ = effect of treatment by trial interaction,

$(WB)_{jk}$ = effect of week by block interaction,

$(TWB)_{ijk}$ = effect of treatment by week by block interaction,

$calf_l$ = random effect of calf l,

e_{ijkl} = residual.

Least squares means were determined using the General Linear Model of SAS 8.2 (2001) for intestinal measurements which included DNA, protein, enzymes and morphology. The statistical model used for analysis was:

$$Y_{ijkl} = \mu + T_i + D_j + A_k + (TD)_{ij} + (TA)_{ik} + (TAD)_{ijk} + e_{ijkl},$$

where:

Y_{ijk} = dependant variables,

μ = overall mean,

T_i = fixed effect of treatment i, where i = C, N or S supplementation,

D_j = fixed effect of day of slaughter j,

A_k = fixed effect of intestinal area k, where k = duodenum, jejunum or ileum,

$(TD)_{ij}$ = effect of treatment by day interaction,

$(TA)_{ik}$ = effect of treatment by area interaction,

$(AD)_{kj}$ = effect of area by day interaction,

$(TAD)_{ijk}$ = effect of treatment by week by block interaction,

e_{ijkl} = residual.

Least squares means for semi-quantitative PCR analysis were obtained using the $2^{-\Delta\Delta CT}$ method (Livak and Schmittgen, 2001) and analyzed using the Mixed procedure of SAS. The threshold cycle, or C(T), was determined from a log-linear plot of the PCR signal compared with the cycle number. To normalize the PCR reaction for the amount of RNA added to the reverse transcription reactions, cyclophilin, a standard housekeeping gene, was used. The C(T) value for experimental groups was subtracted from the cyclophillin C(T) value to normalize the reaction. Because the C(T) was not a linear number but exponential, data was presented for experimental groups relative to the control group set as 1. Data was transformed to ensure normality and P-values were obtained using transformed data.

Least squares means for all analyses were further evaluated if the model was significant at $P < 0.05$; trends were determined as $P < 0.15$.

RESULTS

Feed intake and growth measurements

Initial body weight at birth was significantly lower for C calves ($P < 0.045$) even though treatments were randomized (Table 5-2). Therefore, initial bodyweight was used as a covariate in all growth and feed intake analyses and removed if found not to be significant. Other initial values such as heart girth, withers height and hip height were not different between treatments. All growth measurements were not different for

treatment averages over the 6 week period for C, N and S, including weight (48.9, 48.7 and 49.1 ± 0.6 kg, respectively), heart girth (83.8, 84.7 and 84.8 ± 0.4 cm, respectively), withers height (77.2, 77.7 and 77.9 ± 0.3 cm, respectively) and hip height (82.1, 82.5 and 82.1 ± 0.4 cm, respectively).

Feed intake was not significantly different between treatments however there was a significant treatment by week interaction ($P < 0.048$) due to the C group tending to consume lower amounts of starter grain during week 6 compared to treatment N (Figure 5-2). Feed intake before weaning was not different between treatments although there was a significant treatment by week interaction ($P < 0.03$) at wk 3 where N tended to be higher than S and at wk 4, where S tended to be higher than C. Post-weaning feed intake was not different between treatments.

Fecal Measurements

Fecal water loss was not different between treatments and least squares means for treatments C, N and S were 44.03%, 43.4% and 44.09%, respectively. Only on the eighth day, calves tended to have higher water loss ($P < 0.08$) when provided purified nucleotides (N) which was 53.3% compared to 42.6% for S and 46.2% for C.

Fecal bacteria concentrations were not different among treatments C, N and S (Table 5-3). *C. perfringens* was quantified at 1.1, 2.3 and 1.3 ± 0.5 x 10^7 cfu/ml feces, for treatments C, N and S, respectively. *L. acidophilus* was quantified at 66.4, 79.6 and 110.3 ± 28.8 x 10^7 cfu/ml feces for treatments C, N and S, respectively; *B.* spp measured 114.8, 106.1 and 125.5 ± 27.2 x 10^7 cfu/ml feces, for C,N and S, respectively.

Blood measurements

Least squares means of hematocrit values were 32.5, 35.2 and 32.7 ± 0.8 % for treatments C, N and S, respectively. Treatments N and S were different ($P < 0.03$) as well as N and C ($P < 0.02$). Least squares means of hematocrit (Figure 5-3) were higher for treatment N for weeks 2 and 5 compared to treatments S and C ($P < 0.0205$ and $P < 0.0205$, respectively).

Glucose and BUN least squares means were not different between treatments; results were similar using hematocrit as a covariate. Least squares means of IgG from

week 0 through week 3 were also similar between treatments (Table 5-3) both with and without week 0 as a covariate. Creatinine concentrations were not different between treatments however the BUN:creatinine ratio, where higher values may indicate inferior kidney function, was numerically highest for the N treatment.

Xylose Absorption

Changes in xylose concentrations (mg/dl) for calves by hour are shown in Figure 5-4. Treatment values for xylose concentrations represent least squares means adjusted by hour 0 as well as initial bodyweight, both of which were not significant. There were no differences in xylose concentrations between treatments overall, however treatment S tended to be higher than the control group when slope from hour 3 to hour 4 was investigated ($P < 0.13$).

Health measurements

During the trial, 10 calves died within one week of birth due to bloat or unknown causes but were distributed evenly over treatments (Table 5-6). Averages of the amount of milk replacer refused were similar per treatment as well as number of calves that refused to partially or completely drink milk replacer and that were treated with an oral rehydration solution or antibiotics. The N treatment was highest in number of days that calves refused to drink milk replacer but lowest for number of days treated with oral rehydration solution and antibiotics.

Least squares means for health scores were not different between treatments (Table 5-4). All calves scoured (scour score >3) at least once during the trial but scour scores were not different by treatment; There was a significant week effect ($P < 0.01$) where scour scores decreased over time (Figure 5-5) as well as a treatment by week effect ($P < 0.02$). During weeks 2, 3 and 4, C had significantly higher scour scores than S and N, respectively. During wk 3, S had significantly higher scour scores than N. Respiratory scores had a significant week effect ($P < 0.01$) where scores increased over time as well as a trend for a treatment by week effect ($P < 0.07$) where treatment C had higher scores than treatment N during wks 3, 4 and 5 and treatment S increased at a greater rate over weeks 4 and 5 compared with treatment C. General appearance scores

were similar with a significant week effect ($P < 0.01$). Scores decreased over time and there was a treatment by week effect ($P < 0.01$) where treatment S increased at a higher rate over weeks 1 and 2 and also 5 and 6 compared with other treatments.

Intestinal measurements

Weights and measurements of intestinal tissue were not different between treatments (Table 5-5). The spleen and liver were also not different between treatments although treatment S was numerically higher than the other treatments.

Least squares means of DNA (mg/ml) by area of intestinal tissue tended to be higher for the duodenum compared with the ileum ($P < 0.07$) but treatments were not different (Table 5-6). However, DNA in mg/g wet tissue was significantly different for area of intestine ($P < 0.02$), tended to be significant for treatment ($P < 0.14$) and there was a significant treatment by area interaction ($P < 0.03$). Concentrations of DNA/g wet tissue tended to be higher for treatment S compared to treatment C and significantly higher for duodenum compared to both jejunum and ileum ($P < 0.03$ and $P < 0.01$, respectively). Least squares means of protein were not different between treatments or area of intestinal tissue.

Alkaline phosphatase (Units/ml) was significantly different for area of the intestine ($P < 0.001$) where duodenal tissue activity was much higher than jejunum and ileum activity (Table 5-6). There was also a tendency toward significance of area by treatment where the C and N treatments had higher activity in the duodenum than S treatment ($P < 0.08$). Although no other significant trends were seen, treatment N was numerically lowest for both jejunum and ileum sections in alkaline phosphatase activity. Analysis of alkaline phosphatase activity per g wet tissue showed a significant increase in activity in the duodenum compared with jejunum and ileum ($P < 0.001$ and $P < 0.004$, respectively). In both the jejunum and ileum, activity was numerically highest for C and lowest for N treatments.

Maltase (Units/ml) least squares means were not different between treatments but tended to be higher for duodenum ($P < 0.09$). Maltase per mg protein tended to be significant ($P < 0.06$) for duodenum having the lowest activity. Lactase activity was not significant between areas of intestine or treatments (Table 5-6).

Morphology of intestinal tissue was not different between treatments or area of intestine (Table 5-6). Overall, villi were significantly longer ($P < 0.01$) than crypts (219.64 ± 9.65 and 125.24 ± 9.47 µm, respectively). Villi lengths were only numerically different by area where villi lengths were highest in duodenum and lowest in ileum; crypt depths were only slightly numerically higher in duodenum and lower in ileum.

Nucleoside transporter mRNA abundance

Nucleoside transporter mRNA abundance (Table 5-6) approached significance between treatments ($P < 0.056$). There was also an area by treatment interaction ($P < 0.11$) however mRNA abundance between the duodenum, jejunum and ileum was not significant (Figure 5-6). Treatment S mRNA abundance was significantly higher relative to mRNA abundance of treatment C and N ($P < 0.03$). In the duodenum, S treatment had greater mRNA abundance relative to C ($P < 0.04$) and N ($P < 0.14$). Treatment S had greater mRNA abundance relative to C both in the jejunum ($P < 0.04$) and in the ileum ($P < 0.03$).

DISCUSSION

Nucleotides are known as "semi-essential' nutrients because although the body is able to synthesize them endogenously, disease and rapid growth may increase their requirement. Supplementation of nucleotides in species including rats, pigs and humans has shown increased intestinal integrity, improved intestinal morphology and reduced diarrhea (Brunser et al., 1994; Kulkarni et al., 1986; Uauy et al., 1990).

In the present study, there were no significant differences in intestinal function as indicated by similar xylose uptake from the small intestine between treatment groups Xylose concentrations in the current study peaked at 31 to 38 ± 3.9 mg/dl. Healthy, colostrum fed calves reach peak xylose concentrations of around 55 mg/dl (Schottstedt et al., 2005) whereas in other studies, xylose concentrations in calves with intestinal damage peak at 45 to 75 mg/dl (Holland, 1992; Nappert, 2000). The peak xylose concentrations in this study were lower than other studies indicating that intestinal tissue was challenged as it would have been if calves were purposefully infected. Treatment with yeast cell contents slightly improved intestinal xylose absorption indicating improved function of

enterocytes or perhaps increased surface area due to longer villi and higher amounts of DNA/mg of wet tissue for the S treatment group.

Fecal bacteria concentrations were high during this time although not different between treatments. Others have reported that supplementing piglets with nucleosides during the post-weaning period may positively enhance the intestinal environment by increasing Lactobacilli and Bifidobacteria while decreasing Clostridium (Mateo et al., 2004).

Smith (1965) reported concentrations of *Lactobacilli* in feces for healthy calves approximately 12 d of age ranging from 9 to 10 x $\log_{10}$ which is approximately 1 million cfu/ml. In the current study, lactobacilli for the first two weeks ranged from 30 to 114 x $\log_7$ cfu/ml equaling approximately 2 x 10_{25} to 2 x 10_{96} cfu/ml. These numbers are much higher than concentrations reported by Smith (1965) indicating that the difference between calves kept in a normal dairy environment dramatically increases fecal bacterial concentrations compared with calves kept in a protected, enclosed environment.

All calves scoured between 8 and 16 d and *C. perfringens* concentrations peaked at week 2 for C and S treatments and at week 3 for N treatment. *Bifidobacteria* species peaked at week 1 for S, week 3 for C and week 4 for N. *L. acidophilus* peaked slightly at week 3 for C and S and week 4 for N. The patterns of these bacterial concentrations indicate that the presence of *C. perfringens* inhibited the growth of *Bifidobacteria* species and *L. acidophilus* in all treatments which could have altered the intestinal environment and led to increased scouring. Xylose absorption also could have been affected by the altered intestinal environment due to high bacterial counts.

Bacterial concentrations in the current study also coincided with weaning. During the fourth week of age, concentrations of *L. acidophilus* and *Bifidobacteria* species were high until weaning at 5 weeks of age when bacterial counts decreased dramatically. Pre-weaned calves are most susceptible to enteric diseases and risks decrease as rumen function and starter intake increase.

Volume of milk replacer refused, an indicator of general well being, was not different between treatments. The N treatment had similar number of calves that refused to drink compared to other treatments however calves on the N treatment also had the highest number of days that milk replacer was refused. Similar numbers of calves were

treated with oral rehydration solution but the S group had more calves treated with antibiotics for more days than the N or C treatments. Although the N calves were treated with antibiotics and rehydration therapy the least number of days their status of dehydration and intestinal measurements indicate that this treatment had scours not severe enough to warrant treatment but with a much longer duration compared with the other treatments. Although the number of days treated were less, the higher number of days that calves refused milk replacer on the N treatment indicates that calves were not quick to recover but bordered on illness for longer periods of time.

The lack of effect of nucleotide supplementation on growth measurements is not surprising. When nucleotide–free diets are fed to rats, growth rates are enhanced once nucleotides are reintroduced into the diet (Lopez-Navarro et al., 1996), however rats fed dietary nucleotides compared to rats fed a normal diet without extra supplementation show no enhanced growth rates (Clifford et al., 1976).

Blood measurements, including glucose, BUN and creatinine, were also not different between treatments. Katoh et al. (2005) reported a decrease in plasma glucose levels of Holstein bull calves fed UMP however UMP concentrations fed were higher (2 g/d) than the current study which supplemented at 0.12 g/L twice daily, where calves of average bodyweight received 1.2 g/d UMP. Although significant differences were found in glucose levels, organ weights were not different as reported by Katoh et al. (2005) which support the current lack of differences between treatments for liver and spleen weights.

Enzyme activities were affected by area of the intestine but not by treatments. Enzyme activity is usually the most active in the duodenum and decreases progressively throughout the intestine. Levels of alkaline phosphatase were within normal ranges of calves approximately 5 to 10 d of age however maltase and lactase were higher than previously reported (Le Huerou et al., 1992). Also, lactase activity was lowest in duodenum and highest in jejunum and ileum which is not supported by other work and may have been affected by high bacterial counts which changed the intestinal environment and could have altered enzyme function.

It is difficult to compare the present results with those of other studies since many nucleotide experiments compare supplemented animals to those fed diets deprived of

nucleotides; nucleotide-free diets may enhance differences between treatments. Research studies that determine the effects of animals fed normal, nucleotide containing diets compared to animals supplemented with extra nucleotides, found no differences in enzyme activities (Carver et al., 1993). Other work using calves did not find differences in DNA, protein, or enzyme activities except for higher ileum DNA and lower aminopeptidase N for nucleotide-fed calves (Oliver et al., 2005).

The current study reported significantly higher DNA concentrations per wet tissue weight in duodenum compared with jejunum and ileum which is supported by other work (Buhler et al., 1998; Le Huerou et al., 1992; Uauy et al., 1990). Although not significant, DNA concentrations for the S treatment were numerically higher than both N and C treatments perhaps indicating a greater proliferation of cells which may be due to longer intestinal villi.

The S treatment had numerically higher lengths of villi in the duodenum and ileum. Alkaline phosphatase which is a marker of the maturity of enterocytes reaching villous tips was lower for the S treatment. This may indicate that enterocytes reaching the villous tips were less mature than other treatments which may be caused by a faster turnover rate. The S treatment had longer villi and higher concentrations of DNA/g wet tissue which indicates more enterocytes either due to longer villi containing more cells or higher turnover as indicated by the lower maturity of cells at villous tips. Although this would normally cause increased scours, treatment with yeast cell contents may have ameliorated these effects considering the S group had a slightly improved intestinal xylose uptake, more beneficial intestinal bacteria and normal hematocrit levels.

Calves supplemented with yeast cell contents had the highest expression of the nucleoside transporter. The highest mRNA abundance occurred in the jejunum and ileum which is different than that reported by Theisinger et al. (2002) who found highest nucleoside transporter activity (Vmax) in the duodenum and decreasing activity through to the distal intestine. However, the Theisinger et al. (2002) studies were specific nucleoside uptakes with brush border membrane vesicles conducted in vitro. Furthermore, other studies used purified nucleotides whereas in the current study, the S treatment contained nucleotides within yeast cell contents which may affect differences in transporter expression. The highest gene expression of the nucleoside transporter for

the N group was in the ileum which is different that that reported by Theisinger et al. (2002). In the current study, calves were slaughtered during week 2 to 3 when *C. perfringens* levels were highest which may have altered the intestinal environment and receptor activity.

Our data represents the mRNA abundance and not the presence of the protein whereas the Theisinger study measured protein transport observed in vitro and with individual nucleosides. Comparisons are difficult to interpret, but if mRNA expression is translated into active protein, then the yeast cell extract would be expected to increase transporters expressed in the enterocytes. The S treatment had a 7-fold increase in mRNA abundance compared with the control group which indicates increased transporter protein. However, future work needs to evaluate not only mRNA abundance but also to measure transporter proteins in order to validate an increase in nucleoside transport with yeast cell contents.

Both treatment groups that received nucleotide supplementation had higher fecal bacteria counts of *C. perfringens* during the first week of life compared to calves not receiving any nucleotides. This could indicate that *C. perfringens* bacteria were utilizing part of the purified nucleotide supply from the beginning of supplementation which may explain the higher hematocrit, fecal water loss, and inferior intestinal morphology found in the N treatment compared with other treatments. Although *C. perfringens* counts were also higher during the first week of life for the S treatment, *L. acidophilus* and *Bifidobacteria* species were higher as well which may have somewhat ameliorated the effects of higher Clostridium counts. Although bacteria may have been utilizing part of the nucleotide supply, the increase in expression of nucleoside transporter indicates that there were enough nucleotides present in the intestinal lumen in order to stimulate uptake by increasing expression of the nucleoside transporter.

In milk products used to feed animals, colostrum is considered the gold standard for formulating neonatal nutrition. Therefore, in the current study, amounts of nucleotides fed were based on values determined from 0 to 1 d colostral secretions by Gil and Sanchez-Medina (1981). These values were doubled and fed twice daily which provided calves with four times the amounts found in colostrum per day (160 AMP, 128 CMP and 748 UMP, umol/L). In 1996, infant milk replacer was required to contain 2.99

AMP, 5.35 CMP, 0.96 GMP and 3.73 UMP (umol/L). Recently, some work has been conducted to supplement dairy calves with nucleotides. Oliver *et al.* (2003) fed 0.04 AMP, 1.14 CMP, 0.48 GMP, 0.64 IMP and 10.3 UMP (umol/kg BW) per calf per day. Therefore assuming an average 55 kg calf, amounts fed were 2.2 AMP, 62.7 CMP, 26.4 GMP, 35.2 IMP and 566.5 UMP umol per day. Considering the lack of differences between treatments reported by Oliver *et al.* (2003) these values may have been too low.

Treatment N had significantly higher dehydration incidence during week 2 and 5 compared to C and S. Fecal water loss also tended to be higher for N during day 8 and the BUN: creatinine ratio indicated decreased kidney function due to higher dehydration. Treatment N also had the lowest concentrations of *Bifidobacterium* which never attained as high a peak as the other treatments. Even though treatment S had lower enterocyte maturity and higher sloughing of cells, hematocrit levels were normal, intestinal function was better, intestinal villi were longer and DNA/mg wet tissue concentrations were higher.

With the promising effects of supplementing milk replacer with yeast cell contents, more research needs to be done in order to evaluate how to further enhance these effects. The concentrations fed in the current study were almost double that of colostrum values however further increasing concentrations may increase the supply of nucleotides not utilized by *C. perfringens* bacteria to intestinal transporters. The method of feeding the treatment should also be evaluated whether feeding in milk replacer provides the best results. Nucleoside uptake in the intestine may be decreased in the presence of glucose (Theisinger et al., 2002) however this is the least labor intensive method available to producers. If in fact a different method of supplementation, such as bolusing before or after milk feeding, resulted in lower incidence of scouring, the improvement in calf health may be worth the extra labor cost.

CONCLUSIONS

Neonatal calf diarrhea is a persistent problem on dairy farms. Research with other species has shown that dietary supplementation with nucleotides improves intestinal morphology and decreases diarrhea. In the current study, calves supplemented with purified nucleotides had higher dehydration, lower beneficial bacteria concentrations in

feces and similar xylose absorption to control calves. The nucleoside transporter (N1) expression was shown to perhaps be a means to monitor intestinal activity and that the feeding of dietary nucleotides up-regulates transporter expression in calf intestines. Calves supplemented with yeast cell contents had increased intestinal morphology and a more beneficial intestinal environment due to higher concentrations of *L. acidophilus* and *Bifidobacteria*.

Further evaluation of supplementation with yeast cell contents may lead to better calf health by improving intestinal morphology and function. Results in other species provide positive results in decreasing diarrhea and, from the current study results, yeast cell contents may also provide the same benefit to dairy calves.

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Figure 5-1. Example of the junction of the crypt and villus and how villi heights and crypt depths were measured. Black lines indicate the crypt/villus junction, dashed line indicates villus height and dotted line indicates crypt depth.

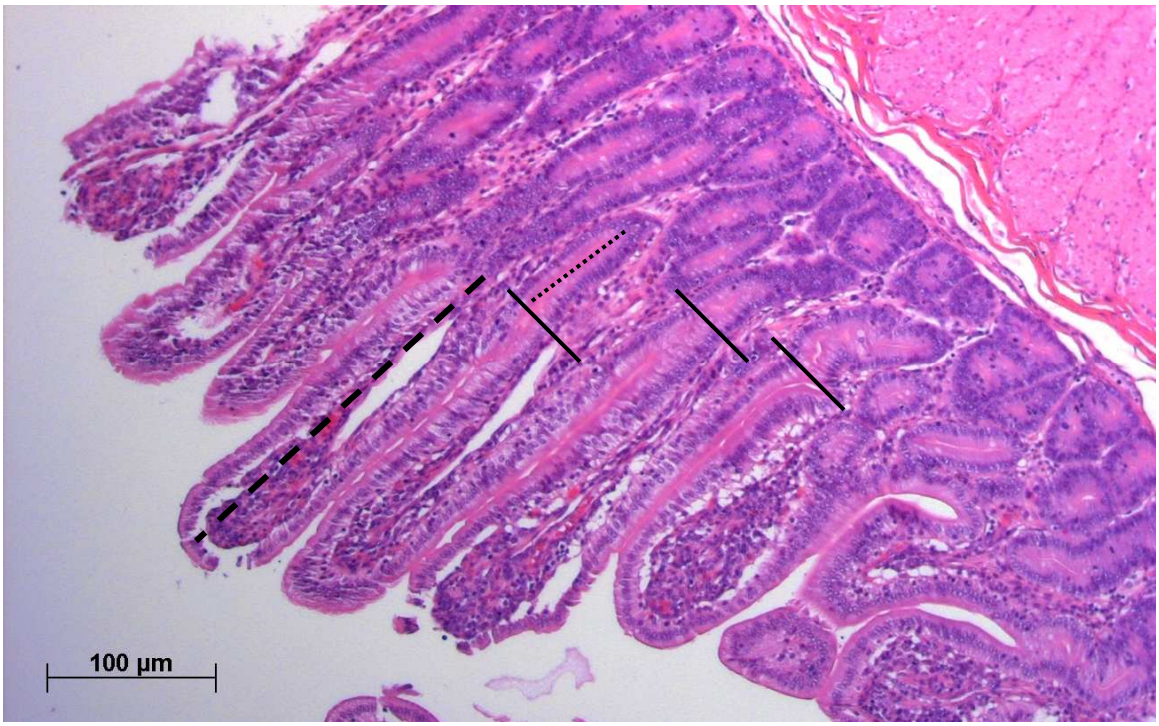

Table 5-1. Nutrient composition of calf starter and milk replacer used.

Item	Calf starter	SEM	Milk replacer	SEM
Crude Protein, %	20.1	2.01	24.11	0.06
Fat, % .	.		16.47	1.11
Acid Detergent Fiber, %	8.15	0.15	.	.
Neutral Detergent Fiber, %	15.15	0.98	.	.
Ash, %	7.6	0.82	7.51	0.05
Calcium, %	0.98	0.05	0.72	0.01
Phosphorus, %	0.61	0.04	0.65	0.03
Magnesium, %	0.45	0.02	.	.
Potassium, %	1.18	0.05	.	.
Sodium, %	0.24	0.05	0.67	0.06
Manganese, ppm	186.50	18.90	.	.
Zinc, ppm	84.15	13.25	.	.
Copper, ppm	54.00	3.2	.	.
Vitamin A, IU/kg[2]	6,600	.	35,140	198
Vitamin D, IU/kg[2]	2,200	.	7,780	311
Vitamin E, IU/kg[2]	22	.	151	0

[1]All values are expressed on a DM basis.
[2]Starter values based on manufacturer's tag.

Table 5-2. Least squares means of initial and final growth measurements and feed intake for calves on C, N and P treatments

Treatments	C	N	S	
Variable				SEM
Weight, kg				
Initial	38.31[a]	41.41[b]	42.09[b]	1.12
Final	57.16	58.09	57.87	1.35
ADG, kg/d	0.45	0.39	0.38	0.01
Heart girth, cm				
Initial	78.42	80.18	79.98	0.86
Final	88.65	89.64	89.71	0.71
ADG, cm/d	0.24	0.23	0.23	0.02
Withers height, cm				
Initial	74.79	75.68	75.83	0.67
Final	79.64	80.38	81.22	0.48
ADG, cm/d	0.12	0.11	0.13	0.01
Hip height, cm				
Initial	78.48	79.58	80.36	0.71
Final	85.06	85.51	84.12	0.97
ADG, kg/d	0.16	0.14	0.09	0.03
Starter intake, DM gm/d				
Pre-weaning	57.41	81.18	58.89	17.32
Post-weaning	803.63	930.94	902.35	89.56
Milk replacer intake, kg/d				
Pre-weaning	4.05	3.96	4.02	0.14
FE[1] (pre-weaning)	0.63	0.67	0.59	0.05
FE[2] (post-weaning)	0.246	0.263	0.239	0.04

[1]FE=Feed efficiency expressed as kg starter and milk replacer intake: kg of gain.
[2]FE=Feed efficiency expressed as kg starter intake and milk replacer: kg of gain.
[a,b]Different postscripts indicate differences ($P < 0.05$) between treatments.

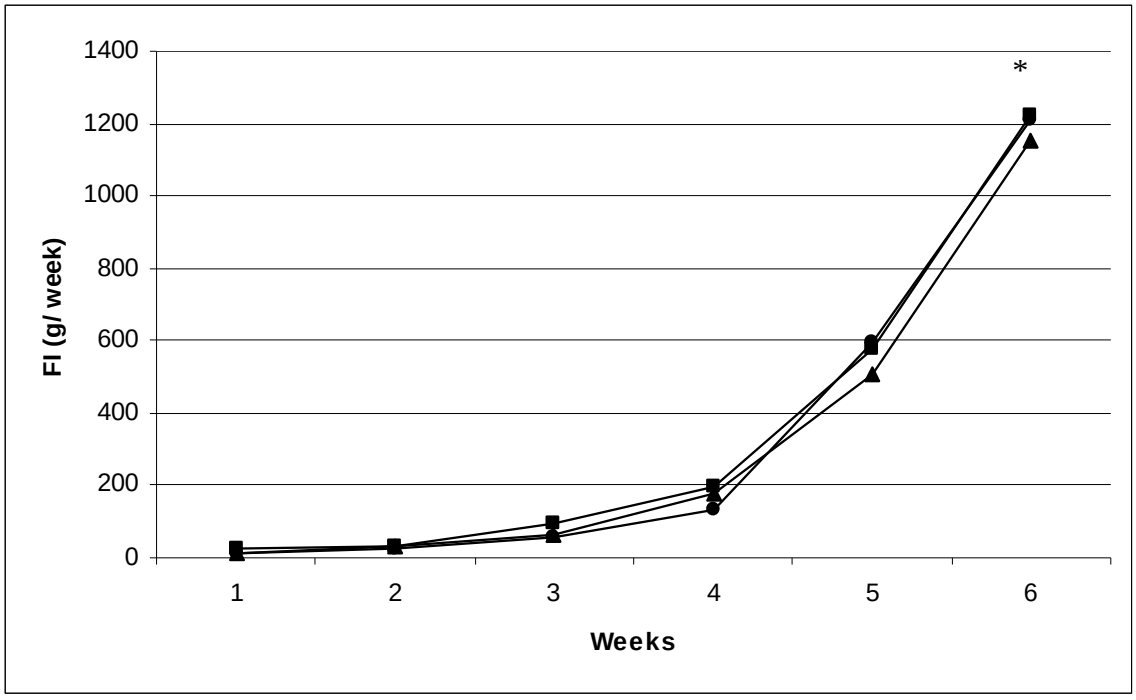

Figure 5-2. Least squares means of weekly calf starter intake in grams over weeks for treatments C (▲), N (■) and S (●). *indicates a significant treatment by week interaction where C consumed less starter than N.

Table 5-3. Least squares means of fecal bacteria counts of Clostridium perfringens, Lactobacillus sp. and Bifidobacteria sp. ($1x10^7$ cfu/ml) for calves on C, N and S treatments.

Treatments	C	N	S	SEM
($1x10^7$ cfu/ml)				
Cl. perfringens				
Week 0	0.57	3.02	1.64	1.23
Week 1	0.34	2.19	2.27	1.38
Week 2	4.35	2.61	4.40	1.93
Week 3	1.01	3.91	0.00	1.54
Week 4	0.04	0.07	0.01	0.05
Week 5	0.05	0.002	0.01	0.03
Lactobacillus				
Week 0	30.95	114.00	281.05	140.24
Week 1	95.03	64.36	100.18	67.14
Week 2	71.68	39.69	89.86	38.35
Week 3	116.75	112.10	147.11	51.43
Week 4	62.49	163.48	53.09	77.84
Week 5	5.99	6.64	2.71	3.05
Bifidobacteria				
Week 0	56.09	152.04	117.87	48.74
Week 1	149.74	95.62	258.32	77.75
Week 2	116.08	62.12	194.78	44.94
Week 3	274.04	128.05	136.63	83.90
Week 4	69.30	160.57	69.01	39.71
Week 5	22.13	15.73	6.18	13.53

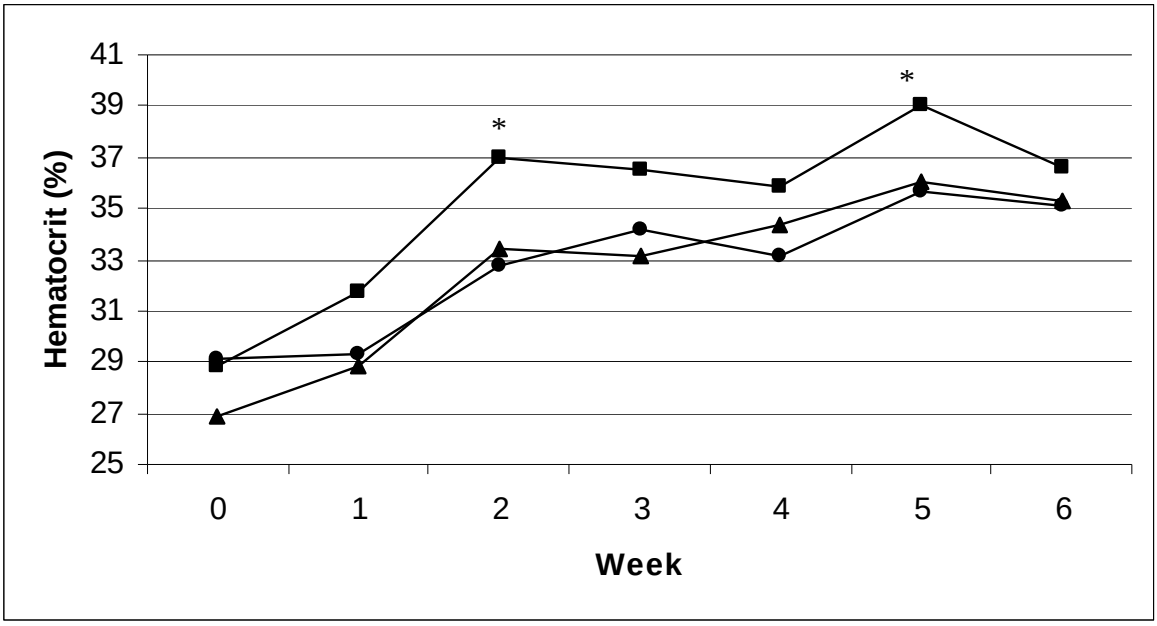

Figure 5-3. Least squares means of hematocrits (%) over time for C (▲), N (■) and S (●). * indicates significance at $P < 0.05$.

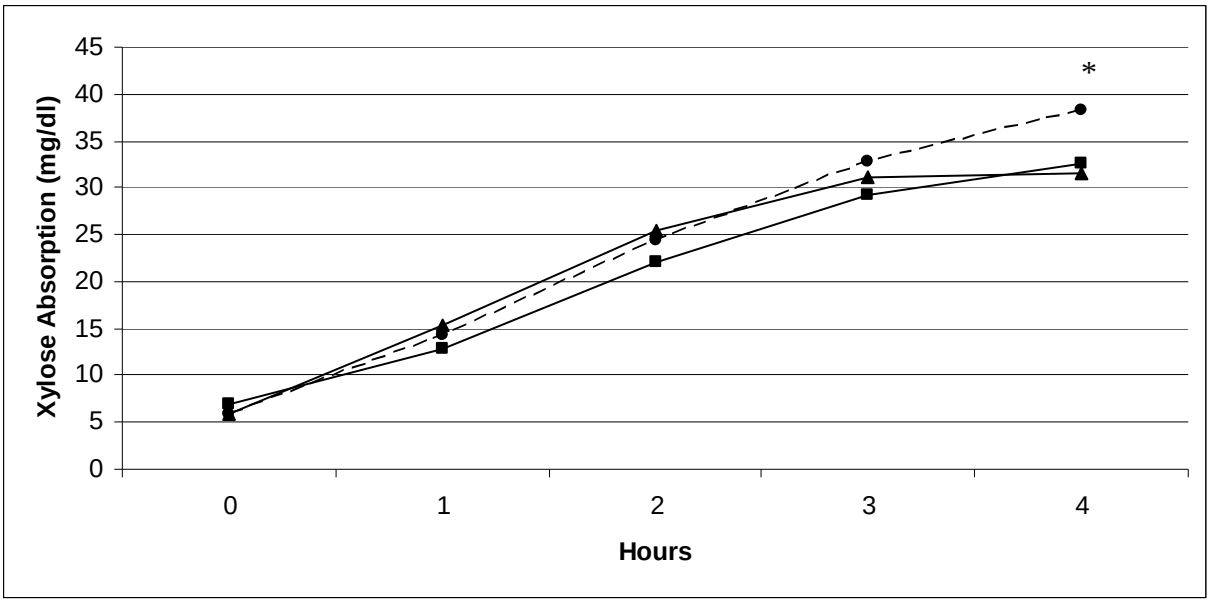

Figure 5-4. Least squares means of xylose concentrations (mg/dl) over time for treatments C (▲), N (■) and S (●). * indicates a trend between the C and S treatments ($P < 0.13$).

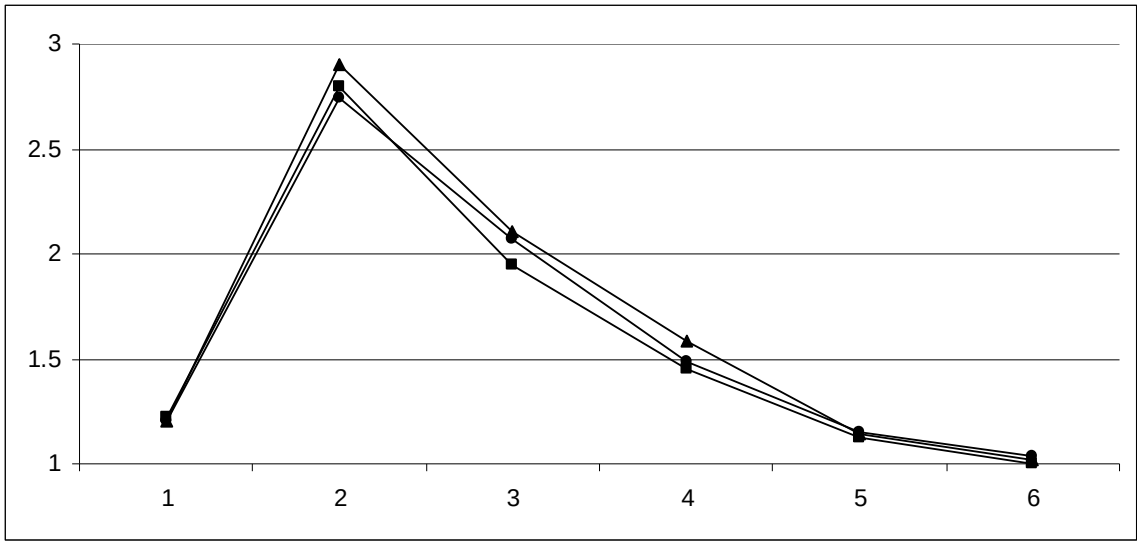

Figure 5-5. Least squares means of weekly fecal scours over time for treatments C (▲), N (■) and S (●).

Table 5-4. Least squares means of blood measurements (mg/dl) and health scores (1-5) for calves on C, N and S treatments.

Treatments	C	N	S	SEM
Variable				
Glucose, mg/dl	227.45	214.75	235.32	13.74
BUN, mg/dl	10.18	8.65	9.02	0.84
Creatinine, mg/dl	7.14	7.11	7.15	0.24
BUN:Creatinine,mg/mg	1.77	1.87	1.75	0.93
IgG, mg/ml				
Week 0[1]	19.29	17.37	14.33	2.13
Week 1	14.48	12.41	12.64	1.48
Week 2	10.51	11.29	8.20	1.11
Week 3	10.14	9.72	8.63	0.83
Respiratory[2]	1.03	1.01	1.02	0.01
General	1.03	1.04	1.05	0.02
MR refusal (ml)[3]	968.4	1114.0	1279.3	141.73
Days refused[4]	23	51	25	--
Calves refused[5]	12	13	13	--
Oral rehydration treatment				
Days treated	68	52	66	--
Calves treated	13	12	14	--
Antibiotic treatment				
Days treated	17	13	24	--
Calves treated	8	7	11	--
Calves that died	3	3	4	--

[1]Samples for week 0 IgG blood were taken at 48 h after birth. All other weeks were taken weekly from day of birth.
[2]Scored on a scale of 1 to 5 as described by Lesmeister et al. (2004). Lower numbers indicate more normal calf health.
[3]Average amount of milk replacer refused in ml per treatment.
[4]Number of days each treatment refused to drink milk replacer and needed to be fed by bottle or forced.
[5]Number of calves in each treatment that refused to drink milk replacer and needed to be fed by bottle or force fed.

Table 5-5. Least squares means of intestinal measurements and weights for calves on C, N and S treatments.

Treatments	C	N	S	SEM
Variable				
Spleen, kg	0.11	0.12	0.13	0.02
Liver, kg	1.33	1.13	1.19	0.09
Small intestine				
Weight, kg	1.00	1.18	1.33	0.14
Length, m	1.27	1.44	1.42	0.86
Large intestine				
Weight, kg	0.34	0.33	0.39	0.07
Length, m	0.24	0.21	0.23	0.02

Table 5-6. Least squares means of intestinal measurements for calves on C, N and S treatments and duodenum (D), jejunum (J) and ileum (I) areas of the intestine.

Treatments			Intestinal Area				
C	N	S	D	J	I	SEM	
Maltase							
U/mg protein[1]	0.157	0.106	0.104	0.036[a]	0.162[b]	0.169[b]	0.028
Lactase							
U/mg protein	0.296	0.202	0.249	0.069	0.341	0.336	0.088
Alkaline Phosphatase							
U/mg protein	14.24[x]	11.52[x]	10.14[y]	29.93[a]	5.76[b]	0.22[b]	4.19
Protein							
mg/g wet tissue	50.49	57.41	50.04	55.64	52.63	49.68	1.07
DNA							
mg/g wet tissue	2.54[x]	3.17[y]	3.37[yz]	3.74[a]	2.79[b]	2.55[b]	0.29
DNA:Protein ratio							
mg/mg	0.43	0.45	0.51	0.52	0.45	0.42	0.06
Relative expression[2]	1.0	3.5	7	1.0	2.8	5.2	
Villus heights,um	228.05	202.75	231.65	245.98	229.45	187.03	20.54
Crypt depths,um	122.38	130.99	121.59	130.09	122.87	121.99	7.91
Duodenum (um)							
Villus heights	244.63	211.96	281.34	--	--	--	37.55
Crypt depths	137.33	126.49	126.44	--	--	--	14.77
Villus:crypt	1.78	1.68	2.23	--	--	--	--
Jejunum (um)							
Villus heights	287.32	182.95	218.08	--	--	--	37.63
Crypt depths	109.89	146.68	112.05	--	--	--	11.83
Villus:crypt	2.61	1.24	1.95	--	--	--	--
Ileum (um)							
Villus heights	152.21	213.35	195.53	--	--	--	32.35
Crypt depths	119.92	119.80	126.97	--	--	--	14.77
Villus:crypt	1.27	1.78	1.54	--	--	--	--

[1]U/ml = Units of activity/min/mg of protein.
[2]Relative expression = Target ΔΔC(T) value of treatments relative to control ΔΔC(T) treatment.
[a,b]Different postscripts indicate differences ($P < 0.06$) between treatments.
[x,y,z]Different postscripts indicate differences ($P < 0.15$) between treatments.

Table 5-7. Primers used for qRT-PCR of bovine Nucleoside Transproter N1

Gene	Sequence	Product Size (bp)	GenBank Accession #
Nucleoside Transporter (N1)			
Forward	TCCCTGCCTGAAACTCTG	248 bp	BC108101
Reverse	ACCCTGGTTCTGTTCTGATGAC		
Cyclophilin			
Forward	GCATACAGGTCCTGGCAT	236 bp	AY247029
Reverse	TGTCCACAGTCAGCAATGGT		

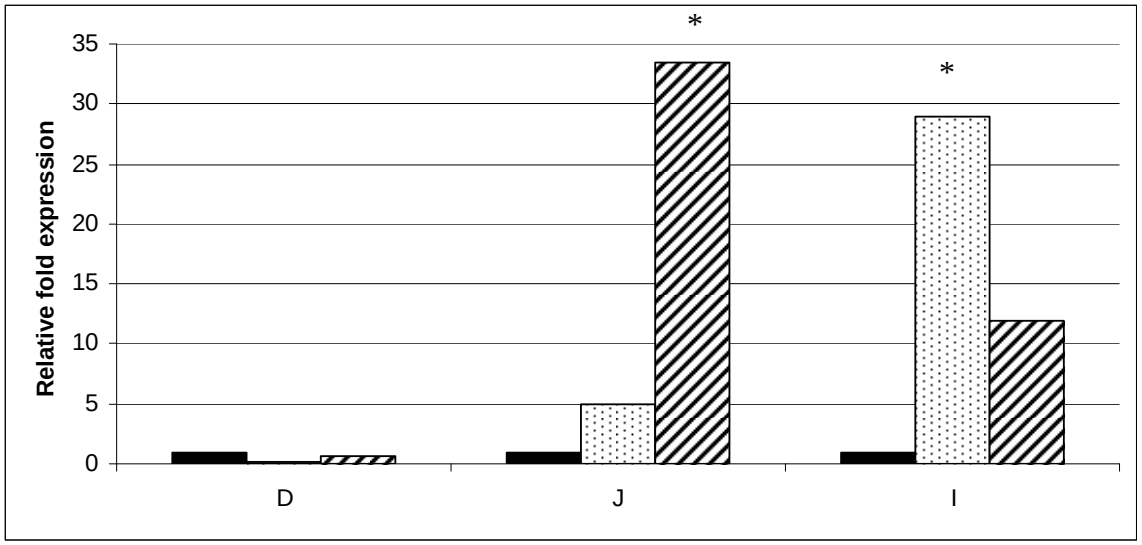

Figure 5-6. Relative fold expression of mRNA for treatments compared to control for the nucleoside transporter (GenBank number: BC108101) by area of intestine. Solid bars represent treatment C set at 1.0; bars with dots represent treatment N; diagonal stripes represent treatment P. * indicates significance at $P < 0.05$.

Appendix

Table A-1. Gel electrophoresis results of product obtained from semi-quantitative RT-PCR. Columns from left to right represent calf 1976 jejunum, 1978 duodenum, 1981 jejunum, 1983 ileum and 1984 ileum. Last column on the right is the molecular weight standard.

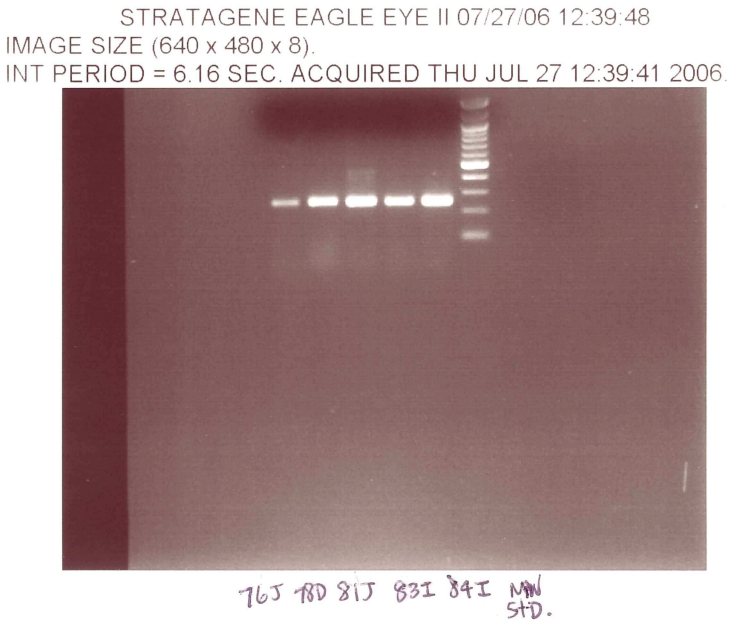

Table A-2. Methodology used for determination of water-soluble vitamins modified from Albala-Hurtado et al. (1997). Analyses were conducted at the Proteomics Facility of the Hucks Institute of Life Sciences at the Pennsylvania State University.

Water-soluble vitamins, including nicotinamide, pyridoxal, pyridoxine, pyridoxamine, folic acid, riboflavin, cyanocobalamin and thiamin, were analyzed using the HPLC method as described by Albala-Hurtado et al. (1997). The samples were allowed to thaw on the bench top until they reached room temperature and shaken vigorously. A 667 uL aliquot of each colostrum sample was pipetted into a 2 mL polypropylene centrifuge tube. The calibration spikes were done at this point, to levels of 1 ug/mL, 2 ug/mL, and 3 ug/mL each of the seven vitamins into four of the samples, chosen randomly.

Next, 333 uL of 50% trifluoroacetic acid was added to each sample. The samples were sonicated for approximately 5 minutes. The samples were then placed on a shaker/vortexer and shaken for 10 minutes after which they were centrifuged at 15000 *x* g for 10 minutes. A 100 uL aliquot of the aqueous layer was pipetted from each centrifuge tube into a 2 mL HPLC vial with a 250 uL polypropylene insert. The sample extracts were then spiked with caffeine as an internal standard at a level of 1 ug/mL. The samples were then analyzed by gradient HPLC on a Waters 2695 HPLC system using a MacMod ACE C18 column (150 x 2.1 mm, 5 um) and a 0.05% TFA in water : 0.05% TFA in acetonitrile (A:B) mobile phase at a flow rate of 0.2 mL/min.

The gradient followed for time 0 was 100% A and 0% B; for time 5 was 97% A and 3% B; for time 6 was 80% A and 20% B; for time 10 was 60% A and 40% B; for time 11 and 16 was 30% A and 70% B. Sample injections used were 5 uL and the eluent was directed into a Waters/Micromass LCT Premier (Milford, MA) electrospray ionization time-of-flight mass spectrometer.

Table A-3. Dots indicate farms that were surveyed and colostrum samples taken.

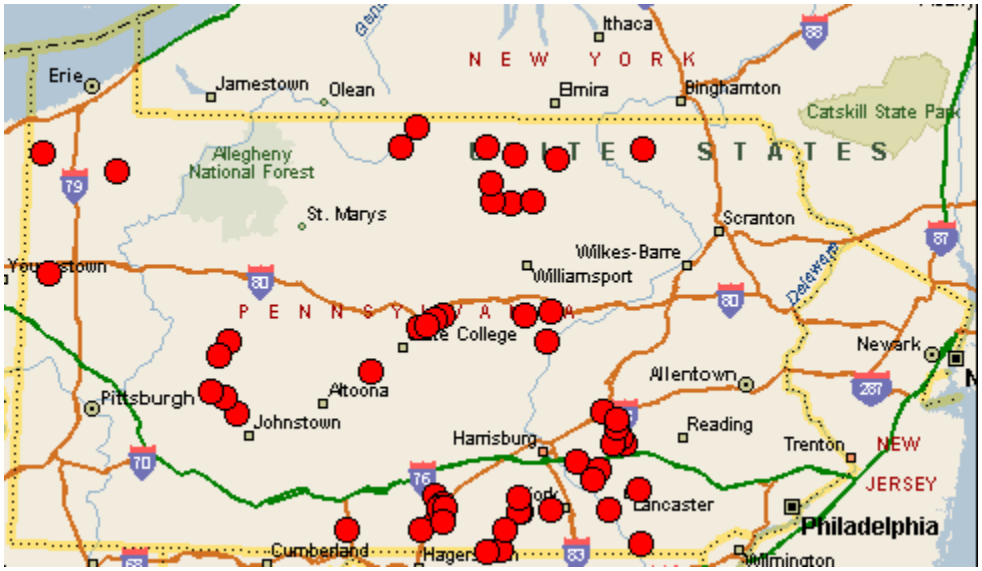

Table A-4. Colostrum survey and the codes used for answers.

Question Number	Answer	Code
1	What is SCC for last month?	
1	<200,000	1
1	>200,000	2
2	What is the herd size?	
2	Under 50	1
2	51-100	2
2	101-200	3
2	>200	4
3	What is the predominant breed (more than 90%)?	
3	Holstein	1
3	Jersey	2
3	Other	3
4	Do you add a vitamin/mineral premix to the dry cow diet?	
4	No	1
4	Yes--What does it contain/what is the brand name?	2
5	Are your cows vaccinated by you or a veterinarian?	
5	You	1
5	Veterinarian--can we contact them if we have additional questions?	2
5	None given	3
6	What vaccinations are given to dry cows?	
6	J-5	1
6	BVD	2
6	Scourguard	3
6	J-5 and BVD	4
6	J-5 and Scourguard	5
6	J-5, BVD, Scourguard	6
6	J-5, Scourguard, other	7
6	None	8
6	Other	9
7	Are cows given any vitamin injections during the dry period?	
7	No	1
7	Yes--Vitamin A	2
7	Vitamin D	3
7	Vitamin E	4
7	Selenium	5
7	Vitamin E and Selenium	6
7	Vitamins A, D, E	7
7	Other	8
7	If MuSE--how many cc's?	
8	What type of bedding are dry cows housed on in the maternity barn at calving?	
8	Straw	1
8	Hay	2
8	Shavings/sawdust	3
8	Sand	4
8	Newspaper	5
8	Pasture	6

8	None-dirt	7
8	Other	8
9	How many cows are grouped in maternity pens?	
9	Individually	1
9	Groups of 2-4	2
9	Groups of 5 or more	3
9	No maternity pen	4
10	Is anything done if a cow begins to leak milk?	
10	Nothing	1
10	Pre-milking	2
11	Are prefresh heifers housed in the same maternity pens near or with older cows?	
11	Yes	1
11	No	2
12	How long are heifers housed with older cows before calving?	
12	>2 month	1
12	1-2 months	2
12	<1 month	3
12	Not housed together	4
13	On average how long after calving, from 5 AM to 8 PM, is a cow milked for first colostrum?	
13	Immediately, <1 hour	1
13	1-2 hours	2
13	2-6 hours	3
13	>6 hours	4
14	Is this longer at night (between 8 PM and 5 AM)?	
14	Yes	1
14	No	2
15	How do you collect colostrum after calving?	
15	Wash udder before milking	1
15	Teat dip before milking	2
15	Hand milk	3
15	Machine milk	4
15	Teat dip after milking	5
15	Dip before, machine milk, dip after	6
15	Wash udder, pre,post dip and machine milk	7
15	Machine milk, dip after	8
16	What do you collect colostrum into?	
16	Open bucket	1
16	Machine milk	2
17	If colostrum is stored, what kind of container is it stored in?	
17	Open top container	1
17	Closed container	2
17	Not stored	3
18	How is colostrum stored after milking?	
18	Frozen	1
18	Refrigerator	2
18	Stored without refrigeration	3
18	Not stored	4
19	If colostrum is stored--how long is it before it is cooled?	
19	Less than 2 hours	1
19	More than 2 hours	2

19	Not stored	3
20	Are calves fed colostrum from their own dam?	
20	Yes	1
20	No	2
21	Is colostrum used from first calf heifers?	
21	Yes	1
21	No--is there a source of stored colostrum (fresh or frozen)?	
21	Yes	2
21	No	3
22	Is first milking colostrum pooled?	
22	Yes	1
22	No	2
23	Is second milking colostrum pooled?	
23	Yes	1
23	No	2
24	Is a colostrum supplement used in addition to colostrum?	
24	Yes	1
24	No	2
25	How much colostrum do heifer calves receive for the first feeding?	
25	2 quarts or less	1
25	2-4 quarts	2
25	4 quarts or more	3
26	How much total colostrum do heifer calves receive (2 feedings)?	
26	2 quarts or less	1
26	2-4 quarts	2
26	4-8 quarts	3
26	More than 8 quarts	4
26	No second feeding	5
27	How is colostrum fed to calves?	
27	Allowed to nurse off the dam	1
27	Hand-fed from bucket or bottle	2
27	Hand-fed using esophageal feeder	3
28	When are heifer calves fed colostrum?	
28	Within the first two hours	1
28	Within 2-6 hours	2
28	After 6 hours	3
29	When are heifer calves fed the second feeding of colostrum?	
29	6-8 hours later	1
29	8-12 hours later	2
29	12-24 hours later	3
29	Not fed second feeding	4
30	In the last year, have you tested for ...?	
30	Johne's	1
30	BVD	2
30	Leukosis	3
30	Johne's and BVD	4
30	Johne's and Leukosis	5
30	Johne's, BVD and Leukosis	6
30	None	7
31	Would you use colostrum from a cow that is positive for Johne's, BVD or leukosis?	

31	Yes	1
31	No	2
32	Do you use a colostrometer?	
32	Yes	1
32	No	2
33	Is a pasteurizer used on the farm?	
33	Yes	1
33	No	2

VITA

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EDUCATION

2003 – Current	Doctor of Philosophy	***Pennsylvania State University***
2000 - 2003	Master of Science	***Iowa State University***
1996-2000	Bachelor of Science	***University of California, Davis***

RESEARCH EXPERIENCE

2003- Current — **Graduate Research Assistant**

- *The Pennsylvania State University*
- Thesis: Nucleotide supplementation affects intestinal morphology in neonatal calves

Additional studies:

- Composition and management of colostrum in Pennsylvania dairy herds
- Differences in composition of heifer and cow colostrum
- Rumen development and feeding practices in dairy calves
- Effects of fat levels of milk replacer on feed intake and health in calves

2000-2003 — **Graduate Research Assistant**

- *Iowa State University*
  - Thesis: Effects of a novel electrolyte solution with added antioxidants on small intestinal absorption
  - Effects of ruminal infusion of electrolyte solutions on calf performance parameters

2000-2002 — **Assistant manager at Animal Facilities Unit at APC, Inc.**

- *American Protein Corporation, Inc.*
  - Assisted in treatment, feeding, and care of Holstein calves and poultry
  - Managed 120 calves/trial and 400 chickens/trial
  - Responsible for data collection and recording
  - Supervision and management of 8-10 personnel