12-2010

BOVINE COLOSTRUM

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A Thesis
Presented to
the Graduate School of
Clemson University

In Partial Fulfillment
of the Requirements for the Degree
Master of Science
Microbiology

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by
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December 2010

Accepted by:
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ABSTRACT

Colostrum is the first milk produced by mammals within a 24 to 72 hour period after parturition. Bovine colostrum is sold commercially as a nutraceutical product and its manufacturers purport numerous health benefits of the product for treating gastrointestinal disorders, respiratory tract disorders, viral and bacterial infections, and promoting tissue repair.

Four commercially available colostrum products, two whey, and one non-fat dry milk were obtained on separate days. These products were analyzed for fat, protein, ash, moisture and dry matter content. One colostrum product did not adhere to label claims in that it contained a mean of 19.1% fat whereas the label claimed the product to be fat free. One whey sample was slightly above 0.5% fat whereas the label claimed the product was fat free.

Immunoglobulin content was analyzed on the dried dairy supplement products using a single radial immunodiffusion method. Results indicated that all four colostrum products contained less immunoglobulin G (IgG) than label or corresponding company website claims.

Bacterial enumeration of dried colostrum products was extremely difficult in methodology due to inability to distinguish white bacterial colonies from white clumps of the dried dairy products. Various experimental techniques were attempted in order to overcome these hurdles; however, a successful methodology was not developed.

Bacterial identification was accomplished on isolates using 16S rRNA analysis to determine the microbial population present in the dried dairy dietary supplements.

Results indicated no lactic acid bacterial genera were identified but several species of primarily *Bacillus* and *Pseudomonas* were identified.

Studies to determine the antimicrobial activity of the dried dairy products were conducted using disk diffusion and well diffusion assays against *Escherichia coli* (ATCC 25922) and *Staphylococcus aureus* (ATCC 25909). Results indicated no antimicrobial activity from any of the dairy products.

DEDICATION

This thesis is dedicated to my family – my mom, my dad, my brother, and my grandparents. Also, I would like to dedicate my thesis to Neko, Bella, Vixen, Hitch, Noki, Ashes, and Precious for their hugs, kisses, and unconditional love.

"If you look deeply into the palm of your hand, you will see your parents and all generations of your ancestors. All of them are alive in this moment. Each is present in your body. You are the continuation of each of these people." --Thich Nhat Hanh

ACKNOWLEDGMENTS

I acknowledge, with much gratitude, my debt of thanks to Annel K. Greene for her advice, support, and foresight. I also would like to thank my committee members, A.B. Bodine and T.A. Hughes, for their constant guidance during my research project. I am eternally grateful to all my friends at Clemson University for being a surrogate family offering continued moral support. Most importantly, I would like to thank my family for all of their love, encouragement and patience, especially my parents who have supported me in all of my pursuits in life.

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LITERATURE REVIEW

Introduction

Colostrum is the nutrient-rich, first secretion produced by mammals within 24 to 72 hours after parturition. Colostrum contains various proteins, carbohydrates, fats, vitamins, and minerals that are essential for the diet of a developing mammalian neonate. Colostrum also includes bioactive constituents such as immunoglobulins, antimicrobial peptides, and growth factors. These bioactive components are not just restricted to colostrum, but are found in a lesser degree in milk and whey products.

Bovine colostrum is commonly used as a raw material to produce immunoglobulin-rich, bioactive commercial products which may sell for up to $250-300 per pound. Bovine colostrum contains nutrients that cannot cross the placental barrier to the calf during pregnancy and, therefore, must be delivered to the newborn following the birthing process. It is sold as a dietary supplement for therapeutic uses in adult humans due to this rich nutrient content. However, there is a lingering question as to the efficiency of absorption of colostrum in the adult human intestinal tract. Recent refereed studies suggest that colostrum and fractions thereof may be useful for treatment of a variety of health conditions, including gastrointestinal disorders, respiratory tract disorders, viral and bacterial infections, and tissue repair.

Colostrum as a Dietary Supplement: Historical Perspective

Colostrum is vital to human development through its role in infant health and nutrition. For many centuries, the benefits of colostrum were debated. In India, during the fourth century B.C., ritual practices dictated that infants were fed a mixture of honey,

clarified butter, plant juices, and gold dust for the first few days after birth instead of colostrum (Stuart-MacAdams, 1995). During the first century A.D., Soranus of Ephesus, advised parents to feed newborn children boiled honey and goat's milk as a first food rather than colostrum (Stuart-MacAdams, 1995). He believed that maternal milk was unhealthy during the first 20 days after birth, so a wet nurse was often used. However, Soranus advised that a child should only be breast fed for several months, but then the child's principal food should be the milk of other animals like camels or cows (Stuart-MacAdams, 1995).

By the end of the seventeenth century A.D and the beginning of the eighteenth century A.D., the beliefs about colostrum began to slowly change (Stuart-MacAdams, 1995). During the eighteenth century, the English physicians William Cadogen and William Moss advocated giving colostrum to an infant after birth. Cadogen and Moss noted that replacement infant foods were correlated with abdominal distress and/or gastrointestinal infections (Stuart-MacAdams, 1995). In 1892, the German scientist Paul Ehrlich confirmed that antibodies are transferred to a newborn animal by colostrum (Stuart-MacAdams, 1995). This finding established the immunological importance of colostrum in mammalian neonates.

Ayurvedic physicians and naturopathic healers have used bovine colostrum for thousands of years for medical treatment of certain skin and eye infections (Stuart-MacAdams, 1995). In Sweden and The Netherlands, a traditional pudding has been made from colostrum for centuries; this pudding is used for both celebration and purported health benefits (Stuart-MacAdams, 1995). In 1950, physician Dr. Albert Sabin

discovered that colostrum contained antibodies against polio and recommended at risk patients consume colostrum (Stuart-MacAdams, 1995).

Current research has indicated that an infant should consume colostrum for the first few days after birth in order to initiate immunological protection and establish gastrointestinal microflora balance (Xu et al., 1996). Colostrum delivers its nutrients in a very concentrated low-volume form. These nutrients are passed to the infant and provide protection against pathogens as well as assistance with development of the gastrointestinal tract (Xu et al., 1996). Since similar biologically active molecules of human colostrum also are found in bovine colostrum, scientific researchers and nutraceutical companies are studying the effect of whole and fractionated bovine colostrum on various human illnesses and for food processing industries.

Bovine Colostrum Harvesting and Processing

Bovine colostrum is sold for use as a nutraceutical product. The concept of a "nutraceutical" originated in Japan during the 1980s with the development of "physiologically functional foods" that are considered, by definition, to be "any food or ingredient that has a positive impact on an individual's health, physical performance, or state of mind, in addition to its nutritive value" (Hardy, 2000). In 1989, the term "nutraceutical" was coined by the Foundation for Innovation in Medicine in order to differentiate nutraceuticals from drugs, medicinal foods, and physiologically functional foods. The term "nutraceutical" was specifically defined as "any substance that may be considered a food or part of a food, and provides medical or health benefits, including the prevention and treatment of disease" (Hayden, 2007). Hardy (2000) further defined

“nutraceuticals” as “bioactive chemicals derived from foods but taken as supplements at much higher concentrations than diet alone could provide. They include antioxidants from fruits and berries, fatty acids found in cold-water fish, and potentially disease fighting compounds from common spices such as cinnamon and turmeric” (Hardy, 2000). The concept of a nutraceutical had to be defined in order to differentiate nutraceutical substances from medical foods in the United States. Medical foods are defined by the U.S. Food and Drug Administration (FDA) as “formulated to be consumed or administered internally under the supervision of a physician and which is intended for the specific dietary management of a disease or condition for which distinctive nutritional requirements, on the basis of recognized scientific principles, are established by medical evaluation” (Hardy, 2000). The range of consumer products sold as nutraceuticals and dietary supplements is broad and often incorrectly categorized. A dietary supplement is defined as a "product taken by mouth that contains a ‘dietary ingredient’ intended to supplement the diet.” They include “vitamins, minerals, herbs or other botanicals, amino acids, and substances such as enzymes, organ tissues, glandulars, and metabolites....." (Food and Drug Administration, 2010). Nutraceuticals were estimated to generate $22.5 billion in consumer sales in the United States in 2006, and sales have increased in recent years (Hayden, 2007).

Bovine colostrum has many purported health benefits. However, as Moore et al. (2005) reported, bovine colostrum should be harvested as soon as possible after calving to maximize colostrum quality. Approximately, 5 - 10 liters of colostrum per milking is normally obtained from a dairy cow (Moore et al., 2005). The typical dairy cow

produces more colostrum than is consumed by the calf and in commercial bovine colostrum operations, this excess colostrum can be processed for nutraceutical products (Moore et al., 2005). In addition to maintaining excellent sanitary conditions, it is essential to collect bovine colostrum within hours of birth (Moore et al., 2005). Immunoglobulin (Ig) concentrations in mammary secretions decrease with time after parturition (Moore et al., 2005). Moore et al. (2005) collected colostrum from randomly selected quarters at 2, 6, 10, and 14 hours after calving. Colostral IgG concentration from each sample was determined with radial immunodiffusion. Results revealed that IgG concentrations rapidly declined with IgG levels measuring at 113, 94, 82, and 76 g/L at 2, 6, 10, and 14 hours, respectively. Moore et al. (2005) recommended that bovine colostrum should be harvested within 6 hours of parturition to obtain a high quality product.

Once bovine colostrum is harvested, it most commonly is dehydrated by either spraying drying or freeze-drying. Spray-drying allows a liquid product to be atomized and contacted with a hot gas to instantaneously obtain a powder. In the food industry, spray-drying decreases water activity to ensure microbiological stability of products, avoid the risk of chemical and/or biological degradation, reduce the storage and transport costs, and finally obtain a product with specific properties like instantaneous solubility (Kudra et al., 2002). Chelack et al. (1993) reported that spray drying bovine colostrum is conducted at a low temperature, thereby, allowing minimal loss of immunoglobulin function and quantity (up to 94%). Although immunoglobulins activity is well conserved, a disadvantage to spray drying is a 25% loss of total solids from the bovine

colostrum (Chelack et al., 1993). The spray-drying of bovine colostrum is the same method used in the manufacture of dried milk. When whole or low fat milk is spray-dried, a mixture of lactose and milk proteins are microencapsulated by milk fat. This outer layer imparts protection against oxidation. Chelack et al. (1993) hypothesized that the butterfat in spray dried bovine colostrum undergoes a similar microencapsulation process. Commercially, spray-dry processing of colostrum is up to 2.5 times less expensive than freeze-dry processing (Chelack et al., 1993).

Chelack et al. (1993) reported the lengthy process of freeze-drying or lyophilization is the most effective method for preservation of immunoglobulin function and minimal loss of total solids. The process of freeze drying is widely used for pharmaceuticals to improve long-term storage of unstable drugs (Chelack et al., 1993). Freeze drying utilizes low temperatures, along with rapid dehydration of a liquid product. This technique allows for a reduction of protein denaturation in the product as compared to spray drying. According to Elfstrand et al. (2002), the process of freeze-drying bovine colostrum concentrate decreased the amount of native IgG_1,IgG_2 and IgA by 25% compared to colostrum whey. In this study, (Elfstrand et al., 2002), colostrum was defined as pooled product collected in three time intervals from 0 to 50 hours postpartum; colostrum whey was defined as a pooled colostrum product collected from seven time intervals from 0 and 80 hours postpartum with additional heat treatment and microfiltration prior to freeze-drying. The freeze-dried colostrum whey had decreased IgG_2 and IgA of 35–40%, while IgG_1 only was reduced by 17%. Elfstrand et al. (2002) also revealed the highly sensitive nature of IgM after freeze-drying of colostrum

concentrate and colostrum whey. After the freeze-drying processing of colostrum whey, only 2% of IgM remained, whereas IgM could not be detected in colostrum concentrate after freeze-drying (Elfstrand et al., 2002). Thus, IgM seems to be the most sensitive of the immunoglobulins during processing of bovine milk products. Elfstrand et al. (2002) also demonstrated that freeze-drying reduced the amount of the immunomodulating agent known as transforming growth factor-beta 2 (TGF-β2), and the hormone known as insulin-like growth factor 1 (IGF-1) in colostrum concentrate by 30%. A minor reduction also was observed in the freeze-dried colostrum whey (Elfstrand et al., 2002). The same researchers hypothesized the layers of fat, casein, lactose and salts play a protective role for immunoglobulins and TGF-β2 in colostrum concentrate and whey during freeze-drying. Due to the negative effects of freeze drying and spray-drying on the content of bioactive substances in colostrum, it is important to note how the bovine colostrum is processed in order to retain the activity of these influential bioactive components in milk.

Bovine Colostrum Bioactive Components and Fractions

Lactoferrin was initially isolated from cow's milk in 1939 by Sørensen and Sørensen (Conesa et al., 2010). It was considered to be a transferrin-like glycoprotein (Lőnnerdal et al., 1995). Lactoferrin is defined as an iron binding glycoprotein that consists of a single polypeptide chain with a molecular mass of 80 kDa (Lőnnerdal et al., 1995). The polypeptide structure of lactoferrin is composed of two homologous domains hypothesized to have been formed by intragenic duplication (Lőnnerdal et al., 1995). These two domains bind to one ferric cation and one carbonate anion, along with one glycosylated site where N-linked glycan residues attach (Lőnnerdal et al., 1995).

Lactoferrin can be found mostly in colostrum and milk, as well as tears, saliva, mucous, and blood (Pakkanen et al., 1997). Lactoferrin is expressed and secreted by the secondary granules of polymorphonuclear neutrophils (Pakkanen et al., 1997). Antimicrobial activity has been documented in lactoferrin inhibition of Gram-negative and Gram positive bacteria, as well as yeasts, fungi, parasites, and viruses. Additionally, multiple studies have reported that lactoferrin appears to be involved in anti-inflammatory and other immune responses. It appears that two different mechanisms involving two separate domains of the protein contribute to the antimicrobial functions of lactoferrin (van Hooijdonk et al., 2000). The first mechanism is a bacteriostatic effect related to the high iron binding affinity of the protein that deprives iron-requiring bacteria of this essential growth nutrient (van Hooijdonk et al., 2000). The second antibacterial property of lactoferrin is due to a direct bactericidal function within the protein (van Hooijdonk et al., 2000). The antimicrobial activity of lactoferrin was demonstrated against *Streptococcus mutans, Listeria monocytogenes, Shigella dysenteria, Salmonella typhimuium, Bacillus subtilis, Bacillus (Geobacillus) stearothermophilis,* and *Vibrio cholera* in various studies (Pakkanen et al., 1997).

Lassiter et al. (1987) suggested a direct bactericidal method of inhibiting growth of bacteria via interaction of lactoferrin with lipopolysaccharide (LPS) of the Gram-negative bacterial membrane of *E. coli.* However, addition of calcium (Ca^{2+}) and magnesium ions (Mg^{2+}) impaired lactoferrin inhibition of bacteria. The presence of Ca^{2+} ions also inhibited the ability of lactoferrin to increase the susceptibility of *E. coli* to the antibiotic rifampicin (Lassiter et al.,1987). Lactoferrin binds to porin molecules in the

outer membrane of *E. coli* and *Salmonella typhimurium* causing permeability changes (Lassiter et al., 1987). Lactoferrin is active at a neutral pH, and in the presence of bicarbonate ions (Pakkanen et al., 1997) which are both conditions in the lumen of the intestine; these conditions allow lactoferrin to exercise antimicrobial activity (Lassiter et al., 1987).

Lactoperoxidase is a basic glycoprotein containing a heme group with Fe^{3+} that catalyzes the oxidation of thiocyanate in the presence of hydrogen peroxide producing a toxic intermediary oxidation product (Pakkanen et al., 1997). This particular product inhibits bacterial metabolism via oxidation of essential sulfhydryl groups in proteins. This single peptide chain of about 612 amino acids contains 15 half-cysteines, 4 N-glycosylation sites, and a high affinity calcium binding site (Kussendrager et al., 2000). Commonly, lactoperoxidase is considered to be a major antibacterial enzyme in colostrum. It is known that this enzyme protects the lactating mammary gland from bacterial infections (Pakkanen et al., 1997). Both Gram positive and Gram negative microbes have been found to be susceptible to lactoperoxidase including *Pseudomonas aeruginosa, Salmonella typhimurium, Listeria monocytogenes, Streptococcus mutans,* and *Staphylococcus aureus* (Pakkanen et al., 1997). Lactoperoxidase and lactoferrin can have an additive effect on the inhibition of microbes, but they are not synergistic, particularly with *Streptococcus mutans* (Pakkanen et al., 1997).

Lysozymes are antibacterial, lytic enzymes discovered by Alexander Fleming in 1922 from his nasal mucosal passages (Pakkanen et al., 1997). Lysozymes are present in multiple mammalian bodily fluids, particularly colostrum. The lysozymes degrade the

peptidoglycan layer of the bacterial cell wall and result in the lysis of the bacterial cells. Lysozymes contain cationic and hydrophobic qualities that interact with the bacterial membranes to lyse bacterial cells (Pakkanen et al., 1997). Milk and colostrum lysozymes are bacteriocidal against numerous Gram positive and Gram negative microbes (Pakkanen et al., 1997). Yamauchi et al. (1993) reported lactoferrin synergistically enhances the antimicrobial activity of milk and colostrum lysozymes against *E. coli.*

Growth factors are present in both milk and colostrum as a mammalian cell growth stimulator. Klagsbrun and Neumann (1979) demonstrated the stimulation of mouse fibroblasts with the addition of bovine colostrum. The most prevalent growth factors in bovine colostrum are the insulin-like growth factors (IGF-1 and IGF-2). IGF-1 and IGF-2 are heat and acid-stable growth factors that consist of approximately 7.6 kDa single-chain polypeptides (Pakkanen et al., 1997). IGF-1 and IGF-2 exist in many mammalian body cells to stimulate glucose uptake, the synthesis of glycogen, protein, RNA, DNA, and lipids (Pakkanen et al., 1997). IGFs in colostrum and milk originate from blood circulation and are not due to local synthesis in the mammary tissues (Pakkanen et al., 1997). Type I and II IGF receptors have been reported to be present in bovine mammary tissue and on intestinal epithelial cells leading to many questions as to the function of IGFs in colostrum and milk. Shamay et al. (1988) demonstrated that dietary based IGF-1 from milk based products are a strong stimulator of mitogenesis and galactopoesis of bovine mammary cells. Xu et al. (1994) and Baumrucker et al. (1994) reported that dietary bovine colostrum stimulated intestinal epithelial cells in the gastrointestinal tract of newborn piglets and calves. Baumrucker et al (1992) reported

that specifically orally-administered IGF-1 promoted the growth of the small intestine of newborn pigs. Baumrucker and Blum et al. (1993) demonstrated that that IGF-1 suppressed insulin secretion and stimulated prolactin secretion. However, research on the effects of dietary IGF-2 from colostrum and milk products has not been well-described in the literature.

Transforming growth factors beta (TGF-β1 and TGF-β2) are highly pleiotrophic peptide growth factors with multiple functions. The two types of TGF- β stimulated cell growth in connective tissues, while inhibiting cell growth of lymphocytes and epithelial cells in other tissues (Pakkanen et al., 1997). The TGF- β substances are involved in embryogenesis, tissue repair, formation of bone and cartilage, and modulation of the immune system (Pakkanen et al., 1997). TGF-β2 is the predominant TGF- β found in milk-based products, and its whole amino acid sequence is identical to the sequence of TGF- β2 found in humans. The function of the two types of TGF- β from colostrum and milk products is largely unknown, but Cox and Bürk (1991) suggested that TGF- β1 and TGF- β2 may be mediators of mucosal immunity and gut epithelial differentiation in a neonate mammal. Several studies have indicated that TGF- β1 and TGF- β2 increase the production of IgG and IgA (Pakkanen et al., 1997). Additionally, McGee et al. (1991) stated that TGF- β stimulated the secretory component in rat epithelial cells, which is primarily responsible for transferring polymeric IgA into the intestinal lumen. It was suggested that IgA plays a major role in the immunological defense systems of mucous membranes in the gut against pathogenic microbes (McGee et al.., 1991); this is particularly important with TGF- β in colostrum or milk based products.

Bovine colostrum is a potent source of immunoglobulins. The absorption of immunoglobulins is essential to the development of passive immunity in calves after birth for protection against enteric and respiratory infections. Initially, calves absorb the maternal immunoglobulins that are concentrated in the colostrum. The colostral immunoglobulins are transferred from the lumen of the calf's intestine into the blood circulation (Pakkanen et al., 1997). IgG_1 is transported from the blood circulatory system in the cow by an active receptor-mediated transfer across the mammary gland epithelium in the cow. Once the IgG_1 diffuses across the vascular endothelium, the IgG_1 binds to specific IgG_1-F_c receptors on the basal membrane of the mammary secretary epithelium. The IgG_1 is then transferred through the epithelial cells that secrete the molecules into the colostrum, prior to suckling.

The total immunoglobulin concentration present in bovine colostrum is approximately a hundred fold higher than in milk. Commercially available colostrum products available for calves and humans usually contain low amounts of immunoglobulins as compared to natural colostrum (Pakkanen et al., 1997, Haines et al., 1990). In fresh colostrum, it is noted that IgG_1 is the predominant immunoglobulin while IgM, IgA, and IgG_2 are present in much lower levels (Table 1) (Mach and Pahud, 1971).

Table 1: The Concentration of Immunoglobulins in Fresh Colostrum and Normal Milk (Mach and Pahud, 1971)

Immunoglobulin	**Colostrum (gL^{-1})**	**Normal Milk (gL^{-1})**
IgG_1	52.0-87.0	0.31-0.40
IgG_2	1.6-2.1	0.03-0.08
IgM	3.7-6.1	0.03-0.06
IgA	3.2-6.2	0.04-0.06

In the newborn calf, the immunoglobulins are absorbed from the colostrum into blood circulation through a non-selective macromolecular transport system across the small intestinal epithelium. This non-selective absorption of immunoglobulins in calves only occurs for 24 to 36 hours after birth (Pakkanen et al., 1997).

Experimental and Clinical Uses of Bovine Colostrum

Throughout history, bovine colostrum was utilized to treat numerous disorders and illnesses. The beneficial and medicinal nature of bovine colostrum was well-known by ancient cultures; however, it is only until recently that scientific research has begun purporting the experimental and clinical uses of bovine colostrum and its bioactive components. In 1986, Tzipori et al. studied use of hyperimmune bovine colostrum to reduce the symptoms of a *Cryptosporidium* parasitic infection in a 3 year old, male patient with hypogammaglobulinemia (Tzipori et al., 1986). The patient was treated with an infusion of hyperimmune bovine colostrum produced against the parasite antigen. The treatment appeared to stop the diarrheal symptoms due to the cryptosporidiosis (Tzipori et al., 1986).

In 1993, Plettenberg et al. conducted a study on 25 male patients infected with human immunodeficiency virus (HIV) with chronic diarrhea to determine the efficiency of the bovine colostrum product Lactobin®. Lactobin® is a standardized concentrate produced from New Zealand bovine colostrum by Biotest Pharma GmbH in Dreieich, Germany for clinical uses (Plettenberg et al., 1993). Since cows are natural hosts of pathogenic *Cryptosporidium*, the *Cryptosporidium*-specific antibodies are naturally present in the Lactobin® preparation. Plettenberg et al. (1993) demonstrated a remission

of *Cryptosporidium*-related diarrheal symptoms in 43% of the studied HIV patients. However, no control group was included in the study.

Additionally, Jenkins et al. (1999) studied hyperimmune bovine colostrum, specific for the recombinant *Cryptosporidium parvum* antigen, in dexamethasone (DEX)-treated, immunosuppressed mice. Pre-partum cows were immunized by injecting the DNA directly into the mammary gland with recombinant plasmid DNA encoding the *Cryptosporidium parvum* CP15/60 antigen (Jenkins et al., 1999). The bovine colostrum was collected after parturition and utilized to examine the presence of passive immunity against *Cryptosporidium parvum* through oral administration in immunosuppressed adult inbred mice (Jenkins et al., 1999). Jenkins et al. (1999) demonstrated that the infected mice receiving immune colostrum had a 50% reduction in the presence of intestinal *Cryptosporidium parvum,* in comparison to immunocompromised mice receiving non-immunized, control colostrum.

In 1998, Sarker et al. further revealed the beneficial nature of hyperimmune bovine colostrum by demonstrating a decrease in diarrheal symptoms and a quicker clearance of rotavirus in infected children in comparison to a placebo group. Although bovine colostrum contains antibodies against enteric pathogens, the level of antibodies is often too low to provide treatment against certain infections (Sarkar et al., 1998). Therefore, Sarkar et al. (1998) utilized hyperimmune bovine colostrum, which contained high levels of antibodies against the four human serotypes of rotavirus. As mentioned previously, the hyperimmune bovine colostrum appeared to be an effective treatment against rotavirus in moderate to severe cases in infected children.

In addition to providing treatment for viral and parasitic infections, bovine colostrum also has been found by many scientific studies to provide protection or eradicate bacterial infections. In 1977, Corley et al. studied the effect of bovine colostrum on *Escherichia coli* O55:B5:H7 transepithelial migration in neonatal calf intestine in tissue culture. One hour after colostrum ingestion, young calves were provided with *E. coli* suspended in saline, *E. coli* suspended in bovine colostrum, or *E. coli* and bovine colostrum in saline. Intestinal tissues of the calves were then collected 24 hours after treatment (Corley et al., 1997). Corley et al. (1977) reported *E. coli* O55:B5:H7 was reduced when dispensed with bovine colostrum. Also, transepithelial migration of *E. coli* O55:B5:H7 ceased when colostrum was administered prior to *E. coli* O55:B5:H7 treatment. In addition to animal studies, human and tissue culture studies have revealed the valuable nature of bovine colostrum.

In 1998, Bitzan et al. reported bovine colostrum concentrate (100 mg/mL) caused in vitro inhibition of *Helicobacter pylori* binding to purified lipid receptors from gastric mucosal and epithelial cells. Receptors studied included the phospholipid phosphatidylethanolamine and the neutral glycolipids gangliotetraosylceramide and gangliotetrasykeramide using thin layer chromatography overlay. Bitzan et al. (1998) demonstrated the potential anti-adhesive, anti-infectivity properties of bovine colostrum for *H. pylori* with selected lipid receptors in vitro. This finding was significant since a critical point in the pathogenesis of *H. pylori* is the bacterial adherence to the gastric mucosa (Bitzan et al., 1998).

In 1999, Warny et al. revealed that bovine immunoglobulin concentrate, also known as hyperimmune colostrum, was obtained from cows that were immunized against *Clostridium difficile*. This bovine immunoglobulin concentrate (BIC) against *C. difficile* resisted digestion in the upper gastrointestinal tract in humans, as well as resisted anti-*C. difficile* toxin A binding (Warny et al., 1999). For the study, 5 grams of reconstituted BIC-*C difficile*, containing 2.1 g of bovine IgG, was administered orally to volunteers in 250 milliliters (mL) of the marker isosmotic polyethylene solution containing 421 mg of sodium bicarbonate along with a standard meal (Warny et al., 1999). After the treatment, the volunteers were required to fast for 4 hours, and then the volunteers were allowed to consume clear liquids until the end of the six hour ileostomy fluid collection period (Warny et al., 1999). The volunteers of this particular study were studied on 4 separate occasions with a 72 hour latent period. Warny et al. (1999) reported 49% of the free bovine IgG from the ileum after oral administration of BIC-C difficile, indicating that this residual anti-*C difficile* BIC may be available in the gut as a useful non-antibiotic treatment for the prevention and treatment of *C. difficile* infections.

In 2006, Xu et al. reported a method to produce bovine colostral antibodies against 17 specific enteric pathogens including 12 *E. coli*, two *Salmonella* and three *Shigella*. The study revealed that purified IgG isolated from cows immunized with multivalent vaccine consisting of whole cells of the 17 bacterial strains had a strong inhibitory effect on in vitro growth and colonization of the pathogens, as well as preventative effect on enteroinvasive *Escherichia coli*/*Salmonella typhi*-infected mice (Xu et al., 2006). Xu et al. (2006) revealed that these purified hyperimmune bovine

colostrum IgGs were effective in preventing pathogen growth in vitro by agglutinating with the bacteria and destroying the bacterial cells walls. However, Xu et al. (2006) found that IgG obtained from colostrum from non-immunized cows significantly increased the growth of the pathogens in vitro. To obtain these results, Xu et al. (2006) transferred 100 µl of each culture of the 12 *E. coli*, two *Salmonella* and three *Shigella* bacterial species suspension into 5 ml of Bacto Synthetic Broth (BSB), along with the hyperimmune colostral IgG or the normal colostral IgG. After 12 hours post-treatment, the bacterial suspensions were examined spectrophotometrically at 570 nanometers (nm); additionally, the bacterial suspensions were serially diluted and plated onto BSB agar plates to be incubated at 37ºC for 24 hours and counted for population growth (Xu et al., 2006). Along with in vitro studies, Xu et al. (2006) performed a mouse study to show the potential use of the hyperimmune colostral antibodies for treatment of enteric pathogens. Mice were infected orally once with 0.5 ml of enteroinvasive *E. coli* and *Salmonella typhi* containing 1×10^8/ml, in addition to being treated with IgG obtained from immunized or non-immunized cows for 10 days (Xu et al., 2006). On the tenth day, the mice were sacrificed and the spleen was examined for immunological factors, such as natural killer cells activity, interleukin-2, and tumor necrosis factor alpha (TNF-α) activity (Xu et al., 2006). Xu et al. (2006) reported the hyperimmune IgG prevented enteroinvasive *Escherichia coli*/*Salmonella typhi*-induced diarrhea and enhanced splenic immune responses by increasing natural killer cell activity, elevating interleukin-2 levels, and inhibiting the release of TNF-α in mice; thereby, the results indicated the potential protective effects of bovine colostral antibodies for enteric pathogens (Xu et al., 2006).

In 2003, Tawfeek et al. examined the use of bovine hyperimmune immuglobulin concentrate containing antibodies against enteropathogenic *E. coli* in preventing diarrheal symptoms in 125 infants in a randomized, double-blind, controlled field trial conducted at the Al-Sheikh Omer Maternal and Child Health Center in Baghdad, Iraq from May to September 1998 (Tawfeek et al., 2003). In the study, Tawfeek et al. (2003) found that infants receiving formula containing hyperimmune bovine colostrum for seven days had a lower incidence of diarrhea than the infants receiving a non-supplemented formula for seven days (Tawfeek et al., 2003). Tawfeek et al. (2003) findings suggested that use of infant formulas supplemented with hyperimmune bovine colostrum could provide an effective method for preventing enteropathogenic *Escherichia coli* (EPEC) infections and diarrheal symptoms.

In 2007, Cesarone et al. examined the effect of orally-consumed, non-immunized bovine colostrum (400 mg, no commercial brand indicated) in the prevention of influenza symptoms with and without influenza vaccination. During a three month observation period, 144 healthy individuals of both sexes ranging in age from 30 to 80 years were either treated with a bovine colostrum tablets alone, an influenza vaccination alone, bovine colostrum tablets plus an influenza vaccine, or no treatment. Cesarone et al. (2007) reported the non-colostrum treated groups had three times longer days of influenza symptoms than those study participants who had received colostrum prophylaxis. The researchers suggested the higher number of influenza episodes in the vaccinated group may have been induced by the vaccination itself in some individuals; however, the authors did not identify the type of vaccine used in the study (Cesarone et

al., 2007). Additionally, Cesarone et al. (2007) indicated that bovine colostrum prophylaxis may be a cost-effective, safe treatment to prevent influenza symptoms but additional evaluation studies in high-risk groups were recommended.

Microbial Aspects of Colostrum Products

Lactic acid bacteria (LAB) comprise a group of Gram positive bacteria that are linked into a single category due to similar morphological, metabolic, and physiological characteristics. Due to these traits, LAB are generally utilized for a variety of industrial food and feed fermentations. These bacteria essentially cause the rapid acidification of food substances through the production of organic acids, mainly lactic acid (Leroy et al., 2004). Additionally, the production of acetic acid, ethanol, aroma compounds, bacteriocins, exopolysaccharides, and several enzymes by LAB improve microbial safety and contribute to the organoleptic properties of the end product (Leroy et al., 2004). Research on the genetics, metabolism and application of LAB for functional properties in foods is an intensifying area of research.

LAB were initially categorized as a group based on the ability to ferment and coagulate milk (Stiles et al., 1997). Since the early 1900s, the influence of selected lactobacilli in various food fermentations has been well established. Orla-Jenson in 1919 stated that "the 'true lactic acid bacteria' form a natural group of Gram-positive, nonmotile, non-sporeforming, rod- and coccus-shaped organisms that ferment carbohydrates and higher alcohols to form chiefly lactic acid" (Stiles et al., 1997). Additionally, in the 20th century, Elie Metchnikoff at the Pasteur Institute in Paris promoted the use of lactobacilli in the human diet for bacterioprophylaxis and

bacteriotherapy, but the proposed practice failed to be recognized due to lack of scientific evidence (Stiles et al., 1997).

Currently, the category of LAB has been further studied and classified according to similar characteristics. The most common genera of food-grade LAB include *Lactobacillus, Lactococcus, Pediococcus, Leuconostoc,* and *Bifidobacterium* (Stiles et al., 1997). Phenotypic characteristics that define the category of LAB include being Gram-positive, low G+C (guanine plus cytosine) content, non-sporeforming bacteria that grow under microaerophilic to strictly anaerobic conditions and are usually catalase-negative (Stiles et al., 1997). Yet, it must be noted that LAB should not be confused with the endospore-forming lactic acid-producing bacteria which are aerobic to facultatively aerobic and are classified in the genera *Bacillus* and *Sporolactobacillus* (Stiles et al., 1997).

The classification of LAB is still fairly controversial since their broad physiological definition could comprise almost 20 genera of bacteria (Stiles et al., 1997). Classification of lactic acid bacteria is usually based on morphology, mode of glucose fermentation, growth at different temperatures, configuration of the lactic acid produced, ability to grow at high salt concentrations, and acid or alkaline tolerance (Caplice et al., 1999). Additionally, fatty acid composition and cell wall components are often used for classification purposes (Caplice et al., 1999).

Ecological niches of LAB can be in the gastrointestinal system of man and animal and also in fermented food. Due to survival in the gastrointestinal system of man and animals, LAB are of interest for their function as probiotics. LAB that serve as probiotics

are able to produce “antimicrobial substances, sugar polymers, sweeteners, aromatic compounds, useful enzymes, or nutraceuticals” (Leroy et al., 2004). The survival of LAB and their classification as a probiotic is based on their resistance to low pH and/or bile, temperature growth ranges, carbohydrate fermentation patterns, resistance to different NaCl concentrations, and resistance against antibiotics.

The 16 S RNA sequencing has improved identification for LAB. Sequencing of the 16 S rRNA has divided lactic acid bacteria into three phylogenetic groups:

1. The group *Lactobacillus delbrueckii* contains this species and other strictly homofermentative lactobacilli;
2. The group *Leuconostoc* is divided into two subgroups: one containing *Lc. paramesenteroides* and heterofermentative lactobacilli; the other containing *Leuconostoc sensu stricto*, to which *Oenococcus oeni* belongs;
3. The group *Lactobacillus casei - Pediococcus* is a more heterogeneous group since it comprises strictly and facultatively heterofermentative species and strictly homofermentative species (Rib´ereau-Gayon et al., 2006).

Grouping by means of 16 S rRNA sequences is based on phylogenetic relationships between bacteria. Analyzing and classifying the conserved 16 S rRNA of LAB does not always follow the grouping pattern revealed by using phenotypes, such as morphology and physiology. Therefore, physiological and biochemical criteria remain useful, but the findings and relationships discovered by molecular taxonomy are significant and are more conclusive since this reveals the genetic evolution of LAB (Rib´ereau-Gayon et al., 2006).

The two main sugar fermentation pathways can be distinguished within the classification of lactic acid bacteria: homolactic fermentation (homofermentative) and heterolactic fermentation (heterofermentative). Homofermentative bacteria such as *Pediococcus, Streptococcus, Lactococcus,* and some lactobacilli produce lactic acid as the sole end product of glucose fermentation (Rib´ereau-Gayon et al., 2006). Under altered growth conditions when pentose is the initial substrate, homofermentative bacteria use the Embden- Meyerhof- Parnas or Glycolysis Pathway to generate two moles of lactate per mole of glucose and derive twice as much energy per mole of energy as heterofermentative bacteria (Caplice et al., 1999). Heterofermentative bacteria such as *Weisella* and *Leuconostoc* (Rib´ereau-Gayon et al., 2006), and some lactobacilli produce equimolar amounts of lactate, carbon dioxide, and ethanol from glucose via the hexose monophosphate or pentose pathway (Caplice et al., 1999). The metabolism of the dissacharide lactose is of primary importance for LAB used in dairy fermentations (Caplice et al., 1999). Lactose may enter the cell by a lactose carrier such as lactose permease. Then, the sugar lactose is cleaved into glucose and galactose via a phophoenolpyruvate-dependent phosphotransferase (PTS). Next, the glucose and galactose are cleaved into glucose and galactose-6-phosphate via the tagatose-6-phosphate pathway (Caplice et al., 1999). The lactic acid produced may be L(+) or , less frequently, D(-), or a mixture of both. It should be noted that D (-) lactic acid is not metabolized by humans (Caplice et al., 1999). End products of these fermentation processes can be altered by stress conditions. These alterations can be attributed to an

altered pyruvate metabolism or the use of external electron acceptors such as oxygen. (Salminen et al., 2004).

Essentially, LAB fermentation pathways are started by creating a proton motive force by utilizing H^+-ATPases located in the cell membrane at the expense of ATP to transport metabolites and ions into the bacterial cell. Extrusion of end-products and transport of certain compounds outside of the bacterial cell may contribute to the proton-motive force, thus not utilizing ATP. Proton motive force-dependent permease systems or phosphoenolpyruvate: sugar phosphotransferase systems primarily modulate sugar transport. Therefore, sugar transport is a coordinated pathway to efficiently bring hexoses and pentoses into the bacterial cell (Salminen et al., 2004).

In addition to carbohydrate fermentation, citrate metabolism is important among *L. lactis* subsp. *lactis* (*biovar diacetylatic*) and *Lc. mesenteriodes* subsp. *cremoris* strains used in the dairy industry, as it results in excess pyruvate in the cell. The pyruvate may be converted via alpha-acetolactone to diacetyl, an important flavor and aroma component of butter and some other fermented dairy products. Moreover, carbon dioxide can be produced due to citrate metabolism, and this gas production can contribute to the formation of "eyes" or holes in Gouda, Danbo and other cheeses (Quintans et al., 2008).

Also, the proteolytic system of lactococcus has been recently examined due to its pivotal role in allowing growth in milk and the development of flavor and texture in cheese (Quintans et al., 2008). With the proteolytic system, casein is degraded by serine proteinase (PrtP) located in the bacterial membrane. The degradation of casein results in

small oligopeptides being transported into the cells via an oligopeptide transport system (Opp), where they are further processed by a variety of intracellular peptidases. Most commercial starter cultures promote proteolysis in cheese and can aid in the development of a consistent cheese flavor (Quintans et al., 2008).

In early 1900s, the interactions of LAB in foods received attention of scientists and resulted in the significant contribution by Pasteur on lactic acid fermentation in 1857, followed by the first isolation of a pure bacterial culture, *Bacterium luctis*, by Lister in 1873 (Stiles et al., 1997). In 1890, Weigrnann in Kiel, Germany and Starch in Copenhagen, Denmark began using starter cultures for cheese and sour milk production (Stiles et al., 1997). This finding opened the door for industrialization of food fermentations utilizing bacteria. However, the association of LAB with food-making dates back several thousand years. Archeological excavations in Switzerland indicated that sourdough bread was made over 5000 years ago. Biblical references mention leavened breads, and ancient texts from around 3200B.C. describe fermented dairy products such as cheese, yogurt and butter in use in the area of modern-day Iraq (Stiles et al., 1997). Additionally, Babylonians utilized fermentation to brew beer which was exported to Egypt around 3000 B.C. (Stiles et al., 1997).

Although LAB are usually involved in a large number of food fermentations, they also are closely associated with the human environment. Recently, claims that LAB are important in human and animal health are receiving renewed attention as health promoting, probiotic agents, and especially strains which are purported to have a

stabilizing or favorable influence on the gastrointestinal tract due to the ability of LAB to survive the gastrointestinal tract (Stiles et al., 1997).

The production of inhibitory substances, like bacteriocins, by LAB is an area of increasing interest by researchers and industry members. Strains, such as *Lc. Luctis*, produce a wide range of bacteriocins such as the antibiotic nisin. Nisin is a broad spectrum bacteriocin that is active against Gram positive bacteria, including *Clostridium botulinum* and its spores (Stiles et al., 1997).

The lactobacilli are strictly fermentative and have complex nutritional requirements. They are able to inhabit various habitats. Lactobacilli are aciduric or acidophilic. *Lactobacillus acidophilus* is considered an important representative of probiotic bacteria. In 1982, Kleeman and Klaenhammer studied the bacterial strain *Lb. acidophilus* BG2 F04 and re-identified the strain as *Lb. johnsonii* (Stiles et al., 1997). This strain adheres to human intestinal cells in vitro (Stiles et al., 1997). Currently, *Lb. johnsonii i*s utilized in yogurts and supports the claims on healthfulness due to population competition (Stiles et al., 1997). This species and other *Lb. acidophilus* strains are currently being studied for their probiotic properties such as microbial antagonism, survival through the stomach passage, growth and metabolic behavior in the small and large intestine, and attachment to the epithelial cells of the gut (Stiles et al., 1997). Previous studies have indicated beneficial health consequences due to LAB and their cellular products. In the study of colostrum, it is unknown if LAB influence health consequences of consuming the nutraceutical or vice versa.

In addition to the lactic acid bacteria, members of the *Bacillus*, *Pseudomonas*, *Kocuria*, *Microbacterium*, and *Enterococcus* genera can be present in dried dairy dietary supplements. *Bacillus licheniformis* is a common industrial, environmental, and dairy contaminant. *B. licheniformis* is closely related to *B. subtilis*, and has been associated with a range of conditions such as septicemia, peritonitis, ophthalmitis, and food poisoning in humans, as well as with bovine toxemia, mastitis, and abortions (Salkinoja-Salonen et al., 1999; Blue et al., 1995; Blowey and Edmondson, 2010). Conditions and food poisoning cases caused by *B. licheniformis* are typically caused by bacterial levels of 10^5 to 10^9 cfu/g (Lund et al., 2000). However, the toxigenic *Bacillus cereus* is responsible for more food poisoning incidents than *Bacillus licheniformis.* However, the involvement of toxins produced by *B. licheniformis* has not yet been demonstrated (Salkinoja-Salonen et al., 1999). Although *B. licheniformis* can be classified as pathogenic, this bacterium is often modified for enzyme production to digest bird feathers or laundry detergent proteases among other commercial applications (Salkinoja-Salonen et al., 1999; de Boer et al., 1994).

Bacillus subtilis is a bacterium commonly found in the environment. This bacterium is not often associated with food-borne illness. However, if food does become contaminated, *B. subtilis* spores can survive the extreme heat during cooking. Additionally, *B. subtilis* can uptake external environmental DNA by recombination and activate sigma factor B, which allows the bacteria to adapt to stressful and harsh conditions (Bandow et al., 2002).

In comparison to the well-understood *B. subtilis* and *B. licheniformis*, *Bacillus massiliensis* was isolated in 2004 from human cerebrospinal fluid. Based on comparative analysis of 16S rRNA gene sequences and phenotypic characteristics, the novel isolate has only been described as being related to the *Bacillus sphaericus*-like group. The isolate is closely related to *Bacillus odysseyi* and *Bacillus silvestris* (Glazunova, et al., 2006).

Bacillus pumilus is the predominant species of the environmentally common *Bacillus* species. This bacterial species is highly resistant to extreme environmental conditions such as low or no nutrient availability, desiccation, irradiation, peroxide and other chemical disinfections. *B. pumilus* is of interest commercially since this bacterial species produces anti-fungal and anti-bacterial compounds (Gioia et al., 2007; Parvathi et al., 2009).

Bacillus badius have been isolated from many environmental and food sources such as feces, dust, marine sources, and antacids (Saghafi and Appleman, 1952; Jutur and Reddy, 2006). Particularly, *B. badius* has been isolated from the intestinal tract of children administered a diet of milk (Batchelor, 1918). *B. badius* colonies characteristically form unique rhizoid outgrowths (Saghafi and Appleman, 1952; Jutur and Reddy, 2006).

Pseudomonas fluorescens is the most common psychotrophic bacteria isolated from the dairy environment. *P. fluorescens* produces heat-stable lipases, proteases, and lecithinases that survive thermal processing techniques (Sillankorva et al., 2008). Commonly, this bacterium can easily form biofilms, thereby, *P. fluroescens* is used as a

model organism for biofilm studies. *Listeria monocytogenes* increase colonization on inert surfaces due to the presence of *P. fluorescens* (Carpentier and Chassaing, 2004). Additionally, Carpentier and Chassaing (2004) reported P. *fluorescens* biofilms protect pathogenic bacteria from disinfection processes.

Kocuria rhizophila, formerly known as *Micrococcus luteus,* (Tang and Gillevet**,** 2003), is a common contaminant of dairy products, which can facilitate its transmission to a host after consumption of milk (Lillo et al., 1997). Lillo et al., 1997 demonstrated the susceptibily of microbes to lactorferrin utilizing *K. rhizophila*. *Enterococcus faecium* is an inhabitant of the mammalian gastrointestinal tract. It is a common probiotic component, yet it is highly resistant to multiple antibiotics *(*Solheim et al., 2009).

MATERIALS AND METHODS

Colostrum Samples

Four brands of dried colostrum and two brands of dried whey were obtained on three different days from the same nutraceutical company over a three month period. One brand of non-fat dry milk was purchased from a local grocery store on three different days over a three month period. Each sample was submitted in duplicate to the Clemson University Agricultural Services Laboratory for analysis of fat, protein, and ash/mineral content analyses.

IgG Concentration- Immunoprecipitation Assay

Concentration of IgG was determined by immunoprecipitation using a VMRD Single Radial Immunodiffusion kit (VMRD, Inc., 240-60, Pullman,WA). Each dairy product (1g into 9 ml) was rehydrated in Class O phosphate and magnesium chloride buffer (Wehr and Frank, 2004) for total IgG determination. Colostrum Brand A was also rehydrated in phosphate and magnesium chloride buffer, along with 1% n-octyl-β-D-glucopyranoside (Sigma-Aldrich Co., O8001, St. Louis, MO) to solubilize membrane bound immunoglobulins due to inability of Colostrum Brand A to go into solution. All samples were brought to room temperature and samples were mixed thoroughly for 1 hour on a shaker before use. Three microliters of each sample and reference standard was applied to serial radial immunodiffusion plates containing agarose gel with anti-bovine IgG. After sample addition, plates were incubated undisturbed for 24 h at room temperature. Samples positive for IgG exhibited opaque rings around the sample.

Resulting ring diameters were measured, and IgG content of samples was calculated by regression analysis. A standard curve was generated with reference sera supplied by the manufacturer.

Bacterial Enumeration

Initial microbiological examination of colostrum products involved standard dairy product enumeration techniques. Whey and non-fat dried milk were not included in the initial bacterial enumeration experiments. Standard plate count agar (SPC) (VWR, 90000-250, West Chester PA), violet red bile agar (VRBA) (VWR, 90000-280,West Chester PA), and Elliker's agar (VWR, 95021-728, 90000-764, West Chester PA) were used and incubated at 32 ± 2°C for 72 h, 32 ± 2°C for 24 h and 32 ± 2°C for 48 h, respectively (Wehr and Frank, 2004). Class O phosphate/magnesium chloride buffer was used as the diluent (Wehr and Frank, 2004) and a lecithin-based buffer were used as the diluents. The lecithin-based buffer contained 10% ADM Ultralec® F Deoiled Lecithin added to standard phosphate/magnesium chloride buffer. For dried dairy products, diluent bottles were pre-warmed to 40 to 45°C. Samples were aseptically weighed into the tempered diluent and mixed by shaking in a 1 foot arc for 20 to 30 revolutions (Wehr and Frank, 2004). Samples were plated using the pour plate method at 10^0 to 10^{-6} dilutions. For the 10^0 dilution, 1 gram of sample was weighed aseptically directly into the sterile Petri dish.

Samples of colostrum, whey, and non-fat dry milk were examined in duplicate for identifying the bacterial species in the products using both Elliker's agar (VWR, 95021-728, 90000-764 West Chester PA) and Standard Plate Count agar (SPC) (VWR, 90000-

250, West Chester PA). One gram of each product was weighed directly into Petri dishes and then adding tempered agar. Plates were incubated for approximately 48 h at 35°C. Individual colonies were selected from plates and then streaked for isolation on Elliker's agar or SPC agar. The Gram reactions and colony morphologies were recorded.

Bacterial Identification via Colony PCR and 16S rRNA

Individual colonies were selected from plates. A small amount of an isolated bacterial colony was added with a sterile inoculating loop to a sterile PCR reaction tube along with 50 µl of Promega nuclease-free water (VWR, PAP1195, West Chester PA). Tube was placed into boiling water for 10 min. A 12.5 µl sample of the boiled mixture to be used as a DNA template was added to a sterile PCR reaction tube. One µl of forward oligonucleotide primer (8F, 5'AGAGTTTGATCMTGGCTCAG 3', Integrated DNA Technologies, Coralville, IA), 1 µl of reverse oligonucleotide primer (1492R, 5'GGYTACCTTGTTACGACTT 3', Integrated DNA Technologies, Coralville, IA), 10 µl of GoTaq® Green Master Mix (VWR, PAM7122, West Chester PA) were added to the PCR reaction tube.

The thermal cycle program consisted of one cycle of 94°C for 30 s, 50.6°C for 30 s, 72°C for 1 min, repeat for 30 cycles, and a final incubation at 4°C. The PCR-amplified fragments were observed by gel electrophoresis in 1.5% agarose gels. Ten µl of each PCR product and the HyLadder™ molecular mass marker (Denville Scientific Inc., CB4225-2, Metuchen, NJ) was examined using agarose gel electrophoresis with subsequent Sybr™ Safe DNA gel (S33102, Invitrogen, Carlsbad, California) staining. The amplified DNA fragments were visualized by UV illumination. The PCR

purification was accomplished using a Promega PCR Clean Up kit (VWR, PAA9281, West Chester PA). The 16S rRNA sequencing was completed by the Clemson University Genomic Institute. Sequences were analyzed with National Center for Biotechnology Information (NCBI) BLAST database (Altschul et al., 1997).

Antimicrobial Disk and Well Diffusion Assays

Antimicrobial activity of each dairy product was determined using disk diffusion and agar-well diffusion assays. For the disk diffusion assays, each dairy product was diluted (1 g in 9 ml) using phosphate and magnesium chloride dilution water (Class O) (Wehr and Frank, 2004) and allowed to rehydrate for approximately 5 min. Autoclaved, sterile, cotton paper disks were suspended into the rehydrated dairy products and were aseptically placed onto Petri plates containing SPC agar (VWR, 90000-250, West Chester PA). The plates were incubated at 35°C and were examined for zones of inhibition at 24 h and 48 h of incubation.

For the agar-well diffusion assays, each dairy product was diluted (1 g in 9 ml) using phosphate and magnesium chloride dilution water (Class O) (Wehr and Frank, 2004). Approximately 50 µl of each rehydrated dairy products were aseptically placed into approximately 5 mm wells in Petri plates containing 0.7% TSA agar (VWR, 90002-700, West Chester PA) (Benkerroum et al., 2000) or BHI Agar (VWR, 90003-262, West Chester PA) (Nowroozi et al., 2004). Both methods were examined with the Gram-positive *Staphylococcus aureus* (ATCC 25922) and Gram-negative *E. coli* (ATCC 25909). The 0.7% TSA plates were incubated at 35°C and were examined for zones of inhibition at 24 h and 48 h of incubation. The BHI plates were incubated at 4°C for 24 h

and then the plates were incubated at 35°C for 24 h. The BHI plates were examined for zones of inhibition at 24 h and 48 h of 35°C incubation.

RESULTS AND DISCUSSION

Colostrum Samples

Samples were labeled Colostrum Brand A, Colostrum Brand B, Colostrum Brand C, Colostrum Brand D, Whey Brand A, Whey Brand B, and Non-Fat Dry Milk Brand A. Colostrum Brand A was loose powder whereas Colostrum Brands B, C and D were powder in gelatin capsules. Whey Brands A and B and Non-Fat Dry Milk Brand A were loose powder. Label claims including immunoglobulin content (percent composition of weight), fat-free claims, and daily dosage recommendations from each sample are recorded from each sampled dried dairy dietary supplement in Table 2 (Symbiotics©, 2010; The Vitamin Shoppe©, 2010; Nutraceutical Corporation©, 2010; FoodScienceCorp©, 2010; DESIGNER WHEY®, 2010; Country Life, LLC, 2010; Ingles Markets Inc©, 2010).

Table 2. Label Claims Presented on Sampled Dried Dairy Dietary Supplements.

Dried Dairy Product	Percent Immunoglobulin	Immunoglobulins per Daily Dosage	Fat-Free Claim	Recommended Daily Dosage (mg)
Colostrum Brand A	25	240	No	32
Colostrum Brand B	40	190	No	475
Colostrum Brand C	30	200	No	2500
Colostrum Brand D	30	180	Yes	30
Whey Brand A	No claim	N/A	Yes	23247
Whey Brand B	No claim	N/A	No	85049
Non-Fat Dried Milk Brand A	No claim	N/A	No	No recommendation

Chemical analyses of the individual dried dairy dietary supplements indicated product variations between and within brands. Results are presented in Table 3.

Table 3. Mean Chemical Composition ± Standard Deviation of Duplicate Sampled Dried Dairy Dietary Supplements.

Dried Dairy Product	Protein ± Std Dev (%)	Fat ± Std Dev (%)	Ash ± Std Dev (%)	Moisture ± Std Dev (%)	Dry Matter ± Std Dev (%)
Colostrum Brand A	48.8±4.0	20.4±11.6	6.6±1.8	8.7±1.1	91.4±1.1
Colostrum Brand B	75.8±4.1	0.1±0.0	6.2±0.2	9.7±1.9	90.3±1.9
Colostrum Brand C	77.5±4.2	0.1±0.0	6.2±0.1	11.8±2.6	88.2±2.6
Colostrum Brand D*	43.8±0.7	19.1±1.8	16.7±0.2	11.5±0.6	88.3±0.4
Whey Brand A*	91.4±2.9	0.6±0.7	2.9±0.0	8.0±3.8	92.0±3.8
Whey Brand B	80.9±4.5	0.5±0.6	4.9±0.0	8.6±2.5	91.5±2.5
Non-Fat Dried Milk Brand A	35.6±0.9	0.5±0.2	7.8±0.0	3.5±0.2	96.6±0.2

(*) indicates the product does not adhere to non-fat label claims.

The legal definition for fat-free dairy products is 0.5% or less fat. Results indicate that Colostrum Brand D did not adhere to label claims regarding fat content and instead of being "fat-free" as listed on the label, the product contained greater than 19% fat. Whey Brand A contained slightly greater than 0.5% fat although label claims indicated the product was fat-free. The Dietary Supplement Health Education Act of 1994 directs that dietary supplement labels must be truthful and not misleading (Food and Drug Administration, 2010). However, the FDA Food Safety Modernization Act, a bill passed

by the US House of Representatives and introduced into the US Senate March 3, 2009 is still under debate. This bill has the potential to expand the power of the FDA, specifically the Secretary of Health and Human Services (HHS), to regulate all foods sold, distributed, or imported within the US, including dietary supplements (Civic Impulse, LLC, 2010). Results of this study indicated significant errors in label claims on one colostrum and one whey product. In addition, the fat content of Colostrum Brand A was determined to be 20.4±11.6% probably due to the biolipid delivery system advertised by the manufacturer. The high standard deviation indicated considerable variability between duplicate samples.

Total IgG Concentration- Immunoprecipitation Assay

The total IgG concentration of colostrum, whey and non-fat dry milk products involved quantification via single radial immunodiffusion kits (VMRD, Inc., 240-60, Pullman, WA). Single radial immunodiffusion is a commonly used time and temperature dependent method for classification and quantification of immunoglobulins (VMRD, Inc, 2010). Specific anti-bovine IgG are incorporated into the commercially made agarose gel plates. When each rehydrated dairy dietary supplement sample is added into wells of the agarose plates, the bovine IgG antigen diffuses into the agarose gel containing the antibody forming a precipitant ring that is proportional to the concentration of the IgG concentration in each product (VMRD, Inc, 2010). Total IgG concentration of the individual dried dairy dietary supplements indicated variations from label claims (Table 2). Results are presented in Table 4.

Table 4. Total IgG Concentration of Sampled Dried Dairy Dietary Supplements.

Dried Dairy Product	Total IgG Concentration (mg)	Total IgG Concentration (mg)	Average IgG Concentration ± Std Error (mg)	Average Variation from Label Claim (%)
	Sample Lot # 1	**Sample Lot # 2**		
Colostrum Brand A	32	>4	18±9.9	13.3
Colostrum Brand A*	24	>4	14±7.1	12.6
Colostrum Brand B	32	24	28±2.9	14.7
Colostrum Brand C	5.2	5.2	5.2±0	2.6
Colostrum Brand D	27	8	17.5±6.7	9.7
Whey Brand A	4	>4	4±0	No label claims
Whey Brand B	4	5.2	4.6±0.45	No label claims
Non-Fat Dried Milk Brand A	0	0	0±0	No label claims

(*) indicates the addition of 1% n-octyl-β-D-glucopyranoside (Sigma-Aldrich Co., O8001, St. Louis, MO)

Results indicate that Colostrum Brands A, B, C, and D contained from 2.6 to 13.3% lower mean immunoglobulin content than label claims. Therefore, the products did not adhere to label claims regarding immunoglobulins and, thereby, did not adhere to the Dietary Supplement Health Education Act of 1994 (Food and Drug Administration, 2010). Chelack et al. (1993) reported that spray dried bovine colostrum conducted at a low temperature can cause loss of immunoglobulin function and quantity. Results of this study indicated significant errors in label claims on all colostrum products.

Bacterial Enumeration

Initial microbiological examination of colostrum, whey and non-fat dry milk products involved standard dairy product enumeration techniques on two lots of dried dairy dietary supplements, not including the whey and non-fat dried milk. However, difficulties in identifying colonies versus dairy product particles were noted (Tables 5 through 18). Various techniques were attempted to resolve difficulties in enumeration. Aseptically grinding of samples, blending, longer rehydration periods, lecithin-based buffers and different dilution rates were attempted but all methods were unsuccessful. Results demonstrate the inconsistencies in the plate counts obtained from serially diluted dried dairy products according to the standard dairy product enumeration techniques on standard plate count agar (VWR, 90000-250, West Chester PA), violet red bile agar (VWR, 90000-280,West Chester PA), and Elliker's agar (VWR, 95021-728, 90000-764, West Chester PA). The plate counts and bacterial growth on all media (Tables 5 through 18) did not always reduce according to the dilution factor. Dried dairy products are white powdered materials that often are difficult to differentiate from the white bacterial colonies formed on the plates. Additionally, the high fat content of the dried dairy products (Table 2) may have encapsulated the bacteria within the dried dairy dietary supplements causing the bacteria to not be enumerated properly. Dried dairy dietary supplements have high protein concentrations (Table 2) that could lead to bacterial attachment to the polystyrene Petri dishes (Fletcher, 1975); thereby, impairing enumeration of bacterial content of the dried dairy dietary supplements. In future studies, silicon treated glass or polylysine Petri dishes may be tested.

In Table 5, the results of the SPC enumeration of Colostrum Brand A indicated Lots 1 and 2 were different. However, since dilutions of 2 replicates in Lot 1 did not indicate proper 1 to 10 reductions and were thought to be in error. Statistical analysis was not conducted. In Table 6, the results SPC results for Colostrum Brand B indicated the two lots were different. However, serial dilutions were obviously in error since counts did not abide by 1 to 10 reductions in numbers. Therefore, since enumeration errors were noted, statistical analysis was not conducted. In the SPC results for Colostrum Brand C (Table 7), Lots 1 and 2 were different. No bacterial colonies were noted on Lot 1, but on Lot 2 counts did not match serial dilutions indicating an error. Since enumeration errors were noted, statistical analysis was not conducted. The SPC results for Colostrum Brand D (Table 8), Lot 1 indicated erratic counts that did not correspond to the dilution. For Lot 2, plates were lost due to a laboratory accident after incubation and after disposal of original samples. Therefore, comparisons between Lots 1 and 2 could not be made or statistically analyzed. However, since all other enumeration results were obviously in error, repeating the experiment was not deemed useful.

Coliform counts from Colostrum Brands A-D are listed in Tables 9-12, respectively. Coliform counts are used by the dairy industry as an indication of proper hygiene and sanitation. According to the US Pasteurized Milk Ordinance (2007), the legal limit for coliform in dried dairy products in 10 CFU/g. In Tables 9, 10, 11, and 12, all Colostrum Brands and Lots, except Brand C, Lot 1 were in violation on coliform count.

Table 5. Standard Plate Count of Sampled Dried Dairy Dietary Supplement Colostrum Brand A.

Colostrum Brand A Dilutions	**Standand Plate Count**			
	Sample Lot #1		**Sample Lot #2**	
	Replicate 1 (CFU/g)	**Replicate 2 (CFU/g)**	**Replicate 1 (CFU/g)**	**Replicate 2 (CFU/g)**
10^{-1}	TNTC	TNTC	0	2
10^{-2}	178	162	0	0
10^{-3}	40	51	0	0
10^{-4}	8	2	0	0
10^{-5}	0	2	0	0
10^{-6}	0	0	0	0

Table 6. Standard Plate Count of Sampled Dried Dairy Dietary Supplement Colostrum Brand B.

Colostrum Brand B Dilutions	**Standand Plate Count**			
	Sample Lot #1		**Sample Lot #2**	
	Replicate 1 (CFU/g)	**Replicate 2 (CFU/g)**	**Replicate 1 (CFU/g)**	**Replicate 2 (CFU/g)**
10^{-1}	220	148	5	6
10^{-2}	42	38	2	0
10^{-3}	210	110	1	0
10^{-4}	0	3	0	0
10^{-5}	0	1	0	0
10^{-6}	0	0	0	0

Table 7. Standard Plate Count of Sampled Dried Dairy Dietary Supplement Colostrum Brand C.

Colostrum Brand C Dilutions	**Standand Plate Count**			
	Sample Lot #1		**Sample Lot #2**	
	Replicate 1 (CFU/g)	**Replicate 2 (CFU/g)**	**Replicate 1 (CFU/g)**	**Replicate 2 (CFU/g)**
10^{-1}	0	0	17	40
10^{-2}	0	0	42	2
10^{-3}	0	0	2	6
10^{-4}	0	0	25	1
10^{-5}	0	0	0	0
10^{-6}	0	0	0	0

Table 8. Standard Plate Count of Sampled Dried Dairy Dietary Supplement Colostrum Brand D.

Colostrum Brand D Dilutions	**Standand Plate Count**			
	Sample Lot #1		**Sample Lot #2**	
	Replicate 1 (CFU/g)	**Replicate 2 (CFU/g)**	**Replicate 1 (CFU/g)**	**Replicate 2 (CFU/g)**
10^{-1}	9	19	Experimental Error	Experimental Error
10^{-2}	101	9	Experimental Error	Experimental Error
10^{-3}	1	1	Experimental Error	Experimental Error
10^{-4}	0	0	Experimental Error	Experimental Error
10^{-5}	0	0	Experimental Error	Experimental Error
10^{-6}	0	0	Experimental Error	Experimental Error

Table 9. Violet Red Bile Agar Coliform Count of Sampled Dried Dairy Dietary Supplement Colostrum Brand A.

Colostrum Brand A Dilutions	**Violet Red Bile Agar Coliform Count**			
	Sample Lot #1		**Sample Lot #2**	
	Replicate 1 (CFU/g)	**Replicate 2 (CFU/g)**	**Replicate 1 (CFU/g)**	**Replicate 2 (CFU/g)**
10^{-1}	1	2	1	4
10^{-2}	1	4	0	0
10^{-3}	1	3	0	0
10^{-4}	4	3	0	0
10^{-5}	9	1	0	0
10^{-6}	1	0	0	0

Table 10. Violet Red Bile Agar Coliform Count of Sampled Dried Dairy Dietary Supplement Colostrum Brand B.

Colostrum Brand B Dilutions	**Violet Red Bile Agar Colony Forming Units**			
	Sample Lot #1		**Sample Lot #2**	
	Replicate 1 (CFU/g)	**Replicate 2 (CFU/g)**	**Replicate 1 (CFU/g)**	**Replicate 2 (CFU/g)**
10^{-1}	TNTC	TNTC	TNTC	TNTC
10^{-2}	85	72	TNTC	TNTC
10^{-3}	8	2	44	100
10^{-4}	0	0	51	27
10^{-5}	0	0	0	0
10^{-6}	0	0	0	0

Table 11. Violet Red Bile Agar Coliform Count of Sampled Dried Dairy Dietary Supplement Colostrum Brand C.

Colostrum Brand C Dilutions	**Violet Red Bile Agar Colony Forming Units**			
	Sample Lot #1		**Sample Lot #2**	
	Replicate 1 (CFU/g)	**Replicate 2 (CFU/g)**	**Replicate 1 (CFU/g)**	**Replicate 2 (CFU/g)**
10^{-1}	0	0	10	83
10^{-2}	0	0	105	3
10^{-3}	0	0	8	15
10^{-4}	0	0	6	10
10^{-5}	0	0	0	0
10^{-6}	0	0	0	0

Table 12. Violet Red Bile Agar Coliform Count of Sampled Dried Dairy Dietary Supplement Colostrum Brand D.

Colostrum Brand D Dilutions	**Violet Red Bile Agar Colony Forming Units**			
	Sample Lot #1		**Sample Lot #2**	
	Replicate 1 (CFU/g)	**Replicate 2 (CFU/g)**	**Replicate 1 (CFU/g)**	**Replicate 2 (CFU/g)**
10^{-1}	16	10	Experimental Error	Experimental Error
10^{-2}	7	9	Experimental Error	Experimental Error
10^{-3}	12	6	Experimental Error	Experimental Error
10^{-4}	12	8	Experimental Error	Experimental Error
10^{-5}	0	0	Experimental Error	Experimental Error
10^{-6}	0	0	Experimental Error	Experimental Error

Coliform counts on Colostrum Brand B (Table 10) were too numerous to count (TNTC), indicating gross contamination. However, results indicated that counts did not match with serial dilutions. In addition in comparing coliform counts to SPC counts for the same Colostrum Brand B, it would be expected that coliform counts would be less since it is a subset of the total plate count. This indicates further error in the enumeration of colostrum and points out the obvious errors on the SPC and/or coliform counts. Therefore, statistical analysis was not performed.

It was hypothesized that lactic acid bacteria may be present in colostrum samples. Growth on Elliker's agar was scored as yes or no across 10^{-1} to 10^{-6} dilutions of each colostrum brand (Tables 13-16). Growth was noted on 5 out of 8 lots.

In an effort to improve dispersion of the colostrum products, a lecithin-based buffer was tested (data not shown). Across all brands, bacterial colony counts were lower on the lecithin buffer. This effect could be due to lecithin inhibiting or killing inherent bacteria. Using the standard phosphate/magnesium chloride buffer (Wehr and Frank, 2004), it was difficult to distinguish between clumps of colostrum powder and colonies. Therefore, lower count on the lecithin could have been due to dispersion of colostrum and the counted "colonies" could have been true colonies. However, lecithin would act to disperse fat, it would be expected that this difference in counts would be noted on the high fat products, Colostrum Brands A and D. No such trend was noted.

Table 13. Elliker's Agar Bacterial Growth of Sampled Dried Dairy Dietary Supplement Colostrum Brand A.

Colostrum Brand A Dilutions	**Elliker's Agar Bacterial Growth**			
	Sample Lot #1		**Sample Lot #2**	
	Replicate 1 (CFU/g)	**Replicate 2 (CFU/g)**	**Replicate 1 (CFU/g)**	**Replicate 2 (CFU/g)**
10^{-1}	+	+	-	-
10^{-2}	-	+	-	-
10^{-3}	-	-	-	-
10^{-4}	+	-	-	-
10^{-5}	+	-	-	-
10^{-6}	+	-	-	-

Table 14. Elliker's Agar Bacterial Growth of Sampled Dried Dairy Dietary Supplement Colostrum Brand B.

Colostrum Brand B Dilutions	**Elliker's Agar Bacterial Growth**			
	Sample Lot #1		**Sample Lot #2**	
	Replicate 1 (CFU/g)	**Replicate 2 (CFU/g)**	**Replicate 1 (CFU/g)**	**Replicate 2 (CFU/g)**
10^{-1}	+	+	+	+
10^{-2}	+	-	+	+
10^{-3}	+	+	+	-
10^{-4}	-	-	-	-
10^{-5}	-	-	-	-
10^{-6}	-	-	-	-

Table 15. Elliker's Agar Bacterial Growth of Sampled Dried Dairy Dietary Supplement Colostrum Brand C.

Colostrum Brand C Dilutions	**Elliker's Agar Bacterial Growth**			
	Sample Lot #1		**Sample Lot #2**	
	Replicate 1 (CFU/g)	**Replicate 2 (CFU/g)**	**Replicate 1 (CFU/g)**	**Replicate 2 (CFU/g)**
10^{-1}	-	-	+	+
10^{-2}	-	-	+	+
10^{-3}	-	-	+	+
10^{-4}	-	-	+	+
10^{-5}	-	-	+	+
10^{-6}	-	-	+	+

Table 16. Elliker's Agar Bacterial Growth of Sampled Dried Dairy Dietary Supplement Colostrum Brand D.

Colostrum Brand D Dilutions	**Elliker's Agar Bacterial Growth**			
	Sample Lot #1		**Sample Lot #2**	
	Replicate 1 (CFU/g)	**Replicate 2 (CFU/g)**	**Replicate 1 (CFU/g)**	**Replicate 2 (CFU/g)**
10^{-1}	-	-	+	+
10^{-2}	-	-	+	+
10^{-3}	-	-	+	+
10^{-4}	-	-	-	-
10^{-5}	-	-	-	-
10^{-6}	-	-	-	-

Late in the study, a reference was located for utilizing 0.5% tetrazolium chloride (TTC) added to the agar in an attempt to stain live colonies to a reddish pink color. Using the hypothesis that TTC would allow differentiation between colostrum powder clumps

and colonies, it was expected that TTC would improve accuracy of the plate counts. However, preliminary experiments (results not shown) did not support this hypothesis. Bacteriological enumeration results indicated that counts across serial dilutions were inaccurate. For many of the results, greater counts were reported for higher dilutions. Clearly, further work is needed on improving bacterial enumeration techniques for these dried dairy supplements. A method is needed to separate the clumps so that bacterial cells can be accurately dispersed and subsequent colonies on agar can be distinguished from clumps of the dried dairy powder.

Bacterial Identification via Colony PCR and 16S rRNA

Bacterial isolates from the dried dairy supplement products were selected from standard plate count agar and Elliker's agar. Isolates were subjected to 16S rRNA sequencing analysis to amplify the 16S rRNA gene using the forward oligonucleotide primer (8F, 5' AGAGTTTGATCMTGGCTCAG 3') and the reverse oligonucleotide primer (1492R, 5' GGYTACCTTGTTACGACTT 3'). Results were submitted for sequencing to the Clemson University Genomics Institute©. Data were analyzed using the BLASTn program on the National Center for Bioinformatics (NCBI) website (Altchul et al., 1997). Bacterial identity was selected from the top twenty five BLAST nucleotide database results with max identity greater than 90%. Gram reaction and morphological characteristics were utilized to confirm the identity of bacterial isolates. Approximately 69% of all bacterial isolates were members the *Bacillus* genus, while approximately 14% of all bacterial isolates were identified as members of the *Pseudomonas genus* (Table 19). Additionally, members of the *Kocuria, Microbacterium, and Enterococcus* genera were

identified as well. *Bacillus licheniformis* and *Pseudomonas fluorescens* are common milkborne organisms. *B. licheniformis* is a sporeformer and consequently has high heat resistance. *B. licheniformis* has been implicated in mastitis in cows (Blowey and Edmondson, 2010) and human catheter implants (Blue et al., 1995). However, *B. licheniformis* also has a number of commercial uses due to the variety of proteases, lipases and carbohydrases the organism produces (de Boer et al., 1994). The Gram negative *Pseudomonas fluorescens* is a common milkborne spoilage organism. It produces heat resistant enzymes and especially proteases. It can cause bitter flavor development due to proteolytic release of peptides and has been associated with formation of ropiness in milk (Michalac et al., 2003).

Although coliform bacteria were enumerated on VRBA, no coliform bacteria were identified using 16S rRNA analysis. Colonies were selected from SPC and Elliker's agar. However, coliform bacterial should have been part of the flora on the SPC plates.

No lactic acid bacteria were identified using 16S rRNA sequencing analysis. Isolates were selected from both standard plate count agar and Elliker's agar. Elliker's agar isolates should have been members of the lactic acid bacterial group. However, since universal primers were used, it is hypothesized that the primers did not properly anneal to the bacterial DNA template during the PCR reaction thereby preventing identification. Of the 50 isolates derived from the dried dairy supplements and submitted for sequencing analysis, only 29 were identified.

Table 19. Bacterial Species Identified from Each Dried Dairy Dietary Supplements Using 16S rRNA Sequencing Analysis.

Dried Dairy Product and Bacterial Isolate Classification		**Gram Reaction & Cell Morphology**	**16S Bacteria Identification (>90% Top Identity Match)**
Colostrum Brand A	**Isolate 1**	Positive Rod	*Bacillus licheniformis*
	Isolate 2	Positive Rod	*Bacillus badius*
	Isolate 3	Positive Cocci	*Kocuria rhizophila*
	Isolate 4	Positive Rod	*Bacillus cereus*
	Isolate 5	Positive Rod	*Bacillus badius*
	Isolate 6	Positive Rod	*Bacillus subtilis*
	Isolate 7	Positive Rod	*Bacillus cereus*
Colostrum Brand B	**Isolate 1**	Positive Rod	*Bacillus licheniformis*
	Isolate 2	Negative Rod	*Pseudomonas fluorescens*
	Isolate 3	Positive Rod	*Bacillus licheniformis*
	Isolate 4	Positive Cocci	*Enterococcus faecium*
	Isolate 5	Positive Rod	*Bacillus massiliensis*
Colostrum Brand C	**Isolate 1**	Negative Rod	*Pseudomonas fluorescens*
	Isolate 2	Negative Rod	*Pseudomonas spp.*
Colostrum Brand D	**Isolate 1**	Positive Rod	*Bacillus subtilis*
	Isolate 2	Positive Rod	*Bacillus licheniformis*
	Isolate 3	Positive Rod	*Bacillus subtilis*
	Isolate 4	Positive Rod	*Bacillus subtilis*
	Isolate 5	Positive Rod	*Bacillus licheniformis*
	Isolate 6	Positive Rod	*Bacillus pumilus*
	Isolate 7	Positive Rod	*Bacillus subtilis*
	Isolate 8	Positive Rod	*Bacillus subtilis*
	Isolate 9	Positive Rod	*Bacillus subtilis*
Whey Brand A	**Isolate 1**	Negative Rod	*Pseudomonas fluorescens*

Table 19 (continued). Bacterial Species Identified from Each Dried Dairy Dietary Supplements Using 16S rRNA Sequencing Analysis.

Whey Brand B	**Isolate 1**	Positive Rod	*Microbacterium hydrocarbonoxydans*
	Isolate 2	Positive Cocci	*Kocuria rhizophila*
Non-Fat Dried Milk Brand A	**Isolate 1**	Positive Rod	*Bacillus subtilis*
	Isolate 2	Negative Rod	*Pseudomonas fluorescens*
	Isolate 3	Positive Rod	*Bacillus licheniformis*

Antimicrobial Disk and Well Diffusion Assays

On both the antimicrobial disk and well diffusion assays, no zones of inhibition were noted against either *Staphylococcus aureus* (ATCC 25909) or *Escherichia coli* (ATCC 25922). For the positive control, 70% ethanol in water was used. This indicated no antimicrobial activity from the dried dairy supplements. This is in contradiction to claims purported by colostrum manufacturers concerning antimicrobial activity (Symbiotics©, 2010, Nutraceutical Corporation©, 2010 FoodScienceCorp©, 2010) and, in previous research studies (Pakkanen et al., 1997, Thapa, 2005, Tzipori et al., 1986, Sarkar et al., 1998, Bitzan et al., 1998, Huppertz et al., 1999, Reiter et al., 1976 Siragusa and Johnson, 1989, Kamau et al., 1990, Gaya et al., 1991, Thomas et al, 1976, Kamau et al., 1990, Solomons, 2002). Previous studies in vivo and in vitro have reported antimicrobial activity of bovine colostrum against *E. coli*, *Salmonella*, *Streptococcus*, *Staphylococcus*, *Listeria*, *Clostridium*, *H. pylori*, rotavirus, and *Crytosporidium* (Pakkanen et al., 1997; Thapa, 2005; Tzipori et al., 1986; Sarkar et al., 1998; Bitzan et al., 1998; Huppertz et al., 1999; Reiter et al., 1976; Siragusa and Johnson, 1989; Kamau

et al., 1990; Gaya et al., 1991; Thomas et al, 1976; and Solomons, 2002). Additionally, bovine colostrum was reported to contain immunoglobulins that can prevent viral infections, bacterial infections, allergies, yeast, and fungus (Thapa, 2005). However, many of the reported studies did not utilize dried commercial colostrum supplements. Hyperimmune colostrum was also used in many of the studies. Hyperimmune bovine colostrum is harvested post-partum from cows that have been inoculated repeatedly with specific pathogens during pregnancy. Therefore, high levels of antibodies or immunoglobulins can develop against specific pathogens (Pakkanen et al., 1997). Lactoferrin, lactoperoxidase, growth factors, and other antimicrobials are present in bovine colostrum. However, Elfstrand et al. (2002) and Chelack et al. (1993) demonstrated the reductions of functionality of bovine colostrum bioactive components post-processing. The antimicrobial activity of the tested dried dairy dietary supplements examined may have been negatively influenced potentially by processing, as indicated by the results.

CONCLUSIONS

Dried dairy dietary supplements, specifically bovine colostrum and whey products, are readily available in health-food stores for human consumption. Their manufacturing is regulated only by the food hygiene standards and the Dietary Supplement Health Education Act of 1994. However, the results of this study indicate that some of the dried dairy dietary supplements did not adhere to content label claims concerning fat content, antimicrobial activity and immunoglobulin levels. Bacterial enumeration of dried colostrum products was difficult due to the inability to distinguish product particles from colonies. Examination of the bacterial content with 16S rRNA sequencing analysis of the dried dairy nutraceutical products indicated the presence of primarily *Bacillus* and *Pseudomonas* species. Additionally, members of the *Kocuria, Microbacterium, and Enterococcus* species were identified as well. The presence of these particular bacterial strains could reveal inconsistencies in the processing and packaging of these nutraceutical products because each should be destroyed via thermal processing. Hayden et al. (2007) claimed the leniency of FDA regulations related to nutraceuticals and dietary supplements has allowed for significant scientific claims to be presented without the necessary research to support those claims. As with bovine colostrum dietary supplements, most evidence supporting the effectiveness of nutraceutical and dietary supplementary are anecdotal (Hayden et al., 2007). The results of this study indicate label claims are not supported for fat, immunoglobulin content, and antimicrobial activity versus *Escherichia coli* (ATCC 25909) and *Staphylococcus aureus* (ATCC 25922).

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