Food Reviews International, 22:225–244, 2006

ISSN: 8755-9129 print / 1525-6103 online
DOI: 10.1080/87559120600694606

Taylor & Francis

Bioactive Compounds of Colostrum and Its Application

VISHAL TRIPATHI AND BHAVANA VASHISHTHA

Dairy Technology Division, National Dairy Research Institute, Haryana, India

Colostrum is the initial milk secreted by bovine during parturition and the first few days after birth. Colostrum is a gift of nature used to protect the newborn's immune system and provides passive immunity against pathogens. The presence of bioactive components such as insulin-like growth factor I and II (IGF-I and IGF-II), lactoferrin, lysozyme, lactoperoxidase, and immunoglobulin make the colostrum active against pathogens such as Escherichia coli, Salmonella typhimurium, Shigella dysenteria, Listeria monocytogens, Streptococcus mutans, Bacillus stearothermophilus, and Bacillus subtilis. *It is also active against* Herpes *simple virus type-I (HSV-I), Human Immuno-deficiency virus-I (HIV-I), and human cytomegalovirus. Lysozyme is an antibacterial and lytic enzyme; whereas lactoperoxidase is a major antibacterial found in colostrum; it is toxic to gram-positive and gram-negative bacteria. Lactoperoxidase inactivates the polio virus, vaccinia, and human immunodeficiency virus type-I* in-vitro. *Immunoglobulins are considered an important bioactive component in colostrum, and it contains high levels of immunoglobulin G (Ig G). Immunosupplementation with bovine milk antibodies has been shown to provide local protection to the gastrointestinal tract against disease. The restricted technical and hygienic problems, along with the unstable physio-chemical nature of colostrum, has resulted in minimal utilization of colostrum on Industrial scale.*

Keywords Colostrum, Immunoglobulin, IGF-I, IGF-II, Growth factor, Lysozyme, Lactoperoxidase

Introduction

Bovine colostrum is the initial milk secreted by cows during parturition and the first few days after birth. Postparturition colostrum is designed by nature as a substance that protects a newborn's immune system and provides passive immunity against a host of microorganisms. It also assists the body with protein synthesis, muscle building, and tissue growth. Colostrum is a rich source of bioactive proteins, which could account for its prescribed actions. Although colostrum might contain proteins with anabolic actions that have not yet been described, a possible mechanism of actions could come from insulin-like growth factor-I (IGF-I). It is the most abundant and well-described growth factor in colostrum and has the same amino acid structure in bovine as well as humans. The growth factors promote the growth and development of newborn calves. These include insulin-like growth factor I and II (IGF-I and IGF-II). IGF-I and IGF-II are heat stable, acid stable and are widely distributed mediators of cellular growth, development, and differentiation.

Colostrum is rich in antimicrobial components such as lactoferrin, lysozyme, lactoperoxidase, and immunoglobulins. Lactoferrin exhibits antagonistic properties against

Address correspondence to Vishal Tripathi, 15/107, HIG Duplex, Vasundhara, Ghaziabad-201 012, U. P. India. E-mail: vt137@yahoo.co.in

pathogens such as *Escherichia coli, Salmonella typhimurium, Shigella dysenteria, Listeria monocytogens, Streptococcus mutans, Bacillus stearothermophilus*, and *Bacillus subtilis*. It is also active against *Herpes* simple virus type-I (HSV- I), Human Immuno-deficiency virus -I (HIV-I) and human cytomegalovirus. Lysozyme is antibacterial and a lytic enzyme, whereas lactoperoxidase is a major antibacterial found in colostrum that is toxic to gram-positive and gram- negative bacteria. In addition, lactoperoxidase inactivates polio virus, vaccinia and human immunodeficiency virus type-I *in-vitro*. Immunoglobulins are considered an important bioactive component in colostrum, and colostrum contains high levels of immunoglobulin G (Ig G). Immunosupplementation with bovine milk antibodies has been shown to provide local protection to the gastrointestinal tract against disease.

The utilization of colostrum is, however, restricted, mainly due to technical and hygienic problems. The low clotting temperature and high protein content leads to problems in industrial processes, whereas high antimicrobial activity decrease the chances of fermentation. This article considers an overview of the bioactive components of colostrum and its effective utilization.

Antimicrobial Components

Lactoferrin

Lactoferrin is an 80kDa iron-binding glycoprotein present in colostrum, milk, and to a lesser extent in other exocrine fluids such as tears. In addition to its antimicrobial activity, it has been proposed that lactoferrin plays an important role in iron uptake in the intestine and the activation of phagocytes and immune responses. Receptors for lactoferrin are found on intestinal tissues, monocytes, macrophages, neutrophiles, lymphocytes, platelets and on some bacteria.[1–3]

The cDNA for bovine lactoferrin has been isolated, and the deduced amino acid sequence (708 amino acids) is homologous with human lactoferrin (68%) and human transferrin (60%), another iron-binding protein predominantly present in serum. The concentration of lactoferrin in bovine colostrum and mature milk is about 1.5–5 mg/mL and 0.1 mg/mL, respectively.[4,5]

Lactoferrin has been shown to inhibit the growth of several microbes, including *Escherichia coli*,[6,7] *Salmonella typhimurium, Shigella dysenteria*,[8] *Listeria monocytogenes*,[9] *Streptococcus mutans*,[10] *Bacillus stearothermophilus*, and *Bacillus subtilis*.[11] In a recent study, it was shown that human and bovine lactoferrin and their N-terminal peptides were giardicidal against *Giardia lamblia in vitro*.[12] It has been proposed that the antimicrobial effect of lactoferrin is based on its capacity to bind iron, which is essential for the growth of bacteria. However, recent studies have shown that in addition to iron chelation, other mechanisms are also involved. In fact, an antibacterial domain of bovine and human lactoferrin distinct from the iron-binding region of the molecule has been characterized.[7,13]

Lactoferrin is active at neutral pH, and in the presence of bicarbonate ions.[14,15] Since bicarbonate is secreted into the lumen of the intestine, the conditions should be favorable for the antimicrobial activity of lactoferrin.[1] Lactoferrin has been shown to bind lipid A of polysaccharides (LPS)[16] and cause the release of LPS from cell walls of bacteria.[17,18] In addition, lactoferrin binds to porin molecules in the outer membrane of *Escherichia coli*[19] and *Salmonella typhimurium*,[20] resulting probably in permeability changes. Lactoferricin, a peptide derived from pepsin digestion of bovine lactoferrin, has antimicrobial

activities against various bacteria and *Candida albicans*,[18,21,22] and the interaction of lactoferrin with the cell surface is necessary for antimicrobial activity. These results suggest that lactoferrin exerts its antimicrobial activity by modifying bacterial cell membranes. In addition to its antibacterial activity, lactoferrin has antiviral effects against herpes simplex virus type-1 (HSV- 1),[23] (HIV-1) and human cytomegalovirus *in vitro*.[24]

Lysozyme

Lysozyme [EC.3.2.1.17] is a well-known antibacterial and lytic enzyme discovered by Fleming.[25] Lysozyme can be found in many mammalian body fluids, including colostrum. Especially rich sources of lysozyme include egg albumen and human milk. The natural substrate of the enzyme is the peptidoglycan layer of bacterial cell wall and its degradation results in lysis of the bacteria. Some recent results indicate that the antibacterial activity of lysozyme is due not only to its enzymatic activity, but also to its cationic and hydrophobic properties.[26] The concentration of lysozyme in colostrum and normal milk is about 0.14–0.7 and 0.07–0.6 mg/mL, respectively. Milk lysozyme is active against a number of gram-positive and gram-negative bacteria, which are completely resistant to egg white lysozyme.[27] The presence of lactoferrin enhances the antibacterial activity of lysozyme against *E. coli*,[18] which also supports the hypothesis that lactoferrin damages the outer membrane of gram-negative bacteria. Several genes encoding lysozymes[28,29] have been found in cow, and the purified lysozymes from cow kidney and stomach are different from cow's milk lysozyme.[30,31] In addition, cow's milk lysozyme is strikingly different in amino acid content from human milk lysozyme.[32] Further studies are required to define the exact functions of different types of lysozymes expressed even in a single animal.

Lactoperoxidase

Lactoperoxidase [EC 1.11.1.7] is a major antibacterial enzyme in colostrum. It is a basic glycoprotein containing a heme-group with Fe^{3+} and catalyzes the oxidation of thiocyanate (SCN^-) in the presence of hydrogen peroxide (H_2O_2), producing a toxic intermediary oxidation product. This product inhibits bacterial metabolism via the oxidation of essential sulfhydryl groups in proteins.[33]

The lactoperoxidase system protects the lactating mammary gland from infections caused by, for example, pathogenic *Streptococcus spp*.[34,35] The lactoperoxidase system is also toxic to other gram-positive and gram-negative bacteria such as *Pseudomonas aeruginosa, Salmonella typhimurium*,[1] *Listeria monocytogenes*,[36,37] *Streptococcus mutans*, *Staphylococcus aureus*,[37] and psychrotrophic bacteria in milk.[38] In addition, the lactoperoxidase system inactivates polio virus, vaccinia,[39] and HIV- 1[40] *in vitro*.

Bovine colostrum and milk contain about 11–45 mg/L and 13–30 mg/L lactoperoxidase, respectively.[4] The gene encoding lactoperoxidase is expressed in epithelial cells of the lactating mammary gland, indicating that these cells secrete lactoperoxidase into milk.[41] The deduced amino acid sequence of the bovine lactoperoxidase gene is homologous to human myelo-, thyro-, and eosinophil-peroxidases. The single peptide chain (612 amino acids) includes 15 half-cystines and 4 to 5 potential N-glycosylation sites, and the heme group is suggested to bind to the peptide chain via a disulfide linkage.[41] The bovine lactoperoxidase also contains a site with high affinity for calcium. A lactoperoxidase-related enzyme devoid of the heme prosthetic group and enzyme activity has been purified from bovine milk, but its function is unknown.[42] The lactoperoxidase is partly activated

by forming a complex with lysozyme and this interaction appears to be quite specific.[43] The lactoperoxidase system and lactoferrin have been known to have an additive, but not synergistic, antibacterial effect against *Streptococcus mutans*.[44]

Immunoglobulins

Maternal immunoglobulins are not transferred across the placenta to the fetus in cattle, and calves are born with very low concentrations of serum immunoglobulins. Bovine colostrum is a very rich source of immunoglobulins, and their absorption is essential to provide passive immunity after birth. These antibodies protect newborn calves against infectious enteric and respiratory diseases, which are principal reasons for mortality of calves. Calves with high serum immunoglobulin concentrations have lower mortality rates than calves with serum IgG < 10 g/l.[45]

IgG_1 is the principal immunoglobulin type in colostrum, whereas IgM, IgA, and IgG_2 are present at considerably lower concentrations. The concentrations of immunoglobulins in colostrum are almost a hundred-fold higher than in milk. Two processes are involved in the transfer of immunoglobulins from the cow to its calf.[45,46] First, maternal immunoglobulins are absorbed from circulation and concentrated in the colostrum. Next, the colostral immunoglobulins are transferred from the lumen of the intestine into the circulation of the newborn calf. IgG is transported from blood into colostrum by an active receptor-mediated transfer across the mammary gland secretory epithelium in the dam. IgG diffuses across the vascular endothelium and binds to specific IgG-F_c receptors on the basal membrane of the mammary receptors on the basal membrane of the secretory epithelium. Pinocytotic vesicles transfer the immunoglobulin molecules through the epithelial cells, which secrete the molecules into colostrum. The transfer of IgG_1 to colostrum begins several weeks before birth and continues until the time of calving. This process results in a concentration of IgG_1 in colostrum that is 5- to 10-fold higher than in maternal serum.[45,46,47] In a newborn calf, the immunoglobulins are absorbed from colostrum into circulation via a nonselective macromolecular transport system across the small intestine epithelium. No specific Ig receptors have been found to be associated with this process, which probably also transfer other macromolecules. This nonselective absorption occurs, however, only within about 24 to 36 hours of birth and provides the transmission of passive immunity from the cow to its calf.[45,48]

Bovine colostrum contains relatively high amounts of Ig in general, and Ig G specifically constitutes the largest contribution to protein content in colostrum. Mach and Pahud[49] estimated Ig concentrations from bovine mammary secretions with ranges for Ig G_1 of 52–87 g/L, Ig G_2 of 1.6–2.1 g/L, Ig M of 3.7–6.1 g/L, and Ig A of 3.2–6.2 g/L. Ig contribution, however, declines substantially in any colostrum collected more than 24 hours post-parturition, and the amount of lactalbumin and casein increases proportionately. Several uncontrolled intervention trials have been conducted using colostrum during the first 10 hours post-parturition. Table 1 provides a breakdown of Ig content for colostrum collected during the first 10 hours post-parturition.

Growth Factors

Both normal milk and colostrum contain several peptide growth factors that stimulate the growth and differentiation of mammalian cells. The first indirect evidence of this was the finding that human milk[51] and bovine colostrum[52] stimulated the growth of cultured mouse fibroblasts. Several reports confirmed these results and showed that bovine colostrum

Table 1
Immunoglobulin composition of Bovine colostrum collected during the first 10 hours post-parturition

Constituent	Concentration (g/l)
Ig G	30.4
Ig A	3.5
Ig M	9.6

Adapted from: Stephan, et al.[50]

and its fractions stimulate the growth of many other types of mammalian cells *in vitro*.[53,54] Normal bovine milk shows much less stimulation, probably due to its lower content of growth factors.[52,54]

Insulin-like Growth Factors (IGF-I and IGF-II)

The most abundant and best-characterized growth factors in bovine colostrum are insulin-like growth factors (IGF-I and IGF-II). Insulin, IGF-I (also known as somatomedin C), IGF-II and Relaxin constitute the insulin family of growth factors. IGF-I and IGF-II are acid- and heat- stable and are widely distributed mediators of cellular growth, development and differentiation. IGFs are single-chain polypeptides of approximately 7.6 kDa. The polypeptide chain is composed of four polypeptide domains, denoted, A, B, C, and D, whereas insulin has no D domain, and the C domain is cleaved post-translationally. Each IGF molecule contains three disulfide bridges.[55,56]

The biological effects of IGF-I and IGF-II are mediated primarily by a specific IGF receptor, type I IGF receptor, which is structurally homologous to insulin receptor. Another IGF receptor, type II IGF receptor (a cation-independent mannose-6-phosphate receptor), has a slightly higher affinity for IGF-II than for IGF-I. Both receptors co-exist in many cells. IGFs, like insulin, stimulate glucose uptake, the synthesis of glycogen, protein, RNA, DNA and lipids and cell proliferation at nanomolar concentrations *in vitro*. *In vivo*, IGF-I and IGF-II are proposed to act both as endocrine hormones via the blood and locally as paracrine and autocrine growth factors. Six structurally related insulin-like growth factor binding proteins (IGFBPs) are known in rats and humans, and they bind IGFs with high affinity and specificity. IGFBPs are probably involved in the regulation of the biological activity of IGFs and two of them, IGFBP-2 and IGFBP-3, have been found in bovine milk.[55,57,58] In a study conducted by Gibson et al.,[59] milk samples from lactating cows at day 1 and 1, 2, 3, 4, and 5 months postpartum were analyzed and characterized with Western Ligand Blot procedures for specific IGFBP. Electrophoresis and ^{125}[I] IGF-I ligand blot analyses of the samples indicated that milk required removal of casein in order to disclose all IGFBP. Immuno-precipitation studies identified IGFBP-2, -3, -4, and -5 in blood, milk and primary cell culture conditioned media. The IGFBP were present at higher concentrations in serum than in milk and milk concentrations were greater than that shown in conditioned media from primary cultures of bovine mammary cells.[59]

IGF-I and IGF-II, together with a truncated form of IGF-I, -3N:IGF-I, have been purified to homogeneity from bovine colostrum. The amino acid sequence of the purified bovine IGF-I is identical to that of human IGF-I,[60,61] and IGF-II was found to differ in the three amino acid residues from human IGF-II. –3N:IGF-I lacks the N-terminal tripeptide, Gly-Pro-Glu.[60] The IGF content of colostrum has been determined in several studies:

~200 μg/L IGF-I and ~200 μg/L IGF-II, 100–450 μg/L IGF-I, 2000 μg/L IGF m, 50–150 μg/L IGF-I, and 100–600 μg/L IGF-II, 450–500 μg/L IGF-I[62] whereas less than 10 μg/L IGF-I and IGF-II[63] has been found in normal bulk milk. Pasteurization of bovine milk (79°C, 45 s) does not alter the concentration of IGF-I, but the required treatment for infant formula, 121°C for 5 minutes, destroys the protein. IGFs in colostrum and milk are supposed to originate from circulation and are not due to local synthesis in mammary tissues, although the exact mechanism of their appearance in mammary secretions is unknown.[55]

Type I and II IGF receptors have been found in bovine mammary tissue and on intestinal epithelial cells,[64,65] raising a question about the biological effects of IGFs in colostrum and milk. IGF-I has been shown to be a potent stimulator of mitogenesis and galactopoesis of bovine mammary cells,[66] suggesting that IGFs may also have effects on mammary tissue itself. On the other hand, dietary IGFs may have direct effects on epithelial cells of the GI tract or be absorbed into circulation and cause systemic effects. In the two latter cases, IGFs have to survive in the GI tract, where they are exposed to low pH and proteolytic enzymes. Dietary IGF-I has indeed been demonstrated to stimulate cell proliferation in the GI tract of newborn piglets and calves,[55] and enhance D-xylose adsorption in newborn calves. Dietary cow colostrum has also been shown to promote the growth of small intestine of newborn piglets.[55,67] It was found that orally administered ^{125}I-IGF-I was transported into the circulation indicating that systemic effects are also possible. In fact, dietary IGF-I has been shown to suppress erratic insulin secretion, stimulate prolactin secretion and cause a latent (4 days' delay after administration) increase in the concentration of IGF-I in calf blood.[57] Xu and Wang[68] showed that total and trichloroacetic acid (TCA) precipitable radioactivity rose significantly in plasma in newborn and 3-day-old piglets one hour after oral gastric administration of ^{125}I-IGF-I. According to chromatographic analysis, ^{125}I-IGF-I represented about 20% of the total plasma radioactivity in the newborn and 10% in the 3-day-old piglets. Radioactivity of different tissues was analysed, with highest concentrations of TCA precipitate radioactivity found in the stomach wall.

The truncated form of IGF-I, -3N:IGF-I, is especially potent *in vivo* and *in vitro*, possibly due to its reduced affinity for several IGFBPs.[69] In bioassays, -3N: IGF-I has usually several-fold higher biological activity (depending on target cell type) than IGF-I.[70] The relative abundance (activity) of the three forms, IGF-I, IGF-II and -3N:IGF-I, was estimated to be about 1:0.05:2, respectively, in bovine colostrum, indicating that most of the IGF activity in colostrum is due to the presence of -3N:IGF-I.

It has been speculated that a bovine colostrum-based diet would provide natural growth stimulants such as IGFs in food. Some *in vivo* and *in vitro* studies support this idea. A dietary sterile-filtered colostrum-based food supplement has been demonstrated to increase serum IGF-I in male athletes during a short-term strength and speed training period,[71] and dietary colostrum has been shown to increase the concentration of blood IGF-I in neonatal calves.[72] Infused IGF-I enhances muscle protein anabolism in human subjects[73] and in rats. Purified IGF-I acts as a survival factor in the protection of cultured Balb/c mouse fibroblasts against death[74] and stimulates amino acid uptake by cultured human placental trophoblasts. Colostrum also significantly inhibits protein degradation in different cultured mammalian cell lines.[75]

In 1997, a peer-reviewed scientific publication reported that 8 days of supplementation with a colostrum product (Bioenervie™) increased serum IGF-I levels in an athletic population.[76] Antonio, et al.[77] determined the effect of 8 weeks of bovine colostrum supplementation on body composition and exercise performance in active men and women. Subjects were randomly assigned to a placebo (whey protein) and colostrum group (20 g/day in possible form). Each subject participated in aerobic and heavy-resistance training at least three times per week. The whey protein group experienced a significant

increase ($P < 0.05$) in body weight (mean increase of 2.11 kg), whereas the colostrum group experienced a significant ($P < 0.05$) increase in bone-free lean body mass (mean increase of 1.49 kg). Daily supplementation with 60 g of bovine colostrum for 4 weeks did not change blood IGF-I and IGF-binding protein 3 levels.[78] Another study has reported a 17% increase in serum IGF-I following 2 weeks supplementation by athletes with 20 g/day of colostrum from yet another colostrum product (Dynamic™).[79] In this investigation, a separate absorption study indicated that most of the IGF-I in the supplement was degraded in the gastrointestinal tract rather than being absorbed, suggesting that the source of the increase in plasma IGF-I following oral intake of colostrum was enhanced stimulus of human IGF-I synthesis. Colostrum is not on the banned substances of the IOC/World Anti-Doping Agency, and one study has reported that 4 weeks of colostrum supplementation (60 g/day) was not seen to cause a positive doping outcome based on urine testing.[78]

Insulin

In addition to IGFs, varying amounts of insulin have been detected in normal bovine colostrum and milk, from 10–50 μg/L, 85–327 μg/L, and 20–25 μg/L depending on post-partum time. The highest concentration (327 μg/L) was found in the first milking, and it fell to about 50% of its initial value 24 hours post-partum. A stable concentration at about 14% of its initial value was reached 7 days post-partum.[80] Insulin is taken up from the maternal circulation by the mammary gland, from which it is probably released into colostrum.[81] Orally administered insulin results in hypoglycemia in calves[82] and newborn pigs, indicating that insulin is absorbed and retains its biological activity in the GI tract. In addition, a colostrum diet increased the level of serum insulin in neonatal piglets and calves.[83]

Transforming Growth Factor Beta (TGF-β1 and TGF-β2)

A very interesting growth factor found in bovine colostrum is transforming growth factor-β (TGF-β). TGF-β is a highly pleiotropic growth factor with several different types of function. It stimulates proliferation of some cells, especially in connective tissue, whereas it acts as a growth inhibitor of some other cells, such as lymphocytes and epithelial cells. TGF-β plays an important role in embryogenesis, tissue repair, formation of bone cartilage, and in the control of the immune system. Three isoforms of TGF-β (TGF-β1, TGF-β2, and TGF-β3) are known in humans and they are members of the TGF-β superfamily of peptide growth factors, including, for example, a family of bone morphogenic factors and a family containing activins and inhibins. Two high-affinity receptors and several other soluble and cell surface TGF-β binding proteins are known to be involved in mediating the numerous effects of TGF-β.[84,85]

The TGF-β2 gene, like other TGF-βs, encodes a large precursor polypeptide chain. This polypeptide contains a large N-terminal domain and a C-terminal domain, which is the mature form of TGF-β2.[86] After proteolytic processing, the N-terminal domain remains non-covalently bound to the C-terminal domain and this complex is known as an inactive latent TGF-β2.[87] Activation of the latent form by removal of the N-terminal domain is induced by changes in ionic strength, acidification or proteolytic enzymes.[88] Most forms of TGF-β are homodimers containing two identical polypeptide chains, although heterodimers such as TGF-β1, 2 and TGF-β2, 3 have also been found. The subunits of the active form of TGF-β are linked by a disulfide bond.[89]

TGF-β1[90] and TGF-β2[91] have been purified from bovine milk. One of the TGF-β-related growth factors detected in colostrum[88] is probably identical to TGF-β2. It was

also found that colostrum contains a much higher level of TGF-β activity than normal milk. TGF-β2 is probably the predominant form in milk and in colostrum.[92]

It was proposed[91] that TGF-βs may act as mediators of mucosal immunity and/or gut epithelial differentiation in the neonate. The first suggestion is supported by findings that TGF-β increases the production of IgG[93,94] and especially of IgA[93] of lipopolysaccharide (LPS)-stimulated murine B-lymphocytes. It has also been demonstrated that TGF-β enhances expression of secretory component in rat epithelial cells, which is responsible for the transport of polymeric IgA into the intestinal lumen.[95] Since it is well known that IgA plays a major role in immunological protection of mucous membranes,[96] it would be interesting to define the role of TGF-β in milk/colostrum in modulation of the immunological defence systems against pathogenic microorganisms in the gut. It should also be noted that intestinal epithelial cells produce TGF-β by themselves.[97] The suggestion that TGF-β is involved in the development of gut epithelium is supported by a finding that TGF-β1 induces terminal differentiation of intestinal epithelial cells *in vitro*.

Epidermal Growth Factor (EGF)

The EGF family of growth factors includes, for example, EGF transforming growth factor-α (TGF-α) and amphiregulin. The most important members of this family are EGF and TGF-α, which can modulate the development of epidemis, mammary gland and gut, and act as angiogenic factors. EGF and TGF-α bind to the same receptor, i.e. a cell surface glycoprotein (molecular weight: 175 kDa) with tyrosine kinase activity.[5,98] There are contradictory results, however, about the presence of EGF in bovine colostrum. It was found that the PDGF (platelet-derived growth factor)-like growth factor from mammary secretions of goats, sheep and cows inhibited the bonding of ^{125}I-labelled mouse EGF to mouse 3T3 fibroblasts, but stimulated cell proliferation. This activity was significantly higher in colostrum than in normal milk. Shing and Klagsburn[99] purified growth factors from both human and bovine milk and found that the major growth factor of human milk, human milk growth factor III (HMGF III), was similar to EGF, but it was not present in bovine milk. Instead of EGF, the major growth factor detected in bovine colostrum, colostrum-derived growth factor (BCGF), was shown to be structurally related to human platelet-derived growth factor (PDGF).[99]

Immune Factors

The natural environment contains a large variety of infectious microbial agents—bacteria, viruses, and fungi. If left unchecked and allowed to multiply, pathogenic species will eventually kill the host. In normal healthy animals most infections are of limited duration and cause little, if any, permanent damage. This is due to the immune system—a natural defence mechanism that helps ward off or combat infectious agents. The immunoglobulins have an integral role in this defence system in that they form *antibodies*. The most prevalent immunoglobulin in all species of animals is IgG. In human trials, it has been demonstrated that specific antibodies exist in bovine milk that are effective against both enteropathogenic and enterogenic organisms.

Passive Immunity

During embryonic development, the unborn animal immune system is not sufficiently developed to ward off potentially harmful microbes. Fortunately, the fetus is protected from harmful environmental factors by its position in the womb; normally potentially

harmful agents will not pass the placental barrier. At birth, the newborn without a complement of antibodies would find its environment very hostile, being quite susceptible to infection from invading organisms. To alleviate this potentially lethal situation, a very interesting phenomenon, known as passive immunization, has evolved. The maternal blood contains a full complement of antibodies to various antigens to which the mother has been exposed to during her lifetime. In humans and apes, the mother passively immunizes her young ***in utero*** by passage of antibodies through the placenta. In animals where the maternal antibodies do not pass the placental barrier (cattle, pigs, and sheep), the young are passively immunized immediately after birth by way of colostrum. In these species, the maternal antibodies present in the colostrum are absorbed directly through the gut in the first few days after birth.[100–104]

IgG is the form in which antibodies occur most abundantly. In all species of mammals, IgG is passed from the mother to its young, although the actual mechanism of transmission varies from species to species. In humans and apes, it has been shown that IgG and its compliment of antibodies pass across the placental barrier from mother to fetus during the second two-thirds of gestation. This passage appears to be selective in that IgG is transferred but not the other immunoglobulins (IgA, IgM, IgE, IgD). Albumin is also transferred, but to a lesser degree. Other plasma proteins are not transferred across the placental barrier. In cattle, it appears that the same type of selection occurs in absorption of antibody through the gut in that there is a preferential passage of IgG and not IgA, IgM, IgD, or IgE.

Passive Local Protection

In humans, passive transmission of maternal antibodies occurs prior to birth and is ***in utero***. After birth, the antibodies present in human milk function in local passive protection. In cattle, pigs, and sheep, passive transmission of maternal antibodies occurs in the first 20 to 48 hours after birth by way of the colostrum. During this time, they absorb intact antibodies via the newborn's digestive tract. After these first few days, the direct absorption of intact antibodies ceases and any antibodies present in the colostrum and milk then function in local passive protection of the gastrointestinal (GI) tract.

The importance of this passive local protection is evidenced in the newborn calf where diarrhea and other enteric infections (scours) can prove fatal.[105] It has been reported that the best source of nourishment for the infant mammal is mother's milk.[106] This has largely been attributed to not only the nutritional benefits of milk but also to the presence of milk immunoglobulins providing local passive protection to the GI tract.

Immuno-Supplementation

Local protection in the form of immuno-supplementation with bovine milk antibodies has been shown to be an effective means of providing local protection to the GI tract against disease. Bovine immunoglobulin in the form of specific antibody has been shown to be effective against various enteric diseases. Although bovine colostrum contains Ig to neutralize enteric pathogens, the titer of Ig present is considered by some researchers too low to afford protection against specific infectious organisms. This limitation is overcome by using one of three methods: collection of bovine colostrum during a more narrow time period post-parturition; concentration of Ig; or, production of hyperimmune bovine colostrum (HBC).

The use of bovine colostrum as prophylaxis or treatment of infectious disease relates to the historical concept of "immune milk" being capable of transferring passive immunity.

With the advances in research relating to the understanding of chemical structure and functions of Ig, the role of bovine colostrum as treatment for enteropathogenic microbes has come into focus. The majority of research trials have been conducted using hyper-immune bovine colostrum products created to have higher antibody titers against the specific microbe. In trials, it has been successfully shown that specific antibodies in bovine milk are effective against both enteropathogenic and enterotoxigenic *Escherichia coli, Cryptosporidium, Helicobacter pylori, Rotavirus*, and *Shigella flexneri*.[107–113]

Cryptosporidiosis. HBC collected from cows immunized with cryptosporidia antigens has been reported to be an effective treatment for cryptosporidia-induced diarrhea resulting in elimination of stool oocysts. In most cases, patients were immune-compromised and had not responded to conventional treatment with HBC preparations. Resolution of diarrhea occurred within 3 to 10 days and stools were no longer positive for oocysts.[114–118] Bovine colostrum from non-immunized cows has also appeared to be beneficial in eliminating oocysts and altering the clinical course of infection.

Helicobacter pylori. Preliminary evidence suggests that total eradication of *H.pylori* through colostrum supplementation is unlikely. However, reports relating to decrease in severity of gastric inflammation and symptoms give encouraging observations. Daily doses of 12 g HBC concentrated for anti-*H.pylori* bovine Ig were given to 20 children positive for *H.pylori* for 3 to 4 weeks. While the severity of symptoms and rate of colonization were decreased, the organism was not eradicated from any of the children.[119] In a study, daily doses of 20 g HBC concentrated for anti-*H.pylori* bovine Ig were given to nine adults with gastritis for 3 to 4 weeks. While treatment reduced the severity of symptoms and inflammation, the organism was eradicated in only one subject.[120]

Rotavirus. Evidence suggests specific HBC preparations might be effective for both prevention and treatment of rotavirus. In existing trials, HBC were produced to have high neutralizing titers of rotavirus Ig against the four human rotavirus serotypes. It is proposed that this type of HBC preparation might offer prophylaxis against infection and promote more rapid recovery in persons already infected with rotavirus. In one study, 120 children aged 3–15 months who had previously been admitted to a hospital for a range of health challenges received either HBC or infant formula as placebo. The dose administered was 50 ml daily for 10 days with a follow-up period of 14 days. Complete protection against rotavirus infection occurred in the children consuming HBC; the rate of infection in children receiving placebo was 13.8%.[50] In another study, 80 children age 4–24 months with acute diarrhea secondary to rotavirus infection were randomly assigned to receive either 10 g HBC (containing 3.6 g of antirotavirus Ig) daily in four divided doses or milk powder as placebo for four days. By the fourth day, diarrhea had completely resolved in 33 children receiving HBC compared with 21 in the placebo group. While 50% of children who received placebo continued to have rotavirus in their stools on the fourth day, rotavirus was no longer detectable in the stool of 95% of those receiving the active treatment. Clearance of rotavirus from the stool was also earlier in the HBC group compared with the placebo group (mean day, 1.5 versus 2.9, respectively).[121]

Shigellosis. Recent research suggests that HBC produced to contain a high neutralizing titer against *Shigella sp*. might have a role in prevention of infection. Two different lots of HBC concentrated for Ig were produced by immunizing pregnant cows against *S. flexneri*.

Ten participants were given a higher antibody titer (1:40, 960) against *S. flexneri* 2a lipopolysaccharide (LPS), 11 participants were given a lower antibody titer (1:2, 560) while the 11 participants of the control group were given a bovine colostrum (BC) preparation containing an antibody titer of 1:40 against this organism. Participants ingested 10 g three times daily of the concentrates for seven days and were then given an oral challenge consisting of *S. flexneri* 2a strain 2457T followed by an additional 10 g dose of the preparations. None of the 10 participants receiving the high antibody titer HBC developed symptoms of acute illness, while three of the 11 participants receiving low antibody titer HBC and five of 11 subjects receiving the control BC preparation developed symptoms of acute illness. The results strongly suggest a dose-response effect of HBC with respect to specific Ig for prevention of *S. flexneri* infection.[112]

Multiple Sclerosis. Preliminary evidence suggests a benefit of HBC from cows immunized with the Schwarz strain of measles virus in persons with multiple sclerosis (MS). Currently, no available evidence supports a role for BC collected from cows not previously immunized with this virus. Three different oral preparations (two of which would be considered HBC) were administered to 20 patients with MS for 30 days at a dose of 100 ml. The preparations were classified based on antibody neutralization (NT) titer to the measles virus as high (512–5120), low (8–32), or negative (< 8). Changes in disability scores from baseline to the end of the treatment were assessed. It was observed that the improvements in participants receiving the high and low NT HBC preparations were statistically significant.[110]

Industrial Utilization of Colostrum

Colostrum is normally produced in amounts that exceed the need of the newborn. Technical and hygienic problems related to collection and processing of bovine colostrum have as yet restricted its utilization by the dairy and food industries at a large scale. In many countries, colostrum must be excluded from the bulk milk collection during the first five days post partum. The low clotting temperatures of colostrum interfere with pasteurization and the high protein content leads to problems in industrial processes. Further, the high content of antimicrobial components in colostrum may slow down or inhibit fermentation processes. This fact also affects the antibiotic residue tests based on microbial growth, causing false positive results.[122]

Bovine colostrum is utilized on minor industrial scale and in households in many countries, e.g. in Scandinavia, in form of cheese baked in an oven. Also, the casein fraction of renneted colostrum is baked and used as fresh cheese. Cheese-whey and colostral whey-based antibody preparations are commercially available in many countries as feed supplements or colostrum substitutes for farm animals. Increasing interest is focused on the development of so-called "immune milk" preparations made from the colostrum of hyper-immunized cows. The research carried out as yet on HBC has established colostrum as a dietary supplement with immune enhancing attributes and positive effects on athletic performance and body building. Success has been gained in some Asian markets by targeting infants and elderly consumers most at risk of infections. Retail products include 100% colostrum powders in tins, sachets, and capsules, flavored powders, products with colostrum and one or more other immune or performance enhancing components (e.g., Immunizen™ capsules, Rexall products), flavored chewable tablets and liquid colostrum whey. A few preparations (e.g., against rotavirus and *E. coli*), are already on the market.[123]

The pharmaceutical and biotechnological industries have recently shown interest in bovine colostrum as a source of growth factors and other specific bioactive components. Colostrum-based growth media have been developed for culturing cells. Also, a multitude of health products and foods made from various colostrum fractions (e.g., drinks and chewing gums for athletes) have been launched on the market. Currently, there is increasing pharmaceutical research under way to examine the potential related to the use of colostrum-derived preparations for a wide range of gastroenterological conditions.[124] GASTROGARD-R™ is indicated and sold for prevention of rotavirus diarrhea in infants and children to 4 years of age at 5 g/day.[121,125] Dose response data is limited to studies of antibody activity in faeces of children taking antirotavirus colostrum.[126] PR_XO IMMUNE™ 99 (Gala Gen Inc., Minnesota, USA) is a product used on young calves to prevent scours caused by *E.coli*. Biotest Pharma GmBh (Frankfurt, Germany) produces Lactimmunoglobulin Biotest, a product for human subjects that contains immunoglobulins from colostrum of nonimmunized cows. It has been tested, for example, in the treatment of severe diarrhea in AIDS patients.[50]

Spray and freeze-dried colostrum powders and filtered colostrum whey liquids are already being produced. Newer techniques are being employed to get sterile long-life colostrum products. According to Jalonen,[127] microfiltration of ultrafiltered colostrum whey permeate leads to a sterile liquid whey fraction. Fractionation of minor components like Lactoferrin and Glycomacropeptides (GMP) from colostral whey may result in profitable returns and hence, more research into effects of fractions or individual components compared to whole colostrum is being undertaken. Individual components may also be cloned and produced as pharmaceuticals.[128]

Safety Concern

Research has shown that bovine colostrum is easily assimilable by humans and is up to 40 times richer in immune factors than human colostrum, and only cows' colostrum contains special glyco-proteins and protease inhibitors, which are extremely effective at protecting the destruction of colostrum's active components by adult human digestive enzymes and stomach acids. The corollary to all this is that bovine colostrum is safe for consumption by humans. According to the Center for Nutritional Research, "Colostrum is so safe, it has been prepared by nature as the first food for infants. It would be hard to imagine any nutritional substance more natural or beneficial".[129] There have been no contraindications, side effects or allergies reported through thousands of years of human use of bovine colostrum and there are no problems for those with lactose intolerance. Also, colostrum is a whole, completely natural substance that has been minimally processed.

Recent research has shown that the body's ability to assimilate Colostrum is greatly enhanced if it is water-soluble. If it is not readily water-soluble, Colostrum would not disperse well in the human bowel, and thus would be minimally effective. Colostrum does not seem to cause any significant side effects. However, comprehensive safety studies have not been performed.[130] Safety in young children or women who are pregnant or nursing is yet to be established.

Future Prospects

Our current knowledge about the *in vivo* efficacy of immune bovine colostral or milk Ig concentrates suggests that these preparations could be effective in the prevention, and to a lesser extent also in the treatment, of specific microbial gastrointestinal diseases. Such

preparations would be of particular importance for those microbial diseases that cannot be cured or are difficult to treat using current chemotherapy, such as rotaviruses, antibiotic-resistant enteropathogens and *Cryptosporidium*. Future aims to utilize bovine antibodies as intervention agents in the prevention or treatment of infection should determine, at an early stage of product development, the specific target for which the intervention product is intended. For example, antibodies that block the H antigen on fimbriae of enteropathogenic *E. coli* strains have proven useful as a prophylactic measure.[131] In many cases there is scope for improvement of the immunization regimes, such that the ensuing bovine response produces high titers of strong binding-affinity antibodies with polyclonal activity against a range of important pathogen determinants. In a related context, regulatory and ethical considerations for the immunization regime should be taken into account, particularly with respect to the use of 'acceptable' immunopotentiating adjuvants and in relation to the frequency of immunization doses.

The immunosupplementation of clinical diets and special infant formulas with specific antibodies appears, therefore, a challenging future approach. Also, the worldwide trend towards the development of health-promoting functional foods offers interesting opportunities for applications that contain specific antibody ingredients derived from hyperimmunised cows. However, the optimization of the dietary regime still needs to be determined in many cases, from the viewpoint of dose, frequency, duration of use and (in the case of prophylactics) time of use prior to likely exposure to the pathogen. It is expected that such detailed information will only come from clinical trials.

The form in which bovine-derived antibodies are delivered to patients is also an area worth further consideration with respect to product development. Bovine IgG I (the predominant colostral Ig) is relatively resistant to the conditions of the human gut and is thought to remain efficacious throughout the GI tract when delivered in colostrum. Contrary to this, it should be borne in mind that colostral proteins can act as immunogens in their own right, and in rare cases patients can present atopic reactions to milk proteins, including sensitivity to bovine IgG.[132] Accordingly, intact colostrum-containing IgG may not be the appropriate treatment or prophylactic vehicle in these patients, and in cases where sensitivity to milk protein has been detected, it may be necessary to develop a purified product based on pepsin-cleaved Ig, comprising two F $(ab)'_2$ fragments, which are less allergenic than the parent molecule.[133]

There is increasing interest within the food and pharmaceutical industries to develop products that are targeted towards the manipulation of oral and intestinal microflora. Apart from specific antibodies, the possible benefits obtained from the application in the diet of specific antibodies together with probiotic bacteria should, therefore, be investigated. In the past, the commercial development of hyperimmune bovine colostral or milk-based preparations has been constrained by technological limitations. Recent developments in membrane separation techniques enable the concentration and isolation of antibodies from bovine colostrum and milk in an active form. There is, however, an obvious need to upgrade the technological processes so as to improve the economics related to the manufacture of hyperimmune colostral or milk preparations or ingredients. From a commercial viewpoint, the profitable exploitation of lacteal products from immunized cows may be limited to early colostrum, which contains the greatest concentration of Ig. A standardized approach to the production of commercial health-intervening bovine Ig products should be undertaken to ensure high product quality and consistency. It is therefore suggested that batch production be monitored by established *in vitro* testing of product efficacy (e.g., by *in vitro* neutralization tests against enteric viruses and bacteria).[124,134] Humanized infant formula has a lot of scope for the use of colostrum because of the presence of lactoferrin, and it can be successfully used in geriatric foods, as well.

The colostrum after ultrafiltration can be converted into colostrum powder and used in low acid products like beverages because of its suitability at low pH. The presence of branched chain amino acids in colostrum can be used to develop a food or beverage for immunochallanged persons.[134]

Conclusions

Bovine colostrum has been known to provide essential food for newborn calves as it is a rich source of immunoglobulins, growth factors, and related molecules. Since it is relatively easy to collect large amounts of bovine colostrum, it has also been used as a raw material for industrial applications. Recent research has proven that colostrum exerts beneficial physiological effects in infants, children, and adults. Several clinical trials have been conducted that prove the role of colostrum in prevention and treatment of gastrointestinal infections with hyperimmune colostrum and improved athletic recovery and performance with concentrated colostrum protein. Laboratory and clinical research continues to explore the efficacy of colostrum and its components in various medical indications and to determine mechanisms of action. A range of colostrum products are already available in the market today, and more of them are expected to become available in near future. Since the safety of immune colostrum preparation has not been studied in the same manner as required for pharmaceuticals, however, further research is needed to assess the potential allergenic, toxic, and hormonal effects of immune milk preparations. A minimum effective dose for all applications needs to be determined as well.

References

1. Reiter, B. Review of the progress of dairy science: Antimicrobial systems in milk. Journal of Dairy Research **1978**, *45*, 131–147.
2. Lonnerdahl, B.; Iyer, S. Lactoferrin: Molecular structure and biological function. Annual Review of Nutrition **1995**, *15*, 93–110.
3. Viljoen, M. Lactoferrin: A general review. Haematologica **1995**, *80*, 252–267.
4. Korhonen, H. Antimicrobial factors in bovine colostrum. Journal of the Scientific Agricultural Society of Finland **1977**, *49*, 434–447.
5. Tsuji, S.; Hirata, Y.; Mukai, F.; Ohtagaki, S. Comparison of lactoferrin content in colostrum between different cattle breeds. Journal of Dairy Science **1990**, *73*, 125–128.
6. Rainard, P. Bacteriostatic activity of bovine milk lactoferrin against mastic bacteria. Veterinary Microbiology **1986**, *11*, 387–392.
7. Saito, H.; Miyakawa, H.; Tamura, Y.; Shimamura, S.; Tomita, M. Potent bactericidal activity of bovine lactoferrin hydrolysates produced by heat treatment at acidic pH. Journal of Dairy Science **1991**, *74*, 3724–3730.
8. Batish, V.K.; Chander, H.; Zumdegni, K.C.; Bhatia, K.L.; Singh, R.S. Antibacterial activity of lactoferrin against some common food-borne pathogenic organisms. The Australian Journal of Dairy Technology **1988**, *5*, 16–18.
9. Payne, K.D.; Davidson, P.M.; Olivier, S.P. Influence of bovine lactoferrin on the growth of Listeria monocytogens. Journal of Food Protection **1990**, *53*, 468–472.
10. Lassiter, M.O.; Newsome, A.L.; Sams, L.D.; Arnold, R. Characterization of lactoferrin interaction with Streptococcus mutans. Journal of Dental Research **1987**, *66*, 480–485.
11. Oram, J.D.; Reiter, B. Inhibition of bacteria by lactoferrin and other iron-chelating agents. Biochimica et Biophysica Acta **1968**, *170*, 351–365.
12. Turchany, J.M.; Aley, S.B.; Gillin, F.D. Giardicidal activity of lactoferrin and N-terminal peptides. Infection and Immunity **1995**, *63*, 4550–4552.

13. Bellamy, W.; Takase, M.; Yamauchi, K.; Wakabayashi, H.; Kawase, K.; Tomita, M. Identification of the bactericidal domain of lactoferrin. Biochimica et Biophysica Acta **1992**, *121*, 130–136.
14. Bullen, J.J.; Rogers, H.J.; Leight, L. Iron binding proteins in milk and resistance to Escherichia coli infection in infants. British Medical Journal **1992**, *1*, 69–75.
15. Griffiths, E.; Humphreys, J. Bacteriostatic effect of human milk and bovine colostrum on *Escherichia coli*: Importance of bicarbonate. Infection and Immunity **1977**, *15*, 396–401.
16. Appelmelts, B.J.; An, Y.; Geerts, M.; Thijs, B.G.; de Boer, M.A. Lactoferrin is a lipid A-binding protein. Infection and Immunity **1994**, *62*, 2628–2632.
17. Ellison, R.T.; Giehl, T.J.; Laforce, F.M. Damage of the outer membrane of enteric Gram-negative bacteria by lactoferrin and transferrin. Infection and Immunity **1988**, *56*, 2771–2781.
18. Yamaguchi, Y.; Semmel, M.; Stanislawski, L.; Strosberg, A.D.; Stanislawski, M. Virucidal effects of glucose oxidase and peroxidase or their protein conjugates on human immunodeficiency virus type 1. Antimicrobial Agents and Chemotherapy **1993**, *37*, 26–311.
19. Erdei, J.; Forsgren, A.; Naidu, A.S. Lactoferrin binds to porions Ompf and Ompe in Escherichia coli. Infection and Immunity **1994**, *62*, 1236–1240.
20. Naidu, A.S.; Arnold, R.R. Lactoferrin interaction with Salmonellae potentiates antibiotic susceptibility *in vitro*. Diagnostic Microbiology and Infectious Disease **1994**, *20*, 69–75.
21. Jones, E.M.; Smart, A.; Bloomberg, G.; Burgess, L.; Millar, M.R. Lactoferricin, a new antimicrobial peptide. Journal of Applied Bacteriology **1994**, *77*, 208–214.
22. Longhi, C.; Conte, M.P.; Bellamy, W.; Seganti, L.; Valenti, P. Effect of lactoferricin B, a pepsin-generated peptide of bovine lactoferrin, on Escherichia coli HB101 (pR1203) entry into HeLa cells. Medical Microbiology and Immunology **1994**, *183*, 77–85.
23. Fujihara, T.; Hayashi, K. Lactoferrin inhibits herpes simplex virus type-1(HSV-1) infection to mouse cornea. Archives of Virology **1995**, *140*, 1469–1472.
24. Harmsen, M.C.; Swart, P.J.; Debethune, M.P.; Pauwels, R.; Declereq, E.; The, T.H.; Meijer, D.K.F.; Swart, P.J. Antiviral effects of plasma and milk proteins: Lactoferrin shows potent activity against both human immuno-deficiency virus and human cytomegalovirus replication *in vitro*. Journal of Infectious Diseases **1995**, *172*, 380–388.
25. Fleming. A. On a remarkable bacteriolytic element found in tissues and secretions. Proceedings of the Royal Society **1922**, *B93*, 306.
26. Pellegrini, A.; Thomas, U.; von Fellenberg, R.; Wild, P. Bactericidal activities of lysozyme and aprotinin against Gram-negative and Gram-positive bacteria related to their basic character. Journal of Applied Bacteriology **1992**, *72*, 180–187.
27. Vakil, J.R.; Chandan, R.C.; Parry, R.M.; Shahani, K.M. Susceptibility of several microorganisms to milk lysozymes. Journal of Dairy Science **1969**, *52*, 1192–1197.
28. Steinhoff, U.M.; Senft, B.; Seyfert, H.M. Lysozyme-encoding bovine cDNAs from different neutrophile granulocytes and mammary gland are derived from a different gene than stomach lysozymes, Gene **1994**, *143*, 271–276.
29. Irwin, D.M. Evolution of the bovine lysozyme gene family: Changes in gene expression and reversion of function. Journal of Molecular Evolution **1995**, *41*, 299–312.
30. White, F.H.J.; McKenzie, H.A.; Shaw, D.C.; Pearce, R.J. Studies on a partially purified bovine milk lysozyme. Biochemistry International **1988**, *16*, 521–528.
31. Ito, Y.; Yamada, H.; Nakamura, M.; Yoshikawa, A.; Ueda, T.; Imoto, T. The primary structures and properties of non-stomach lysozymes of sheep and cow, and implication for functional divergence of lysozyme. European Journal of Biochemistry **1993**, *213*, 649–658.
32. Eitenmiller, R.R.; Friend, B.A.; Shahani, K.M. Relationship between composition and stability of bovine milk lysozyme. Journal of Dairy Science **1976**, *59*, 834–839.
33. Pruitt, K.M.; Reiter, B. Biochemistry of peroxidase system. In *The lactoperoxidase System: Chemistry and Biological Significance*; Pruitt K.M.; Tnovuo, J.; Eds; Marcel Dekker: New York, 1985, 143–178.
34. Todhunter, D.A.; Smith, K.L.; Schoenberger, P.S. *In vitro* growth of mastitis-associated streptococci in bovine mammary secretions. Journal of Dairy Science **1985**, *68*, 2337–2346.

35. Marshall, V.M.; Cole, W.M.; Bramley, A.J. Influence of the lactoperoxidase system on susceptibility of the udder to Streptococcus uberis infection. Journal of Dairy Research **1986**, *53*, 507–514.
36. Siragusa, G.R.; Johnson, M.G. Inhibition of Listeria monocytogens growth by the lactoperoxidase-thiocyanate-H_2O_2 antimicrobial system. Applied Microbiology and Biotechnology **1989**, *55*, 2802–2805.
37. Kamau, D.N.; Doores, S.; Pruitt, K.M. Enhanced thermal destruction of Listeria monocytogens and Staphylococcus aureus by the cultured human placental trophoblast. Journal of Cellular Physiology **1990**, *165*, 83–88.
38. Bjorck, L. Antibacterial effect of the lactoperoxidase system on psychotrophic bacteria in milk. Journal of Dairy Research **1978**, *45*, 109–118.
39. Belding, M.E.; Klebanoff, S.J.; Ray, C.G. Peroxidase-mediated virucidal systems. Science **1970**, *9*, 195–196.
40. Yamauchi, K.; Toimita, M.; Giehl, T.J.; Ellison, R.T. Antibacterial activity of lactoferrin peptide fragment. Infection and Immunity **1993**, *61*, 719–728.
41. Cals, M.M.; Guillomot, M.; Martin, P. The gene encoding lactoperoxidase is expressed in epithelial cells of the goat lactating mammary gland. Cellular and Molecular Biology **1994**, *40*, 1143–1150.
42. Dumontet, C.; Rousset, B. Identification-purification, and characterization of a non-heme lactoperoxidase in bovine milk. Journal of Biological Chemistry **1983**, *258*, 14,166–14,172.
43. Hulea, S.A.; Mogos, S.; Matei, L. Interaction of lactoperoxidase with enzymes and immunoglobulins in bovine milk. Biochemistry International **1989**, *19*, 1173–1181.
44. Soukka, T.; Lumikari, M.; Tenovuo, J. Combined inhibitory effect of lactoferrin and lactoperoxidase system on the viability of Streptococcus mutans, serotype c. Scandinavian Journal of Dental Research **1991**, *99*, 390–396.
45. Besser, T.E.; Gay, C.C. The importance of colostrum to the health of the neonatal calf. Veterinary Clinics of North America- Food Animal Practice **1994**, *10*, 107–117.
46. Biurne, F.J. The mammary gland and neonatal immunity. Veterinary Science Communication **1977**, *1*, 141–151.
47. Butler, J.E. Bovine immunoglobulins: An augmented review. Veterinary Immunology and Immunopathology **1983**, *4*, 43–152.
48. Bush, L.J.; Stanley, T.E. Absorption of colostral immunoglobulins in newborn calves. Journal of Dairy Science **1980**, *63*, 672–680.
49. Mach, J.P.; Pahud, J.J. Secretory Ig A, a major immunoglobulin in most bovine external secretions **1971**, *106*, 552–563.
50. Stephan, W.; Dichtelmiller, H.; Lisner, R. Antibodies from colostrum in oral immunotherapy. J. Clin. Chem. Clin. Biochem. **1990**, *28*, 19–23.
51. Klagsbrun, M. Human milk stimulates DNA synthesis and cellular proliferation in cultured fibroblasts. Proceedings of the National Academy of Sciences of the United States of America **1978**, *75*, 5057–5061.
52. Klagsbrun, M.; Neumann, J. The serum-free growth of Balb/c 3T3 cells in medium supplemented with bovine colostrum. Journal of Supramolecular Structures **1979**, *11*, 349–359.
53. Klagsbrun, M. Bovine colostrum supports the serum-free proliferation of epithelial cells but not fibroblasts in long-term culture. Journal of Cell Biology **1980**, *84*, 808–814.
54. Steimer, K.S.; Packard, R.; Holden, D.; Klagsbrun, M. The serum-free growth of cultured cells in bovine colostrum and in milk obtained later in the lactation period. Journal of Cellular Physiology **1981**, *109*, 223–224.
55. Baumrucker, C.R.; Hadsell, D.L.; Skaar, T.C.; Blum, J.W.; Campbell, P.G. Insulin-like growth factors (IGFs) and IGF binding proteins in mammary secretions: origins and implications in neonatal physiology. In: *Mechanisms Regulation Lactation and Infant Nutrient Utilization*; Picciano, M.F.; Lonnerdal, B.; Eds; Wiley-Liss: New York, 1992; 285–307.
56. Baumrucker, C.R.; Blum, J.W. Secretion of insulin-like growth factors in milk and their effect on neonate. Livestock Production Science **1993**, *35*, 49–72.

57. Froesch, E.R.; Schmid, C.; Schwander, J.; Zapf, J. Actions of insulin-like growth factors. Annual Review of Physiology **1985**, *47*, 443–467.
58. Humbel, R.E. Insulin-like growth factors I and II. European Journal of Biochemistry **1990**, *190*, 445–462.
59. Gibson, C.A.; Staley, M.D.; Baumrucker, C.R. Identification of IGF Binding Proteins in bovine milk and the demonstration of IGFBP-3 synthesis and release by bovine mammary epithelial cells. J.Anim. Sci. **1999**, *77*, 1547–1557.
60. Francis, G.L.; Upton, F.M.; Ballard, F.J.; McNeil, K.A.; Wallace, J.C. Insulin-like growth factors 1 and 2 in bovine colostrum. Biochemical Journal **1988**, *251*, 95–103.
61. Marcotty, C.; Frankenne, F.; van Beeumen, J.; Maghuin-Rogister, G.; Hennen, G. Insulin-like growth factor I (IGF-1) from cow colostrum: Purification and characterization. Growth Regulation **1991**, *1*, 56–61.
62. Vacher, P.Y.; Blum, J.W. Age-dependency of insulin-like growth factor I, insulin, protein and immunoglobulin concentrations and gamma-glutamyl-transferase activity in first colostrum of dairy cows. Milchwissenschaft **1993**, *48*, 423–425.
63. Vega, J.R.; Gibson, C.A.; Skaar, T.C.; Hadsell, D.L.; Baumrucker, C.R. Insulin-like growth factor (IGF-1) and IGF-2 binding proteins in serum and mammary secretions during the dry period and early lactation in dairy cows. Journal of Animal Science **1991**, *69*, 2538–2547.
64. Laburthe, M.; Rouyer-Fessarrd, C.; Gameltoff, S. Receptors for insulin like growth factors I and II in rat gastrointestinal epithelium. American Journal of Physiology **1988**, *254*, 457–462.
65. Schober, D.A.; Simmen, F.A.; Hadsell, D.L.; Baumrucker, C.L. Perinatal expression of type I IGF receptors in porcine small intestine. Endocrinology **1990**, *126*, 1125–1132.
66. Shamay, A.; Cohen, N.; Niwa, M. and Gertler, A. **1988**. Effects of insulin-like growth factor I on deoxyribonucleic acid synthesis and galactopoesis in bovine undifferentiated and lactating mammary tissue. Endocrinology **1990**, *126*, 804–809.
67. Tungthanathanich, P.; Xu, R.J.; Reynolds, G.W.; Simpson, H.V.; Muller, D.J. The effect of milk diets on small intestinal growth in newborn piglets. Proceedings of the Nutritional Society of New Zealand **1992**, *17*, 51–55.
68. Xu, R.J.; Wang, T. Gastrointestinal absorption of insulin-like growth factor-I in neonatal pigs. Journal of Pediatric Gastroenterology and Nutrition **1996**, *23*, 430–437.
69. Ross, M.; Francis, G.L.; Szabo, L.; Wallace, J.C.; Ballard, F.J. Insulin-like growth factor (IGF) binding proteins inhibit the biological activities of IGF-I and IGF-II but does not des- (1–3) IGF-I, a potent IGF analogue, on growth hormone and IGF binding protein secretion from cultured rat anterior pituitary cells. Journal of Endocrinology **1989**, *130*, 93–99.
70. Ballard, F.J. Cell culture as a tool for identifying nutritional disease therapies. Journal of Nutrition **1994**, *124*, S1540–S1545.
71. Mero, A. A dietary supplement based on bovine colostrum increases the serum IGF-1 concentration in male athletes during a short-term strength and speed training period. Congress abstract: The VIIIth FIMS European Congress of Sport Medicine, Granada, Spain, Oct. 23–27 1995.
72. Ronge, H.; Blum, J.W. Somatomedium C and other hormones in dairy cows around parturition, in newborn calves and in milk. Journal of Animal Physiology and Animal Nutrition **1988**, *60*, 168–174.
73. Fryburg, D.A.; Jahn L.A.; Hill, S.A.; Oliveras, D.M.; Barrett, E.J. Insulin and insulin-like growth factor-1 enhance human skeletal muscle protein anabolism during hyperaminoacidemia by different mechanisms. Journal of Clinical Investigation **1995**, *96*, 1722–1729.
74. Tamm, I.; Kikuchi, T. Insulin-like growth factor-I (IGF-I), insulin and epidermal growth factor (EGF) are survival factors for density-inhibited, quiescent Balb/c-3T3 murine fibroblasts. Journal of Cellular Physiology **1990**, *143*, 494–500.
75. Ballard, F.J.; Neild, M.K.; Francis, G.L.; Dahlenberg, G.W.; Wallace, J.C. The relationship between the insulin content and inhibitory effects of bovine colostrum on protein breakdown in cultured cells. Journal of Cellular Physiology **1982**, *110*, 249–254.

76. Mero, A.; Mikkulainen, H.; Riski, J.; Pakkanen, R.; Aalto, J.; Takala, T. Effects of bovine colostrum supplement on serum IGF-I, Ig G, hormone and salive Ig A during training. Journal of Applied Physiology **1997**, *83*, 1144–1151.
77. Antonio, J.; Sanders, M.S.; Gammeren, D.V. The effect of bovine colostrum supplementation on body composition and exercise performance in active men and women. Nutrition **2000**, *17*, 243–247.
78. Kuipers, H.; Breda, E.; Verlaan, G.; Smeets, R. Effects of oral bovine colostrum supplementation on serum Insulin-like Growth Factor-I levels. Nutrition **2002**, *18*, 566–567.
79. Mero, A.; Kahkonen, J.; Nykanen, T.; Parviainen, T.; Jokinen, I.; Takala, T.; Nikula, T.; Rasi, S.; Leppaluoto, J. IGF-1, IgA and IgG responses to bovine colostrum supplementation during training. Journal of Applied Physiology **2002**, *93*, 732–739.
80. Aranda, P.; Sanchez, L.; Perez, M.D.; Ena, J.M.; Calvo, M. Insulin in bovine colostrum and milk: Evolution throughout lactation and binding to caseins. Journal of Dairy Science **1991**, *74*, 4320–4325.
81. Malven, P.V.; Head, H.H.; Collier, R.J.; Buonomo, F.C. Periparturient changes in secretion and mammary uptake of insulin and concentrations of insulin and insulin-like growth factors in milk of dairy cows. Journal of Dairy Science **1987**, *70*, 2254–2265.
82. Petrie, L. Maximising the absorption of colostral immunoglobulins in the newborn dairy calf. Veterinary Record **1984**, *114*, 157–163.
83. Shams, D.; Eispanier, R. Growth hormone, IGF-I and insulin in mammary gland secretions before and after parturition and possibility of transfer into the calf. Endocrine Regulations **1991**, *25*, 139–143.
84. Rosen, S. Transforming growth factor-β. Multiple actions and potential clinical applications. Journal of the American Medical Association **1989**, *18*, 938–941.
85. Lin, H.Y.; Lodish, H.F. Receptors for the TGF-β superfamily: multiple polypeptides and serine/threonine kinases. Trends in Cell Biology **1993**, *3*, 14–19.
86. Madisen, L.; Webb, N.R.; Rose, T.M.; Marquardt, H.; Ikeda, T.; Twardzik, D.; Seyedin, S.; Purchio, A.F. Transforming growth factor-beta 2: cDNA cloning and sequence analysis. DNA and Cell Biology **1988**, *7*, 1–8.
87. Miller, D.M.; Ogawa, Y.; Iwata, K.K.; Ten Dijke, P.; Purchio, A.F.; Soloff, M.S.; Gentry, L.E. Characterisation of the binding protein of transforming growth factor-beta 1, -beta 2 and -beta 3 to recombinant beta 1-latency-associated peptide. Molecular Endocrinology **1992**, *6*, 694–702.
88. Meager, A. Assays for transforming growth factor-β. Journal of Immunological Methods **1991**, *141*, 1–14.
89. Miyazono, K.; Hellman, U.; Wernstedt, C.; Heldin, C.H. Latent high molecular weight complex of transforming growth factor beta 1. Purification from human platelets and structural characterization. Journal of Biological Chemistry **1988**, *263*, 6407–6415.
90. Jin, Y.; Cox, D.A.; Knecht, R.; Rasschdorf, F.; Cerletti, N. Separation, purification and sequence identification of TGF-β1 and TGF-β2 from bovine milk. Journal of Protein Chemistry **1991**, *10*, 565–575.
91. Cox, D.A.; Burk, R.R. Isolation and characterization of milk growth factor, a transforming-growth-factor-β2-related polypeptide from bovine milk. European Journal of Biochemistry **1991**, *197*, 353–358.
92. Tokuyama, H.; Tokuyama, Y. Bovine colostric transforming growth factor-β-like peptide that induces inhibition and changes in morphology of human osteogenic sarcoma cells (MG-63). Cell Biology International Reports **1989**, *13*, 251–258.
93. Coffman, R.L.; Lebman, D.A.; Schrader, B. Transforming growth factor β specifically enhances IgA production by lipopolysaccharide-stimulated murine B lymphocytes. Journal of Experimental Medicine **1989**, *170*, 1039–1044.
94. Chen, S.S.; LI, Q. Transforming growth factor β1 (TGF-β1). Is a bifunctional immune regulator for mucosal IgA responses. Cellular Immunology **1990**, *128*, 353–361.
95. McGee, D.W.; Aicher, W.K.; Eldridge, J.H.; Peppard, J.W.; Mestecky, J.; McGhee, J.R. Transforming growth factor-beta enhances secretory component and major histocompatibilty

complex class I antigen expression on rat IEC-6 intestinal epithelial cells. Cytokine **1991**, *3*, 5543–550.
96. Brown, W.R. Relationship between immunoglobulins and the intestinal epithelium. Gastroenterology **1978**, *75*, 129–138.
97. Koyama, S.Y.; Podolsky, D.K. Differential expression of transforming growth factors α and β in rat intestinal epithelial cells. Journal of Clinical Investigation **1989**, *83*, 1768–1773.
98. Carpenter, G.; Cohen, S. Epidermal growth factor. Journal of Biological Chemistry **1990**, *265*, 7709–7712.
99. Shing, Y.; Klagsbrun, M. Purification and Characterization of a bovine colostrum-derived growth factor. Molecular Endocrinology **1987**, *1*, 335–338.
100. Brambell, F.W.R. *The Transmission of Passive Immunity From Mother to Young*; Am. Elsevier Publishing Company: New York, 1969; Vol. 18.
101. Penhale, W.J.; Logan, E.F.; Selman, I.E.; Fisher, E.W.; McEwan, A.D. Observations on the adsorption of colostral immunoglobulins by neonatal calf and their significance in colibacillosis. Ann. Rech. Vet. **1973**, *4*, 223–229.
102. Smith, T.; Little, R.B. The significance of colostrum to the newborn calf. J. Exp. Med. **1922**, *36*, 181–186.
103. McEwan, A.D.; Fisher, E.W.; Selman, I.E. Observations on the immunoglobulin level of neonatal calves and their relationship to disease. J. Comp. Path. **1970**, *80*, 259–263.
104. McGuire, T.C.; Pfeiffer, N.E.; Weikel, J.M.; Bartsch, R.G. Failure of colostral immunoglobulin transfer in calves dying from infectious disease. J. Am. Vet. Med. Assn. **1976**, *169*, 713–716.
105. Pahud, J.J.; Hilbert, H.; Scwartz, K.; Amster, H.; Smiley, M. Bovine milk antibodies in the treatment of enteric infections and their ability to eliminate virulence factors from pathogenic *E. coli*. In *The Ruminant Immune System*; Butler, J.E. Ed.; Plenum: New York: 1981; 591–600.
106. Hilpert, H.; Gerber, H.; Amster, H.; Pahud, J.J.; Ballabriga, A.; Arcalis, L.; Farriaux, J.P.; de Pryer, E.; Nussle, D. Bovine milk immunoglobulins (Ig) and their possible utilization in industrially prepared infants milk formulae. In *Food and Immunology Swedish National Foundation Symposium XIII*; Hambraeus L.; Hansen L.A.; McFarlane H.; Eds.; Almquist and Wikell: Stockholm, 1977; 182–197.
107. Mietens, C.; Keinhorst, H.; Hilpert, H.; Gerber, H.; Amster, H.; Pahud, J.J. Treatment of infantile E. coli gastroenteritis with specific bovine anti-E. coli milk immunoglobulins. Eur. J. Ped. **1979**, *132*, 239–52.
108. Tacket, C.O.; Losonsky, G.; Link, H.; Hoang, Y.; Guesry, P.; Hilpert, H.; Levine, M.M. Protection by milk immunoglobulin concentrate against oral challenge with enterogenic Escherichia coli. Eng. J. Med. **1988**, *318*, 1240–1241.
109. Tzipori, C.O.; Binion, S.B.; Bostwick, E.; Losonsky, G.; Roy, M.J.; Edelman, R. Remission of diarrhoea due to cryptosporidosis in an immunodeficient child treated with hyperimmune bovine colostrum. Brit. Med. J. **1986**, *293*, 1276–1277.
110. Ebina, T.; Sato, A.; Umezu, K.; Ishida, N.; Ohyama, S.; Oizumi, A.; Kitaoka, S.; Suzuki, H.; Kunno, T. Prevention of rotavirus infection by oral administration of cow colostrum containing antihuman rotavirus antibody. Med. Microbiol. Immunol. **1985**, *174*, 177–85.
111. Brussow, H.; Hilpert, H.; Walther, J.; Sidoti, J.; Meitens, C.; Bachman, P. Bovine milk immunoglobulins for passive immunity to infantile rotavirus gastroenteritus. J. Clin. Microbiol. **1987**, *25*, 982–986.
112. Hilpert, H.; Brussow, H.; Meitens, C.; Sidoti, J.; Lerner, L.; Werchau, H. Use of bovine milk concentrate containing antibody to rotavirus to treat rotavirus gastroenteritis in infants. J. Infect. Dis. **1987**, *156*, 158–66.
113. Tacket, C.O.; Binion, S.B.; Bostwick, E.; Losonsky, G.; Roy, M.J.; Edelman, R. Efficacy of bovine milk immunoglobulin concentrate in preventing illness after *Shigella flexneri* challenge. Amer. J. Trop. Med. Hyg. **1992**, *47*, 276–283.
114. Heaton, P. Cryptosporidiosis and acute leukemia. Arch. Dis. Child. **1990**, *65*, 813–814.

115. Tzipori, S.; Roberton, D.; Chapman, C. Remission of diarrhoea due to cryptosporidiosis in an immunodeficient child treated with hyperimmune bovine colostrum. British Medical Journal **1986**, *293*, 1276–1277.
116. Tzipori, S.; Roberton, D.; Cooper, D.A.; White, L. Chronic cryptosporidial diarrhoea and hyperimmune cow colostrum. Lancet **1987**, *2*, 344–345.
117. Ungar, B.L.; Ward, D.J.; Fayer, R.; Quinn, C.A. Cessation of Cryptosporidium-associated diarrhea in an acquired immunodeficiency syndrome patient after treatment with hyperimmune bovine colostrum. Gastroenterology **1990**, *989*, 486–489.
118. Nord, J.; Ma, P.; DiJohn, D. Treatment with hyperimmune colostrum of cryptosporidial diarrhea in AIDS patients. AIDS **1990**, *4*, 581–584.
119. Oona, M.; Rigo, T.; Maroos, H. *Helicobacter pylori* in children with abdominal complaints: has immune colostrum some influence on gastritis? Alpe Adria Microbiology Journal **1997**, *6*, 49–57.
120. Tarpila, S.; Korhonen, H.; Salminen, S. Immune colostrum in the treatment of *Helicobacter pylori* gastritis. In *Abstract book of 24th International Dairy Congress*; Melbourne, Australia 1995, 293.
121. Davidson, G.P.; Daniels, E.; Nunan, H.; Moore, A.G.; White, P.B.D. Passive immunization of children with bovine colostrum containing antibodies to human rotavirus. Lancet **1989**, *2*, 709–712.
122. Tupasela, T. The functional and biological properties of whey proteins: prospects for the development of functional foods: a review. Agriculture and Food Science in Finland **1998**, *7*, 283–296.
123. Marnila, P.; Korhonen, H. Colostrum. In *Encyclopedia of dairy sciences*; Roginski, H.; Fuquay, J.W.; Fox, P.F.; Eds.; Academic Press: London, **2002**, 473–478.
124. Shah, P.N. Effects of milk-derived bioactives: an overview. British Journal of Nutrition **2000**, *84, Suppl. 1*, S3–S10.
125. Davidson, G.P.; Tam, J.; Kirubakaran, C. Passive protection against symptomatic hospital acquired rotavirus infection in India and Hong Kong. J. Pediatr. Gastroenterol. Nutr. **1994**, *19*, 351.
126. Pacyna, J.; Roberton, E.S.; Siwek, K.; Terry, S.; Whyte, P.B.D.; Davidson, G.P.; Johnson, R.B. Survival of antibodies in the gastrointestinal tract. In *Proceedings of the Australasian Society for Infectious diseases Annual Scientific Meeting*; Cavins, 1999.
127. Jalonen, H. WO Patent no. WO/1995/000155, **1995**.
128. Filipp, D.; Alizadeh-Khiavi, K.; Richardson, C.; Palma, A.; Paredes, N.; Takeuchi, O.; Akira, S.; Julius, M. Soluble CD 14 enriched in colostrum and milk induces B-cell growth and differentiation. Proc. Natl. Acad. Sci. US **2001**, *98* (2), 603–608.
129. Center for Nutritional Research. Is colostrum safe? 2005. Available at http://www.iCNR.org. (accessed May 6, 2006).
130. Korhonen. H. Technology options for new nutritional concepts. International Journal of Dairy Technology **2002**, *55* (2), 79.
131. Tacket, C.O.; Lononsky, G.; Link, H.; Koang, Y.; Guersy, P.; Hilpert, H.; Levine, M.M. Protection by milk immunoglobulin concentrate against oral challenge with enterotoxigenic E. coli. New England Journal of Medicine **1988**, *318*, 1240–1243.
132. Bernhiesel-Broadbent, J.M.; Yolken, R.H.M.; Sampson, H.A. Allergensity of orally adminstrated immunoglobulin preparation in food-allegic children. Pediatrics **1991**, *87*, 208–214.
133. Lefranc-Millot, C.; Vercaigne-Marko, D.; Wal, J.M.; Lepretre, A.; Peltre, G.; Dhulster, P.; Guillochon, D. Comparison of the IgE titres to bovine colostral G immunoglobulin and their Fe(ab)2 fragments in sera patients allergic to milk. International Archives of allergy and Immunology **1996**, *110*, 156–162.
134. Korhonen, H.; Pihlanto, A. Food derived bioactive peptides—opportunities for designing future foods. Current Pharmaceutical Design **2003**, *9*, 1297–1308.