Milk composition changes during mastitis

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Normal function of the mammary gland is disrupted during mastitis occurrences. As a result, milk composition will change, although not always in a predictable manner.

Effects of mastitis on milk composition are determined by severity of infection, extent of infection, secondary physiological changes which alter metabolism of mammary cells and/or the cow, and whether the epithelial cells are disrupted or milk components are destroyed by enzymatic action.

The likely impacts of mastitis on production of milk and milk components, as well as the underlying biology, are discussed in detail.

Introduction

The dairy industry in New Zealand, Australia and some other parts of the world is pasture-based and seasonal. This practice has created an irregular supply of milk to processors in terms of both volume and quality. The manufacturers of dairy products, sourcing milk from pasture-based dairying systems, are challenged by the increasing demand to produce high-quality dairy products from a milk supply that is inconsistent in its processing characteristics across the season. Factors contributing to variations in milk composition, and therefore product quality, include the stage of lactation of the cows, breed, plane of nutrition, seasonal factors and pathological changes associated with mastitis (Auldist et al., 1995; Auldist and Hubble, 1998).In this article, changes in milk composition associated with mastitis will be considered.

Mastitis

Both clinical and sub-clinical mastitis can affect the composition and manufacturing properties of milk (Auldist and Hubble, 1998; Pyorala, 2003). Bacterial infections are by far the most common cause of mastitis in dairy cattle and much is known about the effects of these bacterial intramammary infections (IMI) on milk composition. How these changes occur is less well understood. Moreover, despite the effects of mastitis on the milk of individual cows, these effects are often masked in bulk milk, through dilution effects (Linzell and Peaker, 1971).

For a better description of the hypotheses on how the changes in the milk composition occur, a basic knowledge of the mammary gland morphology and physiology is necessary.

Basics of the mammary gland morphology and physiology

McManaman and Neville (2003) state that a lactating mammary gland is composed of a branching network of ducts formed of epithelial cells ending in extensive lobulo-alveolar clusters which are the sites of milk secretion. A single layer of polarised secretory epithelial cells surrounds each alveolus within these clusters. In turn the alveoli are surrounded by myoepithelial cells that function in milk ejection, and a vascularised connective-tissue stroma.

There is only one type of secretory cell in the mammary gland, according to Linzell and Peaker (1971), but, they vary greatly in shape, which is confidently ascribed to different phases of activity and rest in different alveoli.

Secreting mammary cells show all the signs of immense activity and synthesis (Figure 1). Under an electron microscope the cytoplasm of lactating mammary secretory cell is filled with a spherical nucleus, a set of free ribosomes, rough endoplasmatic reticulum, mitochondria, products of synthesis (e.g. fat globules and protein granules) and a well developed Golgi apparatus (Linzell and Peaker, 1971; McManaman and Neville, 2003). There are a few microvilli on the apical surface and the basal cell membrane is folded, which probably accounts in part, according to Linzell and Peaker (1971), for the efficiency in removing metabolic precursors from the blood. The secretory cells are connected to each other through an apical junction complex composed of adherens - and tight-junctional elements that function to inhibit direct paracellular exchange of substances between vascular and milk compartments during lactation, although the considerable portion of the cells may bulge into the lumen (Linzell and Peaker, 1971; McManaman and Neville, 2003).

Tight junctions, as described by Nguyen and Neville (1998), form a narrow, continuous seal that surrounds each endothelial and epithelial cell at the apical border, and act to regulate the movement of material between the cells. During lactation, the tight junctions of the alveolar epithelial cells are impermeable, allowing milk to be stored between nursing or milking periods while restricting the leakage of milk components from the lumen or leakage of interstitial fluid components into the milk. Nonetheless mammary epithelial tight junctions are dynamic and can be regulated by a number of stimuli. Tight junctions of the mammary gland during late lactation become leaky, undergoing closure around parturition to become the impermeable tight junctions of the lactating gland. In general, changes in tight junction permeability in the mammary gland appear to be the results of a state change and not assembly and disassembly of tight junctions.

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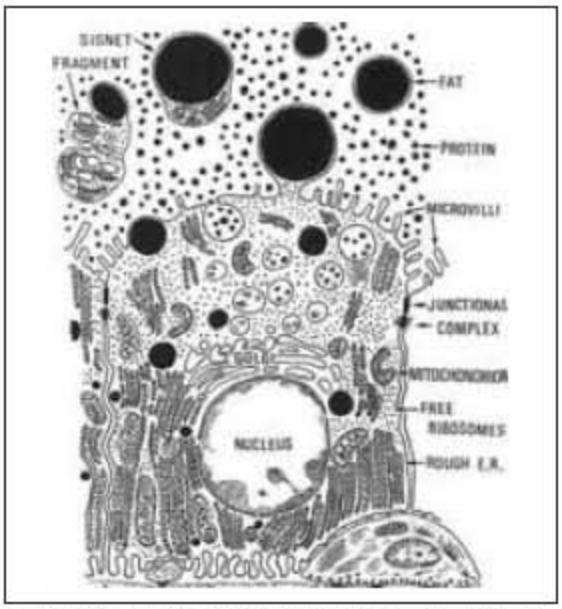

Figure 1 Diagram of secretory alveolar cell as interpreted from electron microscopy (from Linzell and Peaker, 1971)

Secretion, as described by Nguyen and Neville (1998), once initiated at about the time of parturition, is continuous but relatively slow in terms of fluid volume output per unit time. The composition of the mammary secretion begins to change around parturition, and gradually approaches the composition of mature milk. Much of this change in composition, according to the same authors, can be attributed to closure of tight junctions between the mammary epithelial cells during the onset of copious milk secretion, technically called lactogenesis stage II. state Thus, milk is a complex mixture arising from different secretory pathways across, within, and sometimes between the mammary epithelial cells, comprising of membrane-bound fat droplets, casein micelles, and an aqueous phase that usually contains lactose and sometimes complex carbohydrates, minerals, other proteins, and a confusing collection of soluble components (Shennan and Peaker; 2000). So, during lactation, when the ducts and alveoli are filled with milk, the epithelium is positioned between two very different milieu, the milk, containing lactose, milk proteins and low concentrations of sodium and chloride, and the interstitial fluid containing plasma proteins and high concentrations of sodium and chloride. To prevent inter-diffusion of the constituents of these two fluids, the epithelial cells must be sealed tightly one to the other. The mechanism for tight junction closure is not completely understood, but appears to correlate with certain ultra-structural changes in the tight junction network (Nguyen and Neville, 1998).

There are known to be five major pathways of milk constituents' secretion across the mammary secretory epithelium from the blood into the milk (Shennan and Peaker, 2000; McManaman and Neville, 2003).

Four are transcellular, involving transport across at least two membrane barriers:

1) Membrane pathway,
2) Golgi pathway,
3) Milk fat pathway
4) Transcytosis.

The fifth is paracellular that allows direct exchange of interstitial and milk components:

5) Paracellular pathway

The transport through these pathways is affected by the functional state of the mammary gland and regulated by direct and indirect actions of hormones and growth factors. Description of the main pathways below is modified from McManaman and Neville (2003), Nguyen and Neville (1998) and Shennan and Peaker (2000):

In the **membrane pathway**, substances may traverse the cell membranes. Examples are water, ions (Na^+, K^+ and Cl^-) and small molecules such as urea, glucose and amino acids.

In the **Golgi pathway**, secretory products are transported to or sequestered by the Golgi apparatus and secreted into the milk space by exocytosis. Examples are aqueous solutes including the major milk proteins (casein, whey proteins), oligosaccharides (lactose), citrate, phosphate and calcium.

In the **milk fat pathway**, which is unique to mammary secretory cells, milk fat globules are extruded from the apex of the secretory cell surrounded by a membrane (milk-fat-globule membrane); some cytoplasm is sometimes included. Examples of secreting products transported this way are milk fat, lipid-soluble hormones and drugs, lipid-associated proteins, and the product of the Ob gene, leptin.

In **transcytosis**, that involves various organelles, there are vesicular transports of wide range macromolecular substances derived from serum or stromal cells, including: serum proteins such as immunoglobulins (particularly during colostrum formation), albumin and transferrin; endocrine hormones such as insulin, prolactin and oestrogen; and stromal derived agents such as secretory immunoglobulin A, cytokines and lipoprotein lipase.

The **paracellular** pathway provides a direct passage of interstitial fluid and serum components into milk between the cells via leaky tight junctions. The paracellular route of secretion appears during different physiological and pathological states, particularly during colostrogenesis, late lactation and mastitis. As a generalisation, the paracellular pathway is absent during lactation and present during pregnancy (Nguyen and Neville, 1998). A paracellular pathway appears during other physiological states, such as at the cessation of lactation or when supra-physiological doses of oxytocin are given to contract the myoepithelium and obtain a milk sample. During involution and mastitis the epithelial barrier becomes leaky, although under these conditions it is not clear whether the leakiness should be attributed to the state of the tight junctions *per se* or to dead and dying cells which can leave holes in the epithelium. The disruption of the epithelium and the appearance of the paracellular pathway during mastitis are of a particular interest for this paper.

Milk composition changes during mastitis *continued*

Another unusual feature of the mammary gland is that the secretion is stored within the lumen of the gland, either in the secretory alveoli or duct system, until it is removed at intervals by the sucking (Shennan and Peaker, 2000) of the calf or milking. The milk ejection from the alveoli and ducts, as described by McManaman and Neville (2003), requires contraction of myoepithelial cells, stimulated by oxytocin released from the posterior pituitary as part of a suckling induced neuroendocrine reflex or acquired milking reflex in dairy cows.

Pathogenesis of milk composition changes during mastitis

There are a number of functions that can be disrupted during intramammary infection and a number of mechanisms that can be disturbed, the outcome is difficult to predict and will depend on the following infection (Benites et al., 2002; Pyorala, 2003; Bruckmaier et al, 2004):

a) Severity of the infection, varies from very little effect to completely inhibition of milk secretion depending on the mastitis-causing organism, its virulence and resistance of the host.
b) Extent of the infection, which may be localised to a few alveoli or encompass all alveoli. Quantifying this effect is difficult because in most cases, only one gland is infected; therefore, the effect is diluted when measurements are made on a whole-animal basis. There are considerable amount of variability depending on individual animal variation, breed (in the case of mixed breeds in the same herd), age, and stage of lactation.
c) Alteration of the metabolic activity of milk producing cells, including reduction of milk synthesis, and interference with ion balances, either by a reduced concentration of a galactopoietic hormone or by an increased concentration of an inhibitory hormone or/and an inflammatory mediator.
d) Interference with precursor's availability for milk synthesis due to: anorexia, decreased blood flow in the mammary gland or hormonal imbalance.
e) Disruption of epithelial integrity, by opening up paracellular pathway.
f) Decomposition of the milk constituents due to the presence of enzymes originating from leukocytes and mastitis-causing organisms.

Mastitis-causing organisms enter the mammary gland via the teat canal and multiply in the milk in the teat and mammary cisterns. As part of the cow's defence mechanism, the new intramammary infection is quickly followed by an influx of leucocytes into the milk and an increase of the milk somatic cell counts (Auldist and Hubble, 1998; Bruckmaier and Blum, 2004). The increase in tight junction permeability (Holdaway, 1990) across endothelial and epithelial layers is due to the products of the inflammatory reaction such as histamine, TNF, IFN-γ and acute phase proteins (Nguyen and Neville, 1998; Pyorala, 2003). This increase in permeability may be an important part of the inflammatory process as it allows immune components to reach the infection site. The enhanced paracellular diapedesis of leukocytes through the epithelial cells causes reduced tight junction integrity and hence exchange of constituents between the blood and the milk through the paracellular pathway (Auldist et al.,1995; Benites et al., 2002; Pyorala, 2003). The predominant leucocytes present in milk under such circumstances are PMNs. They are responsible for the high somatic cell counts (SCC) that is characteristic of mastitic milk and are associated with many of the changes to milk composition that occurs during mastitis (Auldist and Hubble, 1998; Pyorala, 2003; Bruckmaier and Blum, 2004).

Furthermore, microbial toxins and enzymes from damaged cells cause injury of secretory cells (Nguyen and Neville, 1998; Bruckmaier and Blum, 2004)]. N-acetyl-β-D-glucosaminidase (NAG-ase) concentrations in milk are increased with the onset of mastitis, and histological examination of mastitic glands revealed necrotic mammary epithelial cells. Such cellular damage can produce 'holes' within the mammary epithelium that can lead to changes in milk composition and short-circuit the blood-milk electrical potential in the same manner as opening of tight junctions. For example, lactose, which is synthesised exclusively by mammary epithelial cells, partially leaks into blood circulation through the damaged blood-milk barrier. Simultaneously, there is an increase of the concentrations of blood borne components in the milk of affected quarters, such as serum albumin and sodium and chloride ions; (Bruckmaier and Blum, 2004).

The tight junction leakage of mastitis is accompanied by a decrease in the rate of milk synthesis and secretion of the major specific milk constituents (Nguyen and Neville, 1998), while the secretion of other proteins like lactoferrin is simultaneously up-regulated (Pyorala, 2003; Bruckmaier and Blum, 2004). The concentration of caseins is reduced in infected quarters due to reduced secretion and increased destruction by blood-borne proteases on milk proteins and fat, for example plasmin (Holdaway, 1990; Auldist et al.,1995; Bruckmaier and Blum, 2004).

Some of the mastitis-causing organisms adhere to the surface of the epithelial cells and form colonies. Localised areas are probably exposed to higher concentrations of toxic products than other parts. Due to the inability of microbial endotoxins to affect the mammary tissue directly, Shuster et al. (1991) conclude that the decline in milk synthesis during endotoxin induced mastitis is likely due to the pathophysiological responses of the cow induced by inflammatory mediators (such as cytokines and eicosanoids) produced by mammary leukocytes upon interaction with the endotoxin.

In extreme cases, the cells may be so damaged that they may have to be removed possibly resulting in gaps appearing in the epithelium. The repair process in the damaged mammary gland tissue is accomplished by the proliferation of fibrous tissue, which is termed fibrosis. Fibrosis is the beginning of the formation of the cicatrices, and can start during an inflammatory response or develop from cystic dilatation. The cystic dilatation is a type of repair reaction that forms cysts in the dilated acini of the gland. The repair process replaces permanently the glandular tissue by connective tissue and consequently leads to reduction in milk production (Benites et al., 2002).

In addition, tissue debris, mastitis-causing organisms, fibrinogen leaking from the interstitial spaces is converted to fibrin and leukocytes form clots that occlude ducts draining the areas. In this case the secretion accumulates in the lumen and causes local involution of the affected area and opening of paracellular pathways.
During the inflammatory process, three mechanisms are involved in milk composition change: a decrease in synthesis, an increase in the permeability of the milk barrier and an increase in the proteolytic/ enzymatic activities in milk (Holdaway, 1990; Roux et al., 2003). Thus, the milk from an infected gland will be a composite of secretion from areas that are barely affected through to others that may no longer be producing milk. In other areas, the epithelium may even be destroyed. This complexity of effects means that interpreting the changes in milk composition will be extremely difficult.

Effects of mastitis on the milk production and composition.

Effects on milk production.

Intramammary infection, even if restricted to sub-clinical levels, has a negative effect on milk production.
The reductions in milk production probably are largely due to physical damage to the epithelial cells of the affected mammary gland, and a consequent reduction in the synthetic and secretory capacity of the gland as a whole. Any retardation of the capacity of the mammary gland to synthesise and secrete lactose is of particular importance in this regard, given the key role of lactose as the osmotic regulator of milk volume (Auldist and Hubble, 1998).
However, not all of the suppression of milk production associated with mastitis can be attributed to the damage of the mammary epithelium. Shuster et al. (1991) concluded that the hypogalactia in non-affected quarters is due to systemic effect of mastitis in the affected quarters or by the systemic absorption of an inhibitor of milk production from the affected quarters acting on the un-affected quarters. A number of inflammatory mediators, including cytokines and metabolites of arachidonic acid, changes in stimulatory or inhibitory hormone concentrations, and reduced milk precursor availability, may play a role. The milk production from the affected quarters is more evident than in the un-affected quarters, resulting from the localised inflammation. Other possible explanations for the local suppression of milk production include direct effects of locally produced inflammatory mediators, leukocytosis and localised mammary oedema. The situation is more complex as it is generally accepted that uninfected quarters can increase production and compensate in part for the decrease in production by the infected quarters (Holdaway, 1990). However, this compensation may only occur after the infection is cured.
The increased permeability of the blood-milk barrier in the affected quarters leads to a decrease in the volume or milk component concentrations. Not all of the decrease in milk output is due to reduced synthesis; some is the result of escape from the gland into the circulation (Shuster et al., 1991).

Effects on milk composition.

Effects on protein contents.

It is generally accepted that during mastitis, there is an increase in milk protein, that has been attributed to the influx of blood-borne proteins (such as serum albumin, immunoglobulins, the minor serum proteins) transferring,α-macroglobulin into the milk coupled with a decrease in caseins (Holdaway, 1990; Shuster et al., 1991; Auldist et al.,1995; Auldist and Hubble, 1998).
According to Auldist et al.(1995) and Auldist and Hubble (1998) this increase in proteins of blood serum origin during mastitis is possibly due to a disruption of the integrity of the mammary epithelia by microbial toxins and opening of the tight junctions.
The decrease in casein concentrations during mastitis is largely due to post-secretory degradation of casein by proteinases originating from mastitis-causing organisms, leucocytes or the blood and in part to a reduction in the synthesis and secretion of casein as a result of physical damage to the mammary epithelial cells by microbial toxins during mastitis. This has important implications for the manufacturing potential of the milk, particularly, but not exclusively, for cheese manufacture.
On the other hand, the whey proteins synthesised *de novo* are relatively resistant to proteolytic attack. However, in mastitic and high SCC milk, there is an evident decrease in α-lactalbumin and β-lactoglobulin. This would be, according to Auldist and Hubble (1998), partly due to impaired cellular synthetic and secretory function, and partly due to leakage of these proteins out of the milk and into the extra-cellular fluid via the paracellular pathways that proliferate during mastitis. To support their theory the authors mention that an elevated concentrations of α-lactalbumin in the blood of cows with elevated SCC is registered.
The lactoferrin concentrations are increased during IMI, possibly related to the immune function of this protein.
Mastitis is also associated with increases in the concentrations of many different enzymes in milk (Holdaway, 1990; Auldist and Hubble, 1998; Pyorala, 2003), which can play significant roles in the diagnosis of sub-clinical mastitis. The main caseinolytic enzyme in milk, plasmin, is normally found in milk in small quantities. The plasmin is derived from plasminogen which originates in the blood and probably leaks into the milk in greater amounts due to disruption of the epithelium. The primary function of plasmin in the blood is dissolving clots. Elevated activity of plasmin occurs both in mastitic milk and milk from late lactation. Plasmin is able to rapidly cleave β-casein into the smaller γ-casein and polypeptide fragments (protease peptones) which then diffuse into the whey. Proteolysis by activated plasmin from the blood, proteolytic enzymes from mastitis-causing organisms and phagocytes leads to poor curding, lowered cheese yield, a bitter taste of dairy products etc (Holdaway, 1990; Audlist et al., 1995; Auldist and Hubble, 1998).
Increases during mastitis in the concentrations of proteins in milk that originate in the blood can lead to an increase in the concentration of non-casein nitrogen.

Milk composition changes during mastitis *continued*

Effects on fat contents.

The effect of mastitis on the characteristics of milk fat has not been studied nearly as extensively as milk proteins. There are contradictory results in the literature dealing with this matter. For example, Auldist and Hubble (1998) report a decrease in fat concentration, but the majority of the authors recorded an increase in total fat content of mastitic milk (Holdaway, 1990; Shuster et al., 1991; Pyorala, 2003; Bruckmaier and Blum, 2004).

According to Bruckmaier and Blum (2004) the increase in fat concentration indicates that there is a reduced lactose synthesis and therefore reduced milk volume while the fat synthesis is only slightly depressed. Very similarly, Holdaway (1990) states that over a period of time, the total output of fat from a quarter is likely to be reduced, because of the lower volume of milk. In addition the leakage of lactose from the milk will take with it water and the volume of secretion left in the gland will decrease. The fat droplets however are large relative to the gaps between the cells and are contained within the alveoli and consequently their concentration increases.

Milk fat globule membranes are susceptible to the action of lipase enzymes, produced by leukocytes that invade the mammary gland in response to infection, resulting in breakdown of triglycerides, oxidation of fatty acids and off-flavours. It has been assumed that milk with a high SCC is more susceptible to spontaneous lipolysis. The factors that increase the hydrolysis of triacylglycerols in the fat droplet, during mastitis, are very poorly understood. One possible explanation is that this process may be accentuated by the addition of blood-serum components (Na^+ and Cl^-) to the milk during mastitis (Holdaway, 1990; Audlist et al., 1995; Auldist and Hubble, 1998).

Effects on lactose content.

It is well accepted that mastitis causes a decrease in the concentration of milk lactose.

The decline in milk lactose in affected quarters probably is in part due to the damage to the alveolar epithelial cells. Given the key role of lactose as the osmotic regulator of milk volume, the reduced lactose concentrations are unlikely to be due to reduced synthesis and secretion at the cellular level and can only be depressed; there is an increased influx of electrolytes during mastitis. This is because water would be drawn into the cells only in sufficient quantities to maintain osmotic equilibrium. The more likely reason for depressed lactose concentrations is the leakage of lactose out of milk via the paracellular pathways that proliferate during mastitis (Audlist et al., 1995; Auldist and Hubble, 1998; Bruckmaier and Blum, 2004). Therefore, the low lactose concentrations are dependent on the severity of damage to the tight junctions. Evidence for this exists in the elevated concentrations of lactose in the blood and urine of mastitic cows. Since lactose is synthesised only in the mammary gland and is not secreted through the baso-lateral surface of the mammary epithelium in significant quantities and is not metabolised elsewhere in the body, the plasma level of lactose provides a measure of the leakage rate of material from the lumen of the mammary gland into the blood stream (Shuster et al., 1991; Audlist et al., 1995; Auldist and Hubble, 1998; Nguyen and Neville, 1998; Bruckmaier and Blum, 2004).

The lactose concentration will also be depressed if the mechanism regulating the concentration of the major ions (K^+, Na^+, Cl^- and HCO_3^-) is impeded. This will increase the concentration of ions and by necessity to maintain osmotic equilibrium decrease lactose concentrations. Perhaps fermentation of lactose by mastitis causing organisms may also reduce its concentration (Auldist et al., 1995).

Effects on mineral content.

The ionic content of milk varies markedly from that of extracellular fluid which batches the acini of the mammary gland. Milk contains a high concentration of potassium relative to sodium, the later being actively removed from the secretory calls by an energy dependant ATPase, which is located at the baso-lateral surface of the cell. The concentrations of many minerals are altered during mastitis, and these changes can play significant roles in determining the manufacturing quality of the milk and diagnosis of sub-clinical mastitis. Potassium, the most abundant mineral in milk, leaks out of milk through the paracellular pathway. Consequently, its concentration decreases. Conversely, sodium, found in the blood in high quantities, leaks into the milk increasing concentrations above normal. The concentrations of chloride in milk from cows with sub-clinical mastitis are elevated probably due to the influx of blood constituents into the milk during infection (Holdaway, 1990; Audlist et al., 1995; Auldist and Hubble, 1998).

<u>Coincidental bacterial contamination.</u>

The bacterial contamination could lead to alterations in milk composition through the action of hydrolytic enzymes released by the bacteria (Auldist and Hubble, 1998). Bacterial contamination of bulk milk from sub-clinical mastitis does contribute to total bacteria count, but does not usually exceed 10,000 cfu/mL. More significant bacterial contamination of milk can occur when milk from cows with clinical mastitis is admitted to the bulk supply.

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