THE EFFECT OF *CORYNE BACTERIUM CUTIS* LYSATE TO CONTROL SOMATIC CELL COUNTS IN DAIRY COWS

by

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DEDICATION

This work is dedicated to:

DORIAN, my husband and best friend. For believing in me, all the love and continual encouragement and for the wonderful person you are.

Dad, for believing in me and always wanting me to achieve the highest goal.

Mom, for all your love and support and for being strong for my sake.

My brother, for being just you.

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DECLARATION

I hereby declare that this dissertation submitted by me to the University of the Free State for the degree, **Magister Scientiae Agriculturae,** is my own independent work and has not previously been submitted for a degree to any other university. I furthermore cede copyright of the dissertation in favour of the University of the Free State.

Christa Pretorius
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Chapter 1 General Introduction

The term mastitis generally refers to an inflammation of the mammary gland, regardless of the cause. The classic meaning of the word mastitis is derived form from the Greek word "*matos*" meaning breast or udder and the suffix "*itis*" meaning inflammation (Kehrli & Shuster, 1994). This condition is characterized by physical, chemical and usually bacteriological changes in the milk and pathological changes in the glandular tissue. (Blood & Radostits, 1989). Mastitis has long been recognised as one of the most costly diseases affecting dairy cows and a major cause of economic loss in the dairy industry worldwide. Mastitis is also the most important reason for the culling of cows in the dairy industry (Barkema *et al*., 1999; Chrystal *et al.,* 1999).

Mastitis as such is a disease that occurs in two main forms: clinical and sub-clinical mastitis. Clinical mastitis produces obvious clinical signs that call for the dairy farmer's attention and prompt veterinary care. Sub-clinical mastitis on the other hand often goes unnoticed and can only be detected if specific tests are performed on a milk sample (Nielen *et al*., 1995). Sub-clinical mastitis has an erosive effect on the economy of dairy farms as it causes a direct loss in milk quantity and quality in affected cows/farms.

The presence of high concentrations of somatic cells (white blood cells) in the milk, are generally associated with sub-clinical mastitis. In the vast majority of the cases, an elevated somatic cell count (SCC) is associated with the presence of pathogenic bacteria and the involvement of the immune system. The SCC of milk is thus an indication of the health and hygienic status of the udder and therefore an indirect indication of the overall management on the dairy farm (Nielen *et al*., 1995).

The need to control the average SCC in a dairy herd is an important management aspect for dairy farmers. In South Africa, milk with more than 500 000 somatic cells/ml is not acceptable for human consumption and therefore cannot be sold (Department of Health, 1997). In addition, the value of milk for processing of cheese and yogurt also depends on its physical and chemical characteristics, e.g. lactose,

protein (quantity and quality), pH and calcium content. Furthermore the presence of high concentrations of SCC induces proteolysis in milk and this occurs in milk with a SCC as low as 250 000 cells/ml (Le Roux *et al.,* 1995). Proteolysis in milk causes two major problems, namely a decrease in cheese yield and a bitterness of the processed dairy products (Harwalkar *et al.,* 1993; Le Roux *et al.,* 1995).

The effect of only a few cows with an elevated SCC in the bulk milk (tank) is legible, while if the SCC score is so great, the entire milk from a tank is penalised, although the vast majority of the cows have low cell counts. In order to maintain the bulk milk SCC at low levels, good management and hygiene practises are essential on the diary farm. The SCC can be effectively controlled with antibiotic therapy, but this practice is currently not very well accepted. Strict control over milk quality often penalises the producers that sell milk containing traces of antibiotics, as this milk will be unsuitable for further processing. In addition over the last few years, there has been an increased consumer demand towards chemical free and more natural animal products. There is a need to develop alternative strategies, as part of good management practices (hygiene, good milking techniques) to improve udder health, milk quality and to control SCC in the diary industry.

The potential use of natural immunostimulant substances for human and animal health is gaining interest (Eid *et al*., 1995). Researchers claim that a lysate of *corynobacterium cutis* - a non-specific immunostimulant, boosts the immune system of animals, rendering them more resistant to diseases in general. Amongst its various uses, there are reports of the potential effect of a *Corynebacterium cutis* lysate-Ultra Corn® in reducing the SCC's in dairy cows (Won-Chang Lee *et al*., 1996).

The main aim of this study was to evaluate the effectiveness of repeated inoculations of a *Corynebacterium cutis* lysate (Ultra-Corn®), to reduce the milk SCC in commercial dairy cows. An additional aim was also to evaluate if these inoculations had any detrimental effects on milk quality.

Chapter 2 Literature review: Mastitis and its importance to the dairy industry

2.1 Introduction

The term mastitis may be defined as an inflammation of the mammary gland, almost invariably due to the effects of bacterial or mycotic pathogens (Merck Veterinary Manual, 1986). It is characterized by a physical, chemical and usually bacteriological change in the milk, as well as pathological changes in the glandular tissue (Blood & Radostits, 1989). Mastitis is a disease that affects a large number of dairy of cows throughout the world. A survey conducted in the major milk producing countries indicates that each year, mastitis affects 15 to 20% of cows (Phelps, 1989).

2.2 Classifications of mastitis based on its severity

Since mastitis is a disease that has different levels of intensity and may be caused by different types of organisms, there exists a complete lexicon to describe the disease. It is therefore important to be able to recognise the different types of mastitis in order to know what preventive measures or treatments to use. There are several types of mastitis based on the severity of the disease.

2.2.1 Sub-clinical Mastitis

This type of mastitis does not exhibit clear, visible signs. It is said there are 15-40 sub-clinical cases of mastitis for every clinical case of mastitis. There are two types of sub-clinical mastitis, namely infectious and non-infectious mastitis.

2.2.1.1 Infectious Sub-clinical Mastitis

The most important symptoms of infectious sub-clinical mastitis are elevated SCC's and the presence of pathogenic bacteria in the milk, as well as decreased milk yield from the infected udder.

2.2.1.2 Non-Infectious Sub-clinical Mastitis

Non-Infectious or aseptic sub-clinical mastitis is characterized by an elevated SCC and the absence of pathogenic bacteria in milk, and develop under traumatising conditions. Irrespective of its cause, aseptic mastitis indicates a high risk factor or pre-disposing condition for the development of infectious mastitis.

2.2.2 Clinical Mastitis

This type of mastitis is associated with visible clinical signs of different severity or gravity. According to these signs and the severity thereof, this form of mastitis can be further classified as:

2.2.2.1 Sub-acute Clinical

This form of mastitis is a very mild and gradual progressive form of the disease. Deviations from the healthy conditions of the udder are minimal and frequently amount only to a reduction in milk yield and limited microscopic changes in the milk. Often flaky particles in the milk is observed, especially in initial ejection of milk

2.2.2.2 Acute Clinical Mastitis

Cases of acute clinical mastitis are always associated with distinct symptoms of udder inflammation, e.g. redness, swelling, elevated body temperature (above 39℃) and increased sensitivity of the udder skin and tissue, as well as changes in milk secretion. The secretion of the mammary gland is visible altered; the milk often has a different consistency and appearance than normal milk.

2.2.2.3 Per-acute Clinical Mastitis

This is the most serious form of mastitis. It commonly destroys extensive portions of udder tissue, affects the general well-being of the animal and frequently kills the cow.

Symptoms like pain, fever (above 41°C), swelling, redness, shock, depression, shivering, and dehydration and body weight loss are common in affected cows.

2.2.2.4 Chronic Clinical Mastitis

Inadequate treatment of acute forms of mastitis is often the major reason for the development of chronic clinical mastitis. In this form of mastitis, the disease episode lasts for several weeks or months. Usually it is repeated but mild clinical attacks, generally without fever. The milk seems to have a lumpy texture and the quarters are sometimes swollen. The quarters may become hard (fibrous indurations). Antibiotic treatments often do not work, and this seems to be the most obvious explanation why mastitogenic bacteria frequently survive in chronically affected udders. These are excreted with the milk and spread to healthy and susceptible udders by means of the milker's hands and or the milking machine. Thus, cows with chronic clinical mastitis are very dangerous sources of infection for healthy cows.

2.2.2.5 Environmental Mastitis

This type of mastitis caused by bacteria such as colliform bacteria (e.g. *E.colli*) of which the main cause is a contaminated environment e.g., manure. Dairy cows may lie down in an enclosed area with a lot of manure present; therefore the colliform like bacteria can get easy access to the udder and teat canal.

2.3 Factors contributing and associated with mastitis

Mastitis is a difficult problem to comprehend because it is a disease caused by many factors (Table 2.1). The response of dairy cattle being exposed to stressful conditions is modified by breed, sex, physiological, metabolic and other factors (Giesecke, 1985; Giesecke *et al.*, 1988)

Table 2.1 Main classes of predisposing factors to mastitis in dairy cows

Class	Predisposing conditions
Genetic	Deficiencies of certain characteristics of modern dairy cows e.g. size, shape and suspension of udder, morphology of teat and teat canal, milking ability and milk flow rate that affect the natural defence mechanisms of the udder against infection.
Environmental	Without proper management factors such as reproductive cycles, rearing, sheltering, culling, feeding, milking, hygiene, disease control and prevention can have a huge impact on the occurrence of mastitis. Geographic, seasonal, climatic and weather conditions eliciting stress in dairy cattle
Physiological	Stress, milk stasis, mammary regression, fluctuating activity of the leucocytic udder barrier, peri-parturient oedema, stage of lactation, composition of udder secretion, age.
Pathological	Circulatory disturbances (e.g. haemorrhages, haematoma and oedema). Trauma of udder and teats (e.g. external and internal lesions, penetrating and non-penetrating lesions of the teats). Disease other than mastitis (e.g. febrile diseases, metabolic diseases, disturbances of the digestive tract, genital conditions, skin diseases of udder/teats).

Adapted from Giesecke, et al. (*1994).

2.3.1 Genetic factors (inheritance)

Genetic variations in natural resistance to mastitis have been proven with regard to *Streptococcus Agalactiae* mastitis and high milk cell counts in cows (Grootenhuis, 1981). It is likely that selection for resistance to mastitis will be of very great importance in the near future. The SCC during the first lactation has also been examined as a basis for selection against mastitis. The rate of infection in subsequent lactations is lower in cows with low cell counts during their first lactation.

One of the inherited characteristics which may affect the susceptibility to mastitis is udder conformation and teat shape. Cows with cylindrical teats become affected more easily than those with funnel-shaped teats (Rathore, 1976). Cows with teats with inverted and/or funnel-shaped ends, or with a recessed plate-like end and cows which are habitually fast milkers and presumably have more diluted orifices are also reported to be more susceptible to mastitis. However based on the limited information available, it would seem to be imprudent to select against extremely fast milkers. A less desirable characteristic which should be selected against are deep udders, excessively low hind quarters and widely places teats (Tomas *et al*., 1984). A very important predisposing factor for mastitis is supernumerous teats, which are usually reservoirs for mastitogenic microbes and significantly increase the risk of mastitis.

Cows selected for several traits have higher somatic cell count (higher immune response), requiring almost two times less treatment, and their milk is thrown away half as often as the milk from cows selected for only one trait, although the latter produce more milk (Vaamonde & Adkinson, 1989). Genetically, there is a correlation between the percentage of milk fat and the incidence of clinical mastitis. The more a line of cows to produce milk with an above average fat content, the more it will be susceptible to mastitis.

2.3.2 Environmental factors

The modern dairy cow may often be seen living in an environment with various stressful conditions (stressors), which may affect her udder health. Direct and indirect effects on the animal initiate different types of acute and chronic stresses of a somatic, physiological, actual and anticipated nature.

The response of the lactating udder to stress usually involves mammary tissue regression. This is the cow's ultimate response to various stressful conditions and leads to the premature dying and discarting of milk-secreting alveolar cells and to a high SCC (Giesecke, 1978; Giesecke 1985). The most important environmental factors affecting udder health and milk SCC are nutrition, temperature, humidity, rainfall and hygiene.

2.3.2.1 Nutritional factors

It is commonly believed that the incidence of mastitis increases when milking cows graze lush pastures or are fed diets high in protein. However, according to a Danish study, there was no definite relationship between protein content in the diet and the incidence of mastitis (Madsen & Nielsen, 1981).

Non-protein nitrogen (NPN) is particularly harsh on the leucocytes, which protects the udder. Diets rich in NPN lead to an increase in ammonia in the blood and have a negative effect on the metabolism. In such diets enough fibre should be included to stimulate micro-organisms in the rumen that convert the NPN into bacterial protein. The commonly reported increased incidence of mastitis when cows are turned out to pasture, has led to the suggestion that high intake of estrogenic compounds may precipitate mastitis. However investigations into the role of these substances have yet been inconclusive.

According to a study conducted in Germany (Emmert & Wendt, 1991), there exists a significant relationship between the level of urea in the blood and bacterial colonization in the udder. In another study the addition of urea to diet increased the susceptibility to infection and increased the number of infections by more than 16% (Sterk *et al.,* 1978).

In recent years, several researchers have looked into the use of selenium and Vitamin E in the prevention and treatment of mastitis. Selenium and vitamin E are often considered together, as they have similar functions as anti-oxidants in the animal. Guarding the cells against potentially destructive free radical compounds formed during cellular metabolism (Chamberlain & Wilkenson, 1996). Cows supplemented with both had shorter rates and durations of clinical signs, a more rapid SCC response following microbial challenge, maintained lower colony-forming units, eliminated infections more rapidly and had less clinical signs (Jones, 2000). Vitamin E, supplemented with selenium, should be administered 21 days before the expected calving date as a prophylaxis against mastitis (Ivandija, 1985).

Vitamin E appears to be especially important beginning at 7 to 10 days before calving through to 3 to 5 days after calving. Metabolic diseases and malnutrition (e.g. milk fever, ketosis, sudden dietary changes, mineral deficiencies, etc) erode the general resistance and increases the susceptibility to mastitis. It is recommended that cows fed stored forages need vitamin E, supplemented at a rate of1000 IU/day for dry cows and 500 IU/day for lactating cows (Jones, 2000).

Additionally beta-carotene can be added to the diet as it enhances the destroying ability of somatic cells against bacteria during the late dry and also leads to a decreased SCC during the first 10 days of lactation. This will result in lower rates of new mastitis (Jones, 2000). A lack of copper supplementation in the diet can result in deficiencies in white blood cells, which can impair the ability of the animal to cope with disease.

2.3.2.2 Temperature, rainfall and humidity

Weather and climate is an important predisposing factor to the occurrence of mastitis. The exposure to intense cold, draughts, excessive humidity or heat predisposes dairy cattle to mastitis (Eckles, 1913; Shaldon, 1980). The incidence of clinical mastitis in the summer months is generally higher due to a warm and moist environment that increases pathogen exposure and bacterial numbers. The incidence of mastitis is associated with the prevalence of rain. The time spend by the cow out in the sun protects it against environmental mastitis due to the cleansing effect of the sun's radiation (Smith, 1985; Schukken, 1989).

Under South African conditions, dairy cattle are frequently subjected to chronic nutritional and heat stresses. Efficient water and thermo-regulation are indispensable for the cows' response to heat stress. Dairy cattle living in warm climates therefore require particularly strategic feeding, sufficient fresh drinking water and shade. Unless such requirements are met, fluctuating temperatures and elevated humidity will lead to chronic stress and increased occurrence of mastitis.

Research on how temperatures influence the incidence of mastitis shows how extreme temperatures interact with other factors to cause mastitis, but rarely will

temperature alone cause the disease (Klastrup *et al*., 1987). Temperature extremes may also influence somatic cell counts. Therefore the incidence of mastitis increases with extreme temperatures.

According to Klastrup (1978), research done in Denmark to study the incidence of mastitis in dry and humid environments found a significantly higher incidence of mastitis under humid conditions. In addition to hot and humid conditions, dairy cows in South Africa also experience great temperature variations, from very high during the noon in summer to very cold temperatures at night in winter. These constitute important stressors for the high producing dairy cow.

Wind can also affect the udder health. Under conditions where high temperature and high humidity of air coincide, cows respond by elevating their respiratory rates and lowering the metabolism (heat production), resulting in lower milk production. Cold draughts may cause localised under-cooling of the udder tissue, a weakening of the udder's internal defence mechanisms (e.g. the leukocytic udder barrier) and common bacteria present (e.g. *E. colli)* - to get a better opportunity to multiply and cause mastitis occur (Giesecke, 1978).

This type of stress is also prevalent when a hail storm occurs on an otherwise humid summer day. It is a regular feature of such sudden weather changes and temperate drops, that farmers tend to experience serious outbreaks of clinical mastitis.

2.3.2.3 Hygiene

The purpose of hygiene is to prevent the transmission of bacteria from one teat to another or from one cow to the next. Pasteur admitted at the end of his life that "the terrain is everything, and the microbe is nothing", meaning that pathogenic organisms could not cause disease in a healthy animal or plant source. Although optimum health is always the ultimate goal, it is not easy to attain it in herd management.

Milk SCC levels are usually the lowest in a clean, dry, comfortable environment. Meticulous attention to hygiene in the milking parlour is essential to ensure the

production of clean wholesome milk and to minimize the stress and loss of production due to mastitis and high SCC's. This means that it is necessary to do everything possible to prevent bacteria from entering the udder or bulk milk tank. Before any cow is milked, her teats and adjacent area of the udder must be cleaned and disinfected and milk from each quarter needs to be inspected for signs of mastitis by performing the milk strip test and if necessary the California Milk Test (CMT).

Teat cup liners should also be in perfect working condition as faulty liners or liners with abnormalities in shape and size are likely to cause damage to the teat. These liners also have a porous surface which is difficult to disinfect and is susceptible to filling with milk fat and other milk solids. That is why proper cleaning and disinfection is so important. Liners should be discarded when they loose shape or become rough or cracked.

At the end of each milking, the teats and the udder need to be protected from the conditions outside the parlour. The teat sphincter is the first line of defence a cow has to thwart invasion by mastitis pathogens. Dirty environments can create excessive bacterial contamination of the teat ends. When the cows leave the milking parlour, their teat sphincters are still relatively relaxed and remain open for about 30 minutes. If they lie down on contaminated bedding (with bacteria like *E. coli*) immediately after milking, while the teats are open and udder is unprotected. It is an easy way for bacteria to gain entry into the teat canal and infect the udder. The best way to prevent infection for the time immediately after milking is to dip or spray the teats with an approved disinfectant - before leaving the parlour to accelerate the closure of the sphincters to sanitize the teats and to promote the healing of any injury caused to the teats. Another valuable management aspect is to stimulate the cow to remain standing by means of feeding.

The most important reservoirs of mastitogenic bacteria are infected teat canals, mastitic udders and an unhygienic environment (McDonald, 1969; Natzke, 1977). The bacteria may be readily spread from such sources to uninfected milk glands by means of soiled udder cloths, the milker's hands, teat cup liners of the milking machine or, reverse flowing of milk in the system.

2.3.2.4 Milking machine

Sanitary milking habits are important to avoid the spreading of bacteria or their proliferation. Faulty milking equipment due to poor installation or maintenance can cause tissue trauma, teat damage, poor milking out, erratic vacuum levels and can also transmit infectious agents at milking time (Rice & Bodman, 2004).

It is very important to make sure that the milking machine does not exceed the recommended vacuum pressure recommended by the manufacturer. According to Blood and Radostits (1989) and Du Preez (2002) for most milking machines a pressure of 50 kPa is sufficient and pressures in excess of this are likely to cause injury by exerting excessive pressure on the teat. Large fluctuations in pressure are caused by inadequate vacuum reserve.

2.3.3 Physiological factors

Various physiological factors contribute to the occurrence of mastitis in dairy cows.

2.3.3.1 Mammary regression

If the cause of mammary regression is physiological, it is probably the cow's most effective way of maintaining udder health. Stress in lactating cows promotes premature mammary regression. Premature udder regression during lactation should be considered as a potentially dangerous predisposition to mastitis, because it erodes and reduces the efficiency of the natural defence mechanisms of the udder. Consequently, teat canals and udders become more readily infected with different types of mastitogenic micro-organisms. The same occurs with the healing of lesions because the udder tends to become less effective.

Stage of lactation, also has a great effect on the cow's susceptibility to intra-mammary infections. Particularly susceptible periods are those in which major changes in the functioning of the udder occur, namely the beginning and end of lactation and dry periods (Giesecke *et al.,* 1994).

2.3.4 Pathological factors

The immediate cause of mastitis is usually an udder infection with bacteria, including pathological tissue lesions. The infection develops after the bacteria have successfully passed through the teat canal to enter the teat and the gland's cistern and proliferate in the udder tissue. The bacteria enter through the teat canal and generally invade the udder by means of two ways (Giesecke & Van Heever, 1974; Giesecke *et al.*, 1979):

2.3.4.1 Direct transportation

The aspiration of bacteria from the teat canal deeper into the cistern and mammary tissue, during milking (by hand or machine) without proper disinfecting or cleaning between cows.

2.3.4.2 Indirect transportation

Milk globules contaminated in infected portions of the teat canal and floating upwards into the teat cavity, where bacteria is growing and moving during the milking interval. Teat canal lining averted during and contaminated after milking by the machine and returning to its normal position without post milking disinfectant teat dipping.

2.4 Economic losses due to Mastitis

Mastitis is a disease that leads to reduced milk yield and an increased number of clinical treatments, resulting in early cow culling (Shook, 1989; Gill *et al.*, 1990; Beaudeau *et al.*, 1993). Thus mastitis inflicts heavy losses to the producers in the dairy industry. Numerous reports have been published on the direct economic impact of mastitis.

All over the world attempts are being made to control bovine mastitis due to the huge effect on public health and the changed composition of milk from animals with

mastitis. These may have a harmful influence on the suitability of milk for processing and the quality of the processed products made from it. Mastitis commonly results in some degree of permanent impairment of milk secretion capacity in the cow. As milk from cows with clinical mastitis is unmarketable and milk from cows with sub-clinical mastitis is of inferior quality, an increasing number of milk processing plants and companies are paying much less for milk with a high SCC, than for good quality milk. Furthermore, milk yield decreases following sub-clinical mastitis. Additional economic losses results from the invested labour, feed, replacement costs, antibiotics, antiseptics, and laboratory and veterinary services (Giesecke, 1978).

Several studies have tried to quantify the economic losses associated with mastitis. In South Africa available data (relatively outdated) indicate that out of every 10 cows in a herd, 4 cows are mastitis negative, 1 has clinical mastitis, and 5 cows have sub-clinical mastitis. The elevation of the seriously increased prevalence of sub-clinical mastitis in approximately 75% of herds, has considerable implications for the productivity and economy of dairy farming, dairy processing, public health and the control of clinical mastitis (Giesecke, 1990). The losses from mastitis in South Africa during 1989 were estimated to be approximately R414/cow/year. This amount is without any doubt much higher currently, and will continue to escalate unless each dairy producer makes a determined effort in the prevention and control of mastitis.

Of the total loss, only approximately 18 % is due to clinical mastitis, whereas the major portion (82%) of the loss is associated with sub-clinical mastitis (Giesecke, 1990). For each R1.00 lost due to clinical mastitis, about R4.60 is lost due to sub-clinical mastitis (Giesecke, 1990). There is no doubt that mastitis is costing the average South African farmer a great deal of production, and in both preventative and curative measures (Veary *et al*., 1989). Another source of economic loss to the dairy producer is associated with the early culling of dairy cows (due to mastitis).

The association between milk production and SCC in dairy cattle is increasingly used to estimate the losses in milk production due to sub clinical mastitis. Because important management decisions regarding cost effective prevention and control of mastitis are based on this relationship (Bartlett *et al*., 1990). According to Schweizer (1983), Philpot (1984) and Conradie (1985) SCC's between 750 000 and 1 million

cells/ml are associated with a 13-18% reduction in herd milk production and the milk is unfit for human consumption. According to Harmon (1994) the mastitis or elevated SCC would usually be associated with a decrease in lactose, α-lacto albumin, and fat content of the milk, due to reduced synthetic activity in the mammary tissue. Although it can be noted that the reported losses from mastitis vary considerable in various studies, there is sufficient evidence showing that mastitis is a very costly disease for dairy farmers (Giesecke, 1990).

2.5 What is a milk somatic cell?

Milk somatic cells are primarily leukocytes or white blood cells (which includes macrophages, lymphocytes and neutrophils), which serve as a defence mechanism to fight disease (infection), and assist in repairing damaged tissue. Studies identifying cell types have shown that epithelial cells or the cells which produce milk are infrequently found in udder secretions, including those from the dry gland and range from 0% to 7% (Lee *et al*., 1980). During inflammation the major increase of somatic cells is due to the influx of neutrophils into the milk to fight infection (Harmon, 1994).

The cells found in the milk consist of about 75 % white blood cells or leucocytes and about 25 % epithelial cells. Leukocyte numbers increase in response to bacterial infection, tissue injury and stress. The epithelial cells increase as a result of injury or infection. Since both types of cells originate from the body, they are given the collective name somatic or body cells (Smith, 1985).

Somatic cells are microscopically small building elements found in all tissues and organs of the cow (Giesecke 1979) and are differentiated (e.g. nerve cells, muscle cells, gland cells, red and white blood cells) with specific functions e.g. producing tears, saliva, digestive enzymes, sweat, milk depending on their location. The secretory cells of the milking gland are integral parts of the lining of the secretory alveoli. They secrete the milk into the alveoli from where the milk collects and drains through the duct system to the gland and teat cistern. The milk collection and drainage system of the udder is also lined with somatic cells with special functions which differ from those of the cells lining the alveoli. The somatic cells lining the

udder cavity are known as the epithelial cells of the mammary gland. The teat too is lined with epithelial cells.

The cells of the mammary gland are subject to natural wear and tear and thus have a limited lifespan. The worn and damaged epithelial cells are discarded and excreted with the milk. In addition, in milk there are always some blood cells as well. These are predominantly leukocytes or white blood cells of with specific task of cleaning of the udder, protection and defence against udder infections and removal of damaged cells. The leukocytes phagocytose and destroy bacteria, which have entered the udder cavity. Hence it is apparent that the phagocytic leukocytes on milk are essential to prevent infection and to eliminate intra-mammary tissue damage associated with mastitis (Schalm *et al*., 1971; Dodd *et al*., 1975; Freeman & Clark, 1977). The presence of bacteria is associated with high SCC, particularly *Staphylococcus aureus.*

2.6 Normal cell counts in dairy milk

The normal SCC in milk from a healthy udder is around 100 000 cells /ml of milk for a healthy udder (Giesecke *et al*., 1994). Eberthart (1979) did a study in which it was estimated that 50% of uninfected cows have a somatic cell count below 100 000 and 80% of uninfected cows have a somatic cell count under 200 000 cell/ml of milk. The continuous process of degeneration and regeneration of mammary epithelium and natural leukocytic defence of the udder are associated with normal cell counts in milk. The baseline level of the SCC is not constant, but fluctuates, depending on conditions such as stage of lactation, fraction of milk and others (Giesecke, 1979; Giesecke *et al*., 1988). In standard foremilk quarter samples it ranges from several tens to hundreds of thousands of cells, usually amounting to less than 500 000 cells per ml of milk (Tolle, 1970; Dodd *et al*., 1975).

At SCC values of 350 000 cells per ml of herd milk, in herds with less than 50 cows, one can expect an increased number of cows will show udder disease predominantly due to infectious sub-clinical mastitis. In herds with more than 50 cows in lactation, that udder health situation already becomes critical at 250 000 cells per ml of milk.

The SCC is usually high during the initial first two weeks of production after calving due to presence of colostrums, when the solids in the milk is more concentrated and lots of stress is involved with the parturition and onset of lactation. Various factors other than udder infection can result in increased SCC in milk. Herd differences in the SCC levels of milk also exist that are not attributable to the cows or udder infection (Giesecke & Van den Heever, 1974; Giesecke *et al*., 1988).

2.7 Practical significance of SCC determinations in herd milk

From the point of view of modern herd management on udder health, the SCC in bulk milk is the most practical way available at present to monitor the efficacy of mastitis prevention and a control program. The somatic cell count is a very sensitive indicator of udder health. Increased SCC values of individual cows signal inflammation of the udder and a high bulk milk SCC indicates deficient udder health management (Giesecke *et al*., 1994).

High SCC's result in decreased milk production, changed composition and reduced dairy technological usefulness of the milk, reduced hygienic quality and safety of milk, and most important of all, increased production costs and decreased profits (Giesecke, 1978; 1979; 1985*).* The SCC in a bulk milk tank is of such importance to the dairy industry that milk buyers and legal regulations often demand that each commercial dairy farmer should regularly (monthly) monitor and report the SCC status in the bulk milk produced by his herd. Milk buyers in South Africa penalize farmers who produce milk with more than 300 000 cells per ml (Giesecke, 1985).

Several scientists have discussed the SCC in milk from the point of view of the diagnosis of mastitis (International Dairy Federation, 1987) and modern herd and management of udder health and mastitis control (Giesecke, 1979; Philpot, 1984; Du Preez *et al*., 1989; Giesecke *et al*., 1989; Veary *et al*., 1989).

From data collected it is apparent that the determination of SCC in herd-milk repeated at monthly intervals facilitates the monitoring of the herd's udder health and management situation. Any continuing trend of an increase in monthly SCC values is evidence that the prevalence of sub clinical mastitis in the herd is escalating. This

should prompt the milk producer to call for a veterinary evaluation of the herd management and mastitis control programme.

2.8 Factors affecting somatic cell counts at individual cow level

The differences in SCC is possibly due to genetic factors, udder infection (mastitis), teat or udder injury, age of cow, stage of lactation, season, stress, day to day variations and management factors. Most of these factors are more than in one way related to deficiencies in the management of dairy herds.

2.8.1 Mastitis, teat and udder injury

The most important factor affecting SCC of an individual cow is the infection status of the udder. General agreement rest on the values of less than 100 000 cells/ml for uninfected cows and greater than 300 000 cells/ ml for cows infected with *S. aureus* and *S. agalactiae*. Cows with SCC's between these values may be recovering from a recent infection, have sustained an infection, sustained an injury or be infected with a less important organism such as *Corynebacyrium bovis.* When the udder or teat is severely injured there are large increases in the SCC.

2.8.2 Cow age

General observations indicate that SCC increases with advancing age. This is primarily due to an increased prevalence of mastitis in older cows. The prevalence of mastitis in infected quarters increases with age, peaking at 7 years (Schukken *et al*., 1989). It may also be a result of a greater cellular response to infection or of a greater amount of permanent udder damage after infection in older cows. Older cows, especially after four lactations (Quinn *et al*., 1994) were submitted to more lactations, increasing the risk for mastitis and udder tissue damage (Du Preez, 2002). It is postulated that young animals have diminished susceptibility due to a more effective host defence mechanism (Dulin *et al*., 1988).

2.8.3 Stage of lactation

In general the SCC is elevated immediately after calving and remains elevated for a few weeks regardless of the infection status. This SCC elevation appears to be part of a cow's natural immune system response in preparation for calving and the onset of a new lactation. This enhances the mammary gland defence mechanisms at this critical pre-parturition time. Quarters with no infections generally show a rapid decline in SCC within a few weeks postpartum. Schukken *et al*. (1989) reported that the first month of lactation is the most sensitive period for risk of mastitis in the cow, even in well managed herds.

On the other hand, the SCC of cows late in lactation are higher than the average throughout lactation, but this is due to an increased prevalence of sub-clinical infections late in lactation and a reduced milk flow production. Some cows will also exhibit an increase in SCC at the end of lactation without having mastitis, but this generally only occurs immediately before drying off or after milk production has dropped below 4kg/day. In short the SCC of uninfected cows is high at freshening, lowest from peak to mid-lactation and the highest at drying off. Harmon (1994) suggested that a modest rise in SCC at the end of the lactation period is in fact a dilution effect.

2.8.4 Season

Generally SCC's are the lowest during the winter months and the highest during the summer (Dooho & Meek, 1982). This coincides with an increased incidence of mastitis in the summer months, which has been reported in several studies (Smith, 1985). The reasons for these seasonal variations are as yet, unknown and only speculated to be the effects as housing and temperature changes on infection status. Smith (1985) has shown the rate of infection with environmental pathogens to be highest in the summer and this coincides with the number of Colliform bacteria in bedding material.

2.8.5 Stress and trauma

Stresses of various types, including oestrus (heat cycles) have been also implicated in causing increases in the SCC (Dooho & Meek, 1982; Rice & Bodman 2004). Changes such as the isolation of an individual, mixing groups of cows or being chased by a dog have been shown to increase the SCC in dairy cows in the absence of mastitis.

External trauma such as rough handling of animals is frequently inflicted in cows as they are driven to the milking parlour and could be a risk factor leading to an increase in SCC. This could be as a result of animals suffering from bruises to the teats while running to the milking shed through muddy or unhygienic stretches, thereby predisposing the cows to environmental pathogens (Quinn *et al*., 1994)

2.8.6 Day to day variation

In dairy cattle it has been reported that the cell counts of cows also vary from day to day with up to 25% of the baseline count. The variation is small in uninfected cows, but may also be larger in cows with active infections (Kirk, 1984). There can be considerable differences in SCC of individual cows from day to day, even if samples are taken on successive days. It has been suggested that this is a normal physiological variation and that these periodic large increases in cell counts are due to stress or injury infections that were eliminated before being detected. Donovan *et al*. (1992) suggested that day-to-day variations in milk SCC could be due to other factors affecting the SCC, such as age, stage of lactation, environmental temperature and stress.

2.8.7 Technical factors

The methods of transportation, storage and electronic cell counting of the milk sample may all have an influence on the resultant values. Different labs use different testing equipment and may find different values on the milk sample, especially when the SCC is very low. These minor differences are relatively

unimportant, provided that there is consistency in handling and processing of samples.

2.8.8 Management factors

Mastitis control procedures such a teat dipping, dry cow treatment, milking machine maintenance and the use of single service paper towels have been useful in reducing SCC. There are many advantages for using dry cow therapy to treat sub-clinical mastitis especially when the treatment is long acting. The levels of the specific remedy (antibiotic) must be high enough for long enough periods in the udder to kill mastitis causing organisms and treatment is much easier when there is no milk loss. The dry period should be approximately 60days and should be seen as time for the time for the dairy cow to recuperate.

2.9 Determination of SCC

The methods used to determine SCC in milk includes direct microscopic somatic cell count (DMSCC), electronic somatic cell count (ESCC), bulk milk cell count (BMCC), individual cow cell count (ICCC) and the California Milk Cell Test (CMCT). Due to high costs of laboratory work, the SCC in milk from udder quarters is determined increasingly by means of cow- slide CMCT evaluations. If correctly and regularly applied, the CMCT facilitates the estimation of increased SCC levels at an early stage before symptoms of mastitis are clinically apparent.

As mentioned, udder health problems in a herd are indicated by a SCC level of more then 350 000 cells per ml of bulk milk, on average. At this SCC level udder-diseased cows are present in the herd and represent approximately 20-30% of the lactating cow herd and 10-15% and more of the udder quarters may show a positive CMCT reaction. However, in such herds further escalation of udder health problems can be checked and prevented quickly. Udder health can be improved rather successfully by means of purposefully selected and applied measures of mastitis prevention and control and a professional approach to management of udder health. Obviously, such management depends on regular SCC determinations in herd milk, experienced veterinary interpretation of the SCC data generated, as well as the dairy

farmer's decision to keep the SCC level in his herd milk not only below 350 000 cells per ml milk, but well below 250 000 cells per ml of milk.

Cell counts are usually performed on the same samples used for bacteriology (cultural examination) and serious errors are avoided if the samples are always taken at the same stage of milking. In bulk milk the SCC is usually determined at laboratories equipped with special (and very costly) electronic cell counting instruments. In South Africa such instruments are used at several public health laboratories of the municipalities, diagnostic laboratories of the directorate of animal health, as well as dairy technological laboratories of several major milk buyers.

Such monitoring facilities are available in the main areas of milk production and consumption. It is therefore difficult to understand why S.A. milk producers and the dairy industry as a whole do not fully support and utilize the diagnostic facilities and veterinary knowledge available for monitoring and improving herd management and udder health (Du Preez *et al*., 1989; Giesecke *et al*., 1989; Veary *et al*., 1989).

2.10 Effects of high SCC on milk production

Research has shown conclusively that, in addition to a range of other changes in milk, there is a negative correlation existing between SCC values and milk yield. This correlation means that the higher the SCC value, the lower the milk yield. This relationship extends to include a high prevalence of clinical cases of mastitis and a high CMT result in quarters with high cell counts (Gill & Holmes, 1978).

Mean decreases of milk production related to escalating SCC values in the milk can be determined readily and fairly accurately. Table 2.2 shows average milk production losses for individual cows based on their individual SCC in Ontario DHI herds

Table 2.2 Relationship between somatic cell counts (SCC) in milk and percentage milk losses related to CMT scores of quarter-, cow and herd-milk samples

CMT Score	Range of SCC/ml of milk	Mean SCC/ml of milk	Mean % milk loss related to CMCT scores of milk samples from:					
			Quarters				cows	herds
	A*	B*	A*	B*	C*	D*	A*	B*
0	0-200.000	100.000	-	-	-	-	-	-
Trace	150.000-500.000	300.000	5.9	3	6	7.4	6	5.4
1+	400.000-1.500.000	900.000	13.6	11	19	17.4	10	5.9
2+	800.000-5.000.000	2.700.000	24.5	26	30	27.8	16	14.0
3+	>5.000.000	8.100.000	37.8	46	42	39.8	25	20.4

*Values compiled from: (A) Schneider & Jasper (1964), Janzen (1969), Schalm *et al.* (1971); (B) Philpot(1984); (C) MacKay (1984); (D) Values determined and data referred to by Conradie (1987).

2.11 Milk quality and processed dairy products and the effect of SCC

Quality can be defined as "conformance to requirements." Someone sets the standards; the product or service then has to meets these specifications. Quality then is a value, a philosophy, and a system within which there is a conscious effort to meet goals or requirements. The somatic cell count (SCC) is commonly used as a measure of milk quality. Milk markets routinely rely on the somatic cell counts to help ensure a quality product. The SCC levels are monitored to assure compliance with state and federal milk quality standards.

In any business, standards are always established by the customers. The allied dairy industry processors, manufacturers, and regulators listen to consumers and set performance guidelines for dairy producers. The consumer demands safe and wholesome dairy products that can be purchased without having to "read the fine print." The notion that we have successfully delivered a quality product must be re-evaluated in light of the changing requirements set for dairy foods. Dairy producers need to listen to consumers, recognize their legitimate concerns, and adapt to a new

set of rules. Basically, there are three types of dairy producers: those that are uninformed, those that are trying to circumvent the system, and those that are genuinely interested in producing quality milk. Fortunately, most producers fall into the latter category which leads to bonuses being paid for higher quality milk where the relationship between mastitis (high SCC) and milk composition is understood.

The Pasteurized Milk Ordinance in the United States of America (PMO) requires that the total bacteria count in Grade A milk leaving the farm is <100 000cfu/ml and that the total bacterial count in commingled milk is <300 000cfu/ml. Most milk in the USA has much lower counts than these requirements. The PMO also requires that the SCC of grade A raw milk be <750 000 cells/ml. These requirements are to ensure public health and are not intended as dairy quality standards. In the 1970's and 1990's, raw milk quality payment incentive programs became common, particularly among cheese makers and milk processors (Barbano, 1991).

The adverse effects of using high SCC for cheese making include reduced curd firmness (Politis & Ng-Kwai-Hang, 1988a), decreased cheese yield (Politis & Ng-Kwai-Hang, 1988b; Barbano *et al*., 1991; Klei *et al*., 1998), increased fat and casein loss in whey (Politis & Ng-Kwai-Hang, 1988b) and compromised sensory quality. For these reasons, the cheese industry has provided dairy farmers premium quality payments to encourage reduced raw milk SCC's. Before the incorporation of protein into the regulated milk payment system, protein was also a common bonus payment item in milk quality payment incentive programs by cheese makers. These milk quality payment incentive programs typically have multiple criteria such as no delectable antibiotic and added water, total bacterial count, laboratory pasteurized count, low sediment test and low SCC, usually 300 000 cells/ml. The bonus amount paid usually increased as SCC decreased.

Chemical changes in milk composition, due to mastitis, reduce milk quality. For example, milk with a high SCC causes an increase in the whey proteins and a decrease in casein, resulting in considerably decreased cheese yields. Shorter (or decreased) shelf life and adverse milk flavors are the common results of an elevated

SCC score. High SCC increases the undesirable components and decreases the desirable components of milk.

Since mastitis is usually associated with elevated SCC values in milk and indicate abnormal, reduced-quality milk that is caused by an intramammary bacterial infection (mastitis), it is not surprising that changes in SCC's are associated with compositional changes in milk. The elevated SCC levels in raw milk are related to modifications of a wide variety of chemical, physical, bacteriological, technological and sensorial characteristics of milk, as well as dairy products processed (Giesecke, 1990). Such changes already start at moderate increases of SCC levels from 200 000 to 600 000 per ml of milk and become more pronounced at 750 000 cells per ml milk (Larsen, 1994).

According to Larsen (1994), regulations require that ordinary milk produced commercially must have less than 750 000 cells per ml milk. This threshold value for the cytological quality of milk has been introduced because milk with SCC values of 750 000 cells per ml milk is frankly inferior from a qualitative point of view. According to the 1997 regulations relating to milk and dairy products in South Africa: *"No person shall use or sell any raw milk intended for processing when bovine milk is subjected to the standard method for counting somatic cells in bovine milk, when the SCC exceed 500 000 cells per ml of bovine milk after three successive readings at intervals of at least seven days during the test period."* The inferior quality of milk cannot be improved by pasteurization or other dairy technological processes.

Due to high SCC's a couple of chemical changes in milk composition will take place which will reduce milk quality. So for example, milk with a high SCC causes an increase in whey proteins and a decrease in casein, resulting in considerably decreased cheese yields, affects cheese curd firmness, an increase in fat and casein loss in whey and a compromised dairy product sensory quality. A shorter (or decreased) shelf life and adverse milk flavours are the common results of an elevated SCC score. This means that the efficiency of milk processing and the milk quality of the dairy products manufactured, depends significantly on the initial quality of the fresh milk harvested (Giesecke, 1990). It is thus acknowledged internationally that raw milk produced on the farm must be of high initial quality. This is possible

only if the cows producing the milk have no mastitis so that the milk produced has low SCC values.

From extensive research it is apparent beyond any doubt, that the SCC is correlated with certain changes in milk. Such changes indicate generally that, the higher the SCC, the more undesirable blood components and less desirable milk components are present in the udder secretion (Larsen, 1994). Milk with low somatic cell count milk is useful from the point of view of dairy technology and public health, because of certain desirable characteristics such as elevated levels of lactose, butterfat, casein, calcium, phosphorus and heat stability of milk protein and low levels of sodium and chloride (Barbano *et.al*., 1991; Klei *et al*., 1998). In contrast, milk with a high somatic cell count has reduced levels of lactose, butterfat, casein, calcium, and phosphorus and heat stability of milk protein, as well as increased levels of sodium, chloride, serum protein and bacteria - potentially harmful to humans.

For the reasons discussed above, milk producers should clearly appreciate that, with on the escalation of SCC values in milk, the valuable properties of the milk decrease (Politis & Ng-Kwai-Hang, 1988b; Giesecke, 1990). Hence, milk with 200 000 cells per ml milk is good milk, whereas milk with more than 750 000 cells per ml milk is unacceptable milk from the point of view of dairy technology and public health. Currently, most markets pay a premium for low SCC, good-quality milk. One can appreciate the reasons for paying a bonus for quality milk when the relationship between mastitis (high SCC) and milk composition is understood. High SCC increases the undesirable components and decreases the desirable components of milk. The quality of pasteurized milk decreases when milk with high somatic cell counts is used. The production of quality milk begins with good hygienic practices.

Mastitis can be seen as one of the main factors affecting changes in milk composition, that leads to a lower milk quality (Mattila 1985). The changes result from a reduction in synthesis activity for the main components of milk (fat, lactose and casein), and also form and increase in the presence of blood elements due to inflammatory reaction e.g. proteins (serum albumin and immunoglobin), chloride and sodium.

2.12 Legal implications of milk SCC's

In some countries, such as the United States of America and South Africa, this relates mainly to the legal maximum limit for SCC. The second "legal" issue regarding cell count levels in milk is related to international trade considerations. Milk and milk products are valuable food items traded between various parts of the world. Inevitably any item that is traded becomes subject to standards and milk is no exception. The European Union, New Zealand, Australia and a few other countries have adopted a standard for maximum allowable cell counts in their Grade A type milk of 400 000 cell/ml. Canada presently is at 500 000 while the USA has an allowable legal maximum of 750,000 per ml. In the international trade arena it is likely that major milk exporters will lobby for their standard to be the international standard and may well make it difficult to compete unless that standard is met. If the countries like the USA want to be a player in the international milk export market, it will likely have to debate this issue and move towards a lower upper limit - similar to what may now exist in Europe.

2.13 Pathogens causing elevated SCC that leads to mastitis

Inflammation of the mammary gland that results from the introduction and multiplication of pathogenic micro-organisms in the mammary gland is a complex series of events leading to reduced synthetic activity, compositional changes, and elevated SCC's (Harmon, 1994). There are a great number of micro-organisms on and in cow udders. Watts (1988) identified 137 species and sub-species of microbes that can be associated with the mammary gland of the cow.

To infect an udder quarter, a micro-organism must first enter the quarter and the cow must be able to get rid of it before it multiplies. The following is a typical scenario that leads to mastitis infection. Following exposure to the microbe: The number of micro-organisms multiplies near the orifice of one or several teats. This is where hygiene and milking habits play an important role in preventing microbes from entering the teat.

2.13.1 Entry of microbes into the teat

The entering of micro-organisms, such as bacteria into the teat may be forced by milking machine, especially at the end of milking. Injured teats where the opening is to wide can be easily accessed by the bacteria. Milking habits and the prevention of injuries is thus very important.

2.13.2 Immune response of the cow against udder infection

The cow's first line of defence is to send leukocytes to eliminate the bacteria that may have entered the teat. If the response is insufficient, the bacteria will multiply by mitosis and under optimum conditions, many bacteria can double in number each 20 minutes. This means one bacterium can result in up to 16 000 000 bacteria in just eight hours. The cow will then show other immune responses such as fever. The effectiveness of the cow's immune system however depends on many factors.

The bacteria causing an elevation in SCC, which may lead to mastitis can be divided into 2 groups: minor and major pathogens (Eberhart *et al*., 1987). The most common major pathogens include *Stapylococcus aureus, Streptococcus agalactoctiae*, and colliform bacteria, *streptococci*, and *enterococci* of environmental origin. Certain outbreaks may be caused by *Pseudomonas spp., Actinomyces pyogenes, Serratia spp*., or other unusual pathogens. The major causative pathogens cause the greatest compositional changes of milk and leads to an elevation of SCC, with a big economic impact.

According to Harmon and Langlois (1986) Corynebacterium bovis and co-agulase-negative Staphylococci are considered to be minor pathogens. These organisms cause a moderate inflammation, with a SCC exceeding those of infected glands. Minor pathogens are infrequently associated with mastitis, huge changes in milk composition and dramatic decreases in milk production.

Staphylococcus aureus and *Strep. agalactiae* are considered to be contagious pathogens. These specific pathogens are located in the infected udder and can

easily be spread among cows during the milking process. The infections tend to be chronic and subclinical with clinical episodes (Harmon, 1994).

The colliform bacteria is gram-negative bacteria that include *E.colli*, *Klebsiella spp*., *Enterobacter spp*. and *Campilobacter spp*. *Streptococcus dysgalactiae*, *Streptococcus uberis* and *Streptococcus bovis* are environmental bacteria which are in the surroundings of the diary cow. Approximately 70 to 80% of the colliform bacteria infections become clinical and about 50% of the environmental streptococcal infections display clinical symptoms. About 60 to 70% of environmental infections exist for less than 30 days.

2.14 Vaccination against mastitis

Effective immunisation against mastitis has been a goal for researchers for many years. Several authors have reviewed problems associated with mastitis and high SCC's (Anderson, 1987; Yancey, 1993; Nickerson, 1999).

Mastitis is the inflammation of the mammary gland as mentioned earlier, yet the purpose of a vaccine against high SCC and mastitis should be to enhance the immune response. In the case of mastitis, an enhanced immune response causes the migration of large amounts of somatic cells to the mammary gland. However, the presence of the somatic cells in the milk is undesirable and penalized, as it is known that a high SCC indicates mastitis and reduces milk quality.

The definition of a successful mastitis vaccine may vary depending on the herd situation. Farmers may expect mastitis vaccines to reduce the severity and frequency of mastitis, prevent new infections and eliminate existing infections. The evaluation of mastitis vaccines is complicated by the biology of the various mastitis pathogens (Yancey, 1993).

2.14.1 Mastitis vaccines available and under evaluation

It is very unlikely that one vaccine will be able to fight all the different pathogens because of their different biology; therefore various vaccines were developed in the

attempt to fight the different pathogens. Here is but a few of the vaccines developed against mastitis:

2.14.1.1 Vaccines against *Staphylococcus aureus*

It is generally accepted that commercially available *Staph. aureus* vaccines have limited the ability to prevent new infections *(*Yancey, 1993; Nickerson, 1999). A 3-lactation study failed to show a reduction in the number of new *Staph aureus* infections when cows were vaccinated with a commercial available vaccine, but showed an increase in spontaneous cure rate of the vaccinated dairy cows (Pankey *et al*., 1985).

2.14.1.2 Vaccines against colliform bacteria

The use of vaccines against gram-negative bacterial mastitis has become a standard practice on many dairy farms in the United States *(*Ruegg, 2001). In several studies it has been demonstrated that these vaccines can be efficient (Hogan *et al*., 1992; 1995). These bacteria have the ability to stimulate the production of antibodies directed against common core antigens that the gram-negative bacteria share. These vaccines are considered efficacious even though the rate of intra-mammary infections was non-significantly reduced in vaccinated animals, but they significantly reduced the clinical effects of the infection. Experimental challenge studies have demonstrated that colliform bacteria vaccines are able to reduce bacterial counts in milk and result in fewer clinical symptoms *(*Hogan *et al*., 1992).

2.14.1.3 Vaccines against *Streptococcus uberis*

Due to a higher occurrence of mastitis caused by environmental Streptococci, attempts were made to develop vaccines against these pathogens. There has been a sustained, focused research effort to get vaccines directed against *Streptococcus uberis* (Leigh, 1999). The number of bacteria in the milk of animals that were challenged with the *S. uberis* vaccine were significantly reduced, but no significant reduction in SCC was found. According Ruegg, (2001) there are no vaccines commercially available that protect against *Streptococcus spp*. mastitis.

2.14.1.4 Non-specific immune-stimulants against mastitis

The potential use of natural immuno-stimulant substances for human and animal health is gaining interest (Eid *et al*., 1995). These authors claim that a lysate of *corynobacterium cutis* - a non-specific immuno-stimulant, boosts the immune system of animals, rendering them more resistant to diseases in general. Amongst the various uses, there are reports on the potential effect of a *Corynebacterium cutis* lysate in reducing the SCC's in dairy cows (Won-Chang Lee *et al*., 1996).

2.15 Medicines used against mastitis (a brief overview)

There are many medicines and remedies used worldwide to treat cows with mastitis. These can be divided into several different groups, according to the origin, mode of action and type of medicine.

2.15.1 Antibiotics

These are chemical compounds (natural or synthetic) with anti-bacterial activity (bacteriostatic or bactericidal). Over the past few decades up to now, cows with mastitis (clinical or sub-clinical) are more likely to be treated with antibiotics. These are also used on many dairy farms worldwide as part of the prophylactic control of mastitis and are used when the cows are dried off. Their use increases the risk of the presence of antibiotic residues in the milk, which renders it unfit for processing. Antibiotic residues in the milk are not well tolerated by regulatory agencies, milk processors and consumers (Giesecke *et al*., 1994). Furthermore, increasing evidence of bacterial resistance against a vast range of antibiotics, also limit their sustainable use in mastitis control of dairy cattle (Giesecke *et al*., 1994).

2.15.2 Homeopathic remedies against mastitis

Many farmers are currently focused on organic farming and therefore more natural or homeopathic remedies needed to be developed. While practitioners report good results of homeopathic treatment in clinical mastitis there are few studies showing acceptable results of these remedies

2.15.3 Isopathic and homeopathic remedies

Isopathic-homeopatic treatment is a regulatory therapeutic modality with the aim to promote the natural restoration of destroyed physical processes with the aid of homeopathic remedies.

The isopathic remedies are not directed against the illness and its symptoms, but they support the body's own regenerative ability, thus facilitating the healing processes. Thus isopathic treatment normalises the symbiotic balance of the endobiotic micro-organisms and their host. The healing of the sick cow and its sick udder can only succeed if it is possible for the whole body to be regulated. On the basis of isophathic remedy treatment, the treatment of mastitis is based on these pillars: removal of blockages and improvement of cell respiration; modulation of immune system; isopathic deconstruction of the mastitis-triggering organism back to a form which is non-pathogenic; cleansing of the bacteriological soil (Schneider, 2000).

2.16 Conclusions

Trouble-shooting with high somatic cell count problems require attention at, and evaluating nearly every aspect of milk production. The most important factor affecting the herd or individual cow's SCC level is an intra-mammary microbial infection. The causes of infections are many and the method of transmission involves a combination of many factors. The analyses of these factors combined with the herd history, cow environment, SCC and bacterial culture information provides valuable guidelines for better mastitis control. This should be focused on

prevention rather than treatment of clinical cases. In this regard, SCC provides valuable information to monitor udder health.

To consistently produce high quality milk with low somatic cell counts requires continual attention to numerous details. One of the most recent developments on mastitis control in dairy cattle is the use of vaccines to boost the immune system, to be able to cope with infections. Amongst these vaccines, two types have been experimentally tested. One which includes the use of specific antigens against specific pathogens and the other, non-specific immuno-stimulants, which intend to promote natural immunity against all types of mastitis. The effectiveness of these vaccines thus far is very poor in general, but promising. Therefore more research is needed to develop vaccines against mastitis with acceptable efficacy and compatible to those commonly used for other infectious diseases in livestock.

Dairy producers must become more committed to produce the highest quality milk possible. They and their employees need to focus on routinely following all the practises and guidelines that have been developed by researchers and consultants for promoting cow comfort, healthy udders and high quality milk. The economical impact of mastitis on the milk industry is so great that there is a need to develop better mastitis control methods for dairy cows.

The main aim of this trial was to evaluate the effectiveness of 3 weekly SC inoculations of *Corynebacterium cutis* lysate, to reduce the milk SCC in commercial dairy cows. An additional aim was to evaluate if this treatment has any detrimental effect on milk quality and quantity.

Chapter 3 Materials and Methods

In this study the effects of repeated inoculation of dairy cows with *Corynebacterium cutis* lysate (Ultra-Corn®) on somatic cell count and milk quality were evaluated in two separate trials, conducted at two commercial dairy farms (A and B) using a pasture based system.

Cows from both groups on the same farm were managed under the same conditions for the entire period, when the two trials were conducted. On both farms the cows were milked twice a day, 10 to 12 hours apart (starting more or less at 5h00 and 16h00). On both farms cows were grazed on pastures for the duration of the trial and received some supplementation (dairy meal according to production). Cows on farm A and B were all commercial dairy cows. The only difference was that cows from the treatment group received 3 weekly inoculations of *Corynebacterium cutis* lysate(Ultra-Corn®), while those from the control group received distilled water for injection (the same volume as the cows in the treatment group).

The two farms were evaluated separately, due to the possible differences between general management conditions, which could introduce serious confounding factors if the results from the two farms were combined. However, it can be considered that both dairy farms used an acceptable level of commercial dairy management practices and produced an acceptable yield per cow under South African commercial conditions.

3.1 TRIAL 1

3.1.1 Study Location

This study was conducted between the months of February and April (end of summer to mid autumn) on two farms (A and B) around Bloemfontein (Free State Province). Both farms had an average bulk milk somatic cell count of > 250000 cells/ml.

3.1.2 Experimental Animals

Plate 1 **Some of the experimental cows waiting to be milked**

Seventy commercial Holstein dairy cows at different stages of lactation (20 cows from Farm A and 50 cows from Farm B) with SCC of >250 000 cells/ml were used in this trial. The cows from each farm were paired according to stage of lactation (not more than 10 days apart) and somatic cell count (not more than 10 000 SSC/ml difference).

One cow from each pair was randomly allocated to a control group (C; Farm A, n=10 and farm B, n=25) and to a treatment group (T; Farm A, n=10 and farm B, n=25). This was done in order to obtain 2 similar groups of experimental animals (lactating cows) on each farm and to reduce the confounding effect of stage of lactation and SCC at the onset of the trial, on the final results.

3.1.3 Experimental treatments

Cows from the treated group received 3 weekly inoculations of Ultra-Corn® (4 ml at 20mg/ml, thus 80mg of *Corynebacterium cutis* lysate per cow) injected

subcutaneously (sc), while those from the control group received 3 weekly sc injections of 4 ml distilled water.

3.1.4 Collection of milk samples

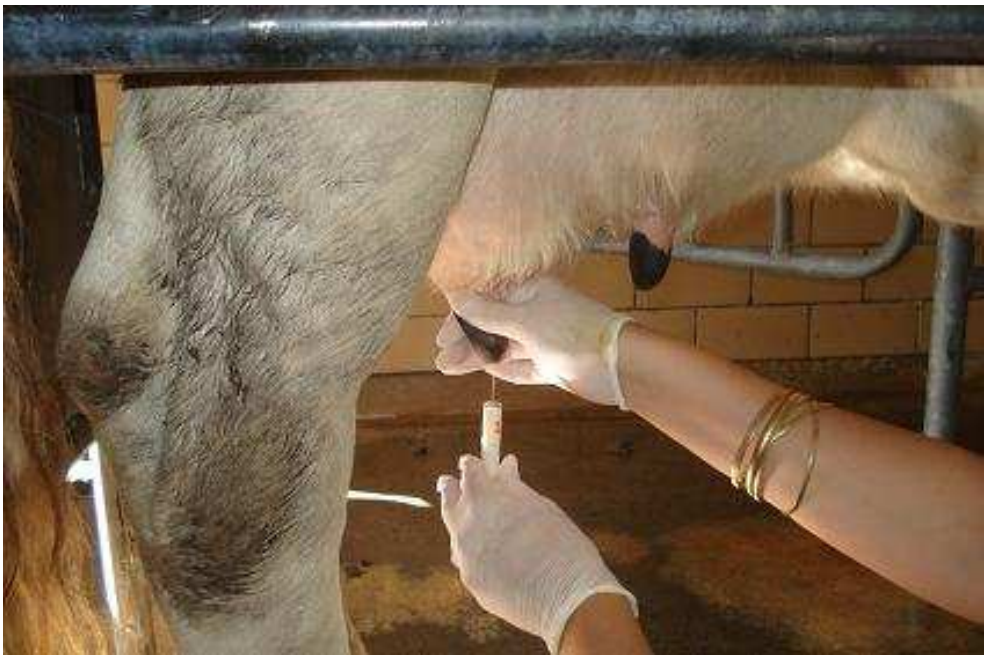

Plate 2 Aseptic collection of a milk sample for SCC analysis

At the beginning of the trial (week 1) and for the following 7 weeks, weekly milk samples were collected aseptically, from each quarter of all the experimental cows, before the morning milking, into 10ml sterile tubes and preserved in a cool box (4-5^{o}C) until delivered within 3 hours to the State Veterinary Laboratory in Bloemfontein.

Plate 3 **Milk samples arrive to the Veterinary Laboratory in Bloemfontein**

Plate 4 **Collection of a milk sample for milk composition analysis**

A second composite milk sample from all four quarters each cow was collected aseptically, directly from the glass measuring bottles in the milking parlour, into a 40ml-plastic milk sample container specifically prepared by the laboratory-MBISI, where the milk quality analyses were performed. The container was filled about 4/5

from the top (±30 ml). Each sample contained an added preservative tablet that is crucial to keep the sample fresh for up to 14 days. Caution was taken not to overfill the container as this would cause the fat to adhere to the lid and cause reduced fat percentages. Also not to under fill, as this would cause the sample to "oil off" as a result of excessive shaking.

The milk sample was left for 10 minutes to react with the preservative and was gently inverted to help the tablet dissolve. This was a very important step. The samples were kept in a cool and dry environment and direct sunlight was avoided. A sample was then taken of all four quarters mixed together. These specific milk samples were sent to the MBISI Laboratory at Irene, for analysis (composition). The protein, lactose, butterfat and urea content of the milk samples were determined and the results analysed statistically and compared between groups.

3.1.5 Processing of the milk samples for SCC

At the State Veterinary Laboratory in Bloemfontein, the milk samples were analysed for somatic cell content using a Coulter Counter (Z1 model). This is a particle counter, capable of rapidly and accurately determining the number of particles in a suspension, in this case milk. It does not specifically count somatic cells in milk but it must be calibrated to count particles of a specific diameter or larger. In order to calibrate the Coulter Counter (Z1 model) for somatic cell determination in milk, the sensitivity of the machine was first set, using the attenuation and aperture current settings. For somatic cells in milk, the diameter of the smallest cells (lymphocytes) was taken as 4.3 micron. Only fresh milk samples were used and were processed making use of the following steps as recommended by the Allerton Mastitis Council Scheme (1990) and the International Dairy Federation (1981; 1984).

3.1.5.1 Fixation

The somatic cells were first stabilized by means of adding Somafix®, a formaline-based fixative, to make them resistant to the subsequent treatment. One drop of fixative was added to a clean 5 ml disposable plastic test tube and then approximately 3 ml of well mixed fresh milk was slowly added. The fixative was an

electrolyte solution which reduces the fat globules to a diameter well below the set Coulter threshold (4.3 micron). The fixed milk sample was then placed in a water bath at 60±1℃ for 5 minutes and later cooled down to room temperature (±25℃).

3.1.5.2 Dilution

The fixed milk sample (at room temperature) was then diluted using a two-step automatic diluter (Hook and Tucker®) automatic diluter, that made a 1:100 dilution of the fixed milk samples using Somaton® as the diluent. Prior to diluting, each tube had to be inverted at least twice to make sure that the samples were well mixed. The automatic diluter was set to draw up 0.07 ml milk and then to dispense a total of 7 ml of the milk sample and Somaton® solution.

3.1.5.3 Dispersion of fat globules

The diluted milk samples were then placed into another waterbath at 80±1℃ for 10 minutes, with the meniscus of the samples situated well below the water level in the water bath. Evaporation was limited by covering the water bath with a lid. The processed milk samples were then cooled to room temperature (±25℃).The milk samples could also be placed in a cold water bath to save time.

3.1.5.4 Counting of the somatic cells

Somatic cell counting was carried out within one hour after the diluted milk samples were removed from the water bath, to ensure that these were cooled down to room temperature. Each sample was then mixed gently (without introducing bubbles) by inverting the tube twice before it was carefully decanted into a Coulter® *acuvette*. This was done shortly before the sample was analysed, otherwise the cells would precipitate. Care was taken not to introduce bubbles as these could be counted as particles.

During the counting procedure, treated milk was drawn automatically through a 100 micron aperture located between two electrodes. When any particle passes through the aperture, a small quantity of highly conductive liquid is displaced by the particle

which is of lower conductivity. The Coulter Counter can be set to count the number of particles in 0.1 ml or 0.5 ml of a milk sample. During counting, the immersion electrode of the aperture tube had to be adequately immersed in the sample.

The somatic cell count is the total of the number of particles in the particular volume that passes through the aperture, as reflected on the digital readout, multiplied by the dilution factor (usually 1:100) and 10 or 2 (depending on the volume of the sample) to bring the result to the number of somatic cells per millilitre. All analyses were performed using standard procedures, as recommended by the Allerton Mastitis Control Scheme (1990).

The analyses of the individual quarters maximized the variability of the measured parameters, in this case the SCC, which was limited when a mixed milk sample from all the udder quarters were analysed.

3.1.6 Processing of the milk for quality analysis (protein, butterfat, lactose and urea)

Composite milk samples were sent (on a weekly basis) from the Taurus branch in Bloemfontein, to MBISI Laboratories at IRENE, RSA. The milk samples were packed in boxes containing 80 or 40 samples each. The milk in 40ml collecting tubes were identified according to each cow's number, farm and date. A package list accompanied every shipment. The samples were received and sorted according to the information on the packing list.

A CL10 MICRO apparatus was used to perform the necessary analyses for the milk quality measurements in terms of urea, butterfat, lactose and protein content. Before analysing each batch of samples, the CL10 MICRO was calibrated by using an urea buffer and solution containing an urease enzyme. The CL10 Micro recorded the analyses results electronically onto a computer disk. These results were later processed by laboratory personnel, to obtain the urea, butterfat, lactose and protein as a percentage of the milk sample.

Plate 5 **Packaging of the milk samples for milk composition analyses**

3.1.7 Statistical analysis

The results in terms of SCC's for each quarter and milk solids composition (protein, butterfat, lactose and urea) from each cow collected weekly during this trial, were compared between the 2 groups (Treatment vs Control) using an analysis of variance ANOVA for repeated measures, at the 95% confidence level.

The results of the milk quality (urea, butterfat, lactose and protein) for each cow were also recorded on a weekly basis and compared between the 2 groups - using an analysis of variance ANOVA for repeated measures analysis, at the 95% confidence level (SAS, 2004).

3.2 Trial 2

Trial 2 was conducted on the same farms (A & B), as for Trial 1. The methodology used in this second trial was very similar to that described for Trial 1, with the

exception that in this trial, less cows were used on both farms and the doses of the *Corynebacterium cutis* lysate administered per cow, differed

3.2.1 Experimental Animals

Forty commercial Holstein dairy cows at different stages of lactation (14 cows from Farm A and 26 cows from Farm B) with SCC's> 250.000 cells/ml were used in this trial. The cows from each farm were paired (treatment and control) according to stage of lactation (not more than 10 days apart) and somatic cell count (not more than 10 000 SSC/ml difference).

One cow from each pair was randomly allocated to a control group (C; Farm A, n=7 and farm B, n=13) and to a treatment group (T; Farm A, n=7 and farm B, n=13). This was done in order to obtain 2 similar groups of experimental animals (lactating cows) on each farm and to reduce the confounding effect of stage of lactation and SCC at the onset of the trial, on the final results.

3.2.2 Experimental treatments

Cows from both groups were managed under the same conditions for the entire trial period (12 weeks). The two farms were evaluated separately, because of the differences between farms in terms of general and management conditions. However, it can be considered that both dairy farms used an acceptable level of commercial dairy management practices and produced an average milk yield per cow for South African conditions.

Cows from the treated group received 3 weekly inoculations of Ultra-Corn® (*Corynebacterium cutis* lysate at 20mg/ml) at a dose of 2ml/100kg (thus 40mg/100 kg) injected subcutaneoulsly (sc), while those from the control group received 3 weekly sc injections of distilled water (2ml/100 kg) as a placebo.

3.2.3 Collection and analysis of the milk samples

The milk samples were collected and analysed in the same laboratories as those used for trial 1. Exactly the same procedures were used as described for Trial 1.

3.2.4 Statistical analysis

Identical statistical procedures were used as described for Trial 1.

Chapter 4 Results and Discussion

4.1 TRIAL 1 - Effects of a low dose of Ultra-Corn® (4 ml per cow, thus 80mg of *Corynebacterium cutis* lysate per cow) on milk SCC and composition

4.1.2 Somatic Cell Counts

The mean (± s.d.) milk SCC for Holstein cows during the 8 weeks of this first trial period, from both groups (treated and control) in Farms A and B are presented in Tables 4.1 and 4.2, respectively. The milk SCC fluctuations during the same period are depicted in Figures 4.1 and 4.2, for the Holstein cows on farm A and B, respectively.

Table 4.1 Mean (± s.d.) somatic cell count (SCC) (x 10^3) in Holstein cows on Farm A during an 8 week trial period following three weekly inoculations with 80mg of *Corynebacterium cutis* lysate per cow

Weeks in trial	Group of cows	
	Control (N=10)	Treated (N=10)
1* ns	356.7 ± 2.4	348.5 ± 9.5
2* ns	390.9 ± 8.0	267.4 ± 3.2
3* ns	286.9 ± 3.7	347.3 ± 7.1
4 ns	327.2 ± 6.9	262.6 ± 3.8
5 ns	448.5 ± 2.5	134.5 ± 3.4
6 ns	303.1 ± 3.8	243.3 ± 5.0
7 ns	635.4 ± 9.1	785.7 ± 5.2
8 ns	232.2 ± 4.3	142.4 ± 2.6

ns- Means within rows do not differ significantly (P>0.05); *- Weeks when *Corynebacterium cutis lysate* was inoculated into the cows in the treated group

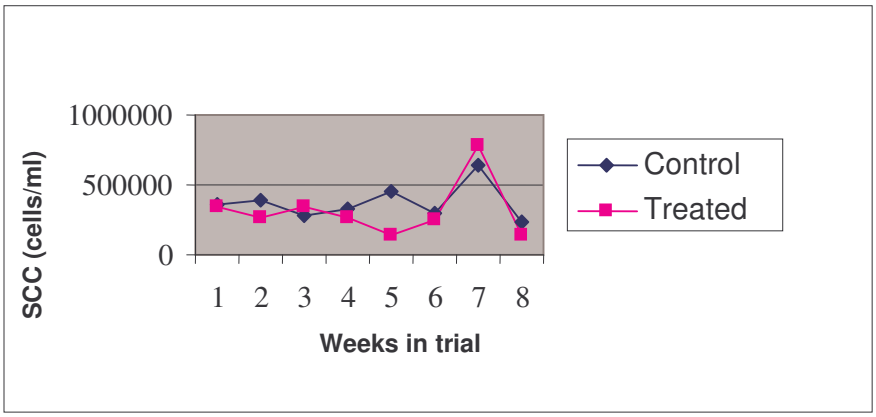

Figure 4.1 **Mean SCC in milk of Holstein cows during an 8 week trial following three weekly inoculations with *Corynebacterium cutis* lysate (80mg/cow) on Farm A**

Table 4.2 **Mean (± s.d.) somatic cell counts (SCC) (x 10^3) in Holstein cows on Farm B during an 8 week trial period following three weekly inoculations with 80mg of *Corynebacterium cutis* lysate per cow**

Weeks in trial	Group of cows	
	Control (N=25)	Treated (N=25)
1* ns	1413.8 ± 1.3	993.0 ± 8.1
2* ns	797.9 ± 1.0	941.4 ± 1.0
3* ns	749.1 ± 9.2	772.5 ± 1.7
4 ns	752.7 ± 1.4	8471 ± 1.5
5 ns	1009.6 ± 1.2	847.1 ± 1.1
6 ns	626.3 ± 9.9	698.7 ± 8.3
7 ns	1641.9 ± 3.1	2023.8 ± 6.3
8 ns	3241.2 ± 7.1	5113.8 ± 1.2

ns- Means within rows do not differ significantly (P>0.05); *- Weeks when *Corynebacterium cutis* lysate was inoculated into the cows in the treated group

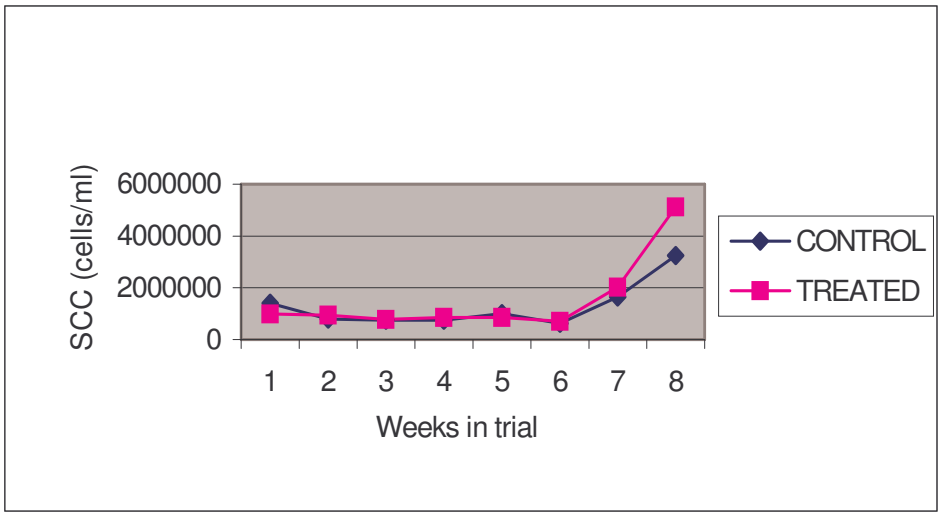

Figure 4.2 **Mean SCC in milk of Holstein cows during an 8 week trial following three weekly inoculations with *Corynebacterium cutis* lysate (80mg/cow) on Farm B**

On both farms (A and B), no significant differences (P>0.05) in terms of mean SCC's were observed between the cows treated with a reduced dose of Ultra-Corn® (80mg of *Corynebacterium cutis* lysate per cow) for the 3 consecutive weeks and those not treated (control), during a 8 weeks trial period.

The results obtained in this trial indicate that a reduced dose of Ultra-Corn® (4 ml/cow), corresponding to 80 mg of *Corynebacterium cutis* lysate is not effective to significantly reduce the SCC in dairy cows. These results are not in agreement with those reported by Won-Chang Lee *et al*. (1996), who reported a significant reduction in SCC in dairy cows treated with 3 weekly inoculations of a *Corynebacterium cutis* lysate *(*Ultra-Corn®) in South Korea. However, these researchers used a much higher dose (2.5 times the dose used in the current trial). However it must be pointed out that in their study, Won-Chang Lee *et al*. (1996) did not include a control group of animals in their trial to adequately compare the effect of *Corynebacterium cutis* lysate and jumped to the conclusion that this inoculant effectively reduced the SCC in dairy cows. These results lead the manufacturers of Ultra-Corn® to claim its immuno-stimulant properties capable of reducing SCC in dairy cows. These expectations were the motivation of at least 2 studies in South Africa to verify Won-Chang Lee's *et al*. (1996) results, and evaluate the potential value of Ultra-Corn® in the South African market.

If one looks only at the treated group of cows on Farm A, a significant ($P<0.05$) reduction in SCC was also observed during the first 6 weeks of this trial, as reported by Won-Chang Lee *et al*. (1996). However, in the present trial, a significant ($P<0.05$) reduction in SCC was also observed in the control group during the same period, making the differences between the treated and the control groups on both farms not significantly statistical.

The only possible explanation for such significant reductions in SCC in both groups on farm A (treated and control), during the first 6 weeks of this first trial is that these reductions in SCC of dairy cows were a result of other unaccounted factors. Most probably environmental factors (i.e. temperature, rainfall and humidity). This trial started at the end of summer and ended in the middle of autumn, a period during which the SCC is known to decrease naturally as a result of less favourable environmental conditions (dryer and cooler day temperatures) for bacteria survival.

During week 7 on Farm A and weeks 7 and 8 on Farm B there was a sharp increase in the mean SCC's from the cows in both experimental groups (treated and control). This was caused by extremely high SCC increases, recorded in some cows from both groups, for reasons unrelated to the effect of the *Corynebacterium cutis* lysate treatment under evaluation. It must be said that during week 7 of the first trial unexpected high rainfall was recorded in the study area, which may be responsible for these sharp increases in SCC of the dairy cows. This demonstrates once again that environmental factors play a major role on the SCC of dairy cows – a much greater one than that of the Ultra-Corn®.dosage used in this study.

Some caution must be taken when interpreting the results of this study, as a much lower dose of Ultra-Corn® was used than that reported by Won-Chang Lee *et al*. (1996). Further research is warranted to properly evaluate the potential use of Ultra-Corn® (*Corynebacterium cutis* lysate), using higher dosages of the inoculant.

4.1.2 Milk Quality

Table 4.2 depicts the overall mean (± s.d.) protein, butterfat, lactose and urea percentages of the solid content of the milk from both dairy farms (A & B) during the

8 weeks of the first trial period, following three weekly inoculations with *Corynebacterium cutis* lysate (80mg/cow).

Table 4.3 Mean (± s.d.) protein, butterfat, lactose and urea content of milk in dairy cows from two farms during an 8 week trial period following three weekly inoculations with *Corynebacterium cutis* lysate (80mg/cow)

Milk Quality Components	Cows in Farm A		Cows in Farm B	
	Control	Treated	Control	Treated
Protein %	$3.085^{a} \pm 0.02$	$3.022^{a} \pm 0.14$	$3.357^{a} \pm 0.08$	$3.415^{a} \pm 0.08$
Butterfat %	$3.644^{a} \pm 0.27$	$3.364^{b} \pm 0.24$	$3.364^{a} \pm 0.21$	$3.398^{a} \pm 0.24$
Lactose %	$4.554^{a} \pm 0.07$	$4.593^{a} \pm 0.07$	$4.791^{a} \pm 0.06$	$4.871^{a} \pm 0.09$
Urea %	$19.302^{a} \pm 0.71$	$17.849^{b} \pm .06$	$15.169^{a} \pm 1.71$	$14.950^{a} \pm 1.7$

a, b - Means within rows for the same farm differ significantly at $P<0.05$

The results obtained in this first trial, in terms of milk quality, showed no significant differences in none of the main milk solid components on Farm B. However, slightly different results were observed on Farm A - where significantly lower ($P<0.05$) butterfat and urea percentages were recorded in the milk of the Holstein cows inoculated with the *Corynebacterium cutis* lysate (80mg/cow). If these reductions are the result of the inoculation these can be considered as adverse effects of the inoculation or vaccination with the *Corynebacterium cutis* lysate. However, as contradictory results were found on the two farms used, no meaningful conclusions can be drawn from these results in terms of any adverse effects of using this inoculant in dairy cows.

Small differences in milk solid composition between the two farms during the 8 weeks of the trial were observed after the milk was analysed. The most possible reasons for these differences may be the differences in feeding diets used in both farms. Small fluctuations were also observed within farms, which are probably caused by small variations in the diets. Particularly in Farm A, the urea content was higher than the acceptable limit of 16% reported by most researchers (Jonker *et al.,*

1999; Kohn, 2007), indicating an energy to protein imbalance in the diet. In simple terms, high milk urea concentrations indicated in general, an excess in protein (N) intake relative to the energy in the diet and/or to the animal's milk production (Kohn 2007). Excess N may be the result of excess protein. A diet with higher energy content may be needed to assure a more efficient usage of protein (the most expensive component of a dairy cow's diet). This would lead to higher milk yield and lower protein wastage.

4.2 TRIAL 2 - Effects of a high dose of Ultra-Corn® (40mg/100kg of Corynebacterium cutis lysate per cow) on milk SCC and composition

Due to the fact that no significant differences were recorded during the first trial using 80mg *Corynebacterium cutis* lysate/cow, irrespective of the weight of the cow, it was decided to repeat the trial, using a higher dose of the *Corynebacterium cutis* lysate. In this case, the same dose as that used by Won-Chang Lee *et al*. (1996) in his trial (40mg/100kg of *Corynebacterium cutis* lysate per cow) was implemented and a prolonged experimental period (observation period) of 12 weeks were used.

4.2.1 Somatic Cell Counts

The mean (± s.d.) milk SCC for Holstein cows during the 12 weeks of this second trial period, from both groups (treated and control) on farms A and B are presented in Tables 4.4 and 4.5, respectively. The milk SCC fluctuations during the same period are depicted in Figure 4.3 and 4.4, for Holstein the cows on farm A and B, respectively.

The results obtained in this trial, using the recommended dose of Ultra-Corn® (2 ml/100 kg or 40mg of *Corynebacterium cutis* lysate per 100kg body weight per cow*)* were very similar to those obtained in Trial 1, using a lower dose (4ml/ cow).

Only at the end of week 5, a significant lower mean SCC was recorded in the treated cows (140 ± 1.4 $x10^3$ cells/ml), when compared to the control non-treated cows (231.7 ± 6.7$x10^3$ cells/ml). However, as this was an isolated observation and no definite trend could be established, this result cannot be linked to the effect of the

Corynebacterium cutis lysate inoculation with certainty. The P value obtained was 0.049, just below the 95% confidence level used in the ANOVA test. From week 5 onwards until the termination of the trial on week 12, no other significant differences in the mean SCC were observed between groups. In fact in some weeks, the treated cows recorded on average higher SCC's than the control group (Table 4.4 and 4.5).

Table 4.4 Mean (± s.d.) somatic cell count (SCC) (x 10^3) in Holstein cows on Farm A during a 12 week trial period following three weekly inoculations with 20mg of *Corynebacterium cutis* lysate per 100kg bodyweight per cow

Weeks in trial	Group of cows	
	Control (N=7)	Treated (N=7)
1* ns	271.8 ± 3.8	246.7 ± 4.2*
2* ns	198.0 ± 5.7	153.8 ± 3.1*
3* ns	198.8 ± 3.1	197.5 ± 2.4*
4 ns	336.0 ± 3.1	415.7 ± 3.0
5 ns	231.7 ± 6.7	140.0 ± 1.4
6 ns	131.4 ± 3.0	111.5 ± 1.3
7 ns	111.6 ± 1.3	119.7 ± 1.9
8 ns	129.3 ± 9.4	914.0 ± 1.2
9 ns	472.9 ± 1.1	1656.1 ± 7.9
10 ns	152.9 ± 7.4	230.0 ± 7.0
11 ns	180.5 ± 2.6	158.2 ± 4.3
12 ns	177.1 ± 2.3	193.0 ± 2.4

ns- Means within rows do not differ significantly (P>0.05); *- Weeks when *Corynebacterium cutis* lysate was inoculated into the cows in the treated group

Table 4.5 Mean (± s.d.) somatic cell count (SCC) (x 10^3) in Holstein cows on Farm B during a 12 week trial period following three weekly inoculations with 20mg of *Corynebacterium cutis* lysate per 100kg bodyweight per cow

Weeks in trial	Group of cows	
	Control N=13	Treated N=13
1* ns	185.8 ± 1.6	726.9 ± 1.0
2* ns	822.5 ± 5.2	724.7 ± 2.7
3* ns	1159.8 ± 2.5	1048.0 ± 2.1
4 ns	673.2 ± 7.1	1729.4 ± 1.0
5 ns	1303.6 ± 6.0	1330.2 ± 7.2
6 ns	615.0 ± 7.9	1250.9 ± 1.0
7 ns	691.6 ± 1.9	668.7 ± 8.8
8 ns	170.3 ± 4.1	1144.2 ± 1.4
9 ns	793.0 ± 1.6	503.3 ± 7.0
10 ns	1489.3 ± 3.0	1644.0 ± 2.5
11 ns	5367.5 ± 1.4	3451.8 ± 7.1
12 ns	992.0 ± 9.8	1675.1 ± 3.3

ns- Means within rows do not differ significantly (P>0.05); *- Weeks when *Corynebacterium cutis* lysate was inoculated into the cows in the treated group

As stated before, the SCC in the dairy cows were affected by many factors. In this case, it was impossible to establish the reasons for the result in week 5 on Farm A. In the dairy cows, the variation in the SCC was large, making it difficult to find statistical significant effects of any treatments intended to reduce SCC. In order to increase the chance of observing significant effects of Ultra-Corn® (*Corynebacterium cutis* lysate inoculations) in the milk SSC of dairy cows, a much higher number of experimental units should be used. Preliminary estimations using the data obtained in this study indicate that the ideal sample size is in thousands of cows, making such a study impractical.

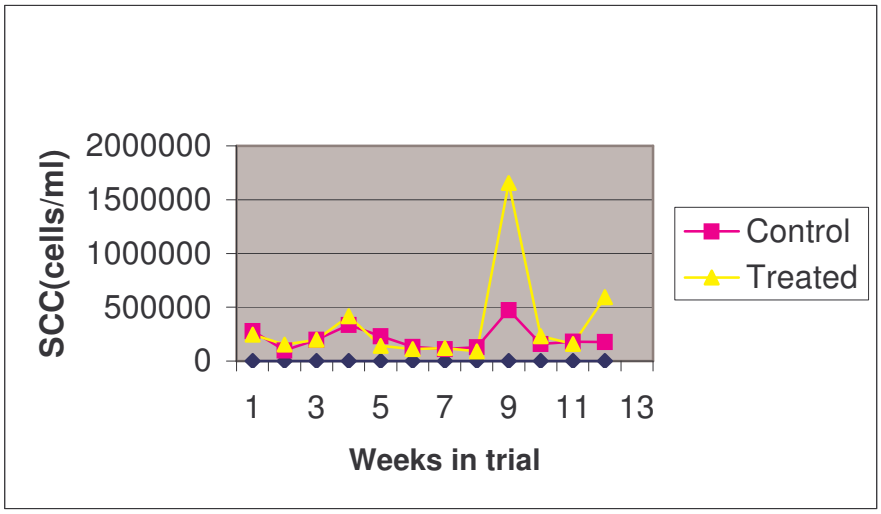

Figure 4.3 **Mean SCC in milk of Holstein cows during a 12 week trial following three weekly inoculations with *Corynebacterium cutis* lysate (20mg/100 kg of body weight per cow) on Farm A**

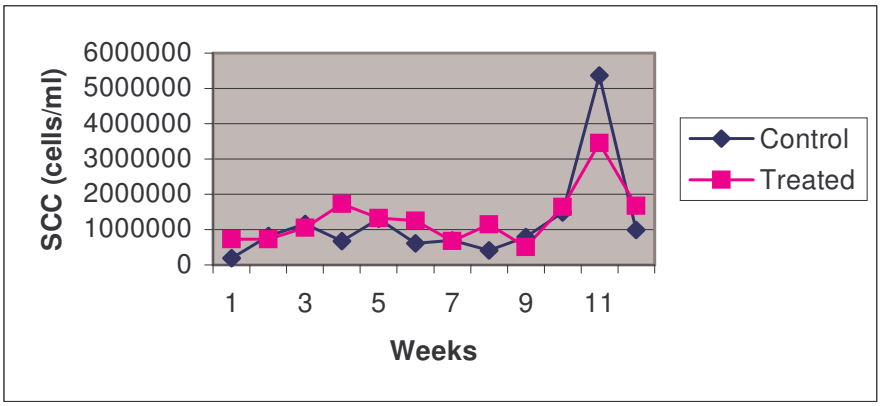

Figure 4.4 **Mean SCC in milk of Holstein cows during a 12 week trial following three weekly inoculations with *Corynebacterium cutis* lysate (20mg/100 kg of body weight per cow) on Farm B**

4.2.2 Milk Quality

Table 4.6 depicts the overall mean (± s.d.) protein, butterfat, lactose and urea percentages of the solid content of the milk from both dairy farms (A & B) during the 12 weeks of the second trial period, following three weekly inoculations with *Corynebacterium cutis* lysate (20mg/100 kg body weight per cow).

Table 4.6 Mean (± s.d.) protein, butterfat, lactose and urea contents of milk in dairy cows from two farms during an 8 week trial period following three weekly inoculations with *Corynebacterium cutis* lysate (20mg/100 kg of body weight per cow)

Milk Quality Components	Cows in Farm A		Cows in Farm B	
	Control	Treated	Control	Treated
Protein% [ns]	3.012 ± 0.09	3.029 ± 0.14	3.357 ± 0.08	3.415 ± 0.08
Butterfat % [ns]	3.344 ± 0.32	3.364 ± 0.24	3.364 ± 0.21	3.398 ± 0.24
Lactose% [ns]	4.612 ± 0.07	4.608 ± 0.07	4.791 ± 0.06	4.871 ± 0.09
Urea% [ns]	16.904 ± 0.09	16.849 ± 0.06	15.169 ± 1.71	14.950 ± 1.7

ns- Means within rows do not differ significantly (P>0.05)

The results obtained in this second trial, in terms of milk quality, yielded similar results to those found in Trial 1. No significant differences were recorded in the main milk solid components between the experimental groups on both farms. These results clearly indicate that there are no detrimental effects of *Corynebacterium cutis* lysate inoculations in the milk quality of dairy cows.

Small differences in milk solid composition (not statistical different) were recorded between the two farms during this 12 week period of the trial. The most likely reasons for these differences could be the differences in nutritional diets used in both farms. Small fluctuations were also observed within farms, which are probably due to small variations in the diets.

Again in Farm A, the urea content was slightly higher than the acceptable limit of 16% reported by most researchers (Kohn, 2007; Jonker *et al.,* 1999), but much lower than that during Trial 1, indicating a small energy to protein imbalance in the diet.

4.3 Conclusions

In this study, the immuno-stimulant effects of Ultra-Corn®, a *Corynebacterium cutis* lysate could not be confirmed in lactating Holstein dairy cows with SCC's over 250 000 cells/ml of milk. Although this inoculant does not seem to have any detrimental effects on the main solids of the milk, its use cannot be justified as it did not significantly reduce the somatic cell counts in lactating cows.

The results of this study also confirmed the large variation in SCC in dairy cows and once again demonstrated that environmental factors play a major role on the SCC of dairy cows – a much greater one than that of Ultra-Corn®. The individual variations in SCC's make it difficult to find statistical effects of any treatments intended to reduce the SCC in dairy cows.

Further research is warranted to develop and evaluate the effectiveness of vaccines against mastitis-causing organisms, in order to control SCC and mastitis in dairy cows. However, when such studies are conducted it is advisable to use very high numbers of experimental units, while proper control trials should be conducted. All efforts should be done to ensure minimum environmental changes during these trials, which can introduce serious confounding effects in the experimental design.

THE EFFECT OF *CORYNE BACTERIUM CUTIS LYSATE* TO CONTROL SOMATIC CELL COUNTS IN DAIRY COWS

by

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ABSTRACT

The main aim of this study was to evaluate the effectiveness of repeated inoculations of a *Corynebacterium cutis* lysate (Ultra-Corn®) - a non-specific immune-stimulant, to reduce the milk SCC in commercial dairy cows. An additional aim was to evaluate if these inoculations had any detrimental effects on milk quality.

This study was performed in two separate trials, using Holstein cows with SCC's over 250 000 cells/ml of milk at different stages of lactation from two commercial dairy farms in the Free State Province. On each farm, cows were paired according days in milk and SCC, in order to obtain two homogeneous groups of experimental animals. The two groups of cows in each farm were randomly allocated to a treatment or a control group. Both groups in the same farm were managed under the same conditions for the entire trial periods. The only difference was that cows from the treatment group received 3 weekly inoculations of *Corynebacterium cutis* lysate(Ultra-Corn®), while those from the control group received distilled water for injection (the same volume as the cows in the treatment group).

Two similar trials were conducted, using the same basic experimental design. Differences were only in the dose of the *Corynebacterium cutis lysate* inoculated per cow treated, number of experimental animals and duration of the observation periods. In Trial 1, cows from the treated group received 3 weekly vaccinations of Ultra-Corn® (4 ml per cow, thus 80mg of *Corynebacterium cutis lysate* per cow) injected subcutaneously (sc), while those from the control group received 3 weekly sc injections of 4 ml distilled water. This was followed by 8 weeks of observation of the effect of treatment on milk SCC and composition. In Trial 2, the three doses of *Corynebacterium cutis lysate* administered weekly per cow for the treated group was 2ml/100kg, thus 40mg *Corynebacterium cutis lysate*/100 kg per cow. This was followed by 8 weeks of observation of the effect of treatment on milk SCC and composition.

Individual quarter milk samples were collected weekly from all cows and analysed for SCC and a combined milk sample (from the measuring bottle in the milk parlour) from each cow was also taken for butterfat, protein, lactose and urea content. The results were compared between the two groups per farm, using ANOVA procedures for repeated measures analysis, using the 95% confidence level (SAS, 2004).

The two farms were evaluated separately, due to the possible differences between general management conditions, which could introduce serious confounding factors if the results from the two farms were combined. However, it can be considered that both dairy farms used an acceptable level of commercial dairy management practices and produced an acceptable yield per cow under South African commercial conditions.

In general no significant differences were recorded between the treated and control groups of cows in both farms in both trials in terms of milk SCC, butterfat, protein, lactose and urea content of the milk. In this study, the immuno-stimulant effect of Ultra-Corn®, a *Corynebacterium cutis* lysate could not be confirmed in lactating cows. Although this inoculant does not seem to have any detrimental effects on the

main solids of the milk, its use cannot be justified as it did not significantly reduce somatic cell counts in lactating cows.

Further research is warranted to develop and evaluate the effectiveness of vaccines against mastitis causing organisms, in order to control SCC and mastitis in dairy cows. However, when such studies are conducted it is advisable to use very high number of experimental units and proper control trials should be conducted. All efforts should be done to ensure minimum environmental changes during these trials, which can introduce serious confounding effects in the experimental design.

Key words: Cows, Dairy, Mastitis, Somatic Cell Counts, Milk Quality, Protein, Butterfat, Lactose, Urea, Holstein, *Corynebacterium cutis* lysate, Ultra-Corn®, Immuno-stimulant, vaccination against mastitis.

DIE EFFEK VAN *CORYNE BACTERIUM CUTIS LYSAAT* OM DIE SOMATIESE SELTELLINGS IN MELKKOEIE TE BEHEER

deur

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OPSOMMING

Die doel van die studie was om die effektiwiteit van herhaalde inentings van *Coryne bacterium cutis* lysaat (Ultra Corn®) te evalueer, 'n nie- spesifieke immuno stimulant, om die melk SST te verander in kommersiële melkbeeste. 'n Addisionele doelwit was om te evalueer of hierdie inentings enige nadelig effek op melkkwaliteit het. Hierdie studie is uitgevoer in twee aparte proewe deur gebruik te maak van SST's van meer as 250 000 selle/ml melk in verskeie stadia van laktasie, op twee kommersiële melkplase in die Vrystaatprovinsie. Op elke plaas was koeie geallokeer volgens dae in melk en SST om 'n homogene groep eksperimentele diere te kry. Die twee groepe diere op elke plaas is ewekansig toegewys aan 'n behandeling en kontrole groep. Groepe op dieselfde plaas is bestuur onder dieselfde toestande vir die proeftydperk. Al verskil was dat die koeie van die behandelingsgroep 3 weeklikse inentings van *Coryne bacterium cutis* lysaat (Ultra Corn®) ontvang het, terwyl die kontrole groep gedistilleerde water as inenting ontvang het (dieselfde volume per koei). Twee soortgelyke proewe is uitgevoer deur dieselfde eksperimentele ontwerp te gebruik. Verskille was slegs die dosis *Coryne bacterium cutis* lysaat per dier, die aantal eksperimentele diere en die tydsduur van die observasieperiode. In die eerste proef het die koeie in die behandelde groep 3 weeklikse inentings van Ultra Corn® (4ml/ koei; dus 80mg van die *Coryne bacterium*

cutis lysaat per koei) onderhuids ontvang. Die kontrole groep het 3 weeklikse inspuitngs van 4 ml gedistilleerde water ontvang. Dit is gevolg deur ‘n 8 weke observasie periode om die effek van die behandeling op melk SST en samestelling te monitor. In die tweede proef is 3 behandelings van *Coryne bacterium cutis* lysaat weekliks toegedien per koei, die dosis was 2 ml/100 kg, dus 40mg/100kg *Coryne bacterium cutis* lysaat/100kg per koei. Dit is gevolg deur 12 weke van waarneming van die effek van behandeling op SST en melksamestelling.

Individuele uierkwart melkmonsters is weekliks geneem van alle koeie en geanaliseer vir SST en gesamentlike monsters (van al die kwarte in die maatbottel in die melkstal), van elke koei is ook geneem vir bottervet, proteien, laktose en urea inhoud. Die resultate is vergelyk tussen groepe per plaas deur gebruik te maak van die ANOVA prosedure vir herhaalde meetings by die 95% sekerheidsvlak (SAS, 2004). Die twee plase is apart geëvalueer a.g.v. verskille in algemene bestuurs praktyke wat moontlike verstrengelde effekte kan veroorsaak as die twee plase se resultate saamgegooi is. Dit moet egter beklemtoon word dat beide melkplase ‘n aanvaarbare vlak van bestuur en produksie gehandhaaf het. Oor die algemeen was geen betekenisvolle verskille waargeneem tussen die behandelde en kontrole groepe op beide plase in terme van SST, bottervet, proteien, laktose en urea inhoud. In hierdie studie kon die immuno-stimulant effek van die *Coryne bacterium cutis* lysaat nie bevestig nie word vir lakterende koeie. Alhoewel die inokulant geen nadelige effek op die bestandele van melk blyk te hê nie, kan die gebruik daarvan nie regverdig word nie, aangesien dit nie die somatiese seltellings in lakterende koeie verlaag nie.

Verder navorsing is geregverdig om die effektiwiteit van entstowwe teen mastisis-vormende organismes te ontwikkel om SST en mastitis in melkkoeie te beheer. As sulke studies uitgevoer word is dit wesenlik om ‘n hoë aantal eksperimentele diere te gebruik en gekontroleerde proewe uit te voer. Alle pogings moet aangewend word om die minimum ongewensde verandering tydens die proewe te hê, wat kan lei tot verstrengelde effekte in die eksperimentele ontwerp.

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